FOOD INNOVATION ASIA CONFERENCE 2022

PROCEEDINGS

The 24th Food Innovation Asia Conference 2022 (FIAC 2022)

THEME :

Innovative and sustainable development of functional ingredients and materials : Benefits, concerns and challenges in human health and well-being

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June 16-17, 2022

The 24th Food Innovation Asia Conference 2022 (FIAC 2022)

Innovative and Sustainable Development of Functional Ingredients and Materials: Benefits, Concerns and Challenges in Human Health and Well-being

June 16-17, 2022

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CONFERENCE PROGRAM

Day 1: June 16, 2022

Opening Remark and Keynote Speeches MC: *Assist. Prof. Dr. Wasaporn Preteseille Chanput* *Assoc. Prof. Dr. Natdanai Harnkarnsujarit* Log In 09.00 - 09.15 09.15 – 12.00	
09.15 - 09.30	**Opening remark** *Assistant Professor Dr. Anadi Nitithamyong, President of FoSTAT* **Welcome remark** *Associcate Professor Dr. Anuvat Jangchud, Dean of Faculty of Agro-Industry, Kasetsart University, Thailand* **Welcome remark** *Associcate Professor Jakraphong Phimphimol, Dean of Faculty of Engineering and Agro-Industry, Maejo University, Thailand*
Group photo	
09.30 - 10.00	**Keynote speech 1:** Potential of targeted prebiotics for improved gut health By *Distinguished Prof. Bruce R. Hamaker, Department of Food Sciences, Purdue University, USA*
10.00 - 10.30	**Keynote speech 2:** Enabling sustainable healthy use of food materials: recent New Zealand food engineering research By *Prof. Richard Archer, School of Food and Advanced Technology, Massey University, New Zealand*
10.30 - 11.00	**Keynote speech 3:** The design of food matrix structure to develop healthier and more sustainable foods By *Prof. Pete Wilde, Quadram Institute, UK*

Day 1: June 16, 2022

Poster Session 1 12.10 - 12.45	
12.10 - 12.15	**AB-P002:** Antioxidative activity of gelatin hydrolysate from threadfin bream skin as influenced by the degree of hydrolysis and concentration By *Acting Sub Lt. Sakonwat Kuepethkaew (Biotechnology program, Thaksin University)*
12.15 - 12.20	**AJ-P009:** Anti-inflammatory effects of snow mushroom (*Tremella fuciformis*) drinks on RAW 264.7 macrophages stimulated with lipopolysaccharide By *Ms. Kwanchanok Hunthayung (Department of Food research, CPF FOOD RESEARCH AND DEVELOPMENT CENTER CO., LTD)*
12.20 - 12.25	**AB-P010:** Effects of packaging method on the chemical and microbiological properties of fermented fish paste (Ka-pi-plaa) produced from beardless barb (*Cyclocheilichthys apogon*) By *Mrs. Pakteera Sripokar (Faculty of Agro and Bio Industry, Thaksin University)*
12.25 - 12.30	**AP-P013:** Optimization of arabica raw coffee beans (*Coffea Arabica* L.) hydrolysate with nitric oxide inhibitory activity using response surface methodology (RSM) By *Ms. Papassara Sangtanoo (The Institute of Biotechnology and Genetic Engineering, Chulalongkorn University)*
12.30 - 12.35	**AB-P014:** Effect of sacha inchi (*Plukenetia volubilis* L.) shell extract concentration on the prevention of lipid oxidation during sacha inchi oil storage By *Ms. Arthittaya Thuanthong (Biotechnology program, Thaksin University)*
12.35 – 12.40	**AB-P015:** Extraction and biochemical properties of cysteine proteinase inhibitors from Sangyod rice (*Oryza sativa* L.) By *Ms. Arthittaya Thuanthong (Biotechnology program, Thaksin University)*

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Poster Session 2 12.10 - 12.45	
12.10 - 12.15	**AP-P020:** Comparison of conventional and ultrasound-assisted extraction techniques of betacyanins from red dragon fruit peels (*Hylocereus polyrhizus* L.) By *Ms. Witavanee Tappreyakunkarn (Department of Food Technology, Chulalongkorn University)*
12.15 - 12.20	**AB-P026:** Effects of drum drying on physical and pasting properties of three different pigmented rice flour By *Ms. Felicia Magdalena Kweesar (Department of Food Technology, Indonesia International Institute for Life Sciences (i3L))*
12.20 - 12.25	**AP-P030:** Identification of physicochemical properties, and bioactive compounds of riceberry rice flour from different cultivation areas in Thailand By *Ms. Yaniss Khidprasert (Institute of Nutrition, Mahidol University)*
12.25 - 12.30	**AP-P031:** Effect of precooking on physiochemical properties, nutritional values, total phenolic compounds and antioxidant activities of durian seed flour By *Ms. Ranchida Tanyawangrat (Nutrition and Dietetics, Mahidol University)*
12.30 - 12.35	**AP-P032:** Encapsulation by spray drying of porcine placenta extract (fat - removed vs non-fat removed): Effects on characteristics and antioxidant activities By *Ms. Pornpassorn Chulalaksananukul (Department of Food technology, Chulalongkorn University)*
12.35 – 12.40	**AB-P040:** Encapsulation properties and digestibility of encapsulated aloe vera bioactive polysaccharides as functional ingredient By *Dr. Waraporn Sorndech (Expert Center of Innovative Health Food, Thailand Institute of Scientific and Technological Research)*
12.40 – 12.45	**AP-P047:** Effects of ultraviolet (UV) irradiation on quality attributes and microorganism's inactivation in mulberry juice By *Ms. Nichakarn Uaphua (Department of Food Technology, Chulalongkorn University)*

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Poster Session 3 12.10 - 12.40	
12.10 - 12.15	**AB-P070:** The protein extraction from three kinds of Thai legumes By *Dr. Chiramet Auranwiwat (Expert Center of Innovative Herbal Products, TISTR)*
12.15 - 12.20	**AB-P071:** Synergistic effects of *Thunbergia lauriforia* Linn. (Rang Chuet) and *Zingiber officinale* (Ginger) through cellular antioxidative activities in HepG2 cells By *Ms. Chattip Sunthrarak (Depsrtment of Food technology, Suranaree University of Technology)*
12.20 - 12.25	**AB-P072:** Effect of defatting process on chemical properties and flavor profile of cricket protein powder By *Dr. Natnirin Booranasakawee (Expert Centre of Innovative Health Food (InnoFood), Thailand Institute of Scientific and Technological Research (TISTR))*
12.25 - 12.30	**AB-P076:** The preparation process of tea from *Cannabis sativa* L. Leaves By *Asst. Prof. Dr. Ratchadaporn Oonsiviali (Department of Food technology, Suranaree University of Technology)*
12.30 – 12.35	**AP-P084:** Valorization of overripe 'Kluai Namwa' banana fruit as a raw material to produce bacterial cellulose and carboxymethylcellulose By *Asst. Prof. Dr. Supaporn Sophonputtanaphoca (Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University)*
12.35 – 12.40	**AP-P093:** Effect of enzymatic hydrolysis by Flavourzyme® on degree of hydrolysis, solubility, and antioxidant properties of freshwater prawn hydrolysates By *Ms. Chatchanok Paisansak (Department of Food Technology, Chulalongkorn University)*
12.40 – 12.45	**AP-P101:** The effect of drying processing on physico-chemical properties in corn silks By *Asst. Prof. Dr. Nicha Kawila (Department of Agricultural Technology, Nakhon Sawan Rajaphat University)*

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Poster Session 4 12.10 - 12.40	
12.10 - 12.15	**AB-P119:** Black rice leaves trigger apoptosis in hepatocellular carcinoma (HepG2) by inducing S-phase cell cycle arrest By *Dr. Chorpaka Thepthanee (Department of food science, National Pingtung University of Science & Technology)*
12.15 - 12.20	**BJ-P039:** Enhanced extraction of xanthone and tannin from mangosteen peel using subcritical fluid technique By *Prof. Mohammad Naghi Eshtiaghi (Department of Chemical Engineering, Mahidol University)*
12.20 - 12.25	**BP-P048:** Effect of pre-treatment on the chemical compositions of skipjack tuna (*Katuwonus pelamis*) bones By *Ms. Sirinya Jaroenchai (New product research and development, T T T HOLDING CO., LTD.)*
12.25 - 12.30	**BP-P064:** Effect of storage temperature on the change of ice crystal size in ice cream for long-term storage By *Ms. Misaki Shiba (Course of Food Science and technology, Graduate School of Marine Science)*
12.30 - 12.35	**EB-P003:** Utilization of Multi-Locus Sequence Typing – MLST for serotyping of *Listeria monocytogenes* in the food supply chain By *Dr. Sudarat Ledlod (Research department, CPF Food and Research Development Center CO., Ltd)*
12.35 – 12.40	**EJ-P054:** Investigation of parameters causing can bulging during pasteurization process of carbonated beverage By *Miss Ruthaikamol Thongon (Faculty of Science, Mahidol University)*

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Poster Session 5 12.10 - 12.40	
12.10 - 12.15	**EJ-P057:** Investigation of glass bottle surface coating quality using contact angle technique By *Mr. Siwat Patamakam (Faculty of Science, Mahidol University)*
12.15 - 12.20	**EJ-P058:** Effects of ground delivery via postal service on integrity of packaging system for food products By *Dr. Thitisilp Kijchavengkul (Faculty of Science, Mahidol University)*
12.20 - 12.25	**EB-P079:** Novel functional packaging containing sodium nitrite and sodium erythorbate to preserve quality of fresh beef By *Ms. Phanwipa Wongphan (Department of Packaging and Materials Technology, Kasetsart University)*
12.25 – 12.30	**EP-P098:** Development of plant-based minced meat from firm tofu and banana blossom By *Ms. Thawankon Khamya (Food Science and Technology Program, Mae Fah Luang University)*
12.30 – 12.35	**EP-P107:** Influence of phenolic from lime peel on xyloglucan-chitosan film properties By *Ms. Wipharat Thaksin (Faculty of Food Industry, King Mongkut's Institute of Technology Ladkrabang)*
12.35 – 12.40	**EP-P113:** Quality changes of fingerroot (*Boesenbergia rotunda* (L.) Mansf.) juice mixed with honey and lime juice as affected by high pressure processing By *Asst. Prof. Dr. Phunsiri Suthiluk (School of Agro-Industry, Mae Fah Luang University)*

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<table>
<tr><td colspan="2">Division A: Oral Presentation
(Food Chemistry, Nutrition, and Analysis)

Chair: Assoc. Prof. Dr. Utai Klinkesorn, Kasetsart University
Co-Chair 1: Assoc. Prof. Dr. Savitree Ratanasumawong, Kasetsart University
Co-Chair 2: Assoc. Prof. Dr. Wichittra Daengprok, Maejo University

Log In 12.45 - 12.55

13.00 – 16.00</td><td colspan="2">Poster Presentation Competition

Chair: Assoc. Prof. Dr. Sopark Sonwai, Silpakorn University
Secretary: Asst. Prof. Dr. Theeraphol Senphan, Maejo University
Assistant secretary: Dr. Chitraporn Ngampeerapong, Maejo University

Log In 12.45 - 12.55

13.00 – 16.00</td></tr>
<tr><td>13.00 - 13.20</td><td rowspan="2">Invited speech 1: Milk intake and cardiometabolic traits - a study in ~2 million people
By Prof. Dr. Vimal Karani, Department of Food and Nutritional Sciences, University of Reading, UK</td><td>13.00 - 13.10</td><td>CP-P059: Study of consumer behavior of tofu from jackfruit seeds and chemical analysis of jackfruit seeds flour from different preparation methods
By Aritsara Chimin (Thammasat University)</td></tr>
<tr><td></td><td></td><td>13.10 - 13.20</td><td>CP-P062: Observation on consumer behavior and market trend for health promoting products with fiber fortification
By Pimpadchara Nuchchareonpaiboon (Department of Product Development, Kasetsart University)</td></tr>
<tr><td>13.20 – 13.40</td><td rowspan="2">Invited speech 2: Water lentil: from duck till dawn of the tiny green superfood
By Assoc.Prof. Dr. Chalat Santivarangkna, The Institute of Nutrition, Mahidol University, Thailand</td><td>13.20 - 13.30</td><td>BP-P064: Effect of storage temperature on the change of ice crystal size in ice cream for long-term storage
By Misaki Shiba (Graduate School of Marine Science, Tokyo)</td></tr>
<tr><td></td><td></td><td>13.30 - 13.40</td><td>AB-P071: Synergistic effects of Thunbergia lauriforia Linn. (Rang Chuet) and Zingiber officinale (Ginger) through cellular antioxidative activities in HepG2 cells
By Chattip Sunthrarak (Suranaree University of Technology)</td></tr>
</table>

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Division A: Oral Presentation		**Poster Presentation Competition**	
13.40 – 13.55	**AP-O018:** Molecular signatures of Thai red wine revealed by a non-targeted 1H-NMR metabolomics approach By *Ms. Suwanan Denchai (Department of Biotechnology, Chulalongkorn University)*	13.40 - 13.50	**EB-P079:** Novel functional packaging containing sodium nitrite and sodium erythorbate to preserve quality of fresh beef By *Phanwipa Wongphan (Kasetsart University)*
13.55 – 14.10	**AB-O024:** Antioxidative and cryoprotective properties of gelatin hydrolysate from salmon (*Oncorhynchus nerka*) skin By *Acting Sub Lt. Sakonwat Kuepethkaew (Biotechnology program, Thaksin University)*	14.00 - 14.10	**CB-P094:** Chocolate ice cream with added silkworm-pupae emulsion has attractive market potential; findings from in-depth interview and formulation optimization By *Teerapong Nantasiri (Mahidol University International College)*
14.10 – 14.20	***Break (10 min)***		
14.20 – 14.35	**AJ-O028:** Optimization of instant cereal beverage utilizing three drum dried pigmented rice using response surface methodology By *Ms. Felicia Magdalena Kweesar (Department of Food Technology, Indonesia International Institute for Life Sciences (i3L))*		
14.35 – 14.50	**AP-O074:** An application of Plackett – Burman design in screening essential amino acids increasing myotube hypertrophy By *Mr. Sukan Braspaiboon*		
14.50 – 15.05	**AP-O081:** Effects of ultrasound-assisted extraction on total phenolics and antioxidant activity of *Wolffia globosa* (Khainam) extracts By *Ms. Tananya Sai-O*		

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Division F: Industrial Session From Farm to Glamorous Coffee Flavor: Sustainable Value Chain of Coffee in Thailand *(This session will be conducted in Thai)* This session is free of charge, please see details and register at link: https://forms.gle/EZBXLxJaaevM4VFi7 Chair: *Assoc. Prof. Dr. Sittiwat Lertsiri, Mahidol University* Co-Chair: *Asst. Prof. Dr. Yaowapa Lorjaroenphon , Kasetsart University* Log In: 12.45 - 12.55 13.00 - 16.30	
13.00 - 13.30	อนาคตกาแฟไทย โดย ผศ.ดร. อัครวิทย์ กาญจนโอภาษ รองผู้อำนวยการศูนย์บริหารจัดการเทคโนโลยี สวทช./ CEO เมืองนวัตกรรมอาหาร
13.30 - 14.30	Panel discussion: ห่วงโซ่มูลค่ากาแฟไทย Moderator: ผศ.ดร. อัครวิทย์ กาญจนโอภาษ ผู้ร่วมเสวนา - ดร. อมร โอวาทวรกิจ สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง - คุณณฐ เตชะวรรณเวคิน ผู้จัดการทั่วไป บริษัท ทรูไลฟ์สไตล์รีเทล จำกัด - ผศ.ดร. อภิชญา ลีลาวนิชกุล คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์
14.30-14.40	**Break**
14.40-15.40	Panel discussion: มุมมอง, การทดสอบกาแฟ, รสชาติเอกลักษณ์กาแฟ Moderator: รศ.ดร. สิทธิวัฒน์ เลิศศิริ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล, Flavor Academy ผู้ร่วมเสวนา - รศ.ดร. ธงชัย สุวรรณสิชฌน์ - รองผู้อำนวยการหน่วยบริหารและจัดการทุนด้านการเพิ่มความสามารถในการแข่งขันของประเทศ (บพข.), Flavor Academy - คุณกรวิกา ยังประภากร - SCA Authorized Trainer, WCE certified judge, Q arabica grader - นพ. สุรเวช น้ำหอม - Q Arabica, Q Robusta Grader, Authorized SCA Trainer, Roast & Learn Coffee Roasters.
15.40-16.10	Flavor Analysis for Coffee - ดร. เจษฎา วิชาพร คณะเกษตรศาสตร์ ทรัพยากรธรรมชาติและสิ่งแวดล้อม มหาวิทยาลัยนเรศวร, Flavor Academy - ผู้แทนจาก Gerstel ประเทศเยอรมนี
16.10-16.40	Safety Concern in Coffee Value Chain - ผศ.ดร. กนิฐพร วังใน คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์

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Division B: Oral Presentation (Food Processing and Engineering) Chair: *Dr. Jatuporn Aroonkamonsri, Rajamangala University of Technology Tawan-ok* Co-Chair 1: *Asst. Prof. Dr. Kornpaka Arkanit, Maejo University* Co-Chair 2: *Assoc. Prof. Dr. Weerachet Jittanit* Log In: 8.45 - 8.55 9.00 - 12.00		**Oral Presentation Competition** Chair: *Assoc. Prof. Dr. Waraporn Boonsupthip, Kasetsart University* Secretary: *Assoc. Prof. Dr. Kitiya Vongkamjan Aurand, Kasetsart University* Assistant secretary: *Assoc. Prof. Dr. Wannasawat Ratphitagsanti, Kasetsart University* Log In: 8.45 - 8.55 9.00 - 12.00	
9.00 - 9.20	**Invited speech 1:** Conventional and novel technologies for extraction of plant protein and impact on structure and functionality By *Prof. Dr. Buddhi Lamsal, Iowa State University, USA*	9.00 - 9.10	Oral competition introduction
9.20 - 9.40	**Invited speech 2:** The solution of rice modification for human health and well-being By *Assoc. Prof. Dr. Nantawan Therdthai, Department of Product Development, Faculty of Agro-Industry, Kasetsart University, Thailand*	9.10 – 9.40	**AP-O018:** Molecular signatures of Thai red wine revealed by a nontargeted 1H-NMR metabolomics approach By *Ms. Suwanan Denchai*
9.40 – 9.55 9.55 – 10.10	**Oral presentation** **BB-O096:** Supercritical carbon dioxide and subcritical liquefied dimethyl ether extraction of policosanol from by-products of sugar mill By *Ms. Sudthida Kamchonemenukool (Department of Agro-Industry, Naresuan University)* **BP-O034:** Comparison of extraction methods of Ardisia elliptica leaves on bioactive compounds and their antioxidant activities By *Mr. Theerawut Tunang (Department of Product Development, Faculty of Agro-Industry, Kasetsart University)*	9.40 – 10.10	**DP-O061:** Altering surface area of fermenters and volume of pineapple juice for enhancing bacterial cellulose production and property with selected strains By *Ms. Chayaporn Thanthithum*

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<table>
<tr><th colspan="2">Division B: Oral Presentation</th><th colspan="2">Oral Presentation Competition</th></tr>
<tr><td>10.10
–
10.20</td><td>Break (10 min)</td><td rowspan="2">10.10
–
10.40</td><td rowspan="2">AB-O024: Antioxidative and cryoprotective properties of gelatin hydrolysate from salmon (Oncorhynchus nerka) skin
By Acting Sub Lt. Sakonwat Kuepethkaew</td></tr>
<tr><td>10.20
–
10.35</td><td>BP-O051: Functional properties of marigold flower extract using ultrasound-assisted extraction
By Ms. Nilar Oo (Department of Food Technology, Chulalongkorn University)</td></tr>
<tr><td>10.35
–
10.50</td><td rowspan="3">BP-O019: CHICKEN BREAST POWDER: Effect of drying processes on physicochemical and functional properties
By Mr. Kiatkhajorn Thassanasuttiwong (Department of Food Technology, Chulalongkorn University)</td><td>10.40
–
11.10</td><td>DP-O049: Growth profiles of probiotic yeast (Saccharomyces boulardii) in different culture media; fruit, vegetable juices, and standard media for development of alternative culture substrate in probiotic cell production
By Ms. Natthanan Buranavanitvong</td></tr>
<tr><td>10.50
–
11.05</td><td>11.10
–
11.40</td><td>BB-O096: Supercritical carbon dioxide and subcritical liquefied dimethyl ether extraction of policosanol from by-products of sugar mill
By Ms. Sudthida Kamchonemenukool</td></tr>
</table>

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Poster Session 1 12.10 - 12.45	
12.10 - 12.15	**CP-P007:** Optimization of gelatin and sorbitol for sugar-infused dried 'Nam Dok Mai Si Thong' Mango (*Mangifera indica* Linn.) Production By *Asst. Prof. Dr. Panida Rattanapitigorn (Department of Food Science and technology, Chiang Mai University)*
12.15 - 12.20	**CP-P027:** Development of high-fiber and gluten-free pasta from cassava flour By *Ms. Palita Udomsirinikorn (Program in Science for Industry, Chulalongkorn University)*
12.20 - 12.25	**CP-P029:** Cereal bar flavored with curried Thai river sprat fish By *Dr. Aoitip Samanros (Nutrition and Dietetics, Institute of Public Health, Suranaree University of Tecnology)*
12.25 - 12.30	**CP-P044:** Development of reduced fat coconut milk ice cream with pea protein isolate By *Ms. Hieng Bopharath (Institute of Nutrition, Mahidol University)*
12.30 - 12.35	**CP-P046:** Effect of chicken protein precipitate and mechanically deboned chicken meat as partial chicken meat substitute on the physicochemical properties and texture of chicken nugget By *Ms. Chanakan Montatipkul (Program of Science for Industry, Chulalongkorn university)*
12.35 – 12.40	**CB-P052:** Optimization of Soft Khanom Piak-Poon formulation containing flour mixture with sucrose substitutes By *Mrs. Kasarin Pedcharat (Department of Product Development, Kasetsart University)*

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Poster Session 2 12.10 - 12.45	
12.10 – 12.15	**CP-P059:** Study of consumer behavior of tofu from jackfruit seeds and chemical analysis of jackfruit seeds flour from different preparation methods By *Ms. Aritsara Chimin (Department of Food Science and Technology, Thammasat University)*
12.15 – 12.20	**CP-P060:** Drinkable fermented RD43 rice jelly with soymilk protein and inulin By *Ms. Ploy Preugpaibul (Marketing Department, Ecovist Intertrade Co., Ltd.)*
12.20 – 12.25	**CP-P062:** Observation on consumer behavior and market trend for health promoting products with fiber fortification By *Ms. Pimpadchara Nuchchareonpaiboon (Department of Product Development, Kasetsart University)*
12.25 – 12.30	**CP-P067:** Effect of egg white hydrolysate on the quality characteristics of gluten-free bread By *Ms. Nopphamas Aungsuttivong (Department of Food science and technology, Thammasat University)*
12.30 – 12.35	**CP-P073:** Using Kano's model for understanding of customer requirements in plant-based drinking yogurt for Generation Y. By *Ms. Kamonwan Pinitkaew (Department of Food science and technology, Thammasat University)*
12.35 – 12.40	**CP-P091:** Effect of rice flour and coconut oil on the physical properties of Thai custard (Khanom Mo Kaeng) By *Ms. Nittayada Bunkaewsuk (Faculty of Food Industry, KMITL)*

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Poster Session 3 12.10 - 12.45	
12.10 – 12.15	**CJ-P092:** Development of instant nutritious beverage from brown rice flavored with coffee for the elderly By *Mrs. Khanittha Punchuklang (Department of Food Science and Technology, Songkhla Rajaphat University)*
12.15 – 12.20	**CB-P094:** Chocolate ice cream with added silkworm-pupae emulsion has attractive market potential; findings from in-depth interview and formulation optimization By *Mr. Teerapong Nantasiri (Food Science and Technology, Mahidol International College)*
12.20 – 12.25	**CP-P097:** Market survey and a study of consumer behavior, attitudes and needs towards plant-based nuggets By *Ms. Patamaporn Onrak (Department of Product Development, Kasetsart University)*
12.25 – 12.30	**CP-P104:** Effect of modified starch and storage condition on the cooking properties and texture of non-gluten chicken pasta By *Ms. Janejira Srithongmai (Faculty of Food Industry, King Mongkut's Institute of Technology Ladkrabang)*
12.30 – 12.35	**CP-P108:** Consumer need analysis and development of healthy jelly from fingerroot (*Boesenbergia rotunda* (L.) Mansf.) By *Ms. Jinjutha Suechawakornkul (Human Resources Development and Food Innovation Product, Thammasat University)*
12.35 – 12.40	**CP-P114:** Study of Thai consumer's behavior in purchasing plant-based meat and dairy alternatives By *Ms. Rudtanagorn Insuk (Research and Development, AGRI PLANT INNOVATION CORPORATION LIMITED)*
12.40 – 12.45	**CP-P115:** Consumer situation on salt reduction product and the effect of flavors and MSG on consumer perception and acceptance By *Ms. TingChen Chang (Department of Product Development, Kasetsart University)*

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Poster Session 4 12.10 - 12.45	
12.10 - 12.15	**CP-P116:** Effect of flour types on qualities and freezing points of frozen plant-based shrimp By *Ms. Supissara Piempol (Department of Food Science and Technology, Thammasat University)*
12.15 - 12.20	**DB-P004:** Effect of polysaccharide and protein compounds on living cell probiotic bacteria and application as microcapsule of *Lactobacillus spp.* in food By *Dr. Sudarat Ledlod (Research department, CPF Food and Research Development Center CO., Ltd)*
12.20 - 12.25	**DB-P012:** Isolation and screening of proteolytic bacteria from Thai traditional fermented fish paste (Ka-pi-plaa) produced from beardless barb By *Mrs. Pakteera Sripokar (Faculty of Agro and Bio Industry, Thaksin University)*
12.25 - 12.30	**DP-P038:** Screening of lignocellulolytic fungi from decayed mango strum By *Ms. Sutthiluck Kwantrairat (Program of Environmental Science and Natural Resources, Khon Kean University)*
12.30 - 12.35	**DP-P069:** Selection of commercial molds and yeasts for Sato production By *Mr. Natthaphong Mungmuang (Department of Food Science and Technology, Maejo University)*
12.35 - 12.40	**DB-P085:** Optimization of ferromagnetic nanoparticles capture and concentration of *Salmonella sp.* from food samples By *Mr. Peerapon Chaisalee (Food safety center, King Mongkut's University of Technology Thonburi)*
12.40 – 12.45	**DB-P086:** Single laboratory method validation for antibody conjugated silica nanoparticles for rapid detection of *Campylobacter jejuni* By *Mr. Peerapon Chaisalee (Food safety center, King Mongkut's University of Technology Thonburi)*

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Poster Session 5 12.10 - 12.45	
12.10 – 12.15	**DB-P087:** Development of lime peels snack using hydrostatic pressure technique By *Ms. Piyarat Khanthawaro (Food safety center, King Mongkut's University of Technology Thonburi)*
12.15 – 12.20	**DB-P088:** Producing virgin aromatic coconut water by direct plunging and purged with nitrogen gas By *Ms. Piyarat Khanthawaro (Food Safety Center, King Mongkut's University of Technology Thonburi)*
12.20 – 12.25	**DB-P089:** Isolation and characterization of probiotics from pig manure on multi-drug resistant pathogens By *Ms. Parichut Rattanapol (Food Safety Center, King Mongkut's University of Technology Thonburi)*
12.25 – 12.30	**DB-P090:** Formation and characterization of Listeria innocua biofilm on stainless steel pits By *Ms. Parichut Rattanapol (Food Safety Center, King Mongkut's University of Technology Thonburi)*
12.30 – 12.35	**DB-P095:** In silico analysis for the design of PCR primer pairs for simultaneous identification *Campylobacter jejuni* and *Campylobacter coli* By *Mr. Rapeepat Tippayawong (Department of Microbiology, Faculty of Science, Kasetsart University)*
12.35 – 12.40	**DB-P099:** Development of a low-cost culture medium for Lactobacillus cultivation By *Asst. Prof. Dr. Sukon Tantipaibulvut (Biotechnology Research for Energy and Bioactive Compounds, King Mongkut's University of Technology Thonburi)*
12.40 – 12.45	**DB-P106:** Element and fundamental property detection in Thai fish sauce from industrial fermentation By *Asst. Prof. Dr. Sureelak Rodtong (Institute of Science, Suranaree University of Technology)*

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Division C: Oral Presentation (Food Product Development, Sensory, and Consumer Research) Chair: *Asst. Prof. Dr. Aussama Soontrunnarudrungsri, Kasetsart University* Co-Chair 1: *Dr. Kunalai Ploydanai, Kasetsart University* Co-Chair 2: *Assoc. Prof. Dr. Wiwat Wangcharoen, Maejo University* Log In: 12.45 - 12.55 13.00 – 16.00		**Division D: Oral Presentation** (Food Microbiology, Food Biotechnology, Fermentation) Chair: *Assoc. Prof. Dr. Pravate Tuitemwong, King Mongkut's University of Technology Thonburi* Co-Chair 1: *Assoc. Prof. Dr. Prakit Sukyai, Kasetsart University* Co-Chair 2: *Asst. Prof. Dr. Kanokwan Tandee, Maejo University* Log In: 12.45 - 12.55 13.00 – 16.00	
13.00 - 13.20	**Invited speech 1:** How to navigate health-biased sweetness intensity when utilizing Just- about-right scale By *Prof. Jeehyun Lee, Department of Food Science and Nutrition, Pusan National University, Korea*	13.00 - 13.20	**Invited speech 1:** Discovery and development of lactic acid bacterial and food protein-derived antimicrobial peptides for food industry By *Dr. Weerapong Woraprayote, BIOTEC (Winner of Ajinomoto – FoSTAT Award 2021 for Young Food Scientist)*
13.20 - 13.40	**Invited speech 2:** Leveraging AI for predictive sensory analysis in food and beverage product development By *Mr. Ryan Ahn, Vice President of Innovation and Application, Gastrograph AI, USA*	13.20 – 13.40	**Invited speech 2:** Natural preservatives: challenges and efforts for future food By *Asst. Prof. Chitsiri Thongson Rachtanapun, Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Thailand*
	Oral presentation		**Oral presentation**
13.40 – 13.55	**CP-O022:** Optimization of maltitol syrup content as glucose syrup replacer in gummy candies by using mixture design method By *Prof. Dr. Nevzat Konar (Food Engineering Department, Eskisehir Osmangazi University)*	13.40 – 13.55	**DJ-O041:** Accelerated fermentation of red beet using pulsed electric field pre-treatment By *Prof. Mohammad Naghi Eshtiaghi (Department of Chemical Engineering, Mahidol University)*
13.55 – 14.10	**CJ-O023:** Influence of sucrose particle size on some physicochemical and sensory properties of compound chocolate By *Prof. Dr. Nevzat Konar (Food Engineering Department, Eskisehir Osmangazi University)*	13.55 – 14.10	**DP-O049:** Growth profiles of probiotic yeast (*Saccharomyces boulardii*) in different culture media; fruit, vegetable juices, and standard media for development of alternative culture substrate in probiotic cell production By *Ms. Natthanan Buranavanitvong (Department of Food Technology, Chulalongkorn University)*

Day 2: June 17, 2022

<table>
<tr><th colspan="2">Division C: Oral Presentation</th><th colspan="2">Division D: Oral Presentation</th></tr>
<tr><td rowspan="5">14.10
–
14.25</td><td rowspan="5">CP-O118: Study of the relationship between meat consumption behavior and the potential to consume cricket protein
By Ms. Pranatun Kaewthanasin (Department of Agro-Industrial Technology Management, Kasetsart University)</td><td>14.10
–
14.25</td><td>DP-O061: Altering surface area of fermenters and volume of pineapple juice for enhancing bacterial cellulose production and property with selected strains
By Ms. Chayaporn Thanthithum (Department of Food Technology, Chulalongkorn University)</td></tr>
<tr><td colspan="2">Division E: Oral Presentation
(Related Food Topics (Food Packaging, Food Safety & Quality, Food Laws & Regulations, Food Policy, etc.))

Chair: Asst. Prof. Dr. Pattarin Leelaphiwat, Kasetsart University
Co-Chair 1: Asst. Prof. Dr. Chananpat Rardniyom, Maejo University

Log In: 14.25 - 14.35

14.35 – 16.00</td></tr>
<tr><td>14.35
-
14.55</td><td>Invited speech 1: Sustainable packaging
By Mrs. Padmaja Bandaru, Senior Packaging Technologist of YFY Jupiter, Australia</td></tr>
<tr><td>14.55
-
15.15</td><td>Invited speech 2: Innovative and sustainable design of packaging materials for health and well-being
By Assoc. Prof. Dr. Natdanai Harnkarnsujarit, Department of Packaging and Materials Technology, Faculty of Agro-Industry, Kasetsart University, Thailand</td></tr>
<tr><td>15.15
–
15.30</td><td>Oral presentation
EP-O033: Effect of UV-C curing on properties of ferulic acid-added soy protein film
By Mr. Md Shakil (Department of Food Technology, Chulalongkorn University)</td></tr>
</table>

Day 2: June 17, 2022

		Division E: Oral Presentation	
		15.30 – 15.45	**EP-O068:** Antimicrobial effect of essential oils with Nisin and Natamycin in combination against *Escherichia coli* O157:H7 By *Ms. Natcha Limpasatirakit (Department of Food Science and Technology, Thammasat University)*
15.50 – 16.15	Poster and oral presentation awards Closing		

ABOUT CONFERENCE

This virtual conference will provide opportunity to meet and share experiences as well as strengthen networking among international food scientists and scientists in related fields from academia, government and food industries. The objective is to highlight significant developments in research and innovations in food science and technology with an emphasis on innovative ASEAN food research towards the World. The conference will feature a series of presentations and discussions in plenary, concurrent and poster sessions, informal gatherings, competitions and exhibitions.

Division (A) Food Chemistry, Nutrition, and Analysis
Division (B) Food Processing and Engineering
Division (C) Food Product Development, Sensory, and Consumer Research
Division (D) Food Microbiology, Food Biotechnology, Fermentation
Division (E) Related Topics (Food Packaging, Food Safety & Quality, Food Laws & Regulations, Food Policy, etc.)
Division (F) Industrial session

Organisers:
Food Science and Technology Association of Thailand (FoSTAT)
Agro-Industry Academic Council Association (AIAC)
Kasetsart University
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OPENING REMARK: FoSTAT PRESIDENT

Assistant Professor Dr. Anadi Nitithamyong

President of Food Science and Technology Association in Thailand (FoSTAT)

Distinguished speakers, FIAC 2022 committees, colleagues, ladies and gentlemen
On behalf of the Food Science and Technology Association of Thailand or FoSTAT, I am pleased to welcome you to the Food Innovation Asia Conference 2022. The conference and competition programs will be convened during 15-18 June 2022 concurrently with ProPak Asia 2022.

In time of the COVID-19 pandemic, today the situation has begun to improve. Hence, the Organizing Committee has decided to go forward with both the virtual conference and the onsite small group workshops. This year we will also organize the final round of 2 undergraduate competitions at BITEC under a strict measure to ensure the safety of all participants as well as to conform with the COVID-19 management policy of the Government. They include Food Innovation Concept Contest and FoSTAT-Nestle Quiz Bowl.

FoSTAT is very proud to be a part of the Food Innovation Asia Conference in 2022. This year, FoSTAT collaborates with the Agro-Industry Academic Council (AIAC), Kasetsart University, Maejo University, Food Innopolis, AOAC Thailand as well as the Federation of the Institute of Food Science and Technology in ASEAN (FIFSTA) and the International Union of Food science and Technology (IUFoST) to bring to you scientific events where there will be numerous technical presentations and workshops along with oral and poster presentation and competition for graduate students.

I wish to thank Informa Markets - Thailand who has been one of the key partners in supporting our activities. We are certain that this year will be another successful year bringing all up-to-date information and innovation to the food science and technology community and the industry. Finally I wish for more successful events of Food Innovation Asia Conference in the future.

WELCOME MESSAGE: KASETSART UNIVERSITY

Assoc. Prof. Dr. Anuvat Jangchud

Dean, Faculty of Agro-Industry
Kasetsart University, THAILAND

Honorable speakers, fellow delegates and distinguished guests

It is my pleasure to welcome you to FOOD INNOVATION ASIA CONFERENCE 2022, which is organized by Food Science and Technology Association of Thailand (FoSTAT), Agro-Industry Academic Council Association (AIAC), Kasetsart University, Maejo University and AOAC Thailand Section.

This year the conference is organized as a virtual conference due to the covid-19 pandemic, during June 16-18, 2022, under the theme of "Innovative and sustainable development of functional ingredients and materials: benefits, concerns and challenges in human health and well-being".

Functional ingredients have recently played important roles in our daily life, especially, during the current covid-19 pandemic. Therefore, it is very important for the scientific community to highlight significant and sustainable developments in research and innovations related to functional ingredients. Through this well-established conference, featuring plenary session, oral and poster presentations, industrial session, and student competitions, I strongly believed that the participants would have great opportunities to update the latest research information, to exchange knowledges as well as to create new scientific networks.

I also would like to thank all staffs and organizing committee, who make this event successful through their hard work and efforts.

Finally, I wish you all a successful and fruitful conference. Hope you enjoy "FOOD INNOVATION ASIA CONFERENCE 2022". Thank you.

WELCOME MESSAGE: MAEJO UNIVERSITY

Assoc. Prof. Jakraphong Phimphimol

Dean, Faculty of Engineering and Agro-Industry
Maejo University, THAILAND

Dear colleagues and friends, ladies and gentlemen,

A warm welcome to all of you. It gives me great pleasure to welcome you all to this important international conference to participate in the virtual conference food innovation asia conference 2022 in the theme of innovative and sustainable development of functional ingredients and materials: benefits, concerns and challenges in human health and well being.

This virtual conference is being co-hosted by Food Science and Technology Association of Thailand (FoSTAT), Agro-Industry Academic Council Association (AIAC), Kasetsart University, AOAC Thailand Section and Maejo university. I also would like to extend my warmest welcome to all the keynote speakers, invited speakers, food professional and guests from different countries.

Many alternative food products have been created on the market for various food groups. Unhealthy eating habits may affect health and cause the development of many diseases. Functional food which is enriched with bioactive components has been developed and increased interest of consumers for their improvement of health and prevention. At the same time, greater awareness of the influence of diet on health has increased. Thus, what we can do in the next generation of research, we need to based on basic, behavioral and social research. It's essential to partner among academia, government and food industries for highlighting the significant developments in research and technology on innovative ASEAN food research towards the world. We will work together for a more sustainable future.

One of the most important aims of this endeavor is to bring people together from across the universities, government and food industries to share ideas and approaches to develop and communicate best practices via a series of presentations and discussions in plenary, poster sessions, competitions and exhibitions.

On behalf of Maejo university, I am very confident the conference will bring the best of the participants and to forester future collaborations between partners.

May you have a very successful conference.

PROCEEDINGS

DIVISION A:
Food Chemistry, Nutrition, and Analysis

ORAL PRESENTATION

Molecular signatures of Thai red wine revealed by a non-targeted ^{1}H-NMR metabolomics approach

Suwanan Denchai[1], Wanwimon Mekboonsonglarp[2], Thanitaporn Phuenpong[1], Suppached Sasomsin[3] and Sarn Settachaimongkon[1,4,5,*]

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[2]Scientific and Technological Research Equipment Center (STREC), Chulalongkorn University, Bangkok, 10330, Thailand

[3]Innovation & Winemaking Division, Siam Winery Company Limited, Samut Sakhon, 74000, Thailand

[4]Omics Sciences and Bioinformatics Center, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

[5]Emerging Processes for Food Functionality Design Research Unit, Chulalongkorn University, Bangkok 10330, Thailand

**Corresponding author: Tel.:0-2218-5535, E-mail: sarn.s@chula.ac.th*

ABSTRACT: Nowadays, metabolomics has been extensively applied for molecular authentication of wine products linked to specific grape variety, geographic origin, terroir and vintage, as well as various vinification techniques applied during winemaking. However, the application of metabolomics for molecular investigation of Thai wines is rather limited. Therefore, this study was aimed to characterize and compare the pioneer non-volatile metabolite profiles of Thai Shiraz wines using a non-targeted proton nuclear magnetic resonance (^{1}H-NMR) metabolomics approach. Non-volatile metabolite profiles of wine samples were analyzed using a high resolution ^{1}H-NMR spectroscopy (500 MHz) and metabolomics data were compared by means of chemometric analysis. As a result, 37 metabolites, including alcohols, amino acids, organic acids, phenolic compounds and sugars, in wine were successfully identified. Multivariate statistical analysis allowed discrimination among wine samples made from Shiraz grapes grown in the country in comparison with commercial products from abroad as well as among Thai wine samples derived from various aging conditions based on their ^{1}H-NMR metabolite fingerprints. Variations in the concentration of formate, fumarate, acetate, pyruvate, γ-aminobutyrate, succinate, tyrosine, gallate and epicatechin were statistically suggested as potential biomarkers linked to geographic origin and aging conditions of the samples. Our findings demonstrated the authenticity of Thai wines from a metabolomics perspective. This information could be further applied to establish strategies for molecular authentication and traceability of Thai wine products in the future.

Keywords: *Thai wine, Molecular fingerprint, Food authentication, Metabolomics, Foodomics*

INTRODUCTION

Wine is a fermented beverage resulted from alcoholic fermentation of grape extracts or grape must by the activity of selected yeast strains, with occasionally followed by malolactic fermentation from specific lactic acid bacteria [1]. Although wine is generally considered as an essential part of western food culture, its global reputation, consumption and trading gradually increase. Undeniably, there is a growing interest in wine products among Thai consumers over the recent decades. According to Thai Industrial Standards Institute, the actual alcohol content of wine products sold in the country shall be ranged between 7% to 15% vol [2].

It is well acknowledged that the quality of wine is predominantly determined by various factors, including viticultural practices and environmental influences at the location of vineyard, along with technological aspects and human interventions related to vinification techniques applied in the winery [3]. Combinations of these parameters result in distinctive biochemical profiles which subsequently provide significant impact on sensory characteristics and therefore the uniqueness of wines [3]. Thanks to recent advanced chemical analysis technologies, molecular characterization and diversity of wines have been progressively unraveled and enabled the authentication and traceability of products according to their geographic origins, particular winemaking and aging processes, as well as contamination and adulteration incidences [3].

Metabolomics is one branch of ~omics technologies focusing on comprehensive characterization of small molecular weight metabolites (<1.5 kDa) present in complex biological systems including food and beverage matrices [4]. The application of mass spectrometry (MS) and nuclear magnetic resonance (NMR) based metabolomic techniques for molecular characterization of wine products have been extensively published [5, 6]. As a result, many classes of volatile and non-volatile metabolites have been reported to be associated with wine authenticity such as type of grape variety used as raw material, geographic identification linked to specific terroir and vintage, biochemical changes related to certain vinification techniques, stages of fermentation and aging as well as product spoilage and contamination [7, 5, 6]. Nevertheless, to our best knowledge, the application of metabolomics for molecular investigation of wine produced in Thailand, is rather limited.

Therefore, the objectives of this study were to characterize and compare non-volatile metabolite profiles of Thai red wine using a high-resolution proton nuclear magnetic resonance spectroscopy (^{1}H-NMR; 500 MHz). Comparative metabolite profilings were performed (i) among samples made from Shiraz grapes grown in the country in comparison with commercial products from abroad as well as (ii) among Thai wine samples derived from various aging conditions by means of chemometric analysis.

MATERIALS AND METHODS

Selection of wine samples

Shiraz is one of the major grape varieties grown and primarily used to produce red wines in many regions around the world. This cultivar can develop well under sufficient sunlight and dry climate condition; therefore, its plantation has been introduced and well adapted with the tropical climate of Thailand [8]. After consultation with co-researchers from Thai wine industry, red wine samples derived from Shiraz cultivar harvested in 2019 vintage were selected in this study. Samples were categorized into 12 groups according to their (i) country of origin, (ii) type of aging vessel, (iii) age and size of aging vessel as demonstrated in Table 1. Thus, a total number of 36 Thai shiraz wine samples and 9 commercial shiraz wine samples from abroad

Table 1. Information of Shiraz wine samples in this study

Group	Code of samples	Origin	Type of barrel	Brand of barrel	Age of oak barrel[a]	Barrel size[b]
1	THA-SS	Thailand	Stainless steel	NA	NA	Large
2	THA-ORD-G7	Thailand	Oak	Radoux	New	Small
2	THA-ORD-G5	Thailand	Oak	Radoux	New	Small
3	THA-ORD-A18	Thailand	Oak	Radoux	New	Medium
4	THA-ORD-E6	Thailand	Oak	Radoux	Medium	Large
5	THA-OSM-A3	Thailand	Oak	Seguin Moraux	New	Medium
6	THA-OSV-A8	Thailand	Oak	Sylvain	New	Medium
7	THA-OTS-A12	Thailand	Oak	Taransaud	New	Medium
8	THA-OFF-FF1	Thailand	Oak	François Frères	Old	Large
9	THA-OAB-ASS1	Thailand	Oak	Assmann Barrel	Old	Large
10	AUS-GW	Australia	NA	NA	NA	NA
11	AUS-BSH	Australia	NA	NA	NA	NA
12	SAF-KWV	South Africa	NA	NA	NA	NA

NA = not applicable; [a]Age of oak barrel: New = 2, medium = 6, old = >10 years.
[b]Size of oak barrel: Large = 2,000, medium = 500, small = 225 L.

were included in this study. After reception, samples were stored under dark refrigerated condition (4±3 °C) and the bottles were uncorked on the day of analysis.

Sample preparation and ^{1}H-NMR analysis

Wine samples were prepared according to the method modified from Mascellani *et al.*, [9] and Luangwilai *et al.*, [10]. In short, insoluble particles were removed by centrifugation under 4,100 ×*g* at 25 °C for 15 min. The pH of all samples was determined and adjusted to 6.0 using 1.0 N NaOH. The clear wine was untrafiltrated through a centrifugal device with 3 kDa molecular weight cut-offs (Pall Nanocep®, Pall Life Sciences, Ann Arbor, MI). The filtrate was then diluted 1:1 (v/v) with phosphate buffer pH 6.0 consisting of 1 mM 3-(Trimethylsilyl) propionic-2, 2, 3, 3-d_4 acid sodium salt (TSP) (Merck, Darmstadt, Germany) as an internal standard. Finally, the mixture (400 µL) and deuterium oxide (D_2O) (Merck, Darmstadt, Germany) (200 µL) were subjected to a high resolution ^{1}H-NMR spectrometer at 500 MHz (Bruker model AVANCE III HD, Rheinstetten, Germany). The temperature was set to 27 °C. ^{1}H-NMR spectra were acquired and processed under the same condition. The Bruker pulse sequence (NOESYGPPR1D) was applied to suppress the residual water signal. For each sample, 256 scans (NS), a time domain (TD) of 65,536 real data point using a 10,245.90 Hz sweep width of proton (SW), a relaxation delay (D1) set to 4 s and an acquisition time requirement (AQ) of 3.198 s, receive gain of approx. Tuning, lock, gain, 90° pulse calibration, and shimming were calibrated automatically for each sample by the standard module routine developed by Bruker [11].

^{1}H-NMR spectra processing, metabolite identification and relative quantification

^{1}H-NMR spectra were corrected, pre-treated and processed through binning technique (0.02 ppm interval) using Bruker TopSpin software (Bruker, Rheinstetten, Germany) [10]. Metabolites present in samples were identified by means of Chenomx NMR suite 8.2 library (Chenomx Inc., Canada), Food Metabolite Database (https://foodb.ca/) and relevant literatures [11, 9].

Statistical analysis

^{1}H-NMR derived metabolomic data were normalized against internal standard, median centering and $\log_2$ scaling before subjecting to multivariate statistical analysis [10]. Heat-map visualization combined with

Pearson's correlation-based hierarchical clustering (HCA) and partial least-squares discriminant analysis (PLS-DA) were performed using MetaboAnalyst 5.0 software (www.metaboanalyst.ca). Finally, PLS-DA derived metabolites with variable importance in projection (VIP) score > 1.0 and $P \leq 0.05$ were considered to be potential biomarkers responsible for the discrimination among red wine samples [10].

RESULTS AND DISCUSSION

^{1}H-NMR data acquisition and metabolite identification

A representative NOESY-1D-^{1}H-NMR spectrum of Thai red wine sample is demonstrated in Figure 1. Binning technique was applied for acquisition of ^{1}H-NMR signal intensities [10]. Metabolite identification labels were assigned to the bins according to their specific chemical shift patterns (Table 2) suggested by NMR library, Food Metabolite Database and relevant literatures [11, 9]. Results revealed that several major metabolites present in grape must and wine, e.g. glucose, fructose, tartrate, malate, citrate, succinate, lactate, acetate, gallate and epicatechin [12, 13], could be simultaneously detected and quantified by a non-targeted ^{1}H-NMR metabolomics approach. In total, 37 metabolites in the group of alcohols, amino acids, organic acids, phenolic compounds and sugars were identified in this study (Fig. 1 and Table 2). This list of compounds corresponded well with those previously reported in red wines using a high resolution ^{1}H-NMR platform (500 - 600 MHz) [11, 9]. The signal intensities of these compounds were normalized and further introduced as variables in chemometric analysis.

Overall comparison of ^{1}H-NMR metabolite profiles of Thai vs. foreign Shiraz wines

A non-supervised Pearson's correlation-based hierarchical clustering combined with heat-map visualization was performed to evaluate overall similarity of the ^{1}H-NMR metabolite profiles of red wine samples in this study (n = 45) (Fig. 2). Results showed that Thai wine samples aged in stainless steel tanks (marked in green) were clearly grouped into different cluster from other wines, either Thai or foreign origins, that have been matured in oak barrels. In addition, a good distinction among oak-aged Thai (marked in blue), Australian (marked in red) and South-African (marked in pink) wine samples could be notably observed. Besides the dendrogram, different color shades in heat-map denote relative quantification, i.e. red color indicates a higher abundance while green color indicates a lower abundance, of respective metabolite detected in the wine samples. Apparently, most metabolites were present in higher relative abundances in Australian, South-African and certain Thai wine samples.

A supervised pattern recognition by PLS-DA was performed to discriminate the ^{1}H-NMR metabolite profiles between Thai and foreign Shiraz wine samples in this study (n = 45) (Fig. 3). An overall PLS-DA score plot was constructed by the first two components with a good accuracy of 91.70%, R^2 = 0.747 and Q^2 = 0.603 (Fig. 3A). Results showed a clear distinction among Thai Shiraz wines, either matured in oak barrels or stainless-steel tanks, and commercial samples made in Australia as well as South-Africa. It is well recognized that distinctive biochemical characteristics of a wine is predominantly

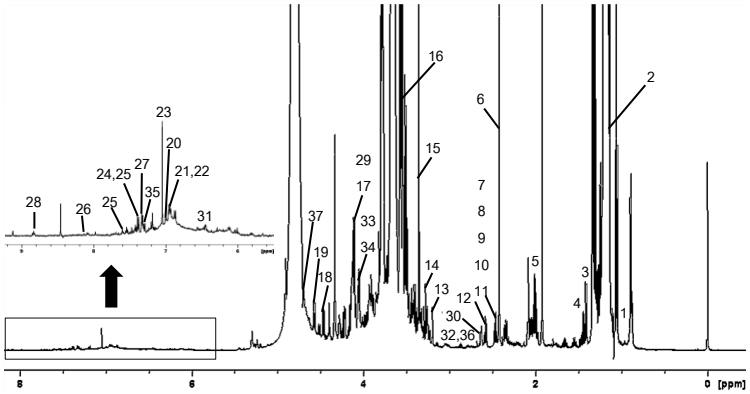

Figure 1. Representative ^{1}H-NMR spectrum of a Thai red wine sample. Numbers correspond with the list of metabolites and their assigned chemical shifts demonstrated in Table 2.

Table 2. List of non-volatile metabolites identified in red wine samples using ^{1}H-NMR technique in this study.

Chemical group	Metabolites	Chemical Shift (ppm)[a]
Alcohol	(2) Ethanol	1.17 (t)[b] 3.65 (q)
	(16) Glycerol	3.55 (m) 3.64 (m) 3.65 (m)
	(34) Mannitol	3.66 (m) 3.76 (m) 3.78 (d) 3.83 (m)
	(15) Methanol	3.35 (s)
	(14) Myo-Inositol	3.26 (t) 3.52 (m) 3.61 (t) 4.05 (t)
Amino acid	(4) Alanine	1.47 (d) 3.77 (q)
	(29) Arginine	3.25 (t) 3.77 (t)
	(11) Glutamate	2.13 (m) 2.32 (t) 2.37 (t) 3.76 (m)
	(27) Histidine	3.24 (m) 3.31 (m) 4.00 (m) 7.27 (s)
	(1) Leucine	0.95 (m) 3.74 (m)
	(7) Methionine	2.12 (s) 2.63 (t) 3.87 (t)
	(24) Phenylalanine	3.15 (m) 3.28 (m) 4.00 (m) 7.31 (d) 7.36 (m) 7.40 (m) 7.41 (m)
	(5) Proline	1.99 (m) 2.03 (m) 2.06 (m) 2.34 (m) 3.33 (m) 3.41 (m)
	(36) Threonine	1.32 (d) 3.60 (d) 4.26 (m)
	(22) Tyrosine	3.04 (m) 3.19 (m) 3.94 (m) 6.89 (d) 7.18 (d)
	(37) Valine	0.96 (d) 1.04 (d) 2.27 (m) 3.60 (d)
Organic acid	(6) Acetate	1.91(s)
	(9) Acetoacetate	2.27 (s) 3.44 (s)
	(8) Acetoin	2.21 (s) 4.43 (m)
	(13) Choline	3.19 (s) 4.05 (m)
	(30) Citrate	2.55 (d) 2.70 (d)
	(26) Formate	8.44 (s)
	(31) Fumarate	6.53 (s)
Organic acid	(32) γ-Aminobutyrate	1.89 (m) 2.29 (t) 3.00 (t)
	(3) Lactate	1.32 (d) 4.10 (m)
	(33) Malate	2.40 (m) 2.68 (m) 4.30 (d)
	(10) Pyruvate	2.36 (s)
	(12) Succinate	2.41 (s)
	(18) Tartrate	4.32 (s)
	(28) Trigonelline	4.42 (s) 8.06 (t) 8.81 (d) 8.83 (d) 8.84 (d) 9.11 (s)
Phenolic compounds	(25) Chlorogenate	2.01 (t) 2.03 (t) 3.88 (m) 4.26 (d) 6.94 (d) 7.18 (d)
	(20) Epicatechin	2.76 (d) 2.93 (m) 4.35 (s) 5.00 (s) 6.95 (t) 7.04 (s)
	(23) Gallate	7.03 (s)
	(21) *p*-Hydroxyphenyl acetate	3.44 (s) 6.85 (d) 7.16 (d)
	(35) Syringate	3.89 (s) 7.27 (s)
Sugar	(19) Glucose	3.24 (t) 3.40 (m) 3.45 (m) 3.48 (m) 3.53 (m) 3.70 (m) 3.71 (m) 3.76 (m) 3.80 (m) 3.83 (m) 3.88 (m)
	(17) Fructose	3.55 (m) 3.58 (m) 3.66 (m) 3.70 (m) 3.78 (m) 3.80 (m) 3.89 (m) 4.00 (m) 4.11 (d)

Note: [a]Chemical shift values are referenced to TSP signal (0.00 ppm) at pH 6.00; [b]Letters indicate singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m) multiplicity of ^{1}H-NMR peaks, respectively.

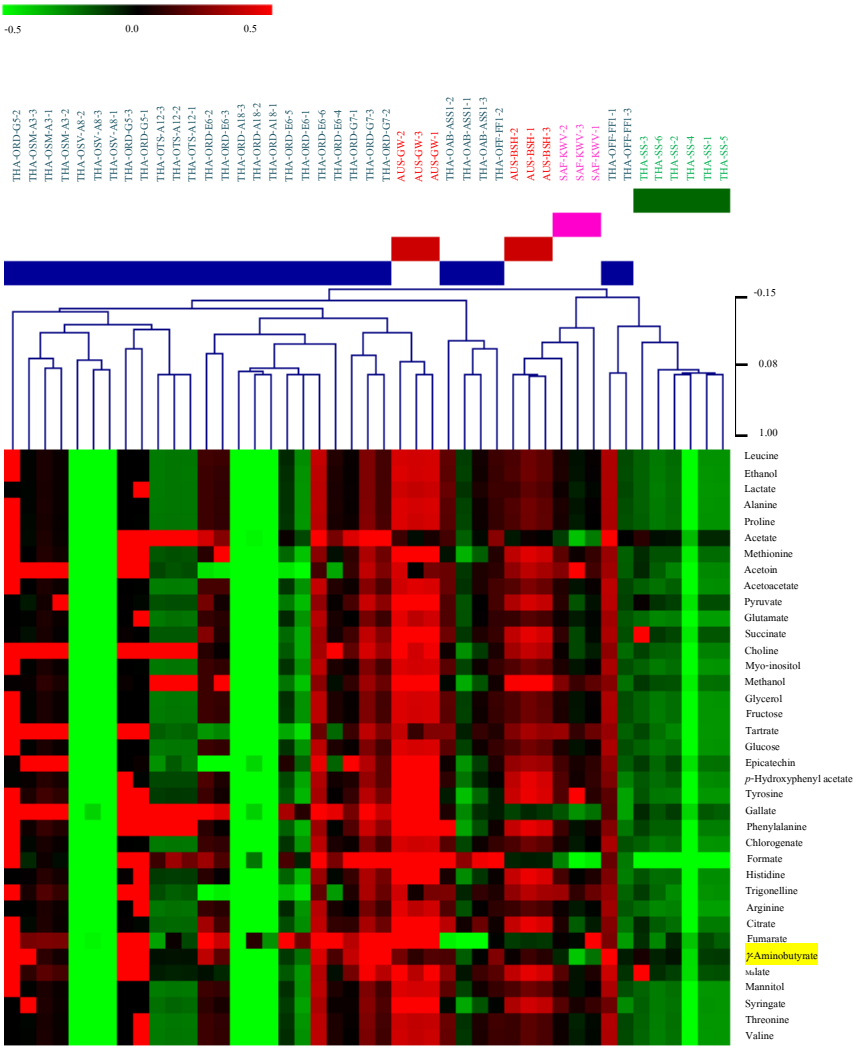

Figure 2. Heat-map visualization and hierarchical clustering of ^{1}H-NMR derived non-volatile metabolite profiles of Shiraz wine samples. The dendrogram represents sample clusters based on Pearson's correlation coefficient with average linkage. Each square in the heat-map expresses.

determined by its production location as the reflection of physiological adaptation of grapevines, environmental and human factors related to a specific terroir [3]. Although Shiraz is the most widely planted winegrape cultivar in Australia, it is well adapted with the tropical climate of Thailand [8]. Our results corresponded with several works [14, 15] which highlighted the distinctive metabolome of Shiraz wines in association with their regional specificity and geographic origins.

VIP scores with a value greater than 1.0 and $P \leq 0.05$ suggested that significant variations in the concentration of formate, acetate, γ-aminobutyrate, gallate and epicatechin could be considered as potential biomarkers accountable for the discrimination between Thai vs. foreign Shiraz wine products in this study (Fig. 3B). Considering the average concentration of these compounds in each group of samples (indicated in different color range; Fig. 3B), formate, acetate, γ-aminobutyrate, gallate were present in significantly higher abundance in Australian, Thai oak-aged, Thai stainless steel-aged and

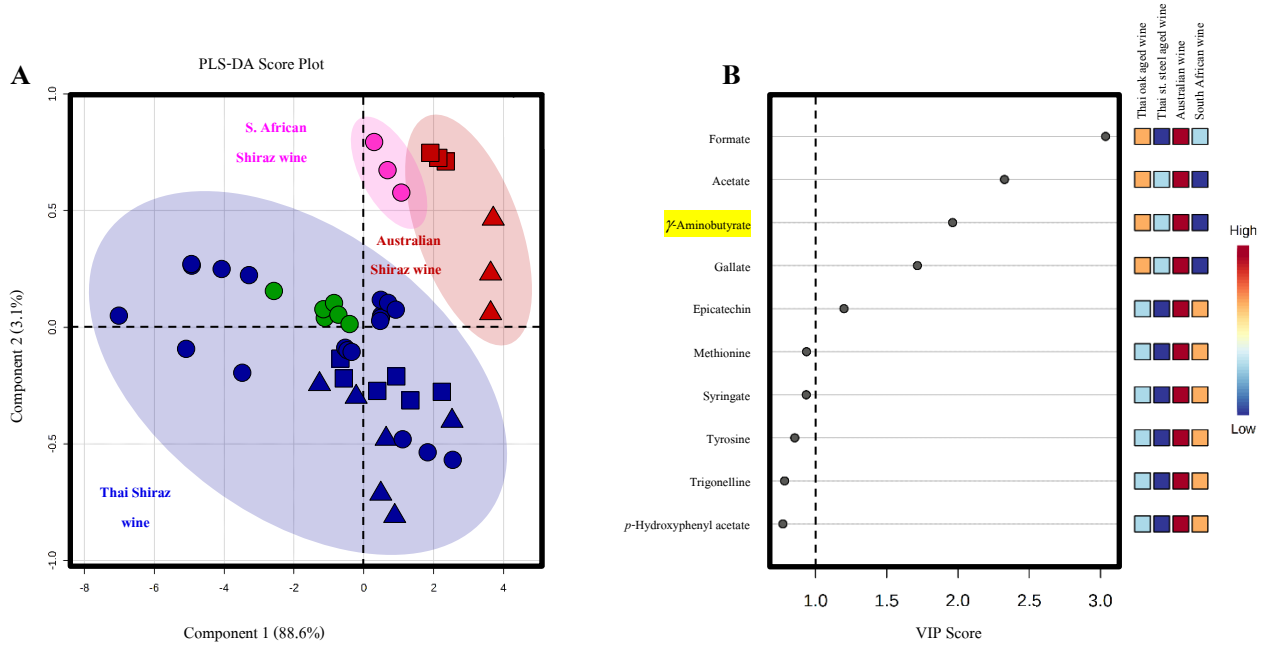

Figure 3. PLS-DA score plot (panel A) and VIP scores (panel B) for an overall comparison of ^{1}H-NMR derived non-volatile metabolite profiles of commercial Shiraz wine samples from Australia (brand A; ■ and brand B; ▲), South-Africa (●) and Thai Shiraz wines aged in stainless steel tanks (●), new used (●), medium used (▲) and old used (■) oak barrels.

South-African wines, respectively. In addition, epicatechin was detected in significantly higher abundance in Australian and South-African wines compared to the Thai samples. This result corresponded well with the color ranges of these metabolites demonstrated in the heat-map earlier (Fig. 2).

Comparison of ^{1}H-NMR metabolite profiles among Thai Shiraz wines aged under different conditions

Another PLS-DA was performed to evaluate the variation of ^{1}H-NMR metabolite profiles among Thai red wines (n = 30) matured under different conditions and identify potential biomarker metabolites responsible for their discrimination. A PLS-DA score plot was constructed by the first two components with a good accuracy of 94.40%, R^2 = 0.625 and Q^2 = 0.520 (Fig. 4A). Results demonstrated a clear distinction between wine samples aged in stainless steel tanks from those matured in wooden oak barrels. Beside this, it should be mentioned that discriminations among wine samples aged in old, medium and new used oak barrels could be remarkably observed (Fig. 4A). The metabolite profiles of wines aged in old used oak barrels (>10 years) were apparently closed to those of samples aged in stainless steel vessels. It has been well documented the intensities of oak flavors and their extraction process decline as barrels age with the times of use [16]. This could result in a more neutral sensory profile due to fewer oak flavors are imparted into the wines [14]. On the other hand, new oak barrels contain large amount and more complexity of flavor compounds

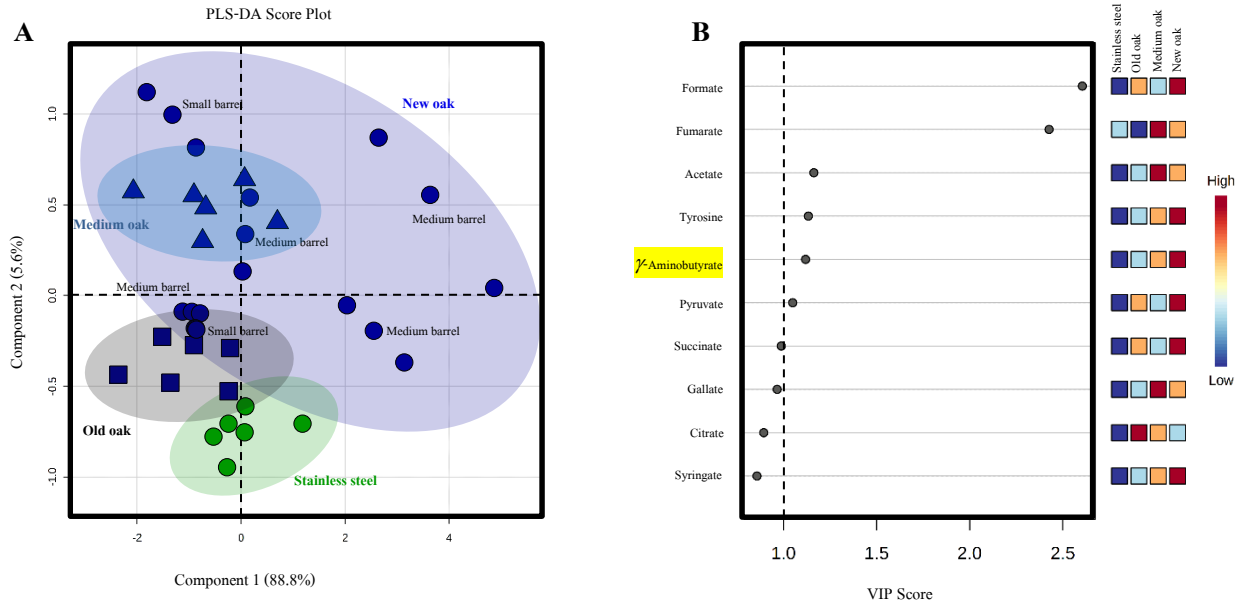

Figure 4. PLS-DA score plot (panel A) and VIP scores (panel B) for a comparison of ^{1}H-NMR derived non-volatile metabolite profiles of Thai Shiraz wine samples aged in stainless steel tanks (●), new used (●), medium used (▲) and old used (■) oak barrels.

that can be extracted into the wines during aging [16]. Furthermore, the origin of woods, manufacturing process and size of the barrels substantially contribute to the biochemical and sensory characteristics of the wines [17]. In this study, although the metabolite profiles of wines aged in medium used oak barrels (6 years) showed a good collective pattern, their distinction from those of samples aged in new used oak barrels (2 years) was not completely evident. A great variation in the metabolite profiles of wines aged in new used oak barrels was remarkably observed together with the influence of the size of barrels (Fig. 4A).

VIP scores with a value greater than 1.0 and $P \leq 0.05$ suggested that significant variations in the concentration of formate, fumarate, acetate, tyrosine, γ-aminobutyrate, pyruvate, succinate and gallate could be considered as potential biomarkers responsible for the discrimination among Thai Shiraz wines matured in stainless steel tanks and wooden oak barrels in which variations in their lifespan were also considered (Fig. 4B). Regarding the average concentration of these compounds in each group of samples (indicated in different color range; Fig. 4B), most of metabolites were present in significantly higher abundance in wine samples aged in wooden oak barrels compared to stainless steel vessels. Moreover, among the groups of wines matured with oak woods, samples derived from new and medium used barrels contained more intensities of these indicative metabolites compared to those attained from old used ones. The relative quantification of metabolites found here was in accordance with the decrease in oak flavor contribution when barrels were aged with the times of use as discussed earlier [16]. It should be mentioned that substantial variations in theses metabolites could be attributed to a series of biochemical reactions, e.g. polymerization of polyphenols, micro-oxygenation, extraction of certain compounds from oak wood to the wine, throughout the entire aging period [18, 14, 16].

Our findings support the effective of using a non-targeted ^{1}H-NMR combined with chemometric analysis to investigate non-volatile metabolite profiles of Thai Shiraz wines in comparison with products made from grapes harvested in the same vintage from Australia and South-Africa as well as within the groups of Thai wine samples derived from various aging conditions. Results suggested significant influences of geographic origins, vinification and aging techniques on the metabolome of products. However, it should be mentioned that the number of samples represented in this study was rather small. Validation with a large number of samples is essentially required. In perspective, extensive investigations of other relevant factors in winemaking could be also of our interests. Eventually, the metabolomic data could be applied to establish strategies for molecular authentication and traceability of Thai wine products in the future.

CONCLUSIONS

This study demonstrates an application of non-targeted ^{1}H-NMR metabolomics approach for molecular characterization and comparison of Thai red wines between samples made from Shiraz grapes grown in the country and products from abroad as well as comparison among Thai wine samples derived from various aging conditions. A total of 37 non-volatile metabolites in wine samples were identified using a high resolution ^{1}H-NMR technique. Chemometric analysis allowed discrimination of Shiraz wine samples according to the country of origin together with aging conditions based on their ^{1}H-NMR metabolite fingerprints. Potential biomarker metabolites accountable for the discrimination were statistically suggested. Variations in the concentration of these indicative compounds could be attributed to a series of biochemical reactions influenced by particular terroir, vinification and aging techniques applied during winemaking.

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An application of Plackett – Burman design in screening essential amino acids increasing myotube hypertrophy

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ABSTRACT: Nowadays, non-meat proteins are gaining in popularity with increasing consumption. Protein is an indispensable source of essential amino acids (EAA). However, many non-meat protein sources lack some EAA, which is known as limiting amino acids. EAA are involved in the normal growth of muscles in the human body. In this study, Plackett - Burman design (PBD) was applied to estimate the effects of EAA on muscle growth through myotube diameter, nuclear fusion index (NFI), and myotube number. The twelve formulas were generated by the experimental design. The results showed that the sixth formula was outstanding. This formula consists of histidine, isoleucine, leucine, methionine, phenylalanine, and tryptophan which provided the highest myotube diameter (20.886 ± 1.821 μm) and NFI (52.73 ± 4.01 %), while myotube number was not significantly different. Considering the type of EAA, leucine was the only EAA that had a significant influence on myotube diameter ($p \leq 0.1$), NFI ($p \leq 0.15$), and myotube number ($p \leq 0.05$). However, the reduced interval confidence in diameter and NFI implies that other EAA were indispensable. This result benefits vegetarians and vegans in choosing non-meat proteins for their consumption.

Keywords: *Plackett-Burman design, Essential amino acids, Leucine, Luscle hypertrophy, myogenesis*

INTRODUCTION

Essential amino acids (EAA) are elementary nutrients important to the body. Especially, muscle is composed of protein the main composition. Nowadays, consumption of non-meat proteins has been increased, replacing meat. Plant proteins and other non-meat proteins are often incomplete proteins that contain some EAA lower than minimum requirements for humans, called the limiting amino acids. Intake of appropriate EAA content can maintain muscle mass, including muscle hypertrophy [1]. Therefore, vegetarians are at greater risk of a deficiency of EAA, which can have negative effects on the body. Because each amino acid affects muscle building differently. Some amino acids may therefore be suitable for athletes or people who exercise to keep in shape.

Identifying a kind of EAA affecting the muscle change encourages them to select appropriate protein consumption.

Plackett-Burman design (PBD) has been popular in studying the influence of numerous factors because it can conduct fewer experimental trials. It can screen all main effects significant from other factors. This method is extensively applied to screen the main influences in the various extraction processes [2]. However, the applications in screening nutrients have limited research.

According to the rationales mentioned above, this research aims to screen the primary essential amino acids influencing the hypertrophy of the muscle cell, planned by the PBD. The hypertrophy was determined by myotube diameter, number of myotubes, and nuclear fusion index (NFI).

MATERIALS AND METHODS

Reagents

Dulbecco's modified Eagle's medium (DMEM) with high glucose powder and antibiotic-antimycotic solution (Pen Strep) were obtained from Gibco (Life Technologies Europe B.V., Kwartsweg 2, Bleiswijk, Netherlands). Fetal bovine serum (FBS; Hyclone) was purchased from GE Healthcare Bio-Sciences Corp. (Piscataway, NJ, USA). Jenner and Giemsa stain solutions were obtained by BioGnost Ltd. (Zagreb, Croatia, EU). Nine essential amino acids were supplied by Himedia Laboratories (Mumbai, Maharashtra, India).

Plackett-Burman design

Twelve formulas consisting of different EAA compositions were formulated by the PBD [3] in Table 1. Two coded levels varied nine EAA and two dummy variables in twelve formulas where the '+' sign denotes the presence of each EAA and the sign '−' denotes the absence of each EAA. The EAA concentration was prepared at 500 µg/mL dissolved in Dulbecco's Modified Eagle Medium (DMEM) and stored at 4°C until use. From the preliminary experiment, this concentration provided cell viability higher than 80% of the control formula according to the MTT assay [4].

Table 1 The twelve formulas planned by Plackett-Burman design

Formulas	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine	Dummy 1	Dummy 2
1	1	-1	1	-1	-1	-1	1	1	1	-1	1
2	1	1	-1	1	-1	-1	-1	1	1	1	-1
3	-1	1	1	-1	1	-1	-1	-1	1	1	1
4	1	-1	1	1	-1	1	-1	-1	-1	1	1
5	1	1	-1	1	1	-1	1	-1	-1	-1	1
6	1	1	1	-1	1	1	-1	1	-1	-1	-1
7	-1	1	1	1	-1	1	1	-1	1	-1	-1
8	-1	-1	1	1	1	-1	1	1	-1	1	-1
9	-1	-1	-1	1	1	1	-1	1	1	-1	1
10	1	-1	-1	-1	1	1	1	-1	1	1	-1
11	-1	1	-1	-1	-1	1	1	1	-1	1	1
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Cell culture

C2C12 myoblast cells were obtained from the ATCC through a Biomedia distributor (Bangyai, Nonthaburi, Thailand). The myoblasts were cultured in a cell-culture flask containing DMEM, 4 mM L-glutamine, and 25 mM D-glucose (Dextrose), and supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) antibiotic-antimycotic solution. The cell cultures were maintained by incubating at 37°C with 5% CO_2. For the differentiation, 1.5×10^4 cells/well of the myoblasts were seeded into a 96-well plate and cultured for 24 h. Upon 90% confluency, the myoblasts were differentiated to myotube by replacing DMEM containing 2% FBS for 6 days [4]. After that, the myotubes were incubated by different twelve EAA formulas for 48 h.

Jenner-Giemsa staining

After incubating for 48 h, the myotube was removed from the culture media and washed with phosphate buffer saline (PBS). The myotubes were fixed with ice-cold methanol for 10 minutes, then air drying in a laminar flow biosafety cabinet. The fixed myotubes were stained by the Jenner-Giemsa solution [5]. The methanol-fixed cells were incubated for 10 min with Jenner staining solution diluted 1:3 in 1 mM sodium phosphate buffer (pH 5.6). The cells were washed with distilled water and incubated for 10 min with Giemsa staining solution diluted 1:20 in 1 mM sodium phosphate buffer (pH 5.6). The cells were washed with distilled water followed by air drying.

Diameter and Nuclear Fusion Index

Each well was randomly selected and photographed at 10X magnification for 5 fields using an inverted microscope (AE 2000, Miotic Asia, Hong Kong). Photographs were analyzed using ImageJ software (US National Institutes of Health, MD, USA). Myotube hypertrophy was determined by the diameter and nuclear fusion index (NFI) [6]. The diameter was measured by five points along each myotube, and at least 30 myotubes were included in the analysis. NFI was defined as the percentage of nuclei incorporated within myotubes per total nuclei in each image. Additionally, myotube numbers were also counted in each image.

Statistical analysis

All Statistical analysis was performed using Minitab 16 All Statistical analysis was performed using Minitab 16 Statistical software (Minitab, Inc., PA, USA). All data were presented by the mean ± standard deviation over five images. Analysis of Variance (ANOVA) evaluated the significant difference among the twelve formulas, while the main effect of EAA was graphically analyzed by the Pareto charts.

RESULTS AND DISCUSSION

Figure 1 shows myotubes images of the twelve formulas stained by Jenner-Giemsa solutions. The myotubes appeared dark tone, while nuclei were a bright circle. The diameters from the twelve formulas stained are shown in Figure 2. The sixth formula was the highest diameter (20.89 ± 1.82 µm) at $p \leq 0.05$. The first and fourth formulas were subordinate (20.38-20.47 µm). The fifth and eleventh formulas were the lowest diameter (17.40-17.42 µm), while the diameter of the rest formulas was higher (18.97-19.34 µm). The sixth formula comprised histidine, isoleucine, leucine, methionine, phenylalanine, and valine. The Pareto chart (Figure 3) shows that there was only leucine significantly affecting the diameter ($p \leq 0.10$). Either first or fourth formulas, the secondarily highest diameter, was also contained by leucine.

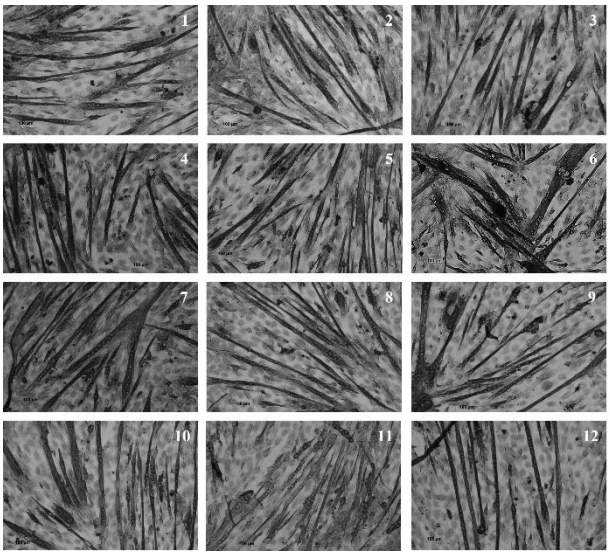

Figure 1 Myotubes of the twelve formulas stained by Jenner-Giemsa solutions

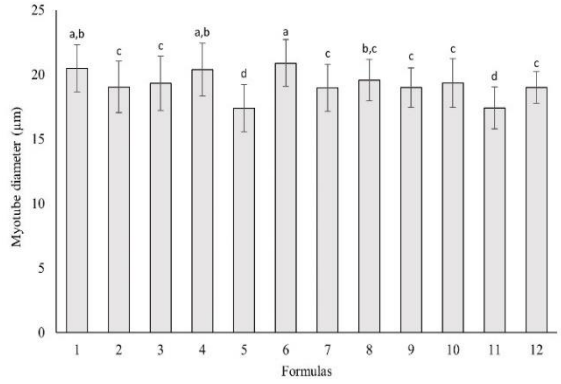

Figure 2 The diameter of the twelve formulas. Superscripts represent the significant difference ($p \leq 0.05$)

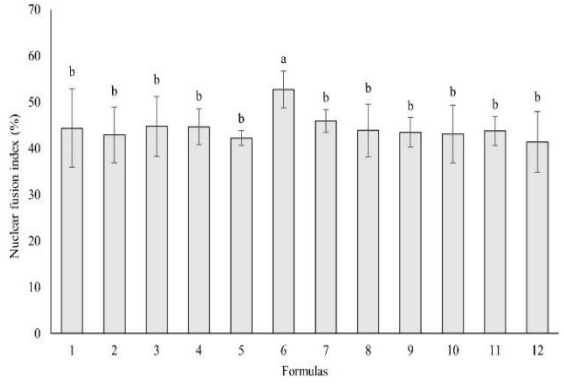

Figure 4 The NFI of the twelve formulas. Superscripts represent the significant difference ($p \leq 0.05$)

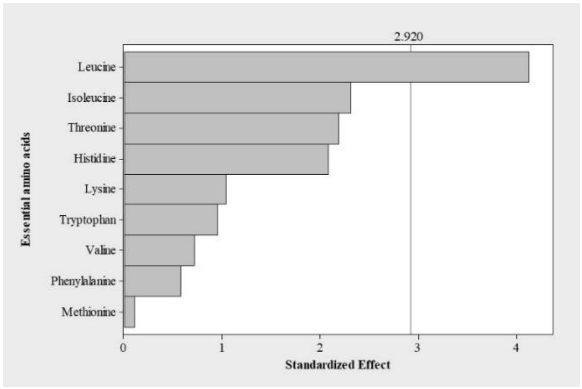

Figure 3 The Pareto chart displays the main effect on diameter ($p \leq 0.10$)

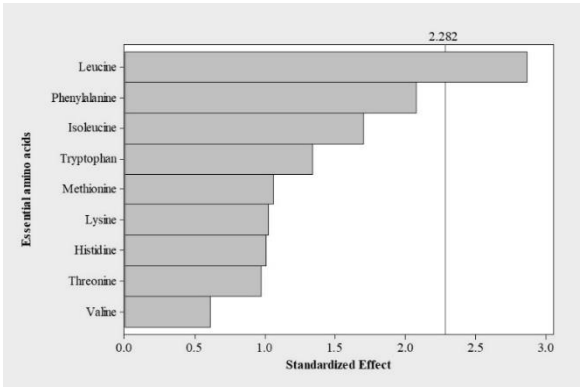

Figure 5 The Pareto chart displays the main effect on NFI ($p \leq 0.15$)

The NFI of the twelve formulas is shown in Figure 4. Likewise, the sixth formula also had an NFI (52.73 ± 4.01 %) higher than all formulas (41.364 - 45.928 %). The main EAA affecting the NFI was visualized in the Pareto chart (Figure 5). Leucine also provided the highest NFI ($p \leq 0.15$), similar to the result of diameter. In addition to diameter and NFI, the effect on myotube number was also observed in myotube differentiation. The difference among the twelve formulas was not significant at $p \leq 0.05$ to the myotube number (data not shown). The data of EAA affecting the myotube number were displayed by the Pareto chart (Figure 6). The effect of leucine on myotube number was superior to the other EAA at a significant difference higher than the effect on diameter and NFI ($p \leq 0.05$).

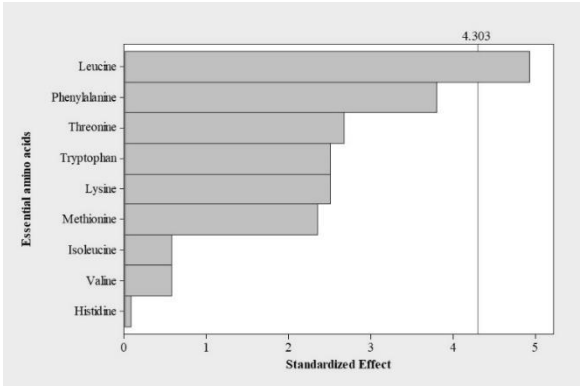

Figure 6 The Pareto chart displays the main effect on myotube number ($p \leq 0.05$)

According to the overall data, leucine demonstrated the potential to encourage myotube hypertrophy. Leucine was significant the only independent variable screened by PBD. On the other hand, the limiting amino acids often lacked in cereals

and legumes, such as lysine, methionine, threonine, tryptophan, were not significantly affected on myotube diameter, NFI, and myotube number of C2C12 myoblast cells. The influence of leucine on myotube differentiation is similar to the effect on the muscle protein synthesis (MPS) rate (%.h^{-1}) determined by the surface sensing of translation (SUnSET). Leucine is reported to have a slight advantage over other EAAs in muscle protein synthesis [7]. Moreover, leucine has been mentioned as the potential to stimulate anabolic signaling activities, represented by the phosphorylation of various signaling proteins. They consisted of mammalian target of rapamycin (mTOR), eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), and ribosomal protein S6 kinase beta-1 (p70S6K1) [8]. Even if the other EAA were not more effective than leucine but they were indispensable. The importance of other EAA was implied by the result of diameter and NFI that leucine was not significantly different at 95% interval confidence. Leucine was more critical in the early MPS stage, during 30-90 min postabsorptive leucine [9]. However, the muscle cell must eventually be composed of all EAA [10]. Consequently, the intake of complete EAA and adequate content is more important than a kind of EAA.

In the aspect of morphological differentiation, the increase in an NFI encourages myotube hypertrophy, represented by the diameter. Increased NFI was caused by numerous nuclei fused into the myotube, included in the NFI calculation. From the result of the correlation between diameter and NFI evaluated by the Pearson correlation (data not shown), the result showed that they had a relationship in the same direction ($p \leq 0.05$). This result demonstrated that expanded diameter resulted from increased NFI. During the early culturing, myoblast proliferated in DMEM. The myoblast cells were differentiated into myocytes containing one nucleus after incubating with 2% FBS. Subsequently, an adhesion between myocytes allows the fusion to differentiate into nascent myotubes (containing two nuclei). Terminally, nascent myotubes fusing to either myocytes or other myotubes form mature myotubes containing many nuclei [11]. Therefore, an increase in NFI was a cause of the expanded diameter. When discussing the leucine effect on increased diameter and NFI, it can promote this myotube hypertrophy through anabolic signaling pathways. Leucine stimulates the phosphorylation of mTOR, 4EBP1, and p70S6K1 proteins, including increased myotube number via the MAPK pathway [12]. These signaling activities were an upstream signaling pathway of myotube differentiation.

In terms of the experimental design, the PBD showed impressive potential in exploring the main effect of numerous complex factors. The main effect was explored by unbias coupled with fewer experimental trials [2]. Nine EAAs with two levels can be conducted by only twelve formulas in this study, while regular full factorial design must be performed by eighteen conditions. Leucine was explored as outstanding among the complex twelve formulas containing random EAAs. Eventually, this method was appropriate to preliminary explore the nutritional elements in the functional foods affecting healthy responses.

CONCLUSIONS

EAAs are limited in some plant sources, called limiting amino acids. The deficiency in these limiting EAAs might affect myotube atrophy. The PBD was an effective tool in screening the main EAA separated from various kinds of EAAs. Leucine was only the main EAA screened by the PBD that can significantly increase myotube diameter, NFI, and myotube number. However, other EAA

were also indispensable to incorporate into the muscle composition. This research could benefit vegans and vegetarians in selecting appropriate plant protein consumption. In an aspect of the experimental design, the PBD showed the potential to distinguish essential leucine from other EAA unbiasedly. The PBD application might be an alternative potential experimental design in exploring nutritional factors for future functional food research. However, PBD has limitations because the interactions between factors cannot be studied.

ACKNOWLEDGEMENTS

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Effects of ultrasound-assisted extraction on total phenolics and antioxidant activity of *Wolffia globosa* (Khainam) extracts

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ABSTRACT: *Wolffia globosa* (Khainam) is one of the most attractive alternatives to protein sources for human consumption and also important sources of biologically active compounds. It can grow quickly and is found in the northeastern and northern Thailand. *Wolffia globosa* is the smallest aquatic edible duckweed, which offers all nine essential amino acids (EAAs), dietary fibres, antioxidants, polyphenols, iron, zinc and B_{12} vitamin. It is interesting to study the extraction of bioactive compounds, especially phenolic compounds, and antioxidants from *Wolffia globosa*. Conventional extraction method is usually not effective to extract all the elements from small aquatic plant cell. Therefore, the main objective of this study was to study the effect of the ultrasonic pre-treatment technique on the total phenolic content and antioxidant activity of *Wolffia globosa* extracts. Ultrasonic pre-treatment at 280 W power and 37 kHz frequency with various extraction times at 15, 30, 45 and 60 min, at temperature of 30±2 °C were employed. After ultrasonication, samples were centrifuged to separate the supernatant from pellet and each part was analyzed for total phenolics content, 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging, ferric reducing/ antioxidant power (FRAP) and metal chelating activity in comparison with the full dispersion and control counterpart. The results showed that *Wolffia globosa* extracted with ultrasonication for 30 min possesses the highest total phenolic content (163.59±3.11 mg GAE/g dry basis) and the FRAP (38.24±2.09 mg Fe (II)/g dry basis). However, the use of ultrasonication did not improve metal chelating activity and inhibition of DPPH.

Keywords: Wolffia globosa (Khainam) extracts, Ultrasonic Pre-treatments, Phenolic content Antioxidant activity

INTRODUCTION

The growing consumer trend of health-conscious is driving plant-based foods. Khainam or Phum, scientific name *Wolffia globosa*, one of the duckweed species, belongs to the botanical family Lemnaceae. It is a small circular floating weed about 0.5-1.5 mm with a high protein content [1]. It is monocotyledons and is classified as a higher plant [2], an important source of bioactive compounds and grown in enriched water containing mineral media. *Wolffia globosa* has been used as a vegetable in northeastern and northern Thailand. It has other essential nutrients that are comparable to green leafy vegetables, such as fiber, calcium, iron, zinc, and several vitamins and rich in powerful

antioxidants [3]. There are also pigments such as chlorophyll, carotenoids, phytochemicals especially flavonoids in high amounts [4]. The bioactivity of each aquatic plant depends on the chemical structure, cultivation area, cultivation and harvesting season, as well as the modification of the molecular structure during the extraction process [5]. For the above reasons, *Wolffia globosa* is an interesting plant-based food alternative.

Numerous research reports have focused on macroalgae as a source of new bioactive compounds. Algal phenolic compounds were reported to be a potential candidate to destroy free radicals, which are harmful to our body and food systems [6]. Freshwater algae are rich in structurally novel and biologically active metabolites. There are antioxidant substances of very different nature. The most powerful water-soluble antioxidants found in algae are polyphenols, phenols, and vitamins [7].

However, it is difficult to get all the elements in a cell from a small aquatic plant effectively [8]. The application of ultrasound for pre-treatments allows the solvent to penetrate better into the extracted raw material. The high-frequency waves have a strength that can destroy plant cell walls equivalent to atmospheric pressure, causing the bubbles to collapse in the cell membrane and tear the cell walls [9]. The collapse of air bubbles causes cavitation, an important phenomenon that occurs when liquids are exposed to ultrasound waves. As soon as the gas bubble bursts, the energy used to accelerate the reaction pathway is released or produces new products that differ in the original reaction from the original state [10]. It is an inexpensive, simple, and efficient alternative to conventional extraction techniques. The apparatus is cheaper and easier to operate compared to other novel extraction techniques such as microwave-assisted extraction and supercritical fluid extraction [11]. It is used to extract important substances from various sources. including small algae. The use of ultrasonic has important variables: time, temperature, and frequency. Ultrasonic extraction studies on the total phenolic content of *Spirogyra sp.* and *Cladophora glomerata.* It was found that the time-temperature ratio of the solvent influenced the total phenolic content of both algae. And the combined use of ultrasonic in the extraction could increase the total phenolic content higher than the non-ultrasonic samples [7].

However, no information is available on the effect of ultrasonic-assisted extraction of *Wolffia globosa* pre-treatments. In this research, the main objective of this study was to study the effect of the ultrasonic pre-treatment technique on the total phenolic content and antioxidant activity of *Wolffia globosa* extracts.

MATERIALS AND METHODS

Frozen *wolffia globosa* from advanced greenfarm co., itd. (Thailand). at 50 °C in a dried oven. The dried *Wolffia globosa* were ground to fine powder using the medium-sized grinder (DXFILL MACHINE: DXM-2000; 220V; 3800W). Packed in an aluminum foil bag and kept at -4 °C until further use.

Ultrasonic Pre-treatments

The dried *Wolffia globosa* powder was mixed with water at a ratio of 1:2% (wt/vol) stirred at room temperature and 60 min for sample control. The ultrasonicated for the duration of 15, 30, 45 and 60 min, respectively using ultrasonic bath at 280W, 37 kHz (Elma Schmid Bauer GmbH) at 30±2 ° C . The mixture was then centrifuged at 10,000 rpm for 30 min using Multi Centrifuge (VARISPIN 15). The samples (full dispersions, pellet, and supernatant) were analyzed.

Determination of total phenolics content in Khainam extracts

The total phenolic content in the extracts was determined based on the method of Yingngam [8]. Briefly, the *Wolffia globosa* extract (0.5 mL), deionized water (9.5 mL),

Folin-Ciocalteu reagent (0.5 mL), and 10% (wt/vol) sodium carbonate solution (2 mL) were mixed and stand in dark for 20 min. The resulting mixture was measured for its absorbance at 765 nm using an ultraviolet visible spectrophotometer (Metash 220V; Thailand). All calculations about the quantitative analysis were compared with the calibration curve of gallic acid and the results were expressed as gallic acid equivalents (mg GAE/g dry basis).

Determination of 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH free radical-scavenging assay was based on the method of Yingngam [8] with some modification. First, prepare the 0.1M sodium phosphate buffer (pH 7) solution containing 1% (wt/vol) Triton-x 100. Prepare the solution (A): 0.2M Sodium phosphate monobasic monohydrate (27.598 g) was mixed with distilled water (100 mL) and prepare the solution (B): 0.2M disodium hydrogen phosphate (2.832 g) was mixed with distilled water (100 mL). Mixed 920 mL of solution (A) with 80 mL of solution (B) adjust pH 7, followed by 1% (wt/vol) Triton-x 100 (10 mL).

A sample extract (0.3 mL) was mixed with buffer (1.5 ml) kept in the dark for 30 min. The mixture was measured spectrophotometrically at 517 nm, against a blank solution without the presence of DPPH. Results were expressed as percentage inhibition of the DPPH as shown in the following equation:

$$\text{Inhibition (\%)} = \frac{(A1-A2)}{A1} \times 100 \qquad (1)$$

Where A1, A2 are the absorbance values of the Control and test sample.

Determination of ferric reducing/ antioxidant power (FRAP) assay

FRAP assay was carried out following the method of Sue-Siang [1 2] with modification. FRAP reagent was made by mixing 25 mL of acetate buffer (0.3M, pH 3.6), 5 mL of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution (10 mM), 5 mL of Iron (III) chloride solution (20 mM). TPTZ and Iron (III) chloride solutions were prepared freshly every day prior to analysis. Extract (0.2 mL) was mixed with 1 mL of FRAP reagent before it was incubated at room temperature for 5 min. The mixture was then measured spectrophotometrically at 593 nm against the blank. Standard solutions consisted of Iron (II) sulfate heptahydrate in ascending concentration order of 0.1–1.0 mM. The results were expressed as micromoles Fe (II)/g dry basis.

Determination of metal chelating activity assay

The metal chelating activity assay was based on the method of Dinis [13] with some modification. A sample extract (5 mL) was mixed with Iron (II) chloride (0.2 ml), followed by 5 mM Ferrozine (0.5 mL) and distilled water (9.2 mL). stand in dark for 10 min. The mixture was measured spectrophotometrically at 562 nm. Results were expressed as percentage metal chelating activity as shown in the following equation:

$$\text{Metal chelating activity (\%)} = \frac{(B1-B2)}{B1} \times 100 \qquad (2)$$

Where B1 and B2 are the absorbance values of the Control and test sample.

Statistical analysis

The student's Analysis of variance; ANOVA was used to determine the level of significance and comparing the mean difference of experimental results by Duncan's new multiple range test (DMRT) method. A p value of less than 0.05 was taken as significant.

RESULTS AND DISCUSSION

Effects of ultrasonic on total phenolics content

The results of the analysis of total phenolic compounds showed that the extraction of the *Wolffia globosa* extract by

water combined with the use of ultrasonic waves increased the total phenolic compounds in the supernatant (163.59±3.11 (mg GAE/g dry basis)) time at 30 min (Figure 1). The high-frequency action during ultrasonication was able to degrade the *Wolffia globosa* surfaces. The rupture of the cell wall allows the solvent to penetrate cell wall and resulting in better mass transfer. However, as the duration of ultrasonic action was increased, the total phenolic compounds decreased.

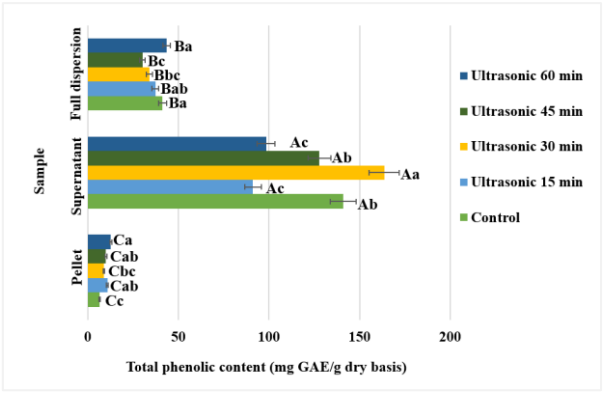

Figure 1 Total phenolic content (GAE mg/g dry basis) of *Wolffia globosa* pre-treatments by ultrasonic and control. Values are mean ± standard deviations of three (n = 3) measurements. Lower case letters (a, b, and c) within bars of the same sample with different ultrasonicated pre-treatments (15, 30, 45 and 60 min) and capital alphabet letters (A, B and C) within bars of the different samples (Pellet, Supernatant and Full dispersion) are significantly different at $p < 0.05$

The use of cold water was used to reduce the temperature of water outside the sample vessel which waves are active throughout the specified period of cavitation in the example by collapsing air bubbles in the cell membrane and causing the cell wall to rupture. Heat energy was released, causing the samples to heat higher.

Pellet samples were also analyzed to see if the total phenolic content remained. It was found that total phenolic content remained. From the analytical values, the ultrasonic pretreated *Wolffia globosa* contained more total phenolic compounds than the non-ultrasonic samples. It was assumed that collapsing air bubbles in the sample generated energy within the cell. The heat energy generated will help to accelerate the reaction pathway or create new products that differ from the original reaction in the original state [10].

Effects of Ultrasonic on 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging

The results showed that the extended time of ultrasonic pre-treatments significantly increased. The amount of antioxidant activity duration of 45 min (16.00±2.51%, 14.68±1.09%) and 60 min (15.46±1.96%, 16.59±2.15%) of the pellet and supernatant (Figure 2). Time had a statistically significant effect on the amount of antioxidant activity of the pellet, supernatant, and full dispersion fractions.

However, the pre-treatments duration of 15, 30 and 60 min showed no statistically different amounts of antioxidant activity of pellet and supernatant (Figure 2).

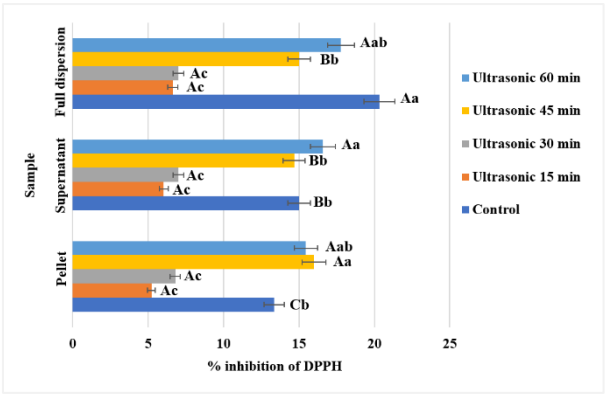

Figure 2 Percentage (%) inhibition DPPH of the *Wolffia globosa* pre-treatments by ultrasonic and control. Values are mean ± standard deviations of three (n = 3) measurements. Lower case letters (a, b, and c) within bars of the same sample with different ultrasonicated pre-treatments (15, 30, 45 and 60 min) and capital alphabet letters (A, B and C) within bars of the different samples (Pellet, Supernatant and Full dispersion) are significantly different at $p < 0.05$

Effects of Ultrasonic on ferric reducing/ antioxidant power (FRAP)

Generally, the supernatant had a higher antioxidant capacity (38.24±2.09 mg Fe (II)/g dry basis) than the pellet and full dispersion. The increase in time to 30 min has the highest antioxidant capacity and decreases gradually as the time increases to 45 and 60 min (Figure 3). The frequency at which the heat energy is released and the longer the duration, the more energy is released. Heat in the sample affected the content of some thermally unstable compounds, and the antioxidant capacity of ferric acid decreased with increasing time.

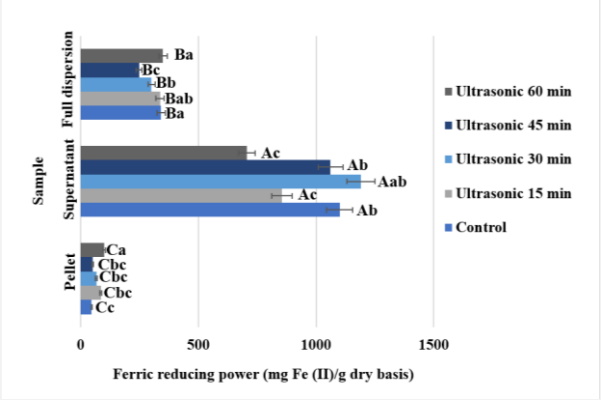

Figure 3 Ferric reducing power (mg Fe (II)/g dry basis) of *Wolffia globosa* pre-treatments by ultrasonic and control. Values are mean ± standard deviations of three (n = 3) measurements. Lower case letters (a, b, and c) within bars of the same sample with different ultrasonicated pre-treatments (15, 30, 45 and 60 min) and capital alphabet letters (A, B and C) within bars of the different samples (Pellet, Supernatant and Full dispersion) are significantly different at $p < 0.05$

Effects of Ultrasonic on metal chelating activity

The results of the metal chelating activity analysis showed that the ultrasonic pretreatment had little effect on increasing the heavy metal binding capacity of the *Wolffia globosa* (Figure 4).

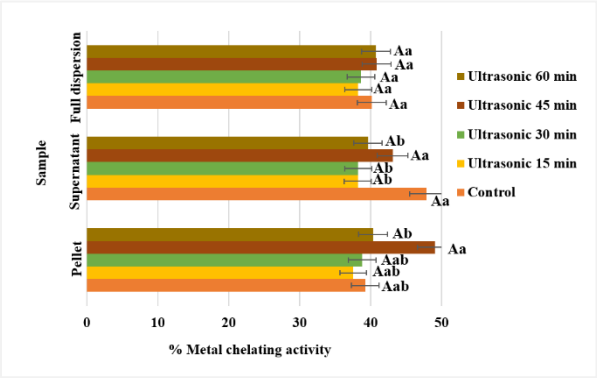

Figure 4 Percentage (%) Metal chelating activity of *Wolffia globosa* pre-treatments by ultrasonic and control. Values are mean ± standard deviations of three (n = 3) measurements. Lower case letters (a, b, and c) within bars of the same sample with different ultrasonicated pre-treatments (15, 30, 45 and 60 min) and capital alphabet letters (A, B and C) within bars of the different samples (Pellet, Supernatant and Full dispersion) are significantly different at $p < 0.05$

The samples were ultrasonication duration of 45 min was not different and extraction time was less when compared to the control. However, metal chelating activity on pellet, supernatant, and full dispersion is less than 50 % of metal chelating activity.

CONCLUSIONS

Ultrasonic-assisted extraction pre-treatment showed an increasing total phenolic content and antioxidant activity of the supernatant fraction at 30 min. The timing was optimal for the total phenolic content and the FRAP on *Wolffia globosa* extracts. The use of ultrasonic did not increase the percentage of metal chelating activity and inhibition of DPPH by less than 50 % in the pellet, supernatant, and full dispersion. Further improvement of the extraction method may be required.

NOMENCLATURE

%wt/vol	Weight/Volume
min	Minute
mL	Milliliter
M	Molarity or Molar
mM	Millimolar
g	Gram
kHz	Kilohertz
W	Watt
V	Volt
mm	Millimeter
nm	Nanometer
mg	Milligram

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POSTER PRESENTATION

Optimization of arabica raw coffee beans *(Coffea Arabica* L.) hydrolysate with nitric oxide inhibitory activity using response surface methodology (RSM)

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ABSTRACT: The aim of this study was to determine the optimal conditions for the production of protein hydrolysate from arabica raw coffee beans *(Coffea arabica* L.) using a microbial protease, Alcalase. Experiments were conducted using the Central Composite Design (CCD) and Response Surface Methodology (RSM) to determine the optimal conditions to obtain the maximum total arabica raw coffee beans protein hydrolysate. The three independent variables were hydrolysis temperature (45, 50, 55°C), hydrolysis time (180, 240, 300 min), and enzyme ratio (E/S ratio; 1, 2, 3 %v/w) while pH was fixed at 8. All data were analyzed using Design-Expert software. The results showed that three factors had a significant effect on degree of hydrolysis (DH) and inhibitory effect of nitric oxide (NO) (p<0.05). The optimal condition for NO inhibitory activity was reached at 3% v/w of E/S ratio, temperature of 50°C, and 257 minutes. Under these conditions, the DH was 98.46% and the IC_{50} for NO inhibitory activity was 2.09 µg protein/mL. The optimal condition of the hydrolysate was fractionated according to molecular weight cut-off membranes (10, 5, 3 and 0.65 kDa) while the greatest NO inhibitory activity was observed in the <0.65 kDa fraction. The fraction below 0.65 kDa showed the highest NO inhibitory activity with IC_{50} of 0.16 µg protein/mL. These results suggest that the protein hydrolysate of arabica raw coffee beans is a good source of bioactive peptides that could serve a target for anti-inflammatory agents.

Keywords: Optimization, Arabica raw coffee beans, Alcalase, NO inhibitory activity, Response Surface Methodology (RSM)

INTRODUCTION

The therapeutic applications of bioactive peptides from natural sources are receiving more attention for their derivatives as safer alternatives, either as functional foods or Dietary supplements [1]. In particular, researchers have focused on the generation, separation, purification and identification of novel peptides from various protein sources, as short peptides (3–20 amino acids) from protein hydrolysis have been shown to have higher nutritional value and are utilized more efficiently than an equivalent mixture of free amino acids [2].

The production of protein hydrolysates involves the use of digestive proteolytic enzymes from food- grade enzymes derived from microorganisms, including Alcalase, flavourzyme and neutrase, which are considered safe for human consumption [3]. Many of these protein peptides have shown to have anti-inflammatory properties [4].

The interaction of reactive nitrogen species and reactive oxygen species (ROS), e. g. NO, with toxic substances is closely associated with inflammation. Currently, there is growing interest in food proteins and the peptides they contain as potential candidates for use as antioxidant and anti-inflammatory agents (5). The ability of these peptides to inhibit nitric oxide (NO) and the anti- inflammatory response has been the subject of numerous experiments based on *in*

vitro studies of RAW264. 7 macrophage cells stimulated with lipopolysaccharide (LPS).

Protein hydrolysis is widely used in the food industry to improve the nutritional quality of proteins and to obtain bioactive peptides [6]. Protein hydrolysate is a mixture of amino acids produced by cleavage of a protein with acid, alkali, or enzyme. This can be defined by a global value known as the degree of hydrolysis (DH), which indicates the proportion of peptide bonds that have been cleaved in the starting protein [7]. It has been shown that short- chain peptides from hydrolyzed proteins have a higher nutritional value and can be utilized more efficiently than a corresponding mixture of free amino acids.

Coffee beans are one of the most consumed beverages in the world. It provides antioxidants and reduces the risk of various medical diseases. Coffee is a rich source of antioxidants that can help prevent diseases caused by oxidative stress [8,9]. The antioxidant properties of coffee may be due to the presence of both phenolic and nonphenolic bioactive compounds. However, there are limited data on the effects of coffee composition or differences in nitric oxide inhibitory activity. The anti-inflammatory of proteins and peptides isolated from the arabica raw coffee beans *(Coffea Arabica* L.) has not yet been reported. Therefore, the aim of the present study was to investigate the anti-inflammatory effect of protein hydrolysate and peptide fractions isolated from arabica raw coffee beans.

Response Surface Methodology (RSM) is an efficient and widely used tool for optimize the enzymatic hydrolysis conditions and other process conditions to achieve the desired response [10].

The aim of this study was to determine the ideal parameters for the hydrolysis of Alcalase in arabica raw coffee beans hydrolysis in order to achieve the maximum degree of hydrolysis (DH) and NO inhibitory activity through the use of the RSM.

MATERIAL AND METHODS

Materials and Chemicals

Arabica raw coffee beans (*Coffea arabica* L.) was purchased from Arabica M Company Limited. Alcalase (3.03 U/mL) was purchased form Affiliate of Merck KGaA Darmstadtm Germany. Phosphoric acid was purchased from Merck (Gibbstown, NJ). Bovine serum albumin (BSA), Sodium nitroprusside (SNP), Sulfanilamide and N-(1-naphthyl) ethylenediamine (NED) All other chemicals used were of analytical grade and were from commercial sources.

Determination of amino acid content

The amino acid content of the arabica raw coffee beans was determined based on the standard AOAC 994.12 methods. In brief, 1 g of arabica raw coffee beans was dissolved in 5mL of 6N HCl in a test tube, and placed in a heating block at 110°C for 24 h to liberate the amino acids. Then, the internal standard (10 mL of 2. 5mM L-α-amino-n-butyric acid in 0. 1M hydrochloric acid (HCl) was added, diluted with deionized water to 250 mL and placed in a heating block at 55°C for 10 min. Amino acid identity and quantity were determined by reverse-phase high- performance liquid chromatography (RP- HPLC) analysis on a Hypersil GOLD column C18 (4.6mm x150 mm, 3 µM), eluted with sodium acetate buffer pH 4.90 and 60% (v/v) ACN at 0. 3 mL/min. These amino acid contents were obtained from ALS Laboratory Group (Thailand) Co., Ltd., Suan Luang, Bangkok, Thailand.

Preparation of hydrolysate from arabica raw coffee beans (crude protein)

Dried arabica raw coffee beans were crushed with a blender and sieved through a 90-mesh sieve and stored in a vacuum-sealed polyethylene bag in a desiccator at room temperature until use. 1 g of arabica raw coffee beans powder was suspended in 50 mL of phosphate buffer saline (PBS) pH 7.2. Then, hydrolysis was carried out with Alcalase (3.03 U/mL) in a shaking incubator at 150 rpm. Hydrolysis was terminated by heating at 90°C for 20 minutes, followed by centrifugation at 10,000 rpm for 20 minutes.

The supernatant was collected and stored at -20 °C until use.

Experimental design

The experimental design and statistical analysis were performed using Design Expert software (version 11, Stat-Ease, Inc., USA) The influences of the three independent variables at five on DH and NO inhibitory activity were examined using the central composite design (CCD), and the coded and actual values of the experimental design are shown in Table 1.

The regression model between dependent variables (Y) and independent variables (x) was according to Equation 1:

$$Y = \beta_0 + \Sigma \beta_i x_j + \Sigma \beta_{ij} x_i x_j + \Sigma \beta_{ii} x_i^2 + e_i \quad \text{(Eq.1)}$$

Where Y is the measured response variable, X_i and X_j are the independent variables. β_0 is the offset term, β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive regression coefficients of the model, respectively, and e_i is the error term.

Enzymatic hydrolysis

Arabica raw coffee beans powder was hydrolyzed by Alcalase to preduce a hydrolysate. The hydrolysate of E/S was dissolved in 50 mL of 20 mM phosphate buffer at a ratio of 0.32 - 3.68 % v/w and the suspension was adjusted to pH 8.0. Hydrolysis was performed at 41.59 - 58.41°C and 139.09 - 340.91 min in a shaker at 150 rpm. Then, the samples were heated at 90°C for 20 min to stop the enzyme activity. The hydrolysates were centrifuged at 10,000 rpm at 4°C for 20 min. The supernatants were collected for further use (Table 1).

Table 1 Experimental range and levels of the independent variables

Independent Variables	Levels				
	(-1.68)	(-1)	(0)	(+1)	(+1.68)
E/S (%v/w), A	0.32	1	2	3	3.68
Temperature (°C), B	41.59	45	50	55	58.41
Time (min), C	139.09	180	240	300	340.91

Degree of hydrolysis (DH)

The reagent o-phthaldialdehyde (OPA) was prepared according to the method described by [11]. 7.62 g di-na-tetraborate decahydrate ($Na_2B_4O_7$) and 200 mg of Na-dodecyl-sulfate (SDS) were dissolved completely in 150 mL DI water (Solvent A); (2) 160 mg OPA was dissolved in 4 mL ethanol and then transferred to the solvent A (solvent B); (3) 176 mg dithiothreitol (DTT) was added to the solvent B, and total volumn was adjusted to 200 mL with DI water. To measure the DH, 400 µL sample or standard solution was mixed with 3 mL of OPA reagent and incubated at room temperature for exactly 2 min. The absorbance of the mixture was then read immediately at 340 nm with a spectrophotometer. The DI water was used as blank, and serine standard solution was prepared as standard. Triplicates of each sample were measured. The degree of hydrolysis was calculated according to [12] as shown in Eq. 2-4.

Calculation

Determination of h:

$$\text{Serine-NH}_2 = \frac{\text{OD sample - OD blank}}{\text{OD STD - OD blank}} * \frac{0.9516 \text{ meqv/L} * 0.1 * 100}{X * P}$$

where serine-NH_2 = meqv serine NH_2/g protein; (Eq.2)
X = g sample; P = protein % in sample; 0.1 is the sample volume in liter (L). h is then:
h = (serine-NH_2 - b) / a meqv/g protein, (Eq.3)
where a and b are shown in Table 2 for specific raw materials.

Calculation of DH:

$$DH = h / h_{tot} * 100\ \% \quad \text{(Eq.4)}$$

Where h_{tot} for specific raw materials

Determination of NO scavenging assay

The sample (25 µL) and 25 µL of 10 mM SNP were mixed and incubated for 2.5 hours. Then 100 µL of 0.33% (w/v) sulfanilamide (in 5% (v/v) phosphoric acid) was added and incubated for 5 minutes. Then, 100 µL of 0.1% (w/v) NED was added and incubated for 30 minutes. Absorbance at 540 nm (A_{540}) was measured using a microplate reader (Multiskan GO, Thermo Fisher Scientific). A control with SNP without the sample was also prepared.

Calculation of the IC_{50}

The percentage of radical scavenging was calculated from equation. (3): as follows

$$\frac{[(\text{Abs control-Abs blank})-(\text{Abs sample-Abs background})]}{(\text{Abs control–Abs blank})} \times 100$$

where Abs control is the A_{540} of the control (no sample), Abs sample is the A_{540} of the sample hydrolysate tested, Abs background is the A_{540} of the sample without the reagents, and Abs blank is the A_{540} of deionized water. The IC_{50} (the concentration of the sample that inhibits 50% of the NO radical scavenging) was calculated using the GraphPad Prism vs. 6.01 for windows software (GraphPad Software Inc., California, USA).

Measurement of protein content

The concentration of protein hydrolysate was determined as reported [13], using BSA as a standard. The respective sample (20 µL) was mixed with 200 µL of Bradford working buffer, incubated for 2 min and then the absorbance was determined at 595 nm (A_{595}) using a microplate reader.

Isolation of peptides by ultrafiltration

The optimal condition (arabica raw coffee beans) obtained from RSM for fractionation of hydrolysate. Ultrafiltration was used for separation through a semipermeable membrane that can classify molecular weights. In this study, peptides were fractionated according to their molecular weight by using four different molecular weight cut off (MWCO) membranes of 10, 5, 3 and 0.65 kDa (Pall Corporation, USA) and calculating the NO inhibitory activity.

Statistical analysis

All data were run in triplicate and are expressed as the mean ± one standard error of the mean. Statistical comparisons of means were performed by analysis of variance (ANOVA), followed by Duncan's multiple- range test using SPSS software version 22 (IBM, New York, USA). A value of $P<0.05$ was considered statistically significant. Regression analysis was performed using GraphPad Prism version 6.01 for Windows (GraphPad Software Inc., California, USA).

RESULTS AND DISCUSSION

Amino acid composition of arabica raw coffee beans

To determine the amino acid composition of the arabica raw coffee beans, the dried arabica raw coffee beans was analyzed according to the AOAC 994.12 methods. The amino acid composition of the arabica raw coffee beans is summarized in Table 2.

Table 2. Amino acid composition in arabica raw coffee beans protein. The results are presented as the mean ± SEM (n=3).

Amino acid	%Content(w/w)
Aspartic Acid (Asp, D)	0.86
Cystine (Cys, C)	0.29
Glutamic Acid (Glu, E)	1.61
Glycine (Gly, G)	0.69
Isoleucine (Ile, I)	0.27
Alanine (Ala, A)	0.41
Arginine (Arg, R)	0.47
Leucine (Leu, L)	0.78
Lysine (Lys, K)	0.76
Methionine (Met, M)	0.11
Phenylalanine (Phe, F)	0.45
Proline (Pro, P)	0.52
Serine (Ser, S)	0.45
Threonine (Thr,T)	0.30
Tryptophan (Trp,W)	0.10
Tyrosine (Tyr, Y)	0.28
Valine (Val, V)	0.40
Total	13.20

The most abundant amino acid in the arabica raw coffee beans was Glu at 1.61% (w/w), followed by Asp at 0.86% (w/w) each, while Trp at 0.10% (w/w) was present in the lowest amount. Hydrophobic (Iso, Leu, Met, Phe, Trp, Tyr and Val) and positively charged (Lys and Arg) amino acids in arabica raw coffee beans accounted for 2.39% w/w and 1.23% w/w of the total amino acids, respectively. These hydrophobic and polar residues were reported to be major components of anti- inflammatory peptides. [14]

Optimization of enzymatic hydrolysis conditions on DH and NO inhibitory activity by RSM

This experimental design was based on 2^3
factorial experimental runs according to CCD with three independent variables; E/S ratio (A,

%v/w), temperature (B, °C) and time (C, min) (Table 3). However, to minimize the error, all of the coefficients were considered in the design. The equation of the predicted model (based on the actual value) used to estimate DH and NO inhibitory activity which represent the response variable (Y_1 and Y_2, respectively) of protein hydrolysate from arabica raw coffee beans is given below.

$$Y_1 = -898.7606+47.3120A+29.8915B+ 1.2180C+0.2527AB -0.0007AC - 0.0027BC- 9.1267A^2- 0.2897B^2- 0.0022C^2$$

$$Y_2 = +451.2848-40.9601A-13.1526B- 0.5194C+0.3917AB -0.0256AC +4.6458BC +4.9970A^2 +0.1094B^2 +7.1981C^2$$

Where; Y_1, Y_2, A, B and C are DH (%), NO inhibitory activity (µg protein/mL; IC_{50}), E/S (%v/w) ratio, temperature (°C), and time (min), respectively. The effects of the variables on the linear (A, B, C), interaction terms (AB, AC, BC) and quadratic (A^2, B^2, C^2) of the model were evaluated for adequacy, fitness and significance by ANOVA (Table 3-4).

Table 3 Actual levels responses of the CCD

Run	Experimental values			Responses	
	A: E/S % (v/w)	B: Temperature (°)	C: Time (min)	Y_1: DH (%)	Y_2: NO activity (µg /mL)
1	1	45	180	33.34	25.59
2	3	45	180	77.91	9.78
3	1	45	300	35.87	26.99
4	3	45	300	79.82	4.68
5	1	55	180	40.44	15.74
6	3	55	180	89.62	7.42
7	1	55	300	39.21	22.37
8	3	55	300	88.66	8.24
9	2	41.59	240	57.62	14.84
10	2	58.41	240	69.91	10.24
11	2	50	139.09	61.07	11.52
12	2	50	340.91	61.88	12.74
13	0.32	50	240	19.71	31.51
14	3.68	50	240	97.29	6.33
15	2	50	240	84.72	4.71
16	2	50	240	85.03	4.78
17	2	50	240	84.55	4.79

Table 4. Analysis of variance for degree of hydrolysis by response surface quadratic model

Source	Sum of square	Degree of freedom	Mean square	F-value	p-value	
Model	9038.41	9	1004.27	2545.42	<0.0001[a]	significant
A-E/S	7387.16	1	7387.16	18723.51	<0.0001[a]	
B-Temp	195.41	1	195.41	495.28	<0.0001[a]	
C-Time	0.9554	1	0.9554	2.42	0.1636	
AB	12.78	1	12.78	32.28	0.0007[a]	
AC	0.0153	1	0.0153	0.0388	0.8494	
BC	5.49	1	5.49	13.93	0.0073[a]	
A^2	935.89	1	935.89	2372.11	<0.0001[a]	
B^2	592.03	1	592.03	1500.56	<0.0001[a]	
C^2	731.76	1	731.76	1854.72	<0.0001[a]	
Residual	2.76	7	0.3945			
Lack of Fit	2.64	5	0.5287	8.93	0.1038	not significant
Pure Error	0.1185	2	0.0592			
Cor total	9041.17	16				
R^2 = 0.9997, Adjusted R^2 = 0.9993, Predicted R^2 = 0.9977						

[a] identified variables with a significant effect on the response (p <0.05)

Table 5. Analysis of variance for NO inhibitory activity by response surface quadratic model

Source	Sum of square	Degree of freedom	Mean square	F-value	p-value	
Model	1184.99	9	131.67	2235.64	<0.0001[a]	signifi-cant
A-E/S	775.59	1	775.59	13169.21	<0.0001[a]	
B-Temp	32.31	1	32.31	548.68	<0.0001[a]	
C-Time	2.47	1	2.47	41.86	0.0003[a]	
AB	30.69	1	30.69	521.17	<0.0001[a]	
AC	18.94	1	18.94	321.63	<0.0001[a]	
BC	15.54	1	15.54	263.87	<0.0001[a]	
A^2	280.73	1	280.73	4766.74	<0.0001[a]	
B^2	84.19	1	84.19	1429.58	<0.0001[a]	
C^2	75.49	1	75.49	1281.84	<0.0001[a]	
Residual	0.41	7	0.059			
Lack of Fit	0.40	5	0.080	12.48	0.0758	not signifi-cant
Pure Error	0.013	2	6.400E-003			
Cor total	1185.40	16				
R^2 = 0.9997, Adjusted R^2 = 0.9992, Predicted R^2 = 0.9973						

[a] identified variables with a significant effect on the response (p <0.05)

The R^2 value of the model for DH value (%). (Y_1) and NO inhibitory activity (Y_2) was 0. 9997 and 0. 9997, respectively, further indicating that the model was adequate to adequately represent the relationships

between the variables. The predicted correlation coefficient values of 0.9977 and 0.9973 are in reasonable agreement with the adjusted coefficient of determination of 0.9993 and 0.9992 (Table 4-5). The non-significant lack of fit is good. The value of R^2 indicates that there is good agreement between the experimental values and the values predicted from the model. In general, the *p*-value determines the significance of each coefficient in the model.

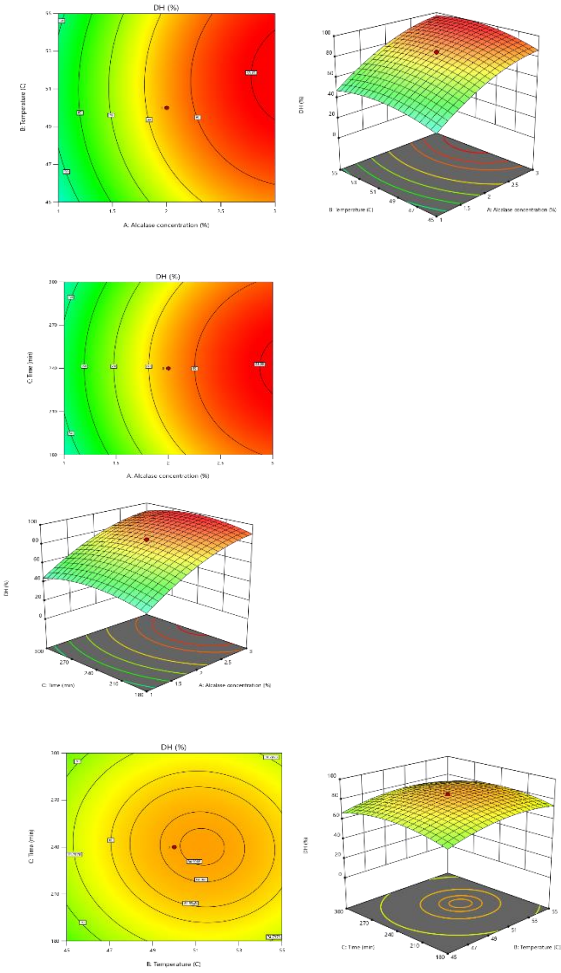

Figure. 1 Contour plot of E/S and temperature (a), E/S and time (b), temperature and time (c), on DH (%)

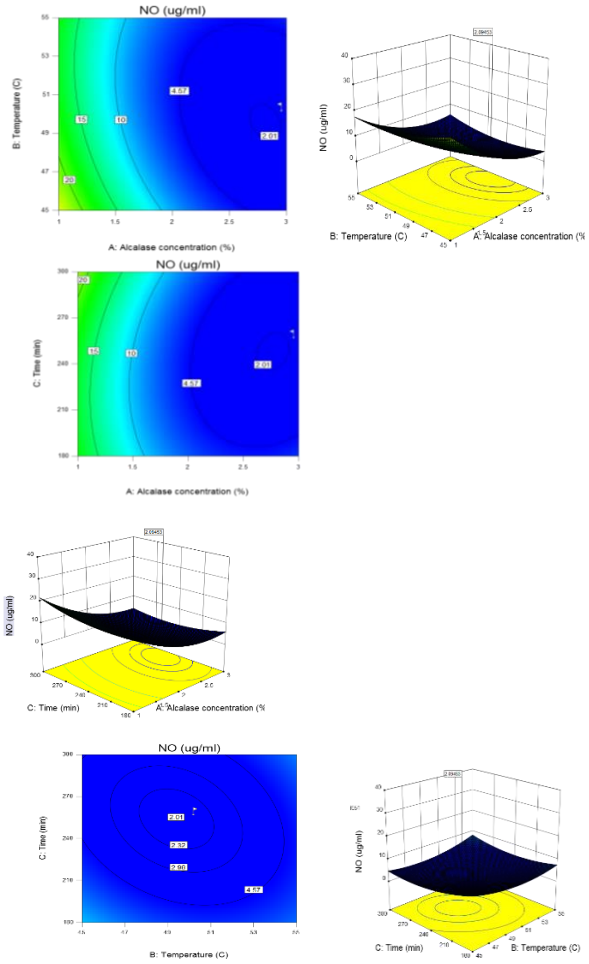

Figure. 2 Contour plot of E/S and temperature (A), E/S and time (B), temperature and time (C) on NO inhibitory activity

Validation of the statistical model and regression equation was performed using the optimal levels of the three factors (E/S 3% v/w, temperature at 50 ℃ and 257 min) . The experimental data were compared with the predicted values. Under these optimized conditions, the predicted results for DH (%) and NO inhibitory activity (IC_{50}; μg/mL) were 98.46% and 2.09 μg protein/mL, respectively, whereas the observed experimental values were 97.91% and 2.06 μg protein/ mL, respectively. These results confirmed the validity of the model and that the experimental designs used in this work were appropriate for predicting the optimized conditions.

The model is significant because the *p*-value is less than 0.05. This result showed that time, temperature and E/S had a strong effect on DH (Y_1) and NO inhibitory activity (Y_2) (Figure.1 and 2). Similar results were reported for the hydrolysis conditions, which also had an effect on the DH value and NO inhibitory activity and depended on the characteristic composition and amino acid sequence of the peptides derived [15].

Response Surface Methodology (RSM) was used to improve the DH and NO inhibitory activity of these hydrolysates. The model was validated and found to be statistically reasonable and accurate in predicting the response.

Isolation of protein hydrolysate from arabica raw coffee beans by ultrafiltration (UF)

The result shows that the low molecular weight fraction (<0.65 kDa) has the strongest NO inhibitory activity (IC_{50} = 0.16±0.01) (Table 5). The low molecular weight of the obtained hydrolysates or peptides was mainly responsible for the NO inhibitory activity [16]. Peptides with low MW showed greater radical scavenging ability than peptides with high MW peptides. It has been previously reported that the peptide fractions obtained from the skin of bluefin leatherjacket [17], heads [18], and blood clams [19] showed their greatest free radical scavenging activity in the fractions with the lowest MW (<1 kDa). These low MW peptides are able to produce this increased antioxidant activity because they can more easily interact with radicals, and hinder the oxidation process [20,21]. Accordingly, the peptide fraction with the lowest MW (<0.65 kDa), which exhibited the greatest antioxidant activity, was selected for further enrichment. The antioxidant activity of peptides isolated from protein hydrolysates depended on the molecular weight, composition and amino acid sequence [22].

Table 5. NO inhibitory activity of hydrolysate from arabica raw coffee beans hydrolyzed by Alcalase after ultrafiltration through membranes with different MWCO

MWCO fraction (kDa)	NO inhibitory activity IC_{50} (µg protein/mL)
>10	1.84±0.06^{e}
10-5	1.46±0.03^{d}
5-3	0.43±0.01^{c}
3-0.65	0.34±0.01^{b}
<0.65	0.16±0.01^{a}

$^{a-e}$ Mean values with different letters in each column are significantly different ($p \leq 0.05$)

CONCLUSIONS

The optimal conditions from the experiments were obtained at E/S 3% v/w, a temperature of 50ºC, and a time of 257 min. Thus, the results indicate that RSM with central composite design proved to be a useful tool for identifying the important factors affecting the statistically optimal strategy as an effective tool for optimising the process parameters on DH and NO inhibitory activity. These results suggest that protein hydrolysate from arabica raw coffee beans is well suited as a source of bioactive peptides that may provide evidence of anti-inflammatory.

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Comparison of conventional and ultrasound-assisted extraction techniques of betacyanins from red dragon fruit peels (*Hylocereus polyrhizus* L.)

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ABSTRACT: Red dragon fruit (*Hylocereus polyrhizus* L.) peels are rich sources of polyphenols, especially betacyanins, and are a by-product of agricultural and juice production. Betacyanins are potent antioxidants, antimicrobial, and have red-violet color that can be utilized as a food coloring agent. However, betacyanins are susceptible to environmental and processing conditions. This study aimed to compare four different extraction techniques, conventional extraction, ultrasound-assisted extraction (bath vs. probe types), and a combination of conventional extraction and ultrasonic-assisted (probe type) techniques for extracting betacyanins from red dragon fruit peels. The betacyanins extract were determined in terms of betacyanins content, total phenolic compound, total flavonoids content, antioxidants capacity (DPPH and FRAP assay), HPLC analysis, and color characteristic. The result showed that the combination extraction method has the highest ($p \leq 0.05$) betacyanins content, total phenolic compound, and total flavonoids content (151.80 ± 1.09, 34.27 ± 0.80, and 4.42 ± 0.18 mg/100ml, respectively). The extract had 25.11 ± 1.72 % inhibition of DPPH and 3287.67 ± 15.28 μmolFe^{2+}/100g of FRAP assay. Furthermore, HPLC analysis revealed that phyllocactin was the most abundant betacyanin in the extract, followed by betanin and butyrylbetanin. The color spectrum of the betacyanin extracts from various extraction procedures was red-bluish/violet. The combined process was found to be effective in extracting betacyanins and could be used as an alternative for food coloring agents.

Keywords: Red dragon fruits (Hylocereus polyrhizus L), Betacyanins, Conventional extraction, Ultrasound-assisted extraction

INTRODUCTION

Red dragon fruit (*Hylocereus polyrhizus* L.) also known as pitaya is one of the tropical fruits varieties that are widely cultivated and consumed in Southeast Asian countries such as Malaysia, Vietnam, and Thailand [1]. The peels of red dragon fruits are a by-product of the fruit juice industry for 33 % of all fruit and constitute approximately 180 tons of agricultural waste per year [2]. The red dragon fruit peel is rich in betacyanins, which is a red-violet color found in the peel more than in the flesh of red dragon fruit. Interestingly, betacyanins have antioxidant properties which are potent inhibitors of cancer cell growth and antimicrobial [3]. Moreover, betacyanins benefits not only for health but also can be used as a food coloring agent. Its color has water-soluble properties and is stable in the widely pH range 4-6. However, these pigments are easily degraded by environmental factors such as light, heat, oxygen, and low or high pH. The extraction method is one of the important causes for reducing the degradation rate of betacyanins. Hence, there is abundant research on extracting betacyanin techniques from various fruits to obtain the maximum yield and the least amount of degradation.

Conventional extraction or solid-liquid extraction using water or organic solvents is the most common method for extracting betacyanins from plants. In addition, this method uses large energy and solvent as well as their long processing time which can cause degradation of betacyanins during processing [4]. Therefore, several authors have been interested in developing innovative alternative extraction processes that utilize less solvent while also saving time and energy.

The ultrasound-assisted extraction is an alternative method for improving the effectiveness of extraction and is an environmentally friendly technique that uses low power consumption, less solvent, and a short extraction time. Ultrasound extraction can be divided into 2 types: ultrasonic bath and ultrasonic probe. The ultrasonic bath is the principle of transmitting energy or sound waves through a medium, which is water. However, the ultrasound extraction method can generate heat during extraction. Thus, the temperature and extraction time must be properly controlled as betacyanin can be degraded.

The extraction of betacyanins from various plants has previously been done using ultrasonic-assisted extraction methods such as *Bougainvillea glabra* flowers [5], red prickly pear [6], quinoa hulls [7], red beet [8], and red dragon fruit [9, 10]. Furthermore, a study demonstrates a comparison between the conventional extraction method and ultrasound-assisted extraction method that was conducted on red dragon fruit [11]. The ultrasound-assisted extraction approach yields more than the conventional extraction method. However, combining ultrasound-assisted extraction with other techniques can elevate the extraction efficiency of bioactive compounds like betacyanins [6, 12, 13]. Nevertheless, it has not appeared that the comparison research of extraction utilizing the conventional extraction, ultrasonic probe, ultrasonic bath, and a combination technique has been conducted. This study illustrates a fascinating comparison of the extraction efficiency of betacyanin using four different techniques.

MATERIALS AND METHODS

Materials

Red dragon fruit was purchased from Kenny Dragon Fruit Garden, Rangsit, Khlong 10, Pathum Thani Province, Thailand, harvested 30 - 40 days after blossoming between May - October 2021. All the other chemicals utilized in this study were analytical grade and obtained from chemical suppliers.

Sample Preparation

Red dragon fruits (*Hylocereus polyrhizus* L.) were properly cleaned and peeled. The peels were cut into 2×2 cm pieces and dried for 36 hours in a hot air oven at 42 °C with a final moisture content of x%. Finally, the dried peels were ground into a fine powder, filtered through a 20-mesh screen (particle size smaller than 0.8 mm), sealed vacuum in an aluminum foil bag, and stored at -20 °C for future extraction.

Betacyanins extractions

Conventional extraction (CE)

Red dragon powder was combined with 50 % ethanol at a ratio of 1:50 % w/v. At 200 rpm for 120 min, the mixture was stirred. After that, a Buchner funnel and Whatman No. 1 filter paper was used to remove mucilaginous material from the extract. Then, the sample was centrifuged for 15 min at 4 °C at 10000 rpm, and the supernatant was separated. Finally, the solvent was removed by using a rotary evaporator at 40 °C. The concentrated betacyanins extract was kept in amber bottle glass at 4 °C for further analysis.

Ultrasound-Assisted Extraction

- Ultrasonic Bath (Ul-B)

The Elmasonic E70H, with a nominal power of 520 W and a frequency of 50 kHz, was used in this extraction method [11]. In the bath, a beaker containing red dragon powder and 50 % ethanol (1:50 % w/v) was placed and extracted for 30 min. At the end of the extraction time, the samples were processed as previously described for conventional extraction.

- Ultrasonic Probe (Ul-P)

This method was slightly modified [9]. Two-hundred fifty mL of the red dragon powder was combined with 50 % (1:50 % w/v). The probe tip (22 mm diameter) is immersed in the liquid and irradiates the liquid with an ultrasonic wave for 5 min. The samples were treated with a frequency of 24 kHz, 400 watts of power, 30 % amplitude. At the end of the extraction time, the samples were processed as previously described for conventional extraction.

Combination of Conventional Extraction and Ultrasonic Probe (CE-P)

Red dragon fruit powder was combined in a 1:50 ratio with 50 % ethanol, stirred for 120 minutes, and extracted with an ultrasonic probe at 30 % amplitude for 5 min. The samples were processed as previously described at the end of the extraction time.

HPLC-DAD analyses

The chromatogram profile of betacyanins extracts was monitored with slight modifications [14]. A C-18 column (4.6 × 280 mm, 5 µm, Zorbax SB-C18) was inserted in the DAD-HPLC system (Agilent, 1100 series, Germany). Betacyanins were separated at 538 nm at a flow rate of 1 ml/min. For their mobile phases, A and B employed 1% formic acid in purified water (v/v) and 80% Methanol in purified water (v/v), respectively. The elution gradient was as follows: (a) 90% A for the first 10 minutes, (b) 80% A for the next 20 minutes, (c) 20% A for the next 30 minutes, and (d) returned to the initial condition after 40 minutes. The relative areas of chromatograms were computed, as well as the specific betacyanin ratios. The phenolic compounds and flavonoids were separated at 280 nm, flow rate of 1 ml/min [15]. A mobile phase of 1% formic acid (A) and methanol (B) was used in a gradient mode of 5–60 % B over 60 min. The retention times and UV spectrum of the compounds were compared to the standards, 4-Hydroxybenzoic acid, syringic acid, gallic acid, sinapic acid, *p*-coumaric acid, ferulic acid, rutin, and epicatechin.

Determination of Betacyanins Content

The betacyanins extracted were diluted with McIlvaine buffer (pH 6.5, Citrate-phosphate) to obtain absorption values of $0.9 \leq A \leq 1.0$ [16]. The number of betacyanins (BC) was calculated by the following equation. (1)

$$BC\ (mg/100ml) = \frac{A \times DF \times Mw \times V \times 100}{\varepsilon \times W \times L} \quad (1)$$

Where A is the absorbance of the sample, DF is dilution factor, MW is molecular weight (betacyanins 550 g/mol), V is volume, ε is molar extinction coefficient, for betacyanins it is 60,000 mol/L × cm, W is the weight of the sample (g) and L is the length of the cuvette (1 cm)

Determination of Total Phenolic Contents

The phenolic compound of the extract was carried out with the Folin–Ciocalteu (FC) method [14]. An amount of 400 µl of Folin-Ciocalteu's phenol reagent was added to the betacyanins extract (200 µl) or standard solutions of gallic acid (0-35 mg/100ml) and was diluted to 4.6 ml with DI water. After 10 min, 1 mL of 20 % Na_2CO_3 aqueous solution was added. After incubation for 90 min, the absorbance versus prepared blank was measured at 765 nm. Total phenolic content was expressed as mg gallic acid equivalents (mg GA)/100 ml.

Determination of Flavonoids Content

1 mL of betacyanins extract or quercetin as standard (25–200 µg/mL) were mixed with 0.2 mL of 10 % (w/v) $AlCl_3$ solution in methanol, 0.2 mL (1 M) potassium acetate and 5.6 mL distilled water. The mixture was incubated for 30 min at room temperature followed by the measurement of absorbance at 415 nm against the blank. The data were equivalents in micrograms per milliliter of extract (mg QE/100ml) [17]

DPPH Radical Scavenging Activity

This method was slightly modified [14]. 0.1 ml of sample was reacted with 3.9 ml of 0.1 mM DPPH solution (0.004 g of DPPH was dissolved in 100 mL ethanol) and was allowed to stand at room temperature for 60 min. Absorbance was measured at 515 nm. Inhibition (%) of DPPH absorbance was expressed by equation (2), absolute ethanol was used as blank.

$$\% \text{ Inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100 \quad (2)$$

$A_{control}$ is the absorbance of the control (DPPH solution without the sample), A_{test} is the absorbance of the sample.

Ferric Reducing Antioxidant Power Assay (FRAP)

The ferric reducing ability was determined by FRAP assay [14]. A 40 µL of betacyanins extracted was added to a 1.8 mL of FRAP reagent (10 mmol TPTZ solution in 40 mmol HCl, 20 mmol $FeCl_3$, and 0.3 mmol acetate buffer with pH 3.6 in ratio 1:1:10). The reagent was freshly prepared and kept at 37 ◦C for 30 min. After 10 mins of incubation at 37 ◦C, the absorbance was measured at 593 nm. Ferrous sulfate ($FeSO_4.7H_2O$) was used as standard calibration. The value was expressed as micromolar of Fe^{2+} equivalent to 100 mg of betacyanins extracted (µmolFe^{2+}/100ml).

Determination of Color

A Minolta CR-400 colorimeter based on the CIE standard was used to determine the color values of betacyanins extract. *L** denotes the degree of lightness, which ranges from 0 (black) to 100 (white), *a** varied from -100 (green) to + 100 (red), and *b** from -100 (blue) to +100 (yellow). Equations (3) and (4) were used to determine hue angle (H°) and chroma (C°) respectively.

$$H^\circ = \tan^{-1}\frac{b*}{a*} \quad (3)$$

$$C^\circ = (a*^2 + b*^2)^{1/2} \quad (4)$$

Statistical analysis

The experiments used a completely randomized design (CRD). All analyses were performed three replicated using IBM SPSS Statistics for Windows (version 22.0) with ANOVA. Fisher's Least-Significant Difference (LSD) to compare data averages and Pearson's Correlation to calculate correlation at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The different extraction techniques for extracting betacyanins from red dragon fruit peels obtained different bioactive compounds in the extracts. From our results, using the combination ultrasound-assisted extraction method (CE-P) contain the significantly ($p \leq 0.05$) highest betacyanins content, total phenolic compound, and total flavonoids content which were 151.80 ± 1.09, 34.27 ± 0.80, and 4.42 ± 1.77 mg/100ml respectively. (Table 1)

Betacyanins content

The betacyanin content of the Combination (CE-P) method had the highest value, followed by Ultrasonic Probe (Ul-P), Conventional (CE), and Ultrasonic bath (Ul-B) which were 126.91 ± 1.33, 120.36 ± 0.56, and 107.20 ± 1.04 mg/100ml, respectively. Our result shows higher content than the previous research in the extraction of betacyanins from red dragon fruits by the ultrasound-assisted extraction technique [9, 11, 18]. Ultrasound-assisted probe-type (Ul-P) can improve the efficiency of betacyanins extraction more than both CE and Ul-B extraction methods due to the source of energy being immersed directly in the sample, resulting in cavitation from high-frequency sound waves, which causes the formation and collapse of bubbles can generate energy and heat that break the cell walls of the solid matrix and solvent can get into a cell to extract bioactive compounds. As a result, the solvent permeability is increased through the cell wall, allowing for more betacyanin extraction. Furthermore, the Ul-B approach yields less betacyanin than the Ul-P and CE methods because the temperature of the sample rises during extraction, and the extended extraction period compared to the probe

method can promote betacyanins degradation. Our results were in accordance with Ramli et al. [11]. It was confirmed CE yielded more betacyanins than Ul-B extraction. In addition, conducted research comparing ultrasound-assisted probe and bath extraction methods for anthocyanin pigment extraction [19]. The ultrasound-assisted probe-type method yielded more anthocyanins than the CE and Ul-B methods. Consequently, the highest concentration of betacyanins, suggests that utilizing a combination of extraction techniques can improve betacyanins extraction efficiency than using a single extraction method [6].

Total phenolic compound and total flavonoids content.

The content of total phenolic compounds and total flavonoids follow a similar pattern. CE-P method produced the highest value, followed by Ul-P, CE, and Ul-B methods. However, there were no significant differences ($p>0.05$) between the CE and Ul-P extraction methods because phenolic compounds have a water-soluble property, the only conventional extraction method can extract a large amount of phenolic compound from red dragon fruit peels. According to previous research [19], the Ul-P approach contributed more to the extraction of anthocyanin pigments than it did to the extraction of phenolic compounds, and in another research [20], the ultrasonic bath extraction method did not improve the extraction efficiency of phenolic compounds from *Delonix regia* tree flowers when compared to the conventional method. Moreover, flavonoids are a subset of phenolic compounds which reflect a similar pattern [11]. As a result, the extraction efficiency can be improved by combining these two techniques

Table 1 The bioactive compound of betacyanins extract by using different extraction techniques.

Extraction techniques	Betacyanins content (mg/100ml)	Total Phenolic compound (mgGA/100ml)	Total Flavonoids content (mgQE/100ml)
CE	120. 36± 0.56 ^{c}	31. 07± 0.14 ^{b}	3.8 4± 0.08 ^{b}
Ul-B	107. 20± 1.04 ^{d}	19. 32± 1.34 ^{c}	2.90 ± 0.11 ^{c}
Ul-P	126. 91± 1.33 ^{b}	29. 63± 0.52 ^{b}	3.64 ±0.07 ^{b}
CE-P	151. 80± 1.09 ^{a}	34. 27± 0.80 ^{a}	4.42 ± 0.18 ^{a}

Different letters (a-d) in the same column mean significantly different ($p \leq 0.05$).

HPLC analysis of betacyanins extracted by combination extraction technique (CE-P)

- Identification of betacyanins

HPLC analysis was used to identified components of the pigment extracts obtained using the CE-P method (Fig. 1). As reported by previous researchers [14, 21, 22] betanin, isobetanin, phyllocactin, and their corresponding C-15 isomers are the main betacyanins components of red dragon fruits. In accordance with our result, there are 8 components were identified in the extracts; betanin (peak 1), isobetanin (peak 1'), phyllocactin (peak 2), isophyllocactin (peak 2'), butyrylbetanin (peak 3), Isobutyrylbetanin (peak 3'), 2'-Apiosyl-phyllocactin (peak 4), and 2'-Apiosyl-isophyllocactin (peak 4'). However, phyllocactin is the highest betacyanins, followed by betanin and butyrylbetanin.

- Identification of phenolic compounds and flavonoids

The HPLC profiles of betacyanins to be 0.70 ± 0.01 mg/100ml in the flavonoid group. Our results were consistent with a prior extract were examined for 7 phenolic compounds, which were divided into 6 phenolic acid groups and 1 flavonoid group (Table 2). The highest phenolic acid is 4-Hydroxybenzoic acid (9.77 ± 0.31 mg/100ml), followed by syringic acid, gallic acid, sinapic acid, p-coumaric acid, and ferulic acid which were 0.80 ± 0.05, 0.33 ± 0.03, 0.21 ± 0.08, 0.12 ± 0.01, and 0.09 ± 0.03 mg/100ml. Rutin was found study on dragon fruit peel betacyanin extracts [15, 23, 24, 25].

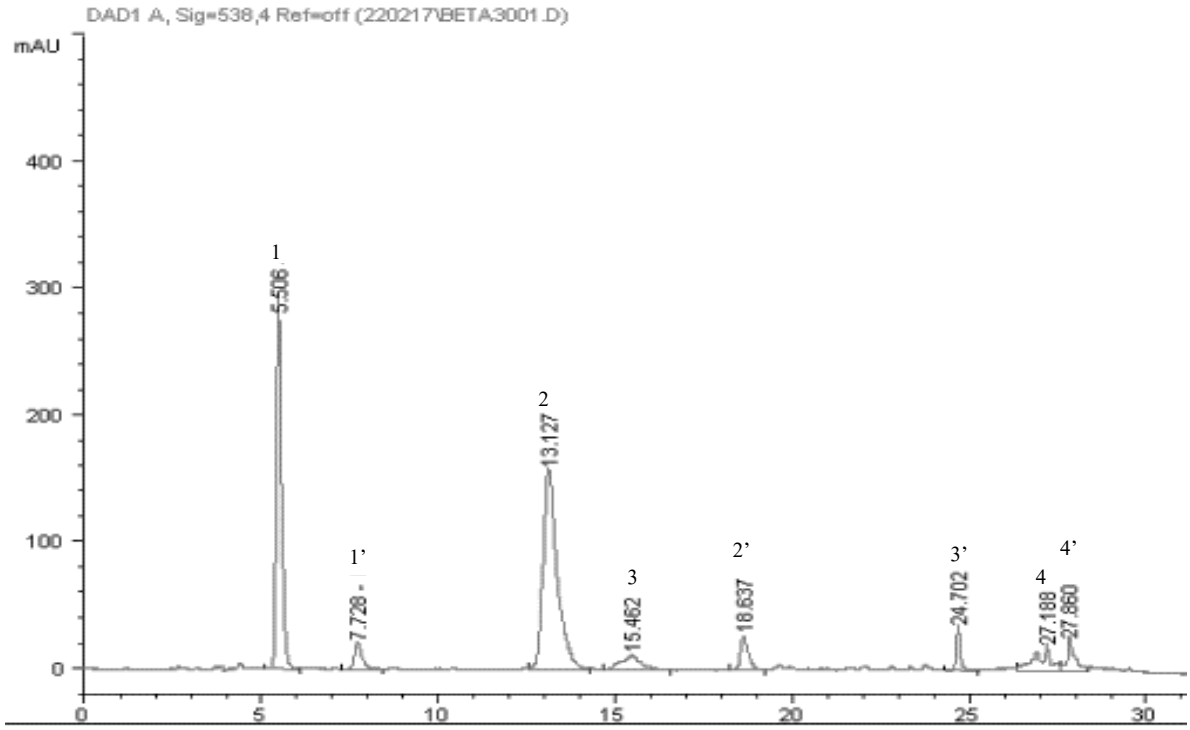

Figure 1 identification of betacyanins in betacyanins extracts by using combination method

Table 2 Concentrations of phenolic compounds in betacyanins extract by using combination extraction technique.

Phenolic compounds	Concentration (mg/100mL)
Phenolic acids	
4-Hydroxybenzoic acid	9.77 ± 0.31
Syringic acid	0.80 ± 0.05
Gallic acid	0.33 ± 0.03
Sinapic acid	0.21 ± 0.08
p-coumaric acid	0.12 ± 0.01
Ferulic acid	0.09 ± 0.03
Flavonoids Rutin	0.70 ± 0.01

Antioxidant capacities

The antioxidant capacities of the betacyanins extract using the CE-P method were evaluated using the DPPH and FRAP assays. As a result, DPPH was inhibited by 25.11 ± 1.73 %, while FRAP was inhibited by 3287.67 ± 15.28 µmolFe2+/100g. Betacyanins from red dragon fruit peel were recovered using Ul-B and CE extraction in previous research [21]. There was no significant difference ($p>0.05$) in the FRAP values between the two extraction processes, which were 255 ± 49.94 and 200.83 ± 38.3 µmol Fe2+/g, respectively. Another study [26], found 6.897 ± 0.127 µmol Fe2+/g in red dragon fruit peel by using liquid biphasic electric flotation extraction. Furthermore, when the sample concentration increased, the reducing power or FRAP value of betacyanins in red dragon fruit increased [27].

The DPPH value in this study was lower than it had been in previous research. Tutunchi et al., [28] used combination extraction to assess the DPPH of red beet extract and found a 35.85 ± 1.49 % inhibition. We analyzed the correlation value (r) between phenolic and flavonoid contents with antioxidant capacities. The correlation of phenolic and flavonoid contents was positively correlated with DPPH of 0.855 and 0.698, respectively.

The betacyanins content was positively correlated with FRAP of 0.866, which confirms previous studies on betacyanins extract from red beet [29]. Since the bioactive compound's mechanisms of antioxidant action may differ. The phenolics appear to be better at scavenging free radicals than the betalains, the betalains have a higher capacity for metallic ion reduction. As a result, the extract in this study contains a lot of betacyanins, which have the antioxidant capacity through FRAP, and a low % inhibition of DPPH could be attributed to the lower content of phenolic compound.

Color characterization

The color characterization of the betacyanins extract of red dragon fruit peels obtained using various extraction procedures was further examined (Table 3). Lightness (*L**), redness (*a**), yellowness (*b**), chroma (C*), and hue angle (h°) are all used to describe their color. The combination extraction technique (CE-P) had a significantly higher degree of *L**, *a**, b*, C*, and h° ($p<0.05$), indicating a stronger red-violet color, which might be attributed to the better extraction efficiency of betacyanins obtained. Despite this, all of the betacyanin extracts from various extraction procedures were red-bluish/violet in color, owing to positive *a** and negative *b** values. They only appeared in the same red-bluish/violet color range, demonstrating that the color of the extract was not affected by the extraction techniques.

Table 3 Color characterization of betacyanins extract of red dragon fruit peel by using different extraction techniques.

Extraction techniques	CE	Ul-B	Ul-P	CE-P
L*	30.05 ±0 .12 ^{bc}	30.39 ± 0.28 ^{ab}	29.78 ± 0.40 ^{c}	30.62 ± 0.07 ^{a}
a*	20.42 ± 0.39 ^{b}	19.20 ± 0.67 ^{c}	20.53 ± 0.53 ^{b}	25.45 ± 0.41 ^{a}
b*	-6.65 ±0 .02 ^{c}	-7.66 ± 0.56 ^{b}	-8.10 ± 0.20 ^{b}	-10.47 ± 0.40 ^{a}
Hue°	359.69 ± 0.01 ^{b}	359.62 ± 0.02 ^{c}	359.62 ± 0.01 ^{c}	359.74 ± 0.02 ^{a}
Chroma*	21.47 ± 0.36 ^{b}	20.67 ± 0.75 ^{c}	22.08 ± 0.48 ^{b}	27.53 ± 0.40 ^{a}

Different letters (a-d) in the same row mean significantly different ($p \leq 0.05$).

CONCLUSIONS

The results confirmed that using a combination ultrasound-assisted extraction (CE-P) method could improve betacyanins extraction efficiency and produced the largest amount of betacyanins, total phenolic compounds, and flavonoids. The ultrasound-assisted extraction with probe type adds to higher extraction efficiency than bath type. As a result, combining extraction techniques is not only suitable for extracting betacyanins but also reduces solvent consumption and extraction time which saves both energy and cost. Furthermore, all extraction techniques produced extracts of the same red-violet color. It can be used as a food coloring agent because it has a great color intensity.

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Identification of physicochemical properties, and bioactive compounds of riceberry rice flour from different cultivation areas in Thailand

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ABSTRACT: This research aims to investigate the physicochemical properties as well as bioactive compounds of riceberry rice flours (RBRF) from five different growing locations of Thailand; north, south, northeast, east, and central region. The physicochemical, proximate compositions, bioactive compounds, and antioxidant activities were analyzed. Results presented that RBRF samples contained various sizes of particles. RBRF from northeastern region presented the highest value of L* while from eastern region possessed the greatest redness and yellowness values. The a_w of RBRF samples ranged from 0.202–0.515. The water absorption index (WAI) and water solubility index (WSI) of RBRF from eastern region showed the highest value (6.26 and 455.69%, respectively). For pasting properties, it found that eastern region RBRF has the lowest peak viscosity, trough, breakdown, final viscosity and setback while RBRF from central region presented the highest trough, final viscosity, and setback. RBRF from central region exhibited the highest protein content but low in fat, carbohydrate, and ash content. The highest total dietary fiber was found in eastern region RBRF. RBRF samples were analysed antioxidant activity using 1,1–diphenyl–2– picryl-hydrazyl, Ferric reducing antioxidant power, and oxygen radical antioxidant capacity assay and found that RBRF from central region had the highest antioxidant activities. In addition, total phenolic content and total anthocyanin content of RBRF from central region also were the highest when compared with RBRF from other regions. Results of this study could help to indicated the characteristics of RBRF grown in different regions.

Keywords: Riceberry rice flour, Physicochemical properties, Total phenolic, Antioxidant activity, Anthocyanin

INTRODUCTION

The food trends of 2022 will be focused on nutrition, health, and wellness. People are more concern about their health and consume pro-ducts that can boost immunity, digestive system, and well-being (1). Riceberry rice is a well-known alternative rice beside Hom Mali rice recently. Riceberry rice is originated from cross-breed between Khao Hom Nin, local non-glutinous purple rice and Khao Dok Mali 105 by Rice Science Center, Kasetsart University, Thailand (2). It is gluten-free rice and provides a complex carbohydrate, protein, fatty acids and micronutrients. The riceberry rice bran mostly remains after milling, and this provides many health benefits for consumers. Pigmented pericarp of rice contains polyphenol compounds, including anthocyanins and these found to be the main bioactive compounds in pigmented rice (3), (4). Moreover, riceberry rice potent to improve hyperglycemia and hyperlipidemia conditions, reducing the oxidative stress, and inflammation (5). Anthocyanins also have many health benefits due to their pharmacological properties such as

antioxidant activity (6), anti-carcinogenic activity (7).

The climate in Thailand is suitable for growing the high-quality riceberry rice, especially in the northeastern and northern part of Thailand, (8) because cold weather is favorable to develop the riceberry rice seed color (9). There are around 20,000 rai of organic riceberry rice registered in organic certification programs (8). Interestingly, the cultivation area is significantly affected on rice qualities in terms of sensory analysis and physical properties of cooked purple rice from 6 different locations as reported by Somthawil and Sriwattana (10). Moreover, literature from Kushwaha (2016) reported the greater anthocyanin content in black rice grown the higher altitude (11).

The riceberry rice is not only popular as function food in Thailand, but a whole grain of riceberry rice flour is used to produce many products. Instead of preparing the riceberry rice flour by themselves, nowadays, consumers can effortlessly purchase the riceberry rice flour from the supermarkets, bakery shops, and online channels. To discover the riceberry rice flour that serve customers' desire in the market, this research aims to investigate the physio-chemical properties as well as bioactive compounds and antioxidant activities of riceberry rice flours from different cultivation areas in Thailand.

MATERIAL AND METHODS

Riceberry rice flour samples

Five brands of riceberry rice flours (RBRF) were purchased from five different shops. All samples were dry milled from rice grains harvested in year 2021. Each brand represented the plantation areas which were northeastern region of Thailand (Selaphum district, Roi-ed province), southern region of Thailand (Wang Wiset district, Trang province), eastern region of Thailand (Mueng chonburi district, Chonburi province), central region of Thailand (Nong Saeng district, Saraburi province) and northern region of Thailand (Thap Khlo district, Phichit province). The RBRF were vacuum-packed into the polyethylene bag, and kept at room temperature (25±1°C) until use.

Physicochemical properties

The RBRF particle size distribution test was determined by sifting 100 g of sample in a particle size analyzer (Retsch, Type Vibro, GmbH & Co., Germany) using the sieves with opening of 420, 250, 170 and 149 μm. The flour fractions passing through each sieve were weight. Color of RBRF were measured in terms of L* (brightness or whiteness), *a** (redness to greenness), and *b** (yellowness to blueness) value by using colorimeter. (Colorflex EZ, HunterLab, Spectrophotometer, USA). Water activity (a_w) was measured by water activity meter (Novasina Model: ms 1, Switzerland) by chilled mirror dew point technique at room temperature (25±1°C).

A Rapid Visco-Analyzer (RVA) was used to measure pasting properties as described by Pongpaiboon *et al.* (12). The 3 g of RBRF sample were mixed with 25 g of distilled water. Sample was held at 50°C for 1 min, then heat to 95°C at 12°C/min, next hold for 2.5 min, cooling down to 50°C at 12°C/min, and then hold at 50°C for 1.5 min. This test measured pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), and final viscosity (FV) based on graph curve. Moreover, breakdown viscosity (BD) and setback viscosity (SB) were calculated as a change between peak viscosity and trough viscosity, and final viscosity and trough viscosity.

Water soluble index (WSI) and Water absorption index (WAI) were determined as described by Anderson *et al.,* (13) with slight modification. Powder 2.5 g were weighed and suspended in 30 mL of distilled water at room temperature (25±1°C). Then, the mixture was suspended in a vortex mixer for 1 min, and centrifuged at 3500 rpm, 25°C, for 20 min. the supernatant was dried at 105°C. The sediment that remains in the tube was weighed and used to calculate for WAI. The WSI and WAI were calculated using equation 1 and 2.

$$WSI\ (\%) = \frac{Dried\ supernatant\ weight}{Initial\ sample\ weight} \times 100 \quad (1)$$

$$WAI\ (\%) = \frac{Weight\ of\ sediment}{Initial\ sample\ weight} \times 100 \quad (2)$$

Proximate analysis

Moisture, ash, protein, fat, total dietary fiber and carbohydrate contents were determined according to AOAC (2019) (14). The calorie content per 100 g sample was calculated based on the contents of protein, fat, and carbohydrate in each sample.

Bioactive compounds and antioxidant activities

The 80-mesh screened RBRF (4 g) was extracted with 20 ml of aqueous ethanol (60% v/v) in a water bath sonicator (model B1510, 40 KHz; Bransonic Bransonic® Ultrasonic, Danbury, (CT) for 10 min. After that, shaking in water bath shaker (Memmert GmbH, Wisconsin, USA) at 50°C for 2 h. Then, centrifuged at 4600 g at 4°C for 15 min by the centrifuge machine (model Z400K; HERMLE Labortechnik GmbH, Wehingen, Germany). The supernatant was collected and filtered through Whatman No.1 filter paper. Filtrate samples were stored at 4°C in darkness until analysis.

Total phenolic compounds (TPCs) were determined using Folin-Ciocalteu colorimetric method according to Ainsworth and Gillespie (2007) (15) which reported as gallic acid equivalents (GAE) per 100 g of dry matter.

Total anthocyanin content (TAC) was measured by using a pH differential method used by Yosphan *et al.* (2020) (16). TAC was calculated by equation 3. TAC was reported as milligrams of cyanidin-3-glucoside per gram of dry matter (mgC3G/g dm).

Total Anthocyanin contents (TACs) =
$(A \times MW \times DF \times 103) / \varepsilon \times 1$ (3)

Where, MW = molecular weight of cyanidin-3-glucoside = 449.2 g/mol, DF = dilution factor,

A = calculate from:

$A = (A520_{nm} - A700_{nm})pH_{1.0} - (A520_{nm} - A700_{nm})pH_{4.5}$

The antioxidant activity was determined using the DPPH (1,1–diphenyl–2– picryl-hydrazyl) radical scavenging assay (17) and express as µmol Trolox equivalents per 100 g dry weight (µmol TE/ 100g dm). FRAP (Ferric reducing antioxidant power) assays and ORAC (oxygen radical absorbance capacity) assay (18) were expressed as µmol Trolox equivalents per gram dry weight (µmol TE/g dm).

Statistical data analysis

All measurements were performed in 3 replicates for all samples. Data was presented as mean ± SD, and data was analyzed by one-way ANOVA. Duncan's multiple comparison was used to analysis significant difference of samples at 95% significant level by using SPSS software (version 18, SPSS Inc., Chicago, II, USA).

RESULTS AND DISCUSSION

Physical properties

The particle size distribution of 5 riceberry rice flour (RBRF) brands from different cultivation areas are shown in the Table 1. The result showed that RBRF contained various sizes of particles. Most of RBRF from northeastern and northern region of Thailand could pass through the sieves with opening of 149 µm (>80 mesh) for 94.69%, and 42.21%, respectively. While, RBRF from southern, eastern, and central region mostly passed through the sieves with opening of 420 µm (40-60 mesh) for 42.04%, 38.72%, and 37.55%, respectively. The higher number of mesh size could represent the higher fineness of the flour. The different in particle size could be affected by the milling machine used by the producer, and this also makes the different in particle shape (19). Many researchers found that the particle size has effect on the characteristics of baking product. The finer fractions seem to obtain the better of overall quality score than coarser fractions (20).

Table 2 shows color of five RBRF samples, and Figure 1 shows their corresponding photographs. For the lightness (L*), all samples are significantly different. RBRF from northeastern region presented the highest value of L*. This could be corresponded from the smaller particle size of sample which reflected lighter and giving brighter color. Results conformed to that reported by Tangsrianugul *et al*. (2019) (21). The redness (+*a**) and yellowness (+*b**) values ranged between 1.85-6.46 and 2.63–10.28, respectively. RBRF from eastern region possessed the greatest redness and yellowness values which is related with the appearance of RBRF (Figure 1). Moreover, the blue color of RBRF samples is responded by anthocyanin pigment which is the main bioactive compound in RBRF (3), (4).

The water activity (a_w) measures the availability of free water in food sample (22). Moreover, a_w affects shelf life, spoilage, and food safety of the products. The lower a_w means lower chance that bacteria and yeast & mold can grow. From Table 2, the a_w of RBRF samples ranged from 0.202–0.515, which was in the category of cereal grains, legumes, and products (23).

The water absorption index (WAI) and water solubility index (WSI) presented in Table 2. The water absorption index indicates the ability of product binding with water which depending on amount of protein and starch while the solubility index shows amount of solid component that can be dissolved in water (24).

The WSI and WAI of RBRF samples ranged from 2.78 – 6.77% and 207.73 – 455.69%, respectively. RBRF from eastern region showed the highest value of WSI (6.26 ± 0.61%) and WAI (455.69 ± 10.48%). This indicated that RBRF from eastern region had better water absorption ability which might be due to the relatively high in carbohydrate and protein content as shown in Table 3.

Pasting properties of RBRF were presented in Table 2. The result showed that there was significant difference in peak viscosity (PV), trough viscosity (TV), breakdown (BD), final viscosity (FV), setback (SB) (p<0.05). Eastern region RBRF has the lowest peak viscosity, trough, breakdown, final viscosity and setback. This could be attributed to the presence of the highest fat, total dietary fiber, and ash, and relatively high protein content. In addition, RBRF from central region presented the highest trough, final viscosity, and setback which might be due to the lowest amount of fat and ash and low total dietary content. It might be said that RBRF from central region could better help provide viscosity and pasting characteristics in food products.

Table 1 The particle size distribution of RBRF from different cultivation areas in Thailand

Particle size (Mesh)	Particle size distribution (%)				
	Northeast	South	East	Central	North
<40	1.21	0.95	6.68	4.76	17.14
40-60	1.51	42.04	38.72	37.55	25.00
60-80	2.59	27.02	30.19	23.26	15.65
>80	94.69	29.99	24.42	34.43	42.21

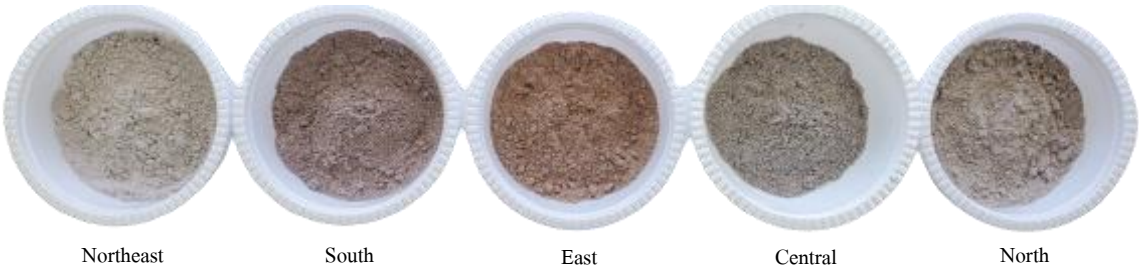

Figure 1 RBRF from different cultivation areas in Thailand

Table 2 Physical properties of RBRF from different cultivation areas in Thailand

Physical properties	Northeast	South	East	Central	North
L*	66.93 ± 0.16^{e}	37.94±0.16^{a}	51.78 ± 0.28^{b}	53.91 ± 0.48^{c}	58.97 ± 0.16^{d}
*a**	2.19 ± 0.01^{b}	3.33±0.07^{d}	6.46 ± 0.05^{e}	1.85 ± 0.01^{a}	2.88 ± 0.01^{c}
*b**	3.62 ± 0.01^{b}	2.63±0.09^{a}	10.28 ± 0.07^{e}	4.79 ± 0.20^{c}	5.99 ± 0.05^{d}
Water activity (a_w)	0.202 ± 0.002^{a}	0.513 ± 0.005^{e}	0.274 ± 0.005^{b}	0.433 ± 0.003^{d}	0.373 ± 0.003^{c}
WSI (%)	4.79 ± 0.41^{b}	3.46 ± 0.62^{a}	6.26 ± 0.61^{c}	4.78 ± 0.40^{b}	4.80 ± 0.41^{b}
WAI (%)	217.95 ± 3.77^{b}	228.19 ± 0.73^{c}	455.69 ± 10.48^{d}	220.87 ± 2.95^{b,c}	207.73 ± 0.58^{a}
Peak Viscosity (RVU)	75.14 ± 0.05^{c}	125.22 ± 4.41^{e}	15.55 ± 1.92^{a}	88.72 ± 1.19^{d}	56.53 ± 0.77^{b}
Trough (RVU)	33.92 ± 0.63^{b}	68.61 ± 2.92^{d}	15.36 ± 1.89^{a}	88.61 ± 1.39^{e}	38.16 ± 0.52^{c}
Breakdown (RVU)	41.22 ± 1.45^{c}	56.61 ± 1.79^{d}	0.19 ± 0.05^{a}	-0.18 ± 0.98^{a}	18.36 ± 1.14^{b}
Final Viscosity (RVU)	76.05 ± 1.03^{b}	148.72 ± 3.65^{d}	29.36 ± 1.41^{a}	217.30 ± 4.81^{e}	109.39 ± 0.59^{c}
Setback (RVU)	42.14 ± 1.30^{b}	80.11 ± 0.92^{d}	14.00 ± 1.30^{a}	128.69 ± 3.83^{e}	71.19 ± 0.62^{c}
Pasting Temp (°C)	87.02 ± 8.90^{a}	86.27 ± 7.56^{a}	81.22 ± 7.57^{a}	88.38 ± 8.59^{a}	89.90 ± 1.64^{a}

Note: Data shown are mean ± SD. Different letters in the same row indicated significant difference among means (p<0.05).

Proximate compositions

The calculated energy, moisture, protein, total fat, total carbohydrate, total dietary fiber, and ash of RBRF from different cultivation areas in Thailand presented in Table 3. The calculated energy of RBRF were in the range of 362.72 – 388.28 kcal/100 g. The RBRF from eastern region expressed the highest energy (388.28 ± 0.14 kcal/100 g). This was probably due to the highest amount of fat content (4.77 ± 0.44%). The moisture contents were statistically different among samples, and ranged from 9.55% – 12.33%. There is no specific standard of RBRF about the moisture content, but the Thai community product standard of rice flour (TCPS 1517/2019) states the moisture content of rice flour as not more than 13% (25). Therefore, the moisture content of all RBRF samples were within the TCPS of rice flour. The protein and fat content of RBRF samples ranged from 6.76 – 8.93% and 3.32 – 4.77%, respectively. RBRF from central region showed the highest protein value (8.93 ± 0.01%) but low in fat values (3.39 ± 0.04%). For total carbohydrate, the values were 76.13 – 78.04 %, and RBRF from northern and northeastern region showed the highest content of total carbohydrate. The RBRF from eastern region had the highest dietary fiber content of 5.25 ± 0.19%. Similar to the ash, only eastern region RBRF that was statistically different from other RBRF and showed the highest value of ash. Overall, results of proximate composition of samples in this study agreed to

those earlier reported by Pannarong *et al.* (2011) (26).

Bioactive compounds and antioxidant activities

Table 4 presents total phenolic contents and total anthocyanin content of RBRF from different cultivation areas in Thailand. Total phenolic and total anthocyanin contents of RBRF samples were in the range of 1.41 - 3.92 mgGE/100 g dm and 10.21 - 69.42 mgC3G/g dm, respectively. Central region RBRF expressed the highest total phenolic and total anthocyanin contents. Anthocyanins is known to show antioxidant activity (6), because it has the ability to capture free radical by donation of phenolic hydrogen atoms. The antioxidant activities of RBRF from different cultivation areas in Thailand had also been determined using DPPH, FRAP, ORAC assays and found that central region RBRF had significantly higher antioxidant activities by FRAP and ORAC assay than other RBRF samples. The result showed a positive correlation between the antioxidant activity and anthocyanin content which were found in many plants (27)

Table 3 Proximate composition of riceberry rice flour from different cultivation areas in Thailand

Sample	Energy (kcal)	Moisture (%)	Protein (%)	Total Fat (%)	CHO (%)	Fiber (%)	Ash (%)
Northeast	374.13 ± 0.42^{c}	9.55 ± 0.01^{b}	7.85 ± 0.04^{b}	3.40 ± 0.08^{a}	78.04 ± 0.12^{d}	3.17 ± 0.15^{b}	1.17 ± 0.02^{a}
South	362.69 ± 0.51^{a}	12.33 ± 0.08^{d}	6.76 ± 0.04^{a}	3.32 ± 0.04^{a}	76.46 ± 0.03^{b}	2.52 ± 0.03^{a}	1.15 ± 0.02^{a}
East	388.28 ± 0.14^{d}	7.16 ± 0.07^{a}	8.58 ± 0.07^{c}	4.77 ± 0.44^{b}	77.72 ± 0.14^{c}	5.25 ± 0.19^{c}	1.75 ± 0.01^{b}
Central	370.72 ± 0.49^{b}	10.39 ± 0.06^{c}	8.93 ± 0.01^{d}	3.39 ± 0.04^{a}	76.13 ± 0.01^{a}	2.93 ± 0.02^{b}	1.16 ± 0.00^{a}
North	374.17 ± 0.42^{c}	9.55 ± 0.01^{b}	7.85 ± 0.04^{b}	3.40 ± 0.08^{a}	78.04 ± 0.12^{d}	3.17 ± 0.14^{b}	1.17 ± 0.02^{a}

Note: Data shown are mean ± SD. Different letters in the same column indicated significant difference among means (p<0.05).

Table 4 Antioxidant activities and bioactive compounds of RBRF from different cultivation areas in Thailand

Parameters	Northeast	South	East	Central	North
Antioxidant Activities					
DPPH (µmol TE/100 g dm)	0.24 ± 0.01^{a}	0.43 ± 0.02^{d}	0.23 ± 0.01^{a}	0.29 ± 0.01^{c}	0.26 ± 0.02^{b}
FRAP (µmol TE/g dm)	9.16 ± 0.78^{b}	13.21 ± 0.21^{d}	7.76 ± 0.40^{a}	18.11 ± 0.41^{e}	9.66 ± 0.52^{c}
ORAC (µmol TE/g dm)	31.59 ± 2.80^{c}	36.20 ± 2.51^{d}	16.58 ± 1.52^{a}	71.05 ± 5.36^{e}	24.56 ± 2.03^{b}
Bioactive Compounds					
Total phenolic contents (mgGAE/100 g dm)	1.89 ± 0.06^{b}	2.97 ± 2.08^{c}	1.41 ± 0.06^{a}	3.92 ± 0.11^{d}	1.87 ± 0.15^{b}
Total anthocyanin content (mgC3G/g dm)	24.94 ± 0.83^{c}	47.19 ± 3.11^{d}	10.21 ± 0.92^{a}	69.42 ± 4.47^{e}	13.42 ± 0.90^{b}

Note: Data shown are mean ± SD. Different letters in the same row indicated significant difference among means (p<0.05).

CONCLUSIONS

Variations were noted among the RBRF samples in their physicochemical properties, proximate composition and level of bioactive compounds and antioxidant activities. RBRF from central region of Thailand presented the highest trough, final viscosity, and setback corresponded to the highest protein but the lowest fat content when compared with other RBRF samples. In addition, central region RBRF expressed the highest total phenolic and total anthocyanin contents which resulted in higher antioxidant activities by FRAP and ORAC assay. Therefore, these results presented the variability in the characteristics of RBRF grown in different regions of Thailand. However, the greater number of samples randomly taken from each cultivation areas should be increased for further study.

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Effect of precooking on physiochemical properties, nutritional values, total phenolic compounds and antioxidant activities of durian seed flour

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ABSTRACT: Durian seed flour has a good potential as new food ingredient. Gelatinization is a physical modification process, changing physicochemical and functional properties of flour which can enhance the utilization of flour for industrial purposes. Therefore, this study aimed to investigate the influences of precooking on nutritional, antioxidant and physio-chemical properties of durian seed flour obtained from the whole and dehulled durian seed. Results showed that whole durian seed flour (WDSF) contained higher amount of energy, fat and fiber than dehulled durian seed flour (DDSF). After precooking, samples showed the higher energy, and ash content by 0.64-5% and 2-18.48%, but greater total dietary fiber content by 11.77-29.70%, respectively. For the physical properties, WDSF appeared to be darker than DDSF while the precooking showed a significant change on the color. WDSF had higher water solubility index (WSI) but lower water absorption index (WAI) than DDSF. However, there was no significant difference on WSI and WAI between the two samples after precooking except for WAI of precooked WDSF. Overall, WDSF had lower pasting viscosities than DDSF. Precooking apparently decreased pasting viscosities of all samples. Total phenolic compounds and antioxidant activities by 1,1–diphenyl–2– picryl-hydroxyl radical scavenging (DPPH), Ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assay were significantly decreased after dehulling while significantly increased after precooking. Precook-WDSF has the highest TPC, FRAP and ORAC of 7.07 mgGAE/100 g DW, 29.58 and 110.33 µmol TE/g DW, respectively. Overall, precooked WDSF could be a good source of dietary flour alternative for common wheat flour in a new food product development.

Keywords: Durian seed flour, Precooking, Physiochemical properties, Nutritional values, Antioxidant activities

INTRODUCTION

Durian (*Durio zibethinus* Murr.) is named as a king of fruit by many countries (1). This seasonal fruit is grown the most in Southeast Asia, including Thailand. However, the edible flesh part is only one third of the whole weight whereas, the rest that was discarded were seeds (20-25%) and the shell (30-40%). Massive amount of these byproducts was discarded annually from the manufacture and lead to environmental pollution. Despite the limited utilization, durian seed showed high potential to improve the quality and added values to products. From previous study, durian seed contain high amount of carbohydrate (83%-

89%) and protein (6%-10%) with very low amount of fat. More than 30% of the carbohydrate is fiber, depends on the species, which benefit to human GI tract (2). Durian seed also has high phenolic content, which is higher than its flesh and peel. These compounds also have functional properties for the body to a certain extent to help to prevent degenerative diseases through their antioxidant and antimicrobial properties (3, 4). In addition, a glycemic index of durian seed flour is 10.90 which is in the low GI criteria category (5), Therefore, durian seed flour has the potential as a source of carbohydrates for Type 2 Diabetes Mellitus (DMT-2) sufferers.

Precooking is a heat treatment process that applied to the foods, ingredients or certain products with somewhat low heat for a long period of time. This heat treatment can affect the starch granule with the high swelling power, solubility, gelatinization temperature and starch paste stability (6-8). Precooking treatment could cause modification in the physicochemical and functional properties of flour which could enhance the utilization of flour for industrial purposes.

Therefore, this study aimed to investigate effect of precooking on physiochemical properties, nutritional values, total phenolic compounds, as well as the antioxidant activities of durian seed flour obtained from both whole and dehulled durian seeds. The result of this study would provide useful scientific information for the production of durian seed flour with better properties to meet the current consumer demands.

MATERIAL AND METHODS

Durian seeds were obtained from Chanthaburi Horticultural Research Center, Department of Agriculture, Chanthaburi province, Thailand. Durian seed was washed with tap water and stored at 4°C until further processes. All reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of Durian Seed flour

Durian seeds were divided into two groups as whole durian seeds (WDS) and dehulled durian seeds (DDS). The dehulled durian seeds were prepared by removing the hard skin (brown color) and cleaning with running water. For precooking treatment, both WDS and DDS were boiled in water at the ratio 1:2 for 40 min after that, it was drained with a filter cloth and thinly sliced (1-2 mm thick) then air dried and oven at 60°C for 8-9 h or the moisture content <10%. Finally, it was blended and sieved with an 80-mesh sieve. The whole durian seed flour (WDSF), dehulled durian seed flour (DDSF), precooked whole durian seed flour (Precook-WDSF), precooked dehulled durian seed flour (Precook-DDSF) were kept in aluminum foil bag at -20°C until study.

Physical properties

The color of the samples was quantified using Hunter Lab Colorflex EZ spectrophotometer using C.I.E. Lab (L*, a*, b*) system. Water activity (a_w) was measured by water activity meter (Nawaid Scientific ms1). Water solubility index (WSI) of the samples was determined using the method described by Ocloo et al. (9). 1 g of sample was added to 10 mL distilled water and magnetically stirred for 5 min. The suspension was then centrifuged at 3500 rpm for 30 min and the volume of supernatant obtained was recorded. The water absorption index (WAI) was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant expressed in percentage. The pasting characteristics of the sample were determined using Rapid Visco-Analyzer (RVA, Newport Scientific Instruments, Starchmaster2, Australia). The suspension was prepared with 3 g of sample in 25 g of distilled water (12% w/w, total weight of 28 g). Peak viscosity (PV, maximum paste viscosity during heating), breakdown (BD, viscosity decrease index while kept at 95°C), final viscosity (FV, paste viscosity at the end of the cooling) and setback (SB, index of the viscosity increase during

cooling) were recorded. The results are displayed as Rapid Visco Units (RVU).

Proximate analysis

Moisture, ash, protein, fat, total dietary fiber and carbohydrate contents were determined according to AOAC (2019) (10). The calorie content was calculated based on the contents of protein, fat, and carbohydrate.

Total phenolic compounds

The durian seed flour samples (1.4 g) at 80-mesh screen were extracted with 20 mL of aqueous ethanol (60% v/v) in a water bath sonicator (model B1510, 40 KHz; Bransonic Bransonic® Ultrasonic, Danbury, (CT) for 10 min. After that, shaking in water bath shaker (Memmert GmbH, Wisconsin, USA) at 50°C for 2 hr. Then, centrifuged at 4600 g at 4°C for 15 min by the centrifuge machine (model Z400K; HERMLE Labortechnik GmbH, Wehingen, Germany). The extracted samples were collected as a supernatant and filtered through Whatman No.1 filter paper. Filtrate samples were stored at 4°C in darkness until analysis.

Total phenolic compounds (TPCs) were determined using Folin-Ciocalteu colorimetric method as reported by Ainsworth and Gillespie (2007) (11). Total phenolic compounds were calculated as gallic acid equivalents (GAE) per 100 g of dry weight (GAE mg/100g DW).

Antioxidant activities

The supernatants of extracted durian seed flour samples which obtained from the same process with total phenolic compounds analysis were used to determined antioxidant activities using the DPPH (1,1–diphenyl–2– picryl-hydroxyl) radical scavenging assay (12), FRAP (Ferric reducing antioxidant power) assays (13) and ORAC (oxygen radical absorbance capacity) assay (14). The results were expressed as µmol Trolox equivalents per 100 g dry weight (µmol TE/ 100g DW) for DPPH and µmol Trolox equivalents per g dry weigh (µmol TE/g DW) for FRAP and ORAC assays.

Statistical analysis

All parameters were analyzed in triplicates. The results were expressed as mean ± standard deviations. One-way analysis of variance (ANOVA) and Duncan's multiple range test were performed to compare mean values of all groups. Average values were considered significantly different at $p < 0.05$. The statistical analysis was performed using SPSS software for Windows version 19.0 (SPSS Inc., lllinois, U.S.A.).

RESULTS AND DISCUSSION

Nutritional values

The nutrient values of different types of durian seed flour (WDSF, precook-WDSF, DDSF and precook-DDSF) are shown in Table 1. WDSF showed that it has higher energy than DDSF which is due to a high fat content. Moreover, DDSF presented a lower total dietary fiber content than WDSF. This might be explained that the skin which is a source of fiber was removed in dehulling. The effect on precooking indicated that there was a significant decrease in total carbohydrate and ash in both WDSF and DDSF which resulted in the decrease of energy. The decrease of total carbohydrate and ash could be attributed to the loss from diffusion into the boiling water. However, it was found that precooking could increase fiber content in both WDSF and DDSF. This could be attributed to the interaction between chemical components during cooking in which from a complex flour structure in which resistant to enzymatic degradation. For instance, the formation of protein polysaccharides complex created by the Maillard reaction during the precooking process or non-degradable polysaccharide from gelatinization and retro degradation of the starch (15). The protein content presented no significant difference in all durian seed flour samples. Moreover, moisture content of all durian seed flour sample ranged from 3.83 – 6.60% which is within Thai community product standard of banana flour (<12%)(TCPS1375/2019) (16).

Physicochemical Properties

Table 2 presented physical properties of WDSF, precook-WDSF, DDSF and precook-

DDSF. The redness (a*) and yellowness (b*) values of WDSF were higher than DDSF while the lightness (L*) value was lower. This was due to the brown color of skin which is still remained in WDSF. Precooking treatment resulted in darker color in WDSF but not in DDSF. Precook-WDSF has more reddish while precook-DDSF has more yellowish tone when compared with other durian seed flour samples. The color changes in precook-WDSF and DDSF samples were possibly caused by the alteration of material surface characteristics when exposed to high temperature during the precooking process. In addition, thermal treatment induced non-enzymatic browning which led to the formation of a brown color. The water activity (a_w) of WDSF, precook-WDSF, DDSF and precook-DDSF ranged from 0.09-0.21%. Carter et al. suggest that the a_w of flour should be 0.62-0.68 for both hard and soft wheat flour (17). The lower a_w limits chance that bacteria and yeast & mold can grow. The water absorption index (WAI) and water solubility index (WSI) were determined. The results expressed that WDSF has higher WAI but lower WSI than DDSF. This could be due to the higher dietary fiber present in WDSF (no skin removed)(18). The results also showed that precook-WDSF had lower WSI than WDSF flour. This can be attributed to its molecules were degraded less during precooking process. WAI of precook-DDSF and WSI of precook-WDSF and precook-DDSF showed no significant change. The result was in accordance with previous research by Kittipongpatana and Kittipongpatana (19).

Pasting properties is used to determine the food application of the starch. The results of different types of durian seed flour (WDSF, precook-WDSF, DDSF and precook-DDSF) are shown in Table 3. DDSF showed significantly the highest peak viscosity (PV), trough viscosity (TV), breakdown (BD), final viscosity (FV) and setback (SB) ($p<0.05$). This could be due to the highest carbohydrate content with the lowest total dietary fiber content (Table 1). Moreover, PV, TV, BD, FV and SB of WDSF were lower than DDSF. PV, TV, BD, FV and SB relate to water binding capacity of starch. The presence of lipid, ash, and fiber can hinder water uptake and swelling of starch granules hence reduce PV, TV, BD, FV and SB (20). After precooking, the results exhibited that PV, TV, BD, FV and SB were significantly lower for precook-WDSF and precook-DDSF than for WDSF and DDSF. Precooking caused starch gelatinization during the process, when the precooked WDSF and DDSF were reheated during the RVA measurement and this caused a decrease in the paste viscosity, leading to "thinning" of the slurry (21).

Table 1 Proximate composition and energy of durian seed flours (values per 100 g dry weight)

Nutritional values	Whole		Dehulled	
	Native	Precooking	Native	Precooking
Energy (kcal)	379 ± 0.50^{d}	360 ± 0.23^{a}	369 ± 0.62^{c}	366 ± 0.20^{b}
Moisture (g)	3.83 ± 0.16^{a}	6.60 ± 0.07^{d}	4.52 ± 0.12^{b}	6.05 ± 0.11^{c}
Protein (g)	6.62 ± 0.01^{a}	6.35 ± 0.21^{a}	6.62 ± 0.08^{a}	6.29 ± 0.09^{a}
Total Fat (g)	1.72 ± 0.01^{d}	0.10 ± 0.00^{a}	0.34 ± 0.03^{b}	0.54 ± 0.06^{c}
Total Carbohydrate (g)	84.24 ± 0.13^{b}	83.45 ± 0.27^{a}	84.76 ± 0.17^{c}	84.05 ± 0.08^{b}
Total dietary fiber (g)	21.03 ± 0.55^{c}	23.50 ± 0.57^{d}	9.39 ± 0.40^{a}	12.18 ± 0.53^{b}
Ash (g)	3.58 ± 0.02^{c}	3.50 ± 0.14^{b}	3.76 ± 0.00^{d}	3.06 ± 0.01^{a}

Values are expressed as mean±SD. Values with different letters superscript within the same row indicate significant difference at $p<0.05$ using one-way ANOVA.

Table 2 Physical properties of durian seed flours

Physical properties	Whole		Dehulled	
	Native	Precooking	Native	Precooking
Color				
L*	75.75±0.49^{b}	67.303±1.109^{a}	86.675±2.531^{c}	86.483±0.774^{c}
a*	5.813±0.096^{c}	6.625±0.309^{c}	2.983±1.013^{b}	1.353±0.235^{a}
b*	13.553±0.059^{b}	13.0440±0.496^{b}	11.251±1.007^{a}	14.198±0.882^{b}
a_w	0.087±0.0193 ^{a}	0.207±0.008 ^{c}	0.104±.0149^{a}	0.149±0.033 ^{b}
WAI	5.504±0.192^{b}	4.290±0.462^{a}	4.184±0.379 ^{a}	3.701±0.467 ^{a}
WSI	7.367±0.511 ^{a}	8.300±0.984^{a}	12.533±0.925^{b}	11.367±0.637^{b}

Values are expressed as mean±SD. Values with different letters superscript within the same row indicate significant difference at $p<0.05$ using one-way ANOVA.

Table 3 Pasting properties of durian seed flours

Pasting properties	Whole		Dehulled	
	Native	Precooking	Native	Precooking
Peak Viscosity (RVU)	94.58±13.29^{b}	23.31±2.08^{a}	142.35±12.62^{c}	16.18±8.38^{a}
Trough Viscosity (RVU)	76.46±5.27^{b}	22.65±2.15^{a}	108.58±12.59^{c}	15.60±8.70^{a}
Breakdown (RVU)	18.12±8.84^{b}	0.65±0.43^{a}	33.76±3.32^{c}	0.58±0.42^{a}
Final Viscosity (RVU)	106.68±12.09^{b}	36.75±2.84^{a}	146.65±13.30^{c}	28.35±9.69^{a}
Setback (RVU)	30.22±7.26^{b}	14.10±0.94^{a}	38.07±3.96^{c}	12.75±1.23^{a}

Values are expressed as mean±SD. Values with different letters superscript within the same row indicate significant difference at $p<0.05$ using one-way ANOVA.

Total phenolic compounds & Antioxidant activities

Total phenolic compounds (TPC) and antioxidant activities using DPPH, FRAP and ORAC assays are shown in Table 4. The total phenolic compounds and antioxidant activities were significantly reduced after dehulling. This probably due to the removal of durian seed skin which is a good source of total phenolic compounds and high antioxidant activities. Precooking caused in the increase of TPC in durian seed flour. This could be explained that higher temperatures can improve the solubility of phenolic compounds leading to the breakdown of cellular structures. This enhance the release of phenolic compounds such as phenolic acids which previously bound to the macromolecules of the cell wall (22). Moreover, it observed that the antioxidant activities of precook-WDSF and precook-DDSF were also increased after precooking. The result was similar to the earlier research in that the strong correlation between total polyphenol contents and antioxidant activities was found (23). The precook-WDSF showed the highest TPC and antioxidant activities using DPPH, FRAP and ORAC assays.

Table 4 Total phenolic compounds (TPC), DPPH radical-scavenging activity, FRAP assay, and oxygen radical antioxidant capacity (ORAC) of durian seed flours

	Whole		Dehulled	
	Native	Precooking	Native	Precooking
Total phenolic content (mgGAE/100 g DW)	7.07±0.67^{c}	7.96±0.74^{d}	1.69±0.17^{a}	2.52±0.21^{b}
Antioxidant activities				
DPPH (µmol TE/100 g DW)	0.59±0.02^{d}	0.53±0.05^{c}	0.28±0.02^{a}	0.34±0.03^{b}
FRAP (µmol TE/g DW)	22.52±1.50^{c}	29.58±2.75^{d}	2.47±0.16^{a}	4.88±0.45^{b}
ORAC (µmol TE/g DW)	91.51±5.23^{c}	110.33±6.58^{d}	23.42±1.75^{a}	41.48±2.72^{b}

Values are expressed as mean±SD. Values with different letters superscript within the same row indicate significant difference at $p<0.05$ using one-way ANOVA.

CONCLUSIONS

Physicochemical properties, proximate compositions, total phenolic compounds and antioxidant activities of durian seed flour was altered by dehulling and precooking process. The precook-WDSF contained the highest fiber content. Precooking increased WSI of WDSF while the pasting properties decreased. Moreover, the precook-WDSF also had the highest TPC and antioxidant activities using DPPH, FRAP and ORAC assays. Therefore, precook-WDSF could be used as a functional ingredient for substituting or replacing other flour such as wheat flour in order to improve the quality of the product along with better nutritional values which benefit the human health.

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Encapsulation by spray drying of porcine placenta extract (fat -removed *vs* non-fat removed): Effects on characteristics and antioxidant activities

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ABSTRACT: The objective of this study was to investigate the effect of encapsulation by spray drying of porcine placenta extracts (fat-removed and non-fat-removed) on antioxidant activities and qualities of microcapsule. Physicochemical properties as well as antioxidant activities including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) were analyzed. Both types of porcine placenta extracts (non-fat-removed and fat-removed) were encapsulated by spray drying and antioxidant activities evaluated by DPPH and FRAP assay were showed to be 21.09±0.09 and 16.28±0.85 mM Trolox equivalent (TE)/g db. and 5.09±0.16 and 8.55±0.63 mM $FeSO_4$ /g db., respectively. Physicochemical characteristics of the both microcapsules were insignificant ($p>0.05$). The microcapsule's yield was between 76.38 – 78.88%, water solubility was more than 90%, moisture content was less than 15% g wb., water activity was less than 0.6, which considered as dry food. Both types of microcapsules showed light yellow to reddish color and the encapsulation efficiency was in the range of 80.13– 82.34 %.

Keywords: Antioxidant activity, Porcine placenta, Fat-removed extract, Microcapsule, Physical-chemical properties

INTRODUCTION

Pig production is increasing rapidly in many countries, with global pork production reaching to 109.2 million tons in 2020 [1]. For more profitability, small-scale pig production systems in Thailand have recently been replaced with more intensive large-scale economically focused pig farms. [2]. Porcine placenta, a by-product from pig production, is frequently utilized in traditional Chinese medicine and for cosmetics purposes [3]. The porcine placenta contains bioactive peptides, enzymes, and nutrients for embryo nourishment such as lipids, carbohydrates, minerals, vitamins, hormone and other potential antioxidants. Porcine placenta

extract has been discovered to be effective as an immunomodulator [4], an anti-aging drug [3], and an adjuvant therapy for hypersensitivity.

In Thailand, Food and Drug Administration had banned the production, import and export of animal organs that still contain hormones in the organs such as placenta, pancreas and ovary etc. then it is necessary to remove steroid which has lipid structure in order to be able to be further used to make food products and others according to the FDA law.

The drying process is a common method of food preservation. Drying provides several advantages, including a longer shelf life, the inhibition of enzyme activity and microbial proliferation, and the slowing of food spoilage [5]. Spray drying, freeze drying, and hot air oven are just a few of the drying methods accessible. Spray drying, on the other hand, is the most extensively used drying method because it is a low-cost, quick, and simple procedure with high drying yield [6].

Encapsulation is also a technique for preventing bioactive compounds from degrading. It is the process of encapsulating biodegradable compounds with additional encapsulants to build a protective wall layer against external environmental conditions, allowing them to be more stable and improve bioactive absorption into the body [7].

Therefore, the objective of this study was to investigate the effects of encapsulation by spray drying of porcine placenta extracts (fat-removed *vs* non-fat removed extracts) on antioxidant activities and qualities of microcapsule. Antioxidant activities were analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) methods. Finally, physicochemical properties (yield, water solubility, moisture content, water activity, color values and encapsulation efficiency) of microcapsules were also assessed.

MATERIALS AND METHODS

Sample preparation and encapsulation procedure

Fresh porcine placenta was provided in Ratchaburi, Thailand. The umbilical cord, as well as the veins and arteries, were cut and removed. The sample was rinsed with 0.9 percent (w/v) saline continuously until no mucus or blood was visible. After that, the sample was combined with deionized water in a 1:1 (w/v) ratio and blended. Supernatant was obtained by centrifugation at 5000 rpm 4˚C for 30 min and the cells and tissues were removed by using a filter cloth No.0 (four layers folded). The fat in the solution was removed using a liquid-liquid extraction process using n-hexane at a 1:1 ratio (v/v) and stored at 4 °C after filtering. For fat extraction, the mixture (fat-containing extract+n-hexane) was stirred by magnetic stirrer for 3 h at 4°C and put into a separate funnel, rest for 16 hours at 4 °C. Then, the two phases became clear with well-defined interface and the fat-removed porcine placenta were then separated by draining from the separatory funnel.

Both types of porcine placenta extract (non-fat removed and fat-removed) were separately mixed with resistance maltodextrin concentration (40% w/v at ratio of 1:2 w/w) by agitator (SCILOGEX, model SCI550-S, USA) for 5 min until getting homogeneous. The mixture was fed into a spray dryer (DK-2860, GEA Niro A/S, Denmark) at inlet air temperature 175 °C, outlet air temperature 100 °C with sample flow rate 8 mL/min. The spray dried microcapsules were packed into an aluminum foil bags until physicochemical and antioxidant activity analyses.

Determination of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity by DPPH assay in porcine placenta extract and microcapsules (fat-removed *vs* non-fat removed) was measured according to the method of Brand-Williams et al. [8] and Teng et al. [9] with slight modifications. For DPPH determination

in microcapsule powders, 1 g was dissolved in 10 mL of distilled water, vortexed for 3 min and centrifuged at 5000 rpm for 20 min. Then, the supernatant was collected for DPPH determination. Briefly, 250 mL of samples (extract *vs* microcapsule) was mixed with 4.75 mL of DPPH methanol solution, vortex-mixed, and incubated in darkness at room temperature for 15 min. The absorbance was read using a UV-Vis spectrophotometer (GENESYSTM 20 Visible, U.S.A) at 517 nm. The Trolox (82-625 µM) standard was also used as standard curve and results were expressed as mM Trolox equivalent (TE)/ g db.

Determination of antioxidant activity by ferric ion reducing antioxidant power (FRAP)

The FRAP was measured according to the method of Benzie and Strain [10] with some modifications. Briefly, 4.75 mL of FRAP solution and 250 µL of sample (extract *vs* microcapsule) was vortex-mixed and left in the dark at room temperature for 30 min. Absorbance was measured at 593 nm using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). The results were calculated using a standard curve built by dissolving Ferrous sulfate in distilled water at different concentration (100-500 µM) and expressed as mM $FeSO_4$ / g db.

Measurement of yield, moisture content, water activity and color values of microcapsule

The percentage of yield was calculated according to Ramakrishnan et al. [11]. The yield (%) was calculated as the following equation:

$$\frac{\text{Mass of microcapsules (g)}}{\text{Total mass of initial substances (g)}} \times 100 \quad (1)$$

The water activity was measured by water activity analyzer (MS1, Novasina, Switzerland) at 25 °C.

The color values was measured by using a colorimeter (Konica Minolta model CR-400, Osaka, Japan). The values were expressed as L* (lightness), a* (redness to greenness) and b* (yellowness to blueness) values.

Moisture content was determined according to AOAC [12]. In brief, 2 g of microcapsule powder were taken in a pre-dried and weighed in aluminium cup, and then placed in the hot air oven (Memmert, Germany) at 105 °C until reaching a stable weight. the amount of moisture content was calculated based on the loss in weight before and after drying process in percent wet basis.

Measurement of water solubility

The water solubility according to the method of Do et al. [13] with some modifications. Briefly, Microcapsule samples (0.1 g) were dispersed in 10 mL distilled water and stirred in a vortex for 5 min. After that the solution was placed in a water bath of 30 °C for 30 min, and centrifuged at 4,000 rpm for 20 min. The supernatant was placed in a pre-dried and weighed aluminium cup and dried in the oven at 105 °C until it reached constant weight. The solubility was calculated based on weight difference, and was expressed as percentage (%).

Encapsulation efficiency

Encapsulation efficiency was determined according to the method of Saénz [14] with some modifications.

For the determination of total antioxidant activity in microcapsules (TAA). Briefly, microcapsules (0.1 g) were accurately weighed and dispersed in 1 mL ethanol, acetic acid and water (50:8:42). This dispersion was mixed using vortex for 1 min and centifuged at 10,000 rpm for 5 min. Then the clear part was filtered by 0.45 µm millipore filter. The amount of antioxidant was quantified by ferric ion reducing antioxidant power (FRAP) method [10].

For the determination of surface antioxidant activity in microcapsules (SAA), microcapsules (0.1 g) were accurately

weighed and dispersed in 1 mL of mix of 95% ethanol and methanol (1:1), respectively. This dispersion was agitated in a vortex at room temperature for 1 min and filtered by 0.45 µm millipore filter. The amount of antioxidant was quantified by ferric ion reducing antioxidant power (FRAP) method. [10]

Encapsulation effiency of antioxidant activity was calculated were calculated following equation:

$$\text{Encapsulation effiency (\%)} = \frac{\text{TAA-SAA}}{\text{TAA}} x\ 100 \quad (2)$$

Statistical analysis

Experimental design was conducted using completely randomized design (CRD) with analysis of variance (ANOVA) by SPSS software version 28.0.0.0 (SPSS Inc., Chicago, IL, USA) and the average was compared with Turkey's (HSD) multiple comparison test at the 95% of confidence level.

RESULTS AND DISCUSSION

Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and by ferric ion reducing antioxidant power (FRAP) of extract and microcapsules

The antioxidant activity of porcine placenta extract and microcapsules was determined using the DPPH and FRAP methods, as shown in table 1. The antioxidant activity by DPPH and FRAP assays of non-fat removed and fat-removed microcapsules was; 16.28±0.85 and 21.09±0.09 mM Trolox equivalents (TE)/g db. and 5.09±0.16 and 8.55±0.63 mM $FeSO_4$ /g db., respectively. The antioxidant activity by DPPH and FRAP assay of porcine placenta extract was also measured to be 2.62±0.08 mM Trolox equivalent (TE) /g db. and 4.80±0.28 mM $FeSO_4$ /g db., respectively.

Microencapsulation appears to be a promising alternative way for creating value-added functional products helping people live healthier lives and prevent disease. According to the current results, when compared with the porcine placenta extract, the use of microcapsules (especially non fat-removed) significantly ($p \leq 0.05$) improved antioxidant activity (DPPH and FRAP assays). Our results were consistent with the previous study [15]. indicating that the encapsulation of bioactive compounds would result in the improved antioxidant activity.

Physicochemical properties of micro-capsules

Percentage of yield

The results showed that the yield (%) of microcapsules was in the range of 76.38±0.03 – 78.88±0.02 % (Table 2) with no significant ($p>0.05$) difference between fat-removed and non-fat removed microcapsules.

Water solubility

The result showed that water solubility of microcapsules was in the range of 99.87±0.02 - 99.91±0.05 % (Table 2). Microcapsule powders showed a high-water solubility because resistance maltodextrin was used as the wall material. The resistant maltodextrin has been modified from the original maltodextrin to have more branches in structure. Therefore, microcapsule using resistance maltodextrin could capture more water molecules [16]. This result was consistent with the previous study [17] showing that microcapsules using resistance maltodextrin as wall material had more water solubility than microcapsule using maltodextrin as wall material.

Moisture content and water activity (aw)

Moisture content of microcapsules was in the range of 2.14±0.01-2.52±0.02 % wb. and water activity (a_w) was in the range of 0.11±0.03-0.13±0.01 (Table 2). The results showed that moisture content and water activity of microcapsules were insignificantly different ($p>0.05$). Microcapsules had moisture content under 15% wb. and water activity less than 0.6 which were acceptable for dry-food products.

Color values

Color values of microcapsules were not significantly different ($p>0.05$). The color values (L*, a* and b*) of microcapsules were in the range of 69.08±0.06 - 69.61±0.80, 1.43±0.02 - 1.45±0.04 and 7.58±0.65 - 7.66±0.01, respectively (Fig 1). According to color values, microcapsules had light yellow to reddish-yellow color.

Encapsulation efficiency

The encapsulation efficiency of microcapsules from different extract was in the range of 80.13±0.32 – 82.34±1.01 with no statistical difference ($p>0.05$).

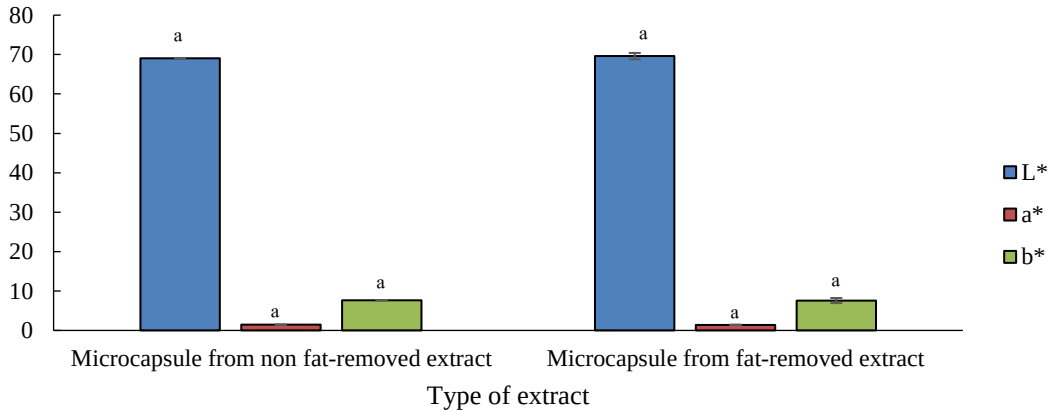

Figure 1 The color values (L*, a* and b*) of microcapsules from porcine placenta extract by spray drying.
The same letters on the column indicate no significant differences ($p>0.05$) in each value (L, a* and b*).

Table 1 Antioxidant activities (DPPH and FRAP) of microcapsules from porcine placenta extract by spray drying.

Samples	Antioxidant activity	
	DPPH (mM Trolox equivalent (TE) /g db.)	FRAP (mM $FeSO_4$ / g db.)
Microcapsule from non-fat removed extract	$21.09±0.09^{a}$	$8.55±0.63^{a}$
Microcapsule from fat-removed extract	$16.28±0.85^{b}$	$5.09±0.16^{b}$
Porcine placenta extract	$2.62±0.08^{c}$	$4.80±0.28^{c}$

*Remark: mean ± SD (from 3 replicates).
*Values in the column with different lowercase ($^{a-c}$) were significantly different ($p\leq 0.05$).

Table 2. The quality parameters of the porcine placenta microcapsules from porcine placenta extract by spray drying.

Samples	Quality parameters				
	Yield (%)	Water activity (a_w)	Moisture content (wb. %)	Water solubility (%)	Encapsulation efficiency (%)
Microcapsule from non-fat removed extract	78.88±0.02[a]	0.13±0.01[a]	2.52±0.20[a]	99.87±0.02[a]	80.13±0.32[a]
Microcapsule from fat-removed extract	76.38±0.03[a]	0.11±0.03[a]	2.14±0.10[a]	99.91±0.05[a]	82.34±1.01[a]

*Remark: mean ± SD (from 3 replicates).

*Values in the column with same lowercase ([a]) were no significantly different ($p>0.05$).

CONCLUSIONS

The effects of encapsulation by spray drying of porcine placenta extract on antioxidant activities and qualities parameters of microcapsules were evaluated in this study. The results demonstrated that encapsulation altered antioxidant activity by DPPH and FRAP assays, with microcapsules (particularly fat-removed) having better antioxidant activity than porcine placenta extracts. Microcapsule powders possessed solubility up to 90%, moisture content and water activity within the requirements of being dry food, and a light yellow to reddish-yellow appearance. In conclusion, encapsulation of porcine placenta extract by spray drying may be advantageous in increasing antioxidant activity which can be used for further application such as food and cosmetic industry.

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Effect of ultraviolet (UV) radiation on quality attributes and microbial inhibition in mulberry juice

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ABSTRACT: Non-thermal methods have shown potential in the production of high-quality fruit juices. The goal of this study was to compare the effects of UV irradiation (0-28 J/cm^2) on physicochemical features and microbiological populations in mulberry juice to no UV treatment (control) and pasteurization (90±1°C, 2 min). Some physicochemical parameters (pH, total soluble solid and color), bioactive compounds (vitamin C, total anthocyanin content and total phenolic compound) and antioxidant activity (DPPH and FRAP assays) were unaffected by UV exposure ($P>0.05$). UV radiation significantly reduced microbiological populations, including total plate count and yeast and mold count ($P\leq0.05$). In summary, the UV treatment could be considered as a potential non-thermal approach according to its ability to reduce microbial count in mulberry juice.

Keywords: *Ultraviolet radiation, Quality attributes, Mulberry juice, Microbial reduction*

INTRODUCTION

Nowadays, consumers are turning their focus on healthy foods and beverages to help them balance their nutritional requirements and reduce the risk of various diseases. This is due to the presence of numerous bioactive components with antioxidant characteristics, such as phenolic compounds, which may help prevent chronic diseases such as heart disease, cancer, and others. The mulberry (*Morus alba* L.) fruit exhibits biological activities such as anticancer effect, according to a previous study [1]. The mulberry has been shown to be a rich source of anthocyanins with antioxidant activities and antimicrobial properties. Mulberry fruits are used in Chinese traditional medicine to cure diabetes, reduce thirst, and as an expectorant and anti-cough agent. Even mulberry leaf extract has antibacterial and antioxidant effects, according to previous study [2]. These properties are mostly due to the presence of wide spectrum of phenolic substances in mulberry fruit as well as mulberry leaf extract which has recently been described in the literatures [3].

Since thermal processing may negatively affect physicochemical characteristics of food products, innovative non-thermal technologies such as exposure to ultraviolet (UV) radiation are under investigation to preserve or even enhance the quality of fruit juices. Some researchers previously reported that using UV radiation could help inactivate microorganisms and preserve nutrition values of some fruit juices during storage [4, 5]. The UV processing is also cost effective compared with most of the other non-thermal processes as it is a simple and appropriate process [4].

This research was conducted to study the changes in the quality attributes and microbial populations of mulberry juice as exposed to UV radiation. The results of the current study could be applied in the industry to maintain nutritional value in mulberry juice.

MATERIALS AND METHODS

Mulberry juice preparation

Fresh mulberry fruits were obtained from Nonthaburi, Thailand. The mulberry juice was then extracted using a juice extractor (Electrolux, EMB3500S, Sweden) from the sample preparation with water at a ratio of 1 to 2 (w/w) mulberry pulp and water, then filtered through a cheesecloth to clean pulp. The mulberry juice was centrifuged (Hettich, Rotanta 460R, England) at 8,000 rpm at 4°C for 10 min. The prepared mulberry juice was packed in a 1 L amber glass bottle covered with aluminum foil and stored at −20°C before the further experiment.

UV-C sterilization system

The UV-C sterilization system consisted of a diaphragm pump (AQ&Q, China) and a UV-C sterilizer (Treatton, Taiwan) through which the diaphragm pump pumped the sample through a flow rate of 19 mL/sec. The UV dose was calculated from the following equation: UV dosage (J/cm^2) = Intensity × Exposure time; Intensity (W/cm^2) = Total UV output / Surface area; Exposure Time (s) = Volume of sample / Flow rate; where, intensity is the intensity of UV rays (W/cm^2), total UV output is the total power of the UV lamp (W), surface area is the surface area of the UV lamp (cm^2), exposure time is the length of time the sample is exposed to UV radiation (s), volume of sample (mL) and flow rate is the sample flow rate (mL/s). The UV dosage for the current study was 0-28 (J/cm^2).

Pasteurization

Pasteurization was accomplished using a water bath. 10 mL of mulberry juice was poured into 15 mL test tubes, which were then placed in a water bath (Memmert, WNB 22, Germany) that had been prepared to 90°C. It was set for 2 min when the temperature of the juice reached 90°C.

Determination of microbial reduction in mulberry juice by UV radiation

To boost the initial microbial load, mulberry juice was incubated for 24 h at room temperature (30±2°C). UV dosages of 0, 4, 8, 12, 16, 20, 24, and 28 J/cm^2 were applied to the incubated mulberry juice. With 0.85 % sodium chloride, an appropriate dilution was performed. Pour plate and spread plate techniques were used for enumeration of total plate count and yeast and mold count, respectively. The plates were then incubated for 48 h at 35±2°C and 48 h at 27±2°C, respectively. The data were given in colony forming units per mL (CFU/mL).

Determination of physicochemical properties of mulberry juice

The physicochemical properties of samples (control, UV-treated and pasteurization) were analyzed as described by Jafari et al. [6]: The pH value was measured using a pH meter (Mettler Toledo, S220, Switzerland). Total soluble solid was measured using a digital hand refractometer (Hanna Instrument, 96801, USA) in the range of 0-30 °Brix. The color values were obtained with a chroma meter (Minolta, Model CR-300 series, Japan) and CIELAB system (L*, a* and b*); where, L* represents the brightness, a* represents the red and green values and b* value represents the yellow and blue.

Determination of Vitamin C, TAC, TPC and antioxidant activity of mulberry juice

The analysis of vitamin C content was conducted by titration, preparing the acidic (metaphosphoric and acetic acid) and dye (2,6 dichlorophenolindophenol) solutions and using ascorbic acid (µg/mL) as standard.

Total anthocyanin content (TAC) was determined following the method of Ren et al.[7]. In brief, 1 mL of juice sample was mixed with potassium chloride (0.025 M) and sodium acetate (0.4 M) buffer to reach the volume of 10 mL and left for 15 min at room temperature. The absorbance was read at 520 and 700 nm. Cyanidin-3- glucoside was used as standard and

data were expressed as mg cyanidin-3-glucosode equivalents per 100 mL of juice.

The total phenolic compound (TPC) was measured using the Folin–Ciocalteau method at the absorbance of 765 nm with spectrophotometer (GENE-SYSTEM 20 Visible, Thermo Fisher Scientific, USA). The result was expressed as mg gallic acid equivalent GAE/100mL.

The antioxidant activity was determined based on the inhibition of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical as described by Jafari et al. [8]. The absorbance was performed at the wavelength of 515 nm with spectrophotometer by using methanol as a blank. The DPPH inhibition (%) was obtained as follows:

$$\text{DPPH inhibition (\%)} = 1 - [\frac{A}{A0}] \times 100$$

where, *A0* was the absorbance of control solution and *A* was the absorbance of DPPH solution containing the juice sample.

For FRAP determination, the absorbance of sample (A_{final}) was determined at 593 nm with spectrophotometer by using distilled water as a blank.

$$A_{difference} = A_{final} - A_{initial}$$

where $A_{difference}$ was the difference between FRAP and sample, $A_{initial}$ was the absorbance of FRAP and A_{final} was the absorbance of sample. $A_{difference}$ was calculated in the above equation and the result was expressed in mM trolox equivalents (TE)/mL.

Statistical analysis

A completely randomized design was adopted in this investigation, and all trials were carried out in triplicate. The mean ± standard deviation was used to express the results. SPSS software version 22 was used for statistical analysis. At a significance level of 0.05, Tukey's (HSD) multiple comparison test was employed to identify a significant difference between mean values using one-way analysis of variance.

RESULTS AND DISCUSSION

Effect of UV radiation on reduction of microorganisms

The presence of microorganisms can cause deterioration in physicochemical properties in fruit juices which results in a short shelf life and harming consumers [9]. Some researchers showed that UV radiation can reduce the number of microorganisms causing food spoilage as well as being an alternative for thermal treatments. The effects of UV radiation on reducing of microbial population in mulberry juice are shown in Table 1. The UV showed a significant effect ($P \leq 0.05$) on reduction of bacteria (total plate count), yeast and mold (yeast and mold count). The total plate count ranged from 3.35 to 5.03 log CFU/mL among the UV group *vs* control (5.51) *vs* pasteurization (3.39). The UV samples showed significantly ($P \leq 0.05$) lower total plate count as compared with the control group. The highest reduction in total plate count (2.17 Log CFU/mL) among the UV-treated group was recorded in the highest UV dose of 28 J/cm^2 (data not shown).

A reduction in yeast and mold count was also observed among all UV group (0-28 J/cm^2) as compared with the control group ($P<0.05$). The highest reduction of yeast and mold count among the UV group observed in UV at dose of 28 J/cm^2 (1.85 log CFU/mL). However, the yeast and mold count in UV treated samples was much higher than pasteurization.

The UV radiation causes the microbial DNA to absorb photons or light energy which cross-links between pyrimidine bases (cytosine and thymine) attached to the same DNA strand forming pyrimidine dimers and causing inactivation [10]. Our results were consistent with Pala [11] studying the effect of UV radiation (12.50-62.40 J/mL) on the microbiological properties of pomegranate juice. It was reported that higher doses of UV-treated pomegranate juice were more effective in reducing microbial population. In addition, it was observed that yeast and mold were more resistant to UV radiation than bacteria (lower

log reduction). It is possible due to the fact that complex composition of cell wall or cell membrane of yeast and mold are more tolerant to UV radiation than those of bacteria [4]. Therefore, higher doses are needed to study whether yeast and mold count can be reduced.

Effects of UV radiation on pH, color and total soluble solid

No significant differences (P>0.05) were recorded among the samples (control, UV-treated and pasteurization) in terms of pH, total soluble solid and color values (Table 2). The pH was in the range of 3.34-3.35, total soluble solid was in the range of 2.43-2.53 °Brix. The color parameters (L*, a* and b*) were in the range of 23.84-24.06, 1.14-1.30 and -0.22 to -0.33, respectively. The color of juice is an important factor affecting the sensory acceptance of consumers and is another important indicator of the quality in the juice, Therefore, any treatment applied to fruit juice should not cause an alteration in its color [12].

Effect of UV irradiation on bioactive compounds and antioxidant activity

The UV effects on vitamin C, TAC, TPC and antioxidant activity by DPPH and FRAP assays were insignificant (P>0.05). Bioactive compounds (e.g., phenolic compounds) are important bioactive substances in fruit juices showing antioxidant activity which have immunomodulatory and antitumor effects. Commercial processing (e.g., thermal treatments) often affect these compounds, since these substances are sensitive to heat, light, and various processes and they cause the loss of their nutritional values [13, 14]. Consistently, Ochoa-Velasco and Beltrán [15] who studied the effect of UV radiation on TPC in dragon fruit juice reported that the UV effect was insignificant. In addition, it was reported that there were no significant differences between mean values of pH, Brix, titratable acidity, and total flavonoid content of lychee juice of UV-treated sample and control [4]. According to TAC, it showed that pasteurization or heat treatment caused a slightly increase in TAC compared to control and UV-treated samples. Several reports showed that anthocyanins in juices were increased by heat processing because heat was able to facilitate the extraction of anthocyanins [16]. Moreover, the polyphenol oxidase (PPO) originally found in anthocyanin-rich fruit juice could be inhibited, which would result in the reduction of enzyme-mediated anthocyanin degradation [16].

Table 1 Effect of UV radiation on total plate count (TPC) and yeast and mold count (YMC) of mulberry juice

Treatments	Microbial count	
	Total plate count (log CFU/mL)	Yeast and mold count (CFU/mL)
Control	5.51±0.16^{a}	6.06±0.06^{a}
Pasteurization	3.39±0.09^{f}	3.27±0.08^{f}
UV 4 J/cm^2	5.03±0.09^{b}	5.79±0.06^{b}
UV 8 J/cm^2	4.85±0.18^{bc}	5.56±0.08^{c}
UV 12 J/cm^2	4.72±0.13^{bcd}	5.43±0.11^{c}
UV 16 J/cm^2	4.42±0.35^{cd}	4.94±0.09^{d}
UV 20 J/cm^2	4.15±0.17^{de}	4.80±0.09^{d}
UV 24 J/cm^2	3.73±0.10^{ef}	4.36±0.08^{e}
UV 28 J/cm2	3.35±0.26^{f}	4.22±0.05^{e}

Data presented as mean ± standard deviation.
* $^{a-f}$ different letter within each column indicates statistically significant differences (P≤0.05).

Table 2 Effect of UV radiation on quality parameters of mulberry juice

Treatment	Quality parameters				
	pH^{ns}	Total soluble solid^{ns}	L*^{ns}	a*^{ns}	b*^{ns}
Control	3.34±0.01	2.53±0.35	23.84±0.54	1.30±0.15	-0.22±0.02
Pasteurization	3.35±0.01	2.33±0.21	23.84±0.12	1.14±0.04	-0.29±0.04
UV 4 J/cm^2	3.35±0.04	2.43±0.25	23.99±0.45	1.20±0.07	-0.28±0.08
UV 8 J/cm^2	3.35±0.04	2.43±0.25	23.96±0.42	1.20±0.08	-0.31±0.09
UV 12 J/cm^2	3.35±0.04	2.43±0.30	24.06±0.44	1.27±0.14	-0.28±0.07
UV 16 J/cm^2	3.35±0.04	2.37±0.25	23.98±0.42	1.23±0.14	-0.33±0.11
UV 20 J/cm^2	3.35±0.04	2.43±0.25	23.96±0.41	1.21±0.13	-0.28±0.19
UV 24 J/cm^2	3.35±0.04	2.47±0.25	23.98±0.28	1.24±0.18	-0.26±0.08
UV 28 J/cm2	3.35±0.04	2.47±0.35	23.97±0.40	1.23±0.11	-0.25±0.11

Data presented as mean ± standard deviation.
ns = not significant (P>0.05).
L* (the brightness value), a* (positive is red, negative is green) and b* (positive is yellow, negative is blue).

Table 3 Effect of UV radiation on bioactive compounds and antioxidant activity of mulberry juice

	Bioactive compounds and antioxidant activity				
Treatment	Vit C^{ns} (mg/100mL)	TAC^{ns} (mg cy-3-glc/100 mL)	TPC^{ns} (mg GAE/ 100 mL	DPPH inhibition^{ns} (%)	FRAP^{ns} (mM TE/mL)
Control	21.68±2.08	11.91±4.74	25.75±4.29	31.15±0.74	2.63±0.37
Pasteurization	21.38±0.47	18.09±3.10	22.60±1.49	30.27±3.30	2.52±0.37
UV 4 J/cm^2	21.48±2.04	13.80±7.35	24.19±5.03	30.62±2.47	2.63±0.50
UV 8 J/cm^2	21.48±2.04	11.74±4.10	24.11±4.61	30.36±1.84	2.51±0.51
UV 12 J/cm^2	21.48±1.91	12.75±4.86	24.67±3.55	31.42±1.76	2.67±0.38
UV 16 J/cm^2	21.58±1.64	12.41±4.26	26.04±5.17	31.34±1.69	2.71±0.39
UV 20 J/cm^2	21.28±1.04	12.36±3.61	24.34±4.97	31.74±1.13	2.63±0.48
UV 24 J/cm^2	21.24±1.95	14.08±4.45	24.71±2.92	31.69±1.06	2.76±0.32
UV 28 J/cm2	21.19±1.84	13.25±4.39	25.30±3.61	31.82±2.34	2.79±0.38

Data presented as mean ± standard deviation.
ns = not significant (P>0.05).
TAC = total anthocyanin content, TPC = total phenolic compound, cy-3-glc = cyanidin-3-glucoside, TE = trolox equivalents.

CONCLUSIONS

The consumer demand for value-added and/or functional products to enhance the health benefits has increased. This study evaluated the effects of UV irradiation on physicochemical properties and inactivation of microorganisms in mulberry juice. The UV irradiation was effective in reducing microbial population including total plate count and yeast and mold count. The higher UV exposure resulted in a higher reduction in microbial count. The results from quality characteristics showed insignificant differences in pH, total soluble solid, color values (L*, a* and b*) and vitamin C, TAC, TPC as well as antioxidant activity (DPPH and FRAP assays) after UV exposure at different doses. In conclusion, the UV treatment could be considered as a potential non-thermal technology since the ability to reduce microorganism counts with no negative effects on quality characteristics in mulberry juice. However, more study on

validation according to USFDA and the storage test are needed.

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Valorization of overripe 'Kluai Namwa' banana fruit as a raw material to produce bacterial cellulose and carboxymethylcellulose

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ABSTRACT: Overripe 'Kluai Namwa' (*Musa sapientum* L.) fruit is a food waste rich in nutrients, especially sugars. Overripe fruit was used as a carbon source for bacterial cellulose (BC) production with no glucose added to the culture medium for *Acetobacter xylinum*. After 15 days of fermentation, a high yield of BC (150 g/L) was achieved, and dried BC was used for carboxymethylcellulose (CMC) synthesis. CMC yield (90% by dry weight) was obtained after the synthesis. CMC derived from bacterial cellulose (CMC_{BC}) was characterized to disclose several characteristics. A low degree of substitution (DS) (0.39) and a low water solubility (38.3%) were investigated. Viscosity of 4% (w/v) CMC_{BC} at 45 °C were found to be 72 cP. Low DS suggests that CMC_{BC} contained fewer hydrophilic groups than carboxymethyl groups. This resulted in low water solubility and low viscosity of the CMC solution. A high average molecular weight (M_w) of 5.16×10^6 and a high degree of polymerization (DP) of 26,708 were determined. X-ray diffraction pattern showed that the crystallinity of CMC_{BC} was comparable to that of a commercial CMC reported in the literature. FT-IR spectra indicated that both BC and CMC_{BC} contain pectin as an impurity. Effect of pectin contamination influenced DS, viscosity, and water solubility of CMC. CMC_{BC} had a smooth surface structure compared to that of BC revealed by SEM images.

Keywords: Biopolymer, Cellulose derivative, Food additive, Food waste, Polymer characterization

INTRODUCTION

'Kluai Namwa' (*Musa sapientum* L.) is an important economic crop of Thailand. Overripe or ugly fruit bananas are undesirable for consumers and markets. This leads to increase in food waste. Reducing food waste by converting food waste into value-added products is a promising problem solution for food waste. Overripe or ugly fruit bananas are still rich in nutrients and can be used as a carbon source and a nutrient source for culture bacteria that produce cellulose. Vatanasuchart et al. [1] revealed three varieties of 'Kluai Namwa' composed of 92-96% carbohydrates by dry weight when ripe. Total sugars significantly increased when the fruit was ripe. The highest glucose detected was 22-34% by dry weight and the fruit contained 17-29% fructose by dry weight. These sugars can be utilized to substitute commercial sugar for bacterial cellulose production.

In general, raw materials for bacterial culture to produce bacterial cellulose are derived from semi-synthetic media, agricultural residues, or food processing waste [2], such as coconut water and pineapple juice [3], corn steep liquor [4], sisal juice [5], yeast lees [6]. Several studies have reported bacterial cellulose production using agricultural or food

processing waste as carbon sources [7]. However, there is no study that investigates using overripe 'Kluai Namwa' fruit as a carbon source for bacterial cellulose production. This study is the first to utilize overripe 'Kluai Namwa' fruit as a raw material for bacterial cellulose production. The outcome of this research aims to increase the value of low-grade banana fruit and reduce the cost of bacterial cellulose production. In addition, reducing food waste is an environmentally friendly approach.

To increase the value of bacterial cellulose, the purity of cellulose produced from bacterial has no contamination from other components, i.e., hemicellulose, lignin, pectin, etc. as cellulose derived from lignocellulosic biomass [8]. Conversion of bacterial cellulose to a cellulose derivative such as carboxymethylcellulose (CMC) is an interesting approach since CMC is an expensive biochemical. CMC is widely used in several industries [9] due to its high water-soluble property, high viscosity, low toxicity, and non-allergenic nature. It can be cast into films or formed into gels used as a stabilizer and water-holding agent [10, 11]. Several commercial products contain CMC ingredients, including food, cosmetics, medicines, adhesives, coating agents, detergents, papers, and textiles [8].

This work focuses on evaluating the potential of 'Kluai Namwa' fruit as a raw material for bacterial cellulose production. The obtained bacterial cellulose was then used to synthesize CMC by alkalization and etherification. CMC was characterized to elucidate its characteristics in terms of physical and chemical characteristics.

MATERIALS AND METHODS

Substrate preparation

Overripe 'Kluai Namwa' (*Musa sapientum* L.) was purchased from a local market in Nakhon Nayok province, Thailand. The overripe fruit was peeled and cut into small pieces. The cut fruit was mixed with distilled water and was ground in a blender until puree was obtained. The puree was filtered through a set of cheese cloth, and the filtrate was diluted with distilled water to obtain total soluble solids at 15 °Brix. The diluted filtrate was kept in a refrigerator for further use in culture medium preparation.

Inoculum preparation

Cellulose producing bacteria, *Acetobactor xylinum* was cultured in Hestrin-Schramm (HS) medium. The inoculum was incubated in the culture medium at 30 °C for 48 h. After that 10% (v/v) of the inoculum was transferred into a fresh HS medium and incubated for 20-24 h. The inoculum was harvested for cellulose production.

Bacterial cellulose production

The inoculum (10%, v/v) was transferred into the diluted filtrate of banana fruit containing 5 g/L peptone, 5 g/L yeast extract, 2.7 g/L Na_2HPO_4, and 1.15 g/L citric acid. Acetic acid was used to adjust pH to pH 4.5. The bacterial culture was incubated at 30 °C for 15 days, and bacterial cellulose was formed as a gel on the surface of the medium. The gel was harvested and washed three times with distilled water, then boiled in distilled water for 30 min to eliminate bacterial residues. Cellulose gel was collected and was kept in a refrigerator for CMC synthesis.

Cellulose sample preparation for CMC synthesis

Cellulose gel was cut into approximately 2×2 cm before drying in a vacuum oven at 45 °C until a constant weight was obtained. The dried film was ground with a blender and sieve through a 20-mesh sieve. Cellulose powder was collected and was kept in a capped scintillation vial that resided in a desiccator at room temperature.

Synthesis of carboxymethylcellulose

Bacterial cellulose powder was used as a raw material for carboxymethylcellulose (CMC) synthesis as described by Biswas et al. [10]. The experiment was done in triplicate. The

yield of CMC was calculated as follows:

$$\%\text{Yield} = \frac{Weight\ of\ CMC,g}{Weight\ of\ cellulose,g}\ x\ 100 \quad (1)$$

Characterization of CMC

Crystallinity

The method was adapted from Rachtanapun et al. [12] and Haleem et al. [13]. X-ray diffraction patterns of CMC obtained from bacterial cellulose (CMC_{BC}) were used to determine its crystallinity compared to that of bacterial cellulose (BC). The sample was dried at 105 °C for 3 h before testing. The scattering angle was 2θ from 20° to 80° with the scanning rate of 5°/min.

Degree of substitution

Determination of the degree of substitution (DS) was performed as described by Haleem et al. [13] by a titration method.

FT-IR analysis

Fourier transform infrared (FT-IR) spectroscopy was performed to determine the functional groups of BC and CMC_{BC}. The fine powder of the samples was put on the instrument probe. The detection was done with a transmission mode in the wave range of 4000 – 500 cm^{-1}.

Water solubility

CMC_{BC} cultured with overripe banana fruit was tested to evaluate its water solubility according to the method described by Abdel-Mohdy et al. [14].

Viscosity

Determination of viscosity was carried out using Rapid Visco Analyzer (RVA). CMC solution (4%, w/v) was prepared by solubilizing CMC_{BC} in distilled water. The solution was allowed to cool to room temperature before measuring the viscosity as described by Sophonputtanaphoca et al. [15].

Average molecular weight

The average molecular weight (average M_w) of CMC_{BC} was determined by column chromatography using gel permeation chromatography (GPC). The sample was prepared by dissolving the CMC sample in a 20-mL scintillation vial containing 50 °C distilled water to obtain 10 mL of 0.2% (w/v) solution. CMC solution was incubated on a rotator at 50 °C for 1 h. After the solution was cool to room temperature, the sample was injected into a Shudex-SB-804 HQ column with water as a mobile phase and a 1 mL/min flow rate. The sample was detected, and the result was reported by a GPC instrument (Shimadzu, LC-10 ADvp).

Degree of polymerization

Determination of degree of polymerization (DP) was calculated from DS and average M_w as follows:

$$DP = \frac{Molecular\ weight}{162+(80\ x\ DS)} \quad (2)$$

Morphological structure

A scanning electron microscope (SEM) was employed to determine the morphological structure of the CMC_{BC} surface compared to BC. The samples were coated with gold and were photographed at the magnification of 100× and1000×.

RESULTS AND DISCUSSION

Bacterial cellulose production

Bacterial cellulose (BC) produced by using overripe 'Kluai Namwa' fruit as a carbon source is shown in Figure 1. After incubation of *Acetobacter xylinum* at 30 °C for 15 days, a disc of cellulose gel with a size of 5.5 cm diameter and 1.3-1.5 cm thickness was obtained (Figure 1a). After drying BC in a vacuum oven at 45 °C until a constant weight was obtained, a dry weight of BC (approximately 3-4%) by weight of fresh cellulose was found. This suggests that fresh cellulose gel contained approximately 96-97% water in its structure. In terms of BC yield compared to the volume of the culture medium, using overripe 'Kluai Namwa' fruit as a carbon source gave 150 g/L fresh BC or equivalent to 0.5-0.6 g/L dry BC. The yield of BC was influenced by several factors, such as medium compositions, cultural conditions, types of microorganisms [6]. Lestari et al. [3] reported that culturing *Acetobacter xylinum* for 12 days using coconut water and pineapple juice gave BC yields of 50 g/L and 30 g/L, respectively. Costa et al. [4] employed alternative sources of

carbon by using 20 g/L sugarcane molasses and 10 g/L corn steep liquor to replace glucose for culturing *Gluconacetobacter hansenii* UCP1619. The obtained BC yield was hydrated (105.8 g/L) or dry (0.90 g/L) mass. Lima et al. [5] studied the production of BC in sisal juice by *Komagataeibacter hansenii* ATCC 23769. Using sisal juice to replace glucose, the highest BC yield of 2.60 g/L was obtained which was 3-fold higher compared to the yield obtained using the standard HS medium. Compared with literatures discussed above, overripe 'Kluai Namwa' fruit can be used as an alternative carbon source instead of glucose for BC production.

Figure 1 Bacterial cellulose after cultured using overripe 'Kluai Namwa' fruit as a carbon source: fresh (a); dried (b)

Characteristics of carboxymethylcellulose

Utilization of BC cultured with overripe 'Kluai Namwa' fruit as a carbon source for the synthesis of a cellulose derivative is an interesting approach to increase the value-added of the BC. CMC synthesized from bacterial cellulose (CMC_{BC}) was characterized by several approaches to elucidate its characteristics. Table 1 shows the CMC_{BC} yield and some of the characteristics investigated in this study. CMC_{BC} had lower degree of substitution (DS) (DS = 0.39) compared to CMC obtained from ten cultivars of rice straw (DS = 0.63-0.87) synthesized by the same method [15]. An increase in DS allows more hydrophilic groups (polar carboxyl groups) to substitute in the cellulose structure, increasing in viscosity [8]. In contrast, fewer hydrophilic groups in the polymer structure result in lower DS [16]. The relationship between DS and water solubility is in the same manner. Generally, CMC is completely soluble at a DS above 0.4. As the DS increases, the water solubility increases [16]. As shown in Table 1, CMC_{BC} had a DS of 0.39 and low water solubility. Thus, DS of CMC affects its water solubility. CMC_{BC} with low water solubility may affect viscosity value. Casaburi et al. [8] produced BC from HS medium and used it to synthesize CMC. In that study, the DS of CMC varied from 0.76 to 1.52. The authors reported that the higher DS values, the higher water solubility values, especially DS ≥ 1.1. Previous studies revealed that CMC synthesized from BC had some difficulties in water solubility. This might be due to the highly heterogeneous derivatization of BC leading to aggregation of unsubstituted anhydroglucose units or due to BC nanofibrils with highly functionalized chains on the outside and insufficiently functionalized core [8].

CMC_{BC} solution (4%, w/v) was tested for viscosity. However, only approximately 38% of the weight of CMC_{BC} was water-soluble. Hence, the viscosity of CMC_{BC} was determined only by dissolution fraction. Theoretically, the viscosity increases as the DS of CMC increases [16]. However, the viscosity of CMC_{BC} was comparable to most of those obtained from rice straw CMC [15]. The average M_w of CMC_{BC} was higher than that of a commercial source (product no. 419303, M_w ~250,000, degree of substitution 0.9, Sigma-Aldrich, Sigma Chemical, Co., St. Louis, MO, USA). In terms of the commercial CMC, the calculated DP according to Eq. (2) was 1068 which was 25-folds lower than the DP of CMC_{BC}. The viscosity of the commercial CMC was tested by the same method. A high viscosity (approximately 600-700 cP) was obtained, and the commercial CMC was completely soluble in water (data not shown). This suggests that high water solubility enhances a viscosity of the solution. Although CMC_{BC} had high average M_w and high DP, low DS and low water solubility led to the low viscosity of CMC_{BC} solution.

Table 1 CMC_{BC} yield and its characteristics. Data represent average ± SD.

%Yield	DS	Water solubility (%)	Viscosity (cP)	Average M_w	DP
90.0±2.28	0.39±0.04	38.3±0.75	72±7.80	5.2×10^6±254	26,708±1.68

Determination of crystallinity of BC and CMC_{BC} was done by means of the X-ray diffraction method. The X-ray diffraction diagram of both samples is shown in Figure 2. CMC_{BC} had lower crystallinity compared to its substrate (BC). Treatment with NaOH during CMC synthesis modified the crystalline structure of BC and increased the chemical accessibility by swelling the material. The reduction of crystallinity of CMC_{BC} is associated with the cleavage of hydrogen bonds by NaOH and the incorporation of NaOH and water in the crystallites. In addition, carboxymethylation cleaved the hydrogen bonds by substituting the hydroxyl groups of cellulose with carboxymethyl groups [8]. CMC_{BC} had comparable crystallinity to that of a commercial CMC reported by Sophonputtanaphoca et al. [15].

Analysis of functional groups present in CMC_{BC}, and BC by FT-IR method was investigated. The FT-IR spectra of BC and CMC_{BC} were compared (Figure 3). CMC was successfully synthesized from BC with the confirmation of the increase in the intensity and structural changes of the peaks at 1603 and 1416 cm^{-1} of CMC_{BC} compared to the peaks at 1624 and 1420 cm^{-1} of BC. These peaks indicate the C=O bond of carboxymethyl group substitution [17, 18]. This result corresponds with the findings reported by Sophonputtanaphoca et al. [15], which used the same method of CMC synthesis but a different substrate.

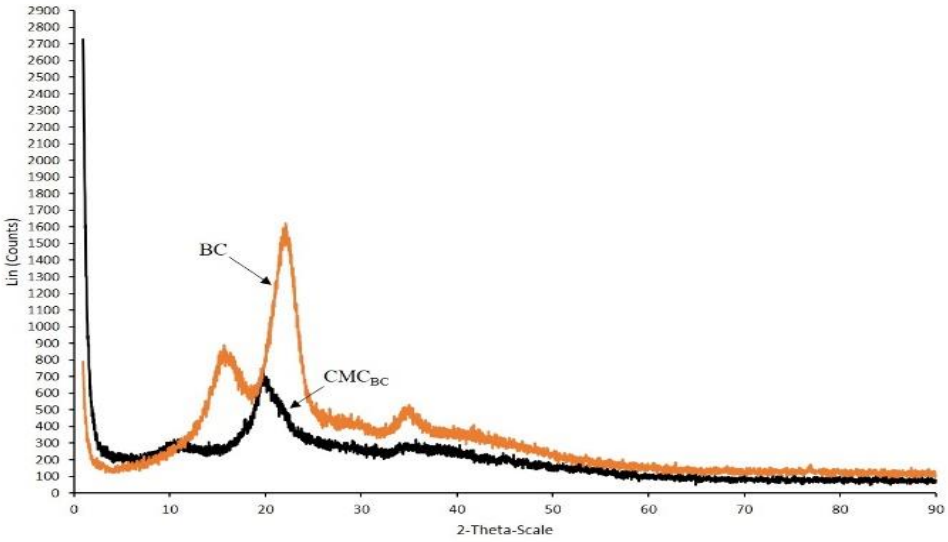

Figure 2 Comparison of X-Ray diffraction patterns of BC and CMC_{BC}

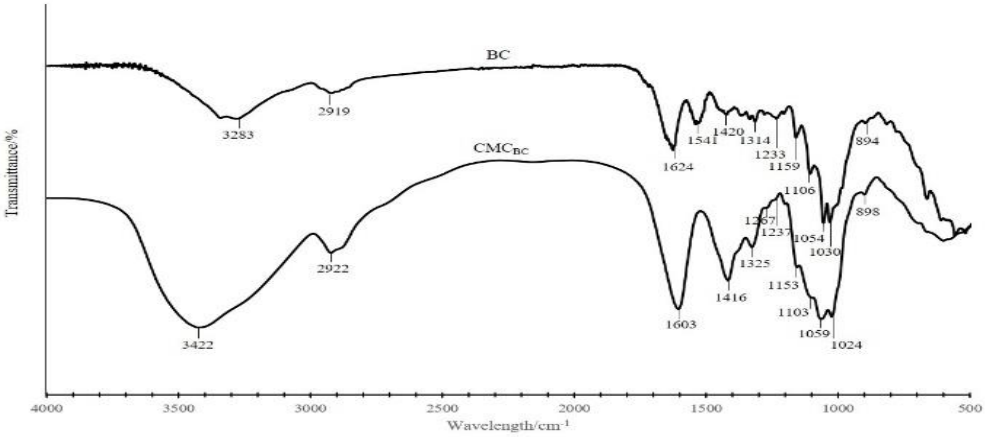

Figure 3 Comparison of FT-IR spectra of BC and CMC_{BC}

The stretching vibrations of –CH and –OH appear in the broad bands at 3600-2800 cm^{-1} of both samples [19]. In BC, these peaks were observed between 3330-3283 cm^{-1} and attributed to the stretching vibration of a hydroxyl group (–OH). However, the broad absorption peak was shifted to 3422 cm^{-1} due to the stretching frequency of the hydroxyl group after carboxymethylation [18]. The peaks at 1159 and 894 cm^{-1} of BC and the peaks at 1153 and 898 cm^{-1} of CMC_{BC} were associated with C–O–C stretching of ß–(1→4)-glycosidic linkage of cellulose [17, 20].

Interestingly, uronic acids such as galacturonic acid were detected in both BC and CMC_{BC}. The peaks at 1106 and 1030 cm^{-1} in BC and at 1103 and 1024 cm^{-1} of CMC_{BC} indicated that the samples were rich in uronic acids [21]. C–O stretching vibrations in the pectin molecule associated with the peak at 1233 cm^{-1} in BC and 1237 cm^{-1} in CMC_{BC} verified the presence of pectin in both samples. The peak at 1325 cm^{-1} confirmed that CMC_{BC} contained pectin as an impurity [21]. To confirm this result, the chromatogram obtained from gel permeation chromatography (GPC) showed the other peak beside the CMC peak with an average M_w of 49,935 (data not shown). This peak was later confirmed as a pectin peak using a standard pectin solution. The contamination of pectin is explained by using overripe 'Kluai Namwa' fruit as a carbon source for the CMC synthesis. Ripe 'Kluai Namwa' contains pectin as the most soluble fiber [1]. Unlike cellulose derived from lignocellulosic biomass, no peak associated with lignin was found in the FT-IR spectra of BC.

A comparison of SEM images between BC and CMC_{BC} revealed that the morphological structure of BC had a rough surface while CMC_{BC} had a smooth surface (Figure 4). The results were in accordance with the findings of Sophonputtanaphoca et al. [15]. According to Haleem et al. [13], the smooth structure of the CMC external surface was due to the intensely carboxymethylated structure.

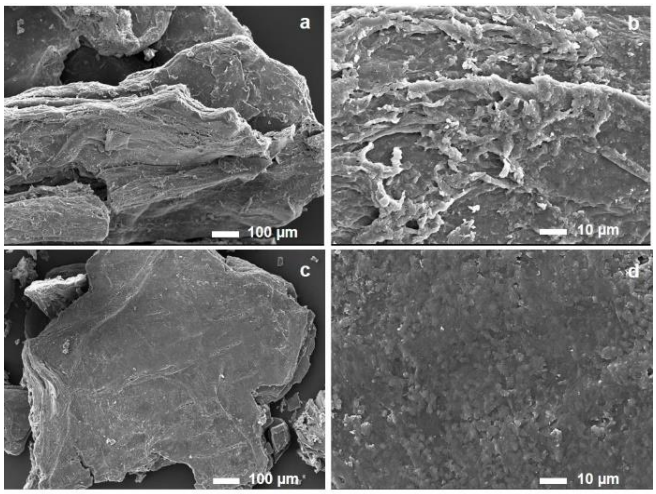

Figure 4 SEM images of BC (a, b) and CMC_{BC} (c, d) at the magnification of 100× and 1000×)

CONCLUSIONS

Overripe 'Kluai Namwa' fruit can be used to substitute glucose as a carbon source to produce bacterial cellulose (BC) by *Acetobacter xylinum*. A high yield of BC with pectin as an impurity was obtained. The presence of pectin was confirmed by the analysis with FT-IR spectra. However, the obtained BC can be used for carboxymethylcellulose (CMC_{BC}) synthesis. CMC_{BC} had characteristics comparable to CMC synthesized using cellulose derived from lignocellulosic biomass. Besides pectin, CMC_{BC} had no other impurities detected in FT-IR spectra. It is worth noting that pectin in BC might play an important role in some characteristics of CMC_{BC}, i.e., low DS, low viscosity, and low water solubility. Further analysis of pectin quantification in both BC and CMC_{BC} in conjunction with using BC after pectin removal for the CMC synthesis as control may help explain this effect. Considering its characteristics, CMC_{BC} can be used as a food additive to increase soluble and insoluble fiber in food products, e.g., jam, jelly, beverages, and fiber supplement products.

NOMENCLATURE

BC	bacterial cellulose
CMC	carboxymethylcellulose
CMC_{BC}	carboxymethylcellulose produced from bacterial cellulose

ACKNOWLEDGEMENTS

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Effect of enzymatic hydrolysis by Flavourzyme® on degree of hydrolysis, solubility, and antioxidant properties of freshwater prawn hydrolysates

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ABSTRACT: The objective of this study was to investigate the effects of enzymatic hydrolysis on degree of hydrolysis (DH), solubility, and antioxidation properties of freshwater prawn (*Macrobranchium lanchesteri*) hydrolysates for potential applications in food products. Flavourzyme® was used in enzymatic hydrolysis and performed at various enzyme/substrate ratios (10, 20, and 30 leucine amino peptidase units/ gram protein) and reaction times (1 to 5 h) under neutral pH (pH=7) and a temperature of 50°C. The results showed that the DH ranged from 6.79 ± 0.55 % to 30.49 ± 0.62 % after enzymatic hydrolysis. In addition, the highest DH (30.49 ± 0.62 %) was obtained by using an enzyme at 30 LAPU/g protein with a reaction time of 4 h. In comparison to the control, increasing enzyme concentration resulted in a significant increase in protein solubility. The solubility of the hydrolysates was above 60% at pH 2, 4, 6, 8, and 10. Moreover, the antioxidation activities, including DPPH and FRAP assays of enzyme-treated samples, were higher than the control sample. From the results, these hydrolysates can be utilized as a potential alternative protein source to improve the nutritional and solubility of food products.

Keywords: Protein hydrolysis, Flavourzyme®, Solubility, Antioxidation, Freshwater prawn

INTRODUCTION

Freshwater prawn (*Macrobranchium lanchesteri*), classified as a species of crustaceans, was found in inland and estuarine areas. It can be consumed as a staple diet, side dish, condiment, and seasoning [1]. The main compositions of the prawn are proteins, carbohydrates, and lipids. In addition, there is a significant proportion of minerals and vitamins in the prawn. The proteins from freshwater prawns are essential sources of amino acids and peptides that can be precursors for flavor development, especially during food processing [2,3].

Protein hydrolysates are of interest to the food industry due to their beneficial effects on food products and human health. Hydrolysate has been used as an important component of flavorings in processed foods such as soups, gravies, snacks, meat products, and other savory products [4,5]. The protein hydrolysates were produced by various physical, chemical, and biological methods. Each method provides a different amount of process control, which is important for the quality and application of the product. Enzymatic protein hydrolysis is a catalytic reaction of a proteolytic enzyme to cleave peptide bonds of the proteins [5,6]. These

obtained peptides and amino acids act as flavor precursors that interact with other components to generate taste and aroma [7]. The advantages of the enzymatic method are that it can be taken place under mild, controllable conditions, and the generation of toxic compounds is minimized. From previous literature enzymes from microbial sources such as Alcalase®, Neutrase®, Protamex®, and Flavourzyme® were used to hydrolyze proteins from marine sources, including seaweed, jellyfish, shrimp, and squid to modify physicochemical, functional, and sensorial properties of the proteins [8-12].

Flavourzyme ® is a commercial protease extracted from the fermentation of *Aspergillus oryzae*. It has a declared activity of 1000 LAPU/g (1 LAPU is the amount of enzyme that can hydrolyze 1 µmol of L-leucine-p-nitroanilide per minute). A combination of endo- and exoproteases of Flavourzyme® results in a selective cleavage at the N- or C-terminals of the proteins and peptides. So, it can provide hydrolysates with low bitter intensity with a high degree of hydrolysis (DH) value [12-14]. The Flavourzyme® can also produce clean-taste and salt-free protein hydrolysates without waste disposal, which usually occurs with acid hydrolysis. Furthermore, the utilization of this enzyme was reported to give a hydrolysate with a strong smell and taste, e.g., shrimp hydrolysate, and to reduce the bitterness compared to other types of hydrolytic enzymes [5,15].

The DH is the most common parameter to differentiate protein hydrolysates functionally [10]. The properties of the hydrolysates mainly depend on enzyme types, substrates, and hydrolysis conditions, including enzyme/substrate (E/S) ratio, reaction time, temperature, and pH [13]. Therefore, the objective of this study was to investigate the effects of enzymatic hydrolysis on the DH, solubility, and antioxidation properties of freshwater prawn hydrolysates using Flavourzyme®.

MATERIALS AND METHODS

Materials

Freshwater prawns (*Macrobranchium lanchesteri*) were obtained from Verasak farm in Phetchaburi province, Thailand. The whole freshwater prawns, sizes ranging from 2 to 3 cm., were packed in an ice box and transported to the laboratory. The prawns were washed, drained, packed into an LDPE bag, and kept at -20°C before further experiments. Flavourzyme® (activity of 1,000 LAPU/g; Novozyme, Denmark) was provided by Brenntag Ingredient (PCL, Bangkok, Thailand). DC protein assay was purchased from Bio-Rad Laboratories (Bio-Rad Laboratories Inc., Hercules, CA, USA). Other chemicals used in all analyses were analytical grade.

Sample preparation

The frozen freshwater prawns were thawed and washed, followed by heating at 90°C for 10 min to inactivate endogenous enzymes. The sample was then cooled down, and drained through a sieve (12 mesh). After that, the sample was ground using a waring blender (7010BU, Waring Laboratory, USA) at high speed (10,000 rpm).

To determinate solubility and antioxidation properties, the hydrolysate samples were prepared by spray-drying following the method of Wongsangasri et al. [9] with some modifications. The hydrolysates were concentrated up to 6°Brix by a rotary evaporator (BUCHI, Rotavapor R114, Switzerland) at 50 °C. Then, the concentrated hydrolysate was mixed with maltodextrin at a ratio of 70:30. The mixture was then dried by spray dryer under inlet and outlet temperatures at 150 °C and 95 °C, respectively. The dried samples were kept in an aluminum foil bag at 4 °C until it was analyzed.

Enzymatic hydrolysis

Enzymatic hydrolysis was performed using the methods described by Laohakunjit et al. [4] and Yang et al. [13] with slight modification. The cooked ground freshwater prawn sample was blended in phosphate buffer (pH = 7) at a ratio of 1:2 and pre-incubated at a temperature of 50°C for 15 min in a shaking water bath (120 rpm). The Flavourzyme® was then added to obtain various activities (10, 20, and 30 LAPU/g protein) and incubated in a shaking water bath (50°C, 120 rpm). The reaction time varied from 1 to 5 h. The hydrolysates were then removed at time intervals and immediately heated to 90°C and stayed at this temperature for 10 min to inactivate the enzyme, then cooled and filtered through a sheath cloth. After that, sediment was separated by centrifuge (Model 6000, Kubota, Japan) with 10,000 rpm at 4°C for 15 min. The supernatant was collected and kept at -20°C until it was analyzed.

Degree of hydrolysis (DH)

The DH was performed according to Laohakunjit et al. [4] and Suppavorasatit et al. [18] with a slight modification. The hydrolysate samples were precipitated by adding 20% (w/v) trichloroacetic acid (TCA) at a ratio of 1:1. The precipitated proteins were refrigerated centrifuge (4°C) at 10,000 rpm for 10 min. Supernatants were subjected to analyze protein content using DC Protein Assay and quantified by using bovine serum albumin (BSA) as a standard. The DH was calculated as:

$$\mathrm{DH} = \frac{\mathrm{N_0}}{\mathrm{NT}} \times 100$$

where N_0 and NT are the amounts of soluble protein in the supernatant and the total amount of total soluble protein, respectively. The total soluble protein was obtained after complete hydrolysis with 2 N sulfuric acid at 100 °C for 4 h.

Solubility

The solubility was performed according to Norman et al. [19]. Two hundred mg of dried hydrolysates were dissolved in 20 mL buffer at various pH (pH 2, 4, 6, 8, and 10). The protein solutions were then incubated for 30 min at 25 °C with stirring. The mixtures were centrifuged (8,000 rpm, 4 °C) for 15 min. The supernatants were collected, and protein contents were determined. The solubility was calculated as the percentage ratio of protein in the supernatant to total protein in the initial sample.

Antioxidation properties

One gram of powder was dissolved in 100 ml deionized water, then centrifuged at 8,000×g for 10 min at room temperature. The supernatant was subjected to analysis.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH assay determined the radical scavenging activity according to the methods of Benjakul et al. [11] and Wu et al. [20] with some modifications. The supernatant (1 mL) was added to 1 mL of 0.1 mmol/L DPPH in 95% ethanol. The mixture was then mixed vigorously and incubated at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). The scavenging activity was determined and calculated as the following equation:

$$\text{DPPH scavenging activity (\%)} = \left[\frac{(\text{blank absorbance} - \text{sample absorbance})}{\text{blank absorbance}}\right] \times 100$$

where blank sample is 1 mL of ethanol mixed with the 1 mL of 0.1 mmol/L DPPH in 95% ethanol.

Ferric reducing antioxidant power (FRAP) assay

The determination of antioxidant activity by FRAP assay was done following Benjakul et al. [11] and Wu et al. [20]. The working solution was freshly prepared by mixing 25 mL of 300 mM acetate buffer, 2.5 mL of 2,4,6-tri (2-pyridyl)-1,3,5-triazine (TPTZ) solution, and 2.5 mL of $FeCl_3.6H_2O$. The mixed solution was incubated at 37°C for 30 min and was referred to as the FRAP solution. The samples (200 µL) were mixed with 4 mL of the FRAP solution and kept for 30 min in the dark. The absorbance of the sample at 593 nm was then measured. The antioxidation activity was expressed as µmol Trolox equivalents (TE)/g sample.

Statistical analysis

A complete randomized design was applied for all experiments. The experiments of each treatment were done in triplicate. Analysis of variance (ANOVA) was performed and mean comparisons were determined using Duncan's multiple range test. The statistical analysis was carried out using IBM SPSS® software (version 19.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Degree of hydrolysis

The DH values of freshwater prawn hydrolysates increased with increasing enzyme concentration and hydrolysis time. All hydrolysates showed the DH ranged from 6.79 ± 0.55 % to 30.49 ± 0.62 %. From Figure 1, it was found that an upward trend in enzyme concentration on the DH of hydrolysates was observed ($p \leq 0.05$). At the same hydrolysis time, a higher DH was observed at higher enzymatic levels. From the result, the highest DH was obtained when using enzyme concentration at 30 LAPU/g protein. It could be explained that there are more molecules of enzymes available per molecule of substrates, various reaction times at the same enzyme concentrations ($p \leq 0.05$). resulting in greater cleavage of the peptide bonds [22]. This result agreed with Klompong et al. [25], who reported increased DH of the proteins from yellow stripe trevally after an increase in Flavourzyme® concentration. In addition, there was also an agreement between shrimp waste hydrolysates [10] and fish protein hydrolysate [15]. Benjakul and Morrissey [26] also suggested that increased enzyme concentration induced DH and soluble peptides.

Figure 1 also demonstrated that the increase in DH was also observed when increasing reaction time. In addition, the sharp increase trends were shown at the first hour of hydrolysis, then gradually increased. At 5 h, the highest DH was obtained when using enzyme at 30 LAPU/g protein (30.49 ± 0.62%). Moreover, the DH at 10 and 20 LAPU/g protein were 23.15 ± 0.98 % and 27.99 ± 0.64 %, respectively.

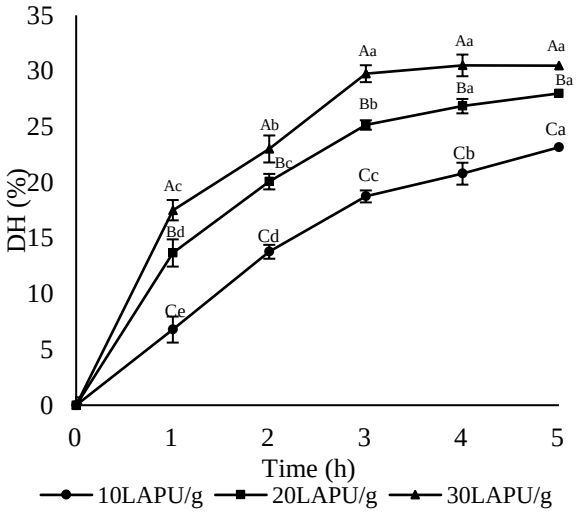

Figure 1. Degree of hydrolysis (DH) of freshwater prawn hydrolysates at various reaction times (1 to 5 hours) and enzyme concentrations (10 to 30 LAPU/g protein). Different uppercase letters (A,B,C) are significantly different in DH with various enzyme concentrations at the same reaction times ($p \leq 0.05$). Different lowercase letters (a,b,c) are significantly different in DH with

From the result, upon extending incubation time, the DH of 20 and 30 LAPU/g protein was not significantly increased after an

incubation time of 4 and 3 h, respectively ($p<0.05$). This finding was supported by Dey et al. [10] who observed the changes in DH of shrimp waste hydrolysate by Alcalase®, Neutrase®, and Flavourzyme®. The reaction curves expressed rapid initial reaction rates, followed by a decrease in reaction rate until reaching a plateau, where apparently hydrolysis was not observed. This would result from the reduction of a substrate, which was hydrolyzed and converted to peptides and amino acids [3, 26]. Furthermore, this phenomenon could be due to the enzymatic steric effect, which prevents interaction between the catalytic site of the enzyme and the active site of the substrate [21]. It might cause by enzyme aggregation, which leads to an increase in substrate diffusion inhibition and saturation of reaction rate. According to these results, the 4 h was chosen as an optimal incubation time for other solubility and antioxidation determinations.

Solubility

The solubility of freshwater prawn hydrolysates with different enzyme concentrations at a pH range of 2 to 10 is shown in Figure 2. An increase in the enzyme concentration corresponded to a considerable significantly increase in solubility compared with the control sample (0 LAPU/g protein) at each pH value ($p\leq0.05$). The influence of enzymatic hydrolysis reduced proteins' molecular weight, the release of the smaller peptides, and an increase in the numbers of polar groups. These could enhance the solubility of the products. For acidic conditions, the solubility of all hydrolysates (treated with the enzyme) was above 60%. At pH 2, the maximum solubility was observed for all enzyme concentrations (95.94± 1.42 %, 90.85 ± 2.71%, and 88.62 ± 1.32% for 30, 20, and 10 LAPU/g protein, respectively). In addition, the solubility was significantly decreased at pH 4, which is closed to the isoelectric point (pI) of the protein. At pI, the net charge of the protein becomes zero; hence more protein-protein interactions and less protein–water interactions would be occurred [21]. Furthermore, the solubility of hydrolysates was sharply increased while the pH was raised from 4 to 8 and slightly decreased at pH 10. It could be explained that the protein's surface is mostly in negatively charged and generates repulsive forces when the pH is over the pI. This result was in agreement with the DH increase shown in Figure 1. The effect of DH on protein solubility depends on protein structure [16]. The increase in solubility of protein samples after hydrolysis was due to the degradation of the proteins into smaller peptides due to an increase in Flavourzyme® concentration. According to this finding, the higher solubility of freshwater prawn hydrolysates in a wide range of pH can make it appropriate for food application, which contributes to improving food products' appearance and gives a smooth mouthfeel [27].

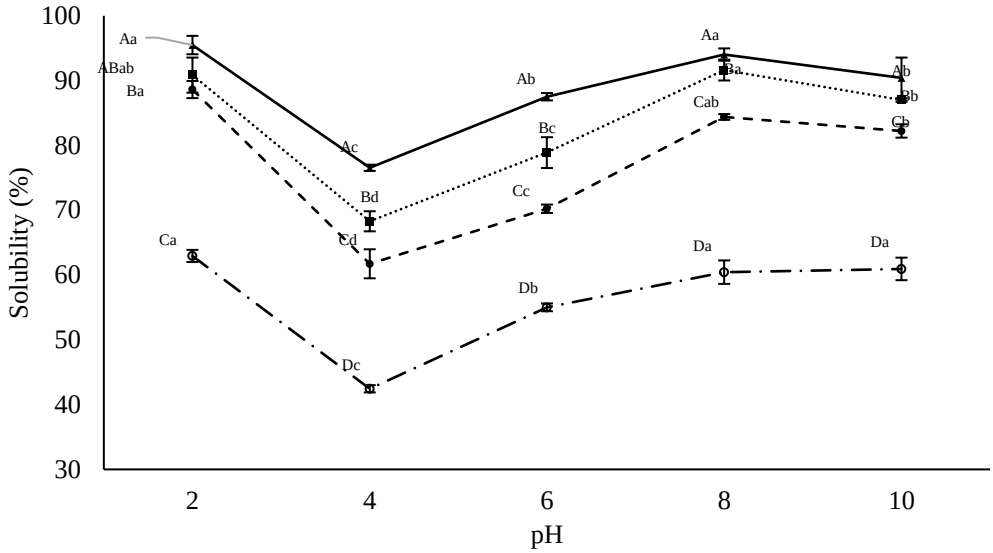

Figure 2. Solubility of freshwater prawn hydrolysates at different enzyme concentrations. 0 LAPU/g protein (control) (o), 10 LAPU/g protein (•), 20 LAPU/g protein (■) and 30 LAPU/g protein (▲). Different uppercase letters (A,B,C) are significantly different in solubility with various enzyme concentrations at the same pH ($p \leq 0.05$). Different lowercase letters (a,b,c) are significantly different in solubility with various pH at the same enzyme concentration ($p \leq 0.05$)

Antioxidation properties

DPPH free radical scavenging activities of the freshwater prawn hydrolysates are demonstrated in Figure 3. This method is based on the hydrogen atom donation ability of the antioxidant to stabilize the DPPH radical. It was found that the scavenging capacity of samples treated with Flavourzyme® at 30, 20, and 10 LAPU/ g protein were 14.79 ± 0.27%, 13.65 ± 0.29%, and 10.95 ± 0.72%, respectively. These values were significantly higher than that observed in the control sample (4.97 ± 0.49%) ($P \leq 0.05$). From the result, enzymatic hydrolysis resulted in a higher DH of protein hydrolysates, which led to improve the radical scavenging activity of the DPPH. Additionally, the DPPH activity values of enzymatic hydrolysis by Flavourzyme® in squid head [13], Nile tilapia [24], and yellow stripe trevally [25] hydrolysates were in agreement with our result. The hydrolysates could interact and donate hydrogen atoms to the free radicals due to the release of peptides and free amino acids during enzymatic hydrolysis [11]. The finding of Pongsetkul et al. [1] also supported that peptides or free amino acids in freshwater prawn salted paste produced by the autolysis process had a higher ability in donating hydrogen atoms to free radicals.

Ferric reducing antioxidant power (FRAP) is commonly used to measure the capacity of substance in reducing TPTZ-Fe (III) complex to TPTZ-Fe (II) complex. The FRAP of freshwater prawn hydrolysates is presented in Figure 4. The FRAP values of all samples were in the range of 1.28 ± 0.27 to 7.52 ± 0.49 µmol TE/g sample. The sample treated with Flavourzyme® at 30 LAPU/g protein showed the highest activity (7.52 ± 0.49 µmol TE/g sample), followed by samples treated at 20 and 10 LAPU/g protein, respectively. In addition, the control sample showed the lowest FRAP activity. This could be explained that the increasing DH (Figure 1) caused the risein smaller peptides, which could act as an electron donors to the free radical, leading to the prevention or retardation of the propagation step in the oxidation reactions [11,24].

The results in the antioxidation ability of hydrolyzed proteins are in agreement with

previous literature, a significant improvement in antioxidant activity was obtained when increasing Flavourzyme® concentration [28]. Benjakul et al. [11] found that the cephalothorax of white shrimp hydrolysates using 0.15% Flavourzyme® provides the highest DPPH, FRAP, and ACE inhibitory activity when compared with the native protein.

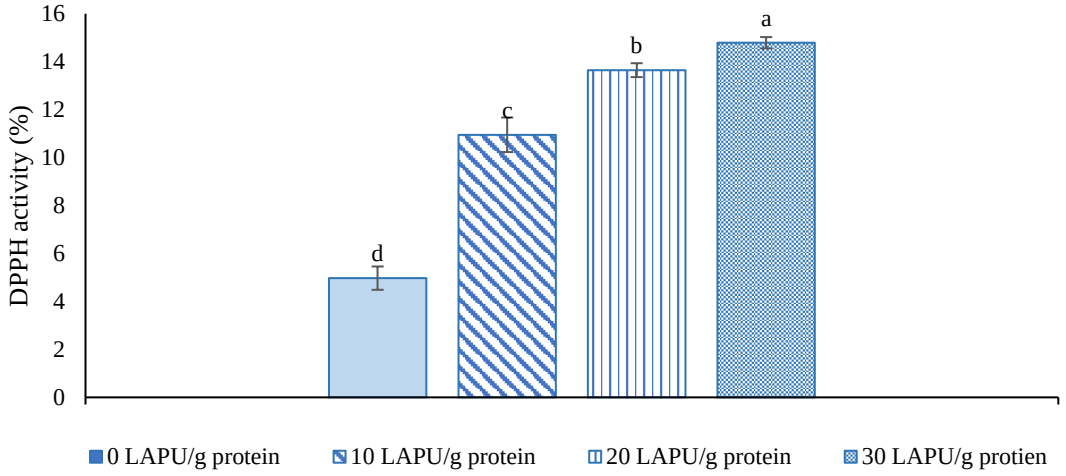

Figure 3. DPPH activity of freshwater prawn hydrolysates. Different lowercase letters (a,b,c,d) are significantly different in DPPH activity value with various enzyme concentrations ($p \leq 0.05$).

In addition, Latorres et al. [16] supported the proteolytic hydrolysis of white shrimp with a higher level of DH (10 and 20%) also increased the DPPH activity. They reported that the hydrolysates by Flavourzyme® could perform as electron donors, resulting in improved antioxidation properties.

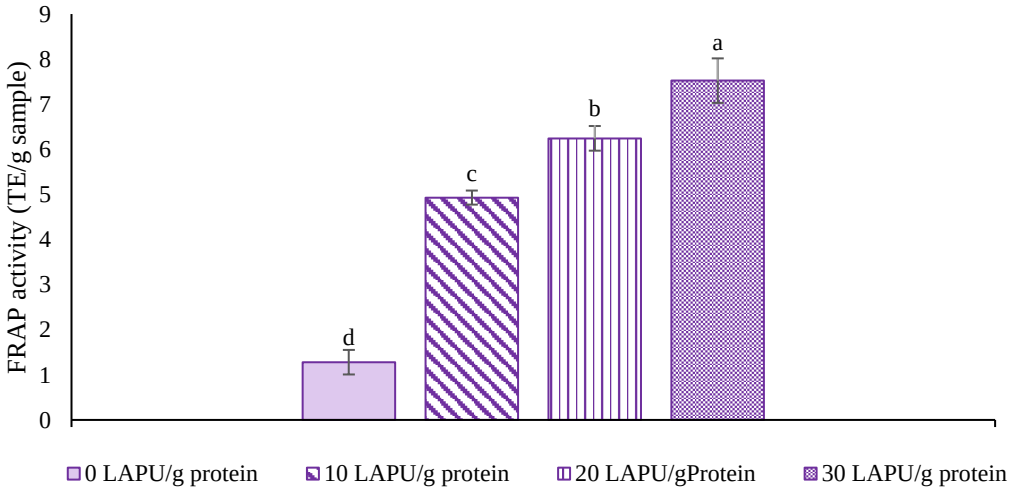

Figure 4. FRAP activity of freshwater prawn hydrolysates. Different lowercase letters (a,b,c,d) are significantly different in FRAP activity value with various enzyme concentrations ($P \leq 0.05$).

CONCLUSIONS

The properties of freshwater prawn hydrolysates were significantly affected by enzymatic hydrolysis using Flavourzyme®. The change in DH and protein properties was influenced by proteins' degradation into smaller peptides and free amino acids. The highest DH, solubility, and antioxidant activities, including DPPH and FRAP were obtained after hydrolysis by 30 LAPU/g protein of Flavourzyme® for 4 h. The results demonstrated that the hydrolysates could be potentially used in the food industry. However, further investigations on amino acid content, taste, and aroma are still required.

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The effect of drying processing on physico-chemical properties in corn silks

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ABSTRACT: Corn silk is the female flower stigma of maize (*Zea mays* L.) and has been utilized as a traditional herb in Asia. Corn silk is also considered as waste in the corn productions including food, processed food and animal feed. This research has been studied on sweet and baby corn silks which is dried at 70, 80 and 90 °C. The objective of this research has been to investigate the effect of drying temperatures using the hot air oven in two types of corn silk (sweet corn and baby corn) on the physicochemical properties. The results indicated that the optimum drying temperature was 80 °C which gave the highest polyphenol content in both types of silk corn. The polyphenol content of baby silk corn was higher by 21.17% than in sweet corn silk. However, anthocyanin content in two types of corn silk were not detected and chlorophyll content was not significantly different between drying temperatures. The results indicated that corn silk can be used as valuable bioactive source of antioxidant beverage

Keywords: **:** *Corn silk, Total polyphenol content, Chlorophyll content, Anthocyanin content*

INTRODUCTION

Corn is one of the mainly grown cereal crop in the world including Africa, America, Australia as well as Asia and has become one of five majors crops in Thailand. In 2020, high corn production reached 501,242 ton/year for sweet corns and 235,195 ton/year for baby corns [11]. At present, Northern Thailand is the largest maize production region, following by the Northeast region and Central region. Sweet corn is the main products of the corn plantation industry and are harvested after 65-75 days of planting while baby corns could be collected earlier after 45-55 days of planting [10]. Sweet corns have been used as foods, feed, health applications and are processed to produce oil, starch and ethanol [14] whereas baby corns are utilized as vegetables. During the corn productions, there are many by-products including corn silks, husks and cobs. Cobs and husks can be used as animal feeds, charcoal, compost, handicraft as well as energy sources while corn silks can be utilised as valued-added products such as cosmetics, medicines, snacks etc. [14].

Corn silks contain many bioactive compounds including protein, carbohydrates, vitamins, minerals (Ca, Mg, Cu, Zn, Fe and Mn), crude fiber, steroids such as stiosterol and stigmasterol [6][7]. It is also a good source of polyphenol compounds as an antioxidant [3]. Corn silk have been utilised in ancient times as traditional remedy and used in several regions to treat urological disorders, prostate problem, edema and gout [10]. However, reports on drying processing on physical properties of corn silks are still lacking. Therfore, the aims of this study were to investigate the effect of drying temperature using the hot air oven in two types of corn silk (sweet corn and baby corn) on the physicochemical properties. The total polyphenol content of corn silk is also determined.

MATERIALS AND METHODS

Two types of corn (sweet corns and baby corns) were purchased from the local market in Nakhon Sawan Province, Thailand.

Corn silk preparations

Corn silk was cut into small pieces (2-3 centimeters) and steamed over the hot water (100 °C) for one minute. After that, corn silk was roasted in the pan for 8-10 minute and dried in hot air oven at the temperature of 70 °C for 1.5 hour, 80 °C for 1 hour and 90 °C for 0.5 hour. Dried corn silks were kept in a sealed bag at room temperature. Prior to analysis, the corn silk was ground into powder form.

Color analysis

Colorimeter measurement of corn silk was determined using a Hunter Colorimeter (Model. CQX 2934, USA) The CIE colour values were recorded as L* (lightness), a* (redness) and b* (yellowness).

Proximate analysis

Dried corn silks were determined for moisture content, protein, lipid, ash, total dietary fiber and carbohydrate contents according to the method of AOAC [2]. Carbohydrate content was determined as the difference between 100 and the sum of moisture, protein, fat, dietary fiber and ash contents.

Determination of pH

Grinded corn silk (0.4 g) were mixed with 20 ml of deionized waster and vortexed for 3 minutes. Mixtures were held at ambient temperature for 1 hour, after that the supernatants were measured using a pH meter (Sartorius PB10, Germany) [19].

Determination of total sugar

Grinded corn silk was diluted with distilled water (1:10000). The extracted solution (2 ml) was pipetted into a test tube. Then, 2 ml of distilled water, 0.1 ml of 80% phenol and 5 ml of concentrated sulfuric acid were added and shaked . The tube was allowed to stand 10 minutes and placed for 20 minutes in a water bath at 25 °C. After that, the absorbance was measured by using spectrophotometer at 490 nm. Blanks were prepared by subsituting distilled water. The amount of sugar was determined by reference to a standard curve using 0.01% glucose solution [6].

Determination of Total polyphenol content

Five grams of grinded corn silk was shaked in waterbath shaker (110 rpm) at 25 °C for 16 hour with 100 ml of 80% acetone (v/v). The solution was centrifuged at 10000g for 10 minutes, the supernatant was collected for total phenolic determination. The total phenolic of corn silk was determined using the Folin-Ciocalteu spectrophotometric method. The extracted solution (0.5 ml) was mixed with 2.5 ml of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 minutes. After that, 2 ml of 7.5% Na_2CO_3 solution was added, the final volume was made up to 10 ml with deionized water and kept at room temperature for 1 hour. Then, the absorbance was measured at 760 nm and compared to a standard curve of prepared gallic acid (GA) solution [8].

Determination of chlorophyll contents

The determination of chlorophyll content was adapted from the previous study with some modification [8]. One gram of grinded corn silk was extracted in 20 ml of acetone: hexane in a proportion of 8:12. The solution was homogenized and centrifuged at 4500g for 5 minutes. The absorbance of supernatant was measured by using spectrophotometer at 645 nm and 663 nm, respectively. The chlorophyll content was calculated by using the following equation;

$$\text{Chlorophyll content } (\mu g/g) = (0.999 \times A_{663nm}) - (0.0989 \times A_{645nm}) \quad (1)$$

Determination of total anthocyanin contents

The corn silk was dispersed in a 1:1 water:methanol solution containing 2% HCl and sonicated for 20 minutes. A solution was passed through a 0.45 µm PTFE filter prior HPLC injection. Sample test solutions were analysed using HPLC in conjunction with a DAD detector. The separation was performed on a Beckman Ultrasphere ODS column (5 µm, 4.6 ×250 mm). The mobile phases A consisted of 0.4% TFA in water and mobile phase B consisted of 0.4% TFA in acetronitrile. The flow rate was 1 mL/min with the column temperature of 35°C [18].

Statistical analysis

All tests and analysis were done in triplicate. Statistical analysed were carried out with a Turkey's multiple test ($P<0.05$) using statistical software.

RESULTS AND DISCUSSION

Effect of thermal processing on chemical and physical properties

Two types of corn silk (sweet corns and baby corns) were investigated the optimum temperature of drying using hot air oven at the temperature of 70 °C for 1.5 hour, 80 °C for 1 hour and 90 °C for 0.5 hour. The results of chemical properties were shown in Table 1 and 2

Table 1 and 2 show the chemical properties of two types of dried corn silks. The total polyphenol content in baby corn silks was significantly higher than sweet corn silks. The drying temperature of 80°C gave the highest polyphenol content in both types of corn silk. The results indicated that decreasing heat treatment time is more useful to reducing the loss of phenolic compounds than lowering the heat temperature [5] and the loss of polyphenol compounds is affected by the heating time and temperature. This was due to the high moisture content of samples during the heat treatment which cause faster water migration and loss as well as can accelerate the decomposition of polyphenols [4]. Moreover, water plays a significant role because of it allows polyphenols to dissolve and after that to be migrated to the surface [1]. Another reason for the increase in total polyphenol at 80 °C might be the higher drying temperature, in which oxdative enzymes have been completely inactivated so the polyphenol compounds remain intact at elevated levels at these high temperatures as found in banana leaves [12]. In accordance with these previous studies, the phenolic compounds losses during the thermal processing period in mulberry juice-enriched dried minced pork slices [4]. Thus, the total polyphenol was the indicator of the antioxidant activity of corn silk. Because of the previous studies found the polyphenol compounds have attracted attention for being the main sources of antioxidant activity [13].

Table 1 Chemical properties (dry basis) of dried sweet corn silk at different drying temperature

	Drying temperature (°C)		
	70	80	90
pH	5.15± 0.02^{b}	5.10± 0.02^{a}	5.10± 0.19^{a}
Total sugar (%)	1.10± 0.023^{a}	1.16± 0.030^{b}	1.28± 0.015^{c}
Chlorophyll content (µg/g)^{ns}	0.034± 0.01	0.034± 0.01	0.034± 0.01
Total polyphenol (mg eq GA/100g)	2,100.58 ± 34.62^{a}	2,428.43 ± 12.03^{b}	2,353.61 ± 23.67^{b}
Anthocyanin content (mg/100g)	n.d.	n.d.	n.d.

Note: In each column, different superscripts represent significant differences ($p \leq 0.05$). ns represents non-significant difference ($p \leq 0.05$). n.d. represents not detected

Table 2 Chemical properties (dry basis) of dried baby corn silk at different drying temperature

	Drying temperature (°C)		
	70	80	90
pH	5.07± 0.02^{b}	6.06± 0.02^{b}	5.00± 0.19^{a}
Total sugar (%)	1.19± 0.02 ^{a}	1.17± 0.03^{b}	1.13± 0.02^{c}
Chlorophyll content (µg/g)^{ns}	0.034± 0.01	0.034± 0.01	0.034± 0.01
Total polyphenol (mg eq GA/100g)^{ns}	2,802.42 ± 13.61	3,080.65 ± 16.88	2,752.99 ± 18.73
Anthocyanin content (mg/100g)	n.d.	n.d.	n.d.

Note: In each column, different superscripts represent significant differences ($p \leq 0.05$). ns represents non-significant difference ($p \leq 0.05$). n.d. represents not detected

The presence of anthocyanins was not detected in two types of corn silk. Our results are in well accordance with the previous studies which found that the content of anthocyanins was not found in medical herbs and corn silks [19]. On the other hand, the purple silks with dark colour had higher anthocyanin contents than waxy corns, sweet corns and baby corns [13]. In addition, the chlorophyll contents were not significantly different between drying temperature. In accordance with these previous studies, the chlorophyll contents in corn silks was low compared with medical herbs [19]. It can be due to chlorophyll can degrade during the silk senescence to undesirable grey-brown compounds such as pheophorbide and pheophytin. More studies reported that the chlorophyll contents in the mature-brown corn silks were lower by about 4.8-fold in relation to that in fresh silks [18]. Percentage of total sugar in dried sweet corn silks and dried baby corn silks ranged from 1.10-1.28 and 1.13-1.19, respectively. The colour of dried corn silk at different drying temperature was measured using the Hunter Colorimeter. The results are shown in Table 3.

Table 3 Colour parameters of sweet dried corn silk

Drying temperature (°C)	Colour parameter		
	L*	a*	b*
70	48.55 ± 0.83^{c}	6.11 ± 0.26^{c}	13.19 ± 0.27^{b}
80	44.64± 0.68^{b}	4.68 ± 0.37^{b}	9.93 ± 0.94^{a}
90	43.51 ± 2.40^{a}	4.28 ± 0.85^{a}	9.15 ± 0.17^{a}

Note: In each column, different superscripts represent significant differences ($p \leq 0.05$).
ns represents non-significant difference ($p \leq 0.05$).

Table 4 Colour parameters of baby dried corn silk

Drying temperature (°C)	Colour parameter		
	L*	a*	b*
70	39.29 ± 0.90^{ab}	4.50 ± 0.11^{ab}	4.70 ± 0.85^{a}
80	38.41 ± 1.72^{a}	3.68 ± 1.25^{a}	3.86 ± 1.49^{a}
90	41.72 ± 2.70^{b}	4.97 ± 0.41^{b}	7.93 ± 1.00^{b}

Note: In each column, different superscripts represent significant differences ($p \leq 0.05$). ns represents non-significant difference ($p \leq 0.05$).

Table 3 and 4 show the colour parameters of dried corn silk produced at different drying temperature. The lightness (L*) shows a significant change at higher drying temperature in both types of corn silk. The visualized colour of baby corn silk tends to get darker than sweet corn silk. Therefore, the colour of corn silk depends on the varieties of corn and drying temperature. It can be due to the polyphenol compounds in corn silks undergo enzymatic oxidation as result of condensing among themselves or with proteins to produce brown-coloured complexes [19]. This research also found the correlation between polyphenol contents and the colour of corn silks. The higher

polyphenol contents correlated with the greater color of corn silk. Our results are in well accordance with previous studies which found the significant correlation between phytochemicals and the levels of color of corn silk [13].

Nutrition compositions of dried corn silks

The nutrition compositions of two types of corn silks were determined following the AOAC (2000) method. The results obtained are presented in Table 5.

Table 5 Nutrition composition (%, dry basis) of dried sweet and baby corn silk

Nutrition composition (%)	Sweet corn silk	Baby corn silk
Moisture content	10.01 ±0.72	10.87 ±1.06
Protein	16.22 ± 0.01	14.82 ± 0.21
Fat	0.72 ± 0.02	0.52 ± 0.05
Ash	5.13 ± 0.07	4.41 ± 0.01
Crude fiber	15.04 ± 1.20	9.26 ± 0.01
Carbohydrate	52.88 ± 1.97	60.12 ± 1.28

The main composition in corn silk is carbohydrate. Sweet corn silks contained higher compostion of crude fiber than baby corn silks. Our finding supported previous finding, dried sweet corn silk powder had 17.94% protein, 0.91% fat, 4.60% ash, 16.11% crude fiber and 51.37% carbohydrate [3]. Variations are also affected by numerous factors including varieties, climate and soil fertility [14].

CONCLUSIONS

The optimum temperature of drying process is 80 °C in two types of corn silk which gave the highest polyphenol content in both types of silk corn. The main composition in corn silk is carbohydrate and it contains high polyphenol contents and phytochemicals that have health benefits. Moreover, the utilisation of corn silk could reduce agricultural waste and could be used as an alternative source of antioxidant as well as produce further value - added products.

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DIVISION B
Food Processing and Engineering

ORAL PRESENTATION

CHICKEN BREAST POWDER: Effect of drying processes on physicochemical and functional properties

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ABSTRACT:

Chicken breast powder is an upcoming alternative high protein source to replace the consumption of whey protein powder in some particular people, especially Asian people who are lactose intolerant. As chicken breast can be prepared into dried powder, it can offer more varieties of uses in food products than the fresh chicken breast meat. Therefore, this study aimed to prepare and compare three types of dried chicken breast powder, to determine their proximate composition and functionalities including water holding capacity and oil absorption capacity, and also to compare them with commercial whey protein concentrate. There are three drying methods operated at 65°C that are used to prepare chicken breast powder including conventional hot air drying, vacuum drying and low pressure superheated steam drying. For the preparation process, after cooking and drying process, it was sieved by using laboratory test sieve **(**50 Mesh**)** for the equal particle size of chicken breast powder. For the result, low pressure superheated steam dried chicken breast powder **(**CBL**)** showed significantly higher water activity (0.427±0.002) than conventional hot air dried **(**CBH**)** (0.288±0.012) and vacuum dried chicken breast powders **(**CBV**)** (0.280±0.002) due to the steam involved in drying process; nevertheless, all chicken breast powder samples showed significantly higher moisture content and water activity than whey protein concentrate which are (2.03±0.13) and (0.196±0.005), respectively. However, for both water and oil absorption capacity, all CB samples exhibited higher capacity than whey protein powder. For color, all chicken breast powder samples are significantly darker and more yellowish than whey since it could come from the browning reaction during the process of pressure cooking and drying. It was found that different drying techniques significantly influences proximate compositions of CB samples especially on moisture content that can further affect protein content in dry basis. Additionally, all CB samples have significantly higher water absorption capacity and oil absorption capacity than commercial whey protein concentrate due to possibly more porous structure and larger particle size. Data from this study is crucial for further development of dried chicken breast powder and application in potential food products.

Keywords*: Chicken breast powder, Drying process, Proximate composition, Water holding capacity, Oil absorption capacity*

INTRODUCTION

Since nowadays there are many protein sources including animal protein and plant protein sources, the former tends to have more proper amount of essential amino acids and easier to be digested than the latter. Although one of the most popular protein powders is whey protein powder, there are still some people who are lactose-intolerant and allergic to certain whey protein. Furthermore, whey protein concentrates could also cause increase in blood sugar level due to the fact that lactose sugar can be broken into glucose and galactose by the enzyme lactase, and also, they usually applied in most desserts and beverages including pancake, ice cream and protein shake. Also, in terms of regulation, there is no specific level of lactose claimed as "low-lactose" product; however, there is the common threshold level for low-lactose product varying between countries which is 1 gram of lactose per 100 grams of the final product, so this could be considered and claimed as low-lactose product (1). On the other hand, according to Thai FDA, to be claimed as "high-protein" product, the food must contain 20% or more of the recommended daily intake (RDI) of protein ((No. 182) B.E. 2541 (1998) Re: Nutrition Labelling). Thus, an alternative animal protein source is recommended to be high protein, low fat, low lactose content along with high availability, quality and low cost. To clarify alternative protein source, in terms of the availability and quality, chicken is the most commonly found in mass production in poultry market with its high protein score of 0.95 (2). Additionally, for the cost, it also is the cheapest white meat compared to other types of white meat including turkey, goose and duck and also red meat including pork and beef. Furthermore, chicken breast is the chicken part that has lower cholesterol, lower saturated fats and higher protein content compared with other chicken edible components which are leg and wing. Hence, chicken breast has gained more popularity and interest to consumers for weight loss and muscle gain, especially who have the problems of consuming whey protein powder. It is also high antioxidant capacity due to histidyl dipeptides including carnosine and anserine in Thai indigenous chickens, low cost ranging 40 to 60 per kilogram, low risk of cardiovascular disease compared with red meat including pork and beef. Nevertheless, there are some limitations of chicken including strong chicken flavor, tough texture of lean meat, hormones, antibiotics and also high content of uric acid due to high consumption of chicken, especially people with gout. Also, drying process also definitely extend the shelf life of product and provide convenience to consumers for transport perspective with higher percent of protein content and less total weight. Therefore, it is crucial to compare different drying methods and investigate the effect on functional properties of chicken breast.

Furthermore, the United Nations reported that there are over 821 million of people around the world who are undernourished. Thus, we need to reduce this huge number as suggested by UN Sustainable Development Goal number 2, which is Zero hunger. Moreover, one of the most crucial factors of hunger is food price because some people cannot afford it. To clarify, it takes approximately 4 months to ship food aid and the shipping cost contributes more than 40 percent of the total cost (3). Moreover, most of the people could not afford the food cost after its surpluses in the market. Hence, if the shipping cost can be reduced by reducing the shipping food weight, this could help lower overall food price. Hence, to help solve this problem, one of the basic processing steps to reduce the weight of food is dehydration or drying into the powder form. Also, as the product development needs to develop a new target market and follow the trend of the food, finding a way to improve the product to be better including high nutritional value along with good sensory and functional properties of the product are needed. Therefore, the aim of

the study is to determine physicochemical and functional properties of chicken breast powder, to compare the effect of conventional, vacuum, and low pressure superheated steam drying on functional properties of chicken breast powder, and to compare whey protein concentrate with chicken breast powder as an alternative protein source.

Basically, protein powder can be done in many processes. For example, for the process of chicken breast powder followed by Ilansuriyan method (4), they reported that firstly chicken lean meat is chopped and minced into tiny pieces of meat. The minced meat is then cooked at a high temperature to eliminate pathogens, especially by contamination of Salmonella spp., which can be normally found in the poultry industry. In addition to microbial count reduction, cooking can also help to stop the enzymatic activity in the chicken meat. After that, the cooked meat was dried in the hot air oven to remove the water out the sample, resulting in less moisture content and water activity of the final product. Then, the dried meat was ground by grinder until the fine powder of chicken meat was obtained. Additionally, the ground meat powder was sieved using sieve in order to remove any contaminant, coarse and large particle size of powder or even foreign material. Lastly, it was kept tight packaging to prevent the powder from humidity and oxygen which can affect the quality of the chicken powder and its shelf life.

To begin with, in terms of functional properties of food products, different food biopolymers have different roles on functional properties in the same food matrix. Functional properties of foods can be affected by concentration, configuration, size and polydispersity of a certain biopolymer of interest; thus, it is very critical and significant to understand those factors and how they affect the overall properties of the product

Vacuum drying is another drying method that is used to alleviate the drawbacks of the conventional drying method by providing higher drying rate at lower drying temperature. Jha reported that the drying temperature of vacuum drying can be ranging from 30°C to 60°C, depending on the vacuum pressure used (5). The aim of this drying technique is to remove moisture under vacuum; thus, it operates at a lower drying temperature in an oxygen-deficient processing environment, resulting in reducing the chance of oxidation reactions in the food during the drying process. Moreover, Jiang reported that vacuum drying operated at lower drying temperature, so it could help to reduce thermal stresses and the problem of over-drying (6). As a result, it is crucial and suitable for both thermolabile materials and oxygen sensitive food in order to enhance the quality and maintain nutritional value of those particular products. Principally, in terms of processing of vacuum drying, the surface of the vacuum dried product is heated mostly by conduction and radiation, since there is less convection in the vacuum atmosphere. To clarify, the boiling point of water is decreased due to the higher vacuum pressure, resulting in more effective hydraulic conductivity of the food matrix, less the resistance of mass transfer at the food surface, higher rate of evaporation, and less drying time. To exemplify, the use of vacuum drying at 25°C can dry the food product three times faster than the conventional method drying with warm air at 30°C and humidity at 50%. In terms of production costs, Parikh reported that the vacuum drying method is cheaper than freeze drying and spray drying methods due to its less energy requirements (7). Thus, the microwave vacuum drying is developed for the extended application.

Low pressure superheated steam drying (LPSSD) is also one of useful drying techniques that is applied in many types of food products since it is the slow drying process that operates at drying temperature lower than 100°C with the assist of reduced pressure. Unlike superheated steam drying (SSD) which applied higher pressure that could make product melt, undergo glass transition, or degrade some bioactive compounds, LPSSD can help reserve

production energy, preserve food quality, and retain nutrients, especially bioactive components in heat sensitive food products. To exemplify, in terms of food quality evaluation, LPSSD exhibits higher retention of bioactive components, total phenol content and antioxidant activity in dried mango cubes than vacuum and hot air-drying methods (8). Nevertheless, in case of applying higher drying temperature, it can affect the color, appearance and texture of the food as well. For example, by comparing low pressure superheated steam drying with conventional hot air drying, LPSSD provides better quality benefits to potato chips than hot air-drying including result in lower browning index, retain product color, and also take shorter drying time (9). Additionally, the drying temperature and time for obtaining the desired final moisture content depends on the product size or surface area, hence chicken breast powder will definitely require lower temperature and shorter time than potato chips. Moreover, with the use of heated saturated steam and reduced pressure, it can also operate at a lower drying temperature in an oxygen-deficient processing environment, resulting in reduce the chance of oxidation reactions in the food and also yield dried product with high porosity (10).

MATERIAL AND METHODS

Materials

Chicken breast meat

Deboned lean chicken breast meat was sourced from Tops Supermarket in Bangkok, Thailand.

Chemical reagents

All chemical reagents were analytical grade including anhydrous copper sulfate (Ajax Finechem Co., Ltd, New Zealand), anhydrous sodium carbonate (Lons Chemie Pvt Ltd., India), boric acid (Ajax Finechem Co., Ltd, New Zealand), ethanol (Qrec Chemicals, New Zealand), hydrochloric acid (Qrec Chemicals, New Zealand), kjeblet catalyst (Oskon Co. Ltd, Thailand), methyl red indicator (Merck, Germany), petroleum ether (Qrec Chemicals, New Zealand), reagent grade concentrated. sulfuric acid (Qrec Chemicals, New Zealand), and sodium hydroxide (Ajax Finechem Co., Ltd, New Zealand).

Laboratory Equipment

All laboratory equipment and instrument in the experiment include centrifuge (Model6000, Kubota Corporation, Japan.), colorimeter (Chroma meter CR-400, Konica Minolta Sensing, Inc., Japan), digestion Unit (K-424) (Buchi, Switzerland), distillation Unit (K-324) (Buchi, Switzerland), drying oven (Memmert UN 30 plus, Germany), low pressure superheated steam dryer (Department of Food Engineering, King Mongkut's University of Technology Thonburi, Thailand), muffle furnace (CWF 1200, Scientific Promotion Co. Ltd, Thailand), seven compact pH meter (Mettler Toledo Co. Ltd, Victoria, Australia), soxhlet extractor (Gerhardt, Germany), and vacuum oven (Model 273600, Hotpack, The United States)

Preparation of chicken breast powder

For chicken breast powder preparation processes, the deboned frozen lean chicken breast was ground by the meat mincer. After mincing, chicken meat was boiled in a pressure cooker for 15 minutes. The minced and pressure-cooked chicken meat was dried by either conventional hot air drying (at 65°C), vacuum drying (at 65°C, 0.09 MPa) (Figure 2) (11), and low pressure superheated steam drying (at 65°C, 0.09 MPa) (Figure 2) (9) with slight modifications to the dried chicken breast meat (Figure 1), and also the drying time is determined by the time each drying method reaching the similar level of final moisture content as the conventional method. The dried chicken breast meat was

grinded into chicken breast powder, and it was sieved by using a sieve for the equal particle size of chicken breast powder.

Figure 1. Chicken breast meat after drying in the oven

Determination of drying time and temperature

The determination of drying time and temperature for cooked chicken breast meat will be performed and followed by the drying methods including conventional hot air drying (at 65°C), vacuum drying (at 65°C, 0.09 MPa) (11), and low pressure superheated steam drying (at 65°C, 0.09 MPa) (9) with slight modifications, and also the drying time is determined by the time each drying method reaches the similar level of final moisture content as the conventional method to compare the effect of those drying techniques on chicken breast powder in further steps. The target final moisture content in this experiment should be controlled to be ranged from 4±2% since the maximum moisture content in powder is 8% according to Regulation (EC) No 1069/2009 and Regulation (EU) No 142/2011 of the food standard (12). Furthermore, in accordance with international standards, in terms of microbiological standard, *Salmonella* (ISO 6579), *enterobacteriaceae* (NEN-EN-ISO 21528-2), *Escherichia coli* (ISO 16649-2), yeasts and moulds (ISO 7954) need to be limited to be lower than standards limits by ISO methods.

Determination of physicochemical properties

Moisture content determination

Moisture content determination is determined by conventional oven drying method at 105°C (13).

Water activity determination

The water activity of chicken breast powder was analyzed by using AquaLab Dew point Water Activity Meter 4TE. 1 gram of chicken breast powder was placed in a sample cup covered with the lid and ensure that the powder covers all the surface area in the bottom as possible to maximize the water level coming out from the samples. The analysis was done in triplicate and reported in mean.

Proximate analysis

Protein determination

Protein content is determined by Kjeldahl method (13)

Fat determination

Fat content is determined by Soxhlet method (13)

Ash determination

Ash content is determined by the method of AOAC (13)

pH determination

The pH of chicken breast powder was analyzed by using a pH meter. 15 grams of chicken breast powder was used and diluted in 30 ml of distilled water in %w/v (1:2).

Color determination

The color of chicken breast powder was analyzed by using the chroma meter. The colorimeter was calibrated using a white reference plate before chicken breast powder measurement. The results were done in triplicate and shown as the value of L^*, a^*, and b^*. (14). Additionally, chroma (c^*), hue angle (h^*), whiteness index (WI), yellowness

index (*YI*), and brownness index (*BI*) were also calculated according to the equations as shown in Table 1 (14).

Table 1. Quantification of colour and equations (14).

Quantification of colour	Equations
Chroma (c^*)	$c^* = \sqrt{a^2 + b^2}$
Hue angle (h^*)	$h^* = \tan^{-1}(\frac{b^*}{a^*})$
Whiteness index (*WI*)	WI = L - 3b + 3a
Yellowness index (*YI*)	$YI = \frac{142.86b^*}{L^*}$
Brownness index (*BI*)	$BI = 100 \text{ x } \left(\frac{X-0.31}{0.17}\right)$, where, $X = \frac{(a+1.75\,L)}{(5.645L+a-3.012b)}$

Determination of functional properties

Water holding capacity

For water holding capacity, followed by Elsohaimy method with some changes (15), whey protein concentrate and chicken breast powder were used in water holding capacity analysis in order to compare its holding capacity. 5 grams of the sample and 10 ml of distilled water were weighed and put in a 50-ml centrifuge tube, then thoroughly mixed and allowed to stand for 30 minutes. The centrifuge tubes were centrifuged at 4°C for both whey protein concentrate and chicken breast powder, at 7000 rpm for 30 minutes each. The supernatant or liquid part was poured from the tube and weighed.

Oil absorption capacity

For oil absorption capacity (OAC), followed by Elsohaimy method with some changes, whey protein concentrate and chicken breast powder were used in oil absorption capacity analysis in order to compare its absorbing capacity. 5 grams of the sample and 10 ml of soybean oil were weighed and put in a 50-ml centrifuge tube, then thoroughly mixed and allowed to stand for 30 minutes. The centrifuge tubes were centrifuged at 4°C for both whey protein concentrate and chicken breast powder, at 9000 rpm for 30 minutes each. The supernatant or liquid part was poured from the tube and weighed.

Statistical analysis

Statistical analysis will be performed by using SPSS for Windows with Duncan test at 95% confidence to compare means. Also, analysis of variance (ANOVA) will be used to determine the significant differences from the testing.

RESULTS AND DISCUSSION

Preparation of chicken breast powder

Figure 2. Vacuum dryer and low pressure superheated steam dryer

Yield of dried chicken breast powder samples

Table 2. % Yield of three types of chicken breast powder

Types of drying method	Weight of raw chicken breast (g)	Weight of pressure-cooked chicken breast (g)	Weight of chicken breast powder (g)	Yield (%)
Conventional hot air drying (CBH)	920.2	551.11	154.95	16.84
Vacuum drying (CBV)	829.8	501.10	136.25	16.42
Low pressure superheated steam drying (CBL)	766.7	462.70	163.11	21.27

According to Table 2, chicken breast powder dried by low pressure superheated steam dryer (CBL) gives higher yield than both powders dried by vacuum dryer (CBV) and conventional hot air dryer (CBH), respectively. This might be due to higher moisture content in CBL remaining in the sample because CBL could better rehydrate than other samples. The reason behind this is that low pressure superheated steam drying can reduce the degree of shrinkage and also improve shrinkage by simultaneously involving vapor inside the sample and expanding into the cell, leading to uniform shrinkage. Since the drying chamber in low pressure superheated steam dryer is more humid than vacuum dryer and conventional hot air dryer, this milder condition could prevent case hardening in the sample. To exemplify, in case of vacuum and conventional hot air drying, the outer surface of the sample tends to be dried and rigid faster than the center, taking surface water by the dry air, resulting in the non-uniform shrinkage blocking the surface to rehydrate vapor from the surrounding. Consequently, due to possible rehydration during cooling and storage of CBL (17), it could definitely result in higher yield of the sample due to higher water rehydration into the CBL, resulting in higher weight of the sample than CBL and CBH, respectively.

Determination of drying time and temperature

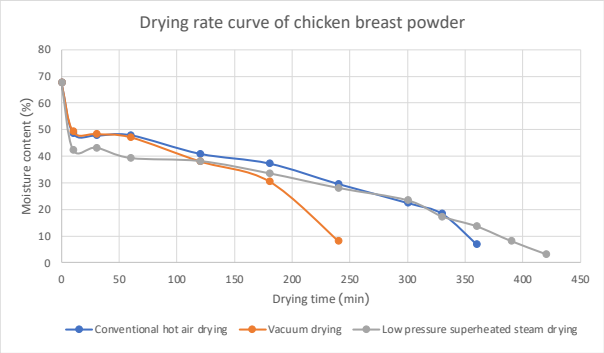

Figure 3. Drying rate curve of chicken breast powder (Moisture content vs. Drying time)

For the determination of drying time and temperature, all chicken breast samples were dried at 65°C. In addition, to determine drying time of each drying method, the powder needed to reach the similar level of final moisture content as the conventional method. As the chicken breast powder dried by conventional hot air oven method is dried for 6 hours, vacuum dried and low pressure superheated steam dried chicken breast powders required about 4 hours and 7 hours, respectively. In terms of drying curve, as shown in Figure 3, by determining the drying time until the weight of cooked chicken breast tends to remain constant, the vacuum drying method requires the shortest drying time than others because it is dried under vacuum providing higher drying rate at lower drying temperature. Unlike vacuum drying, low pressure superheated steam drying requires the longest drying time than others due to the fact that the superheated steam continuously input during drying, so there will be the cycle of some moisture reuptake of the sample and vapor dried out during drying, even though it is a slow drying process that operates at drying temperature lower than 100°C with the assist

of reduced pressure similar to vacuum drying method.

Table 3. Proximate compositions of dried chicken breast powder samples (db.)

Physical properties	Conventional hot air drying	Vacuum drying	Low pressure superheated steam drying
Moisture content (%)	3.92±0.24^{b}	3.62±0.09^{b}	5.58±0.01^{a}
Water Activity	0.288±0.012^{b}	0.280±0.002^{b}	0.427±0.002^{a}
pH	6.28±0.008^{b}	6.19±0.012^{c}	6.33±0.005^{a}
Protein content (%)	91.88±0.45^{b}	94.56±0.55^{a}	89.91±0.12^{c}
Fat content (%)	8.59±0.28^{b}	7.66±0.16^{c}	9.81±0.38^{a}
Ash content (%)	2.00±0.008^{b}	2.67±0.47^{a}	1.86±0.13^{b}
Color			
*L**	77.60±0.24^{c}	78.53±0.45^{b}	80.06±0.06^{a}
*a**	-0.89±0.05^{c}	-0.56±0.05^{b}	0.40±0.01^{a}
*b**	15.33±0.13^{c}	16.48±0.19^{b}	17.88±0.16^{a}
*c**	15.36±0.16^{c}	16.49±0.24^{b}	17.89±0.20^{a}
*h**	86.67±0.21^{c}	88.05±0.20^{b}	88.73±0.02^{a}
WI	28.92±0.34^{a}	27.42±0.53^{b}	27.60±0.50^{b}
YI	28.23±0.22^{c}	29.97±0.29^{b}	31.91±0.33^{a}
BI	20.62±0.21^{c}	22.45±0.21^{b}	25.06±0.21^{a}

*Mean values ± standard deviation (n=3) with different superscript letters (a-c) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

Proximate analysis of chicken breast powder

In terms of physicochemical properties, as shown in Table 3, the pH of chicken breast powder from all drying methods are about 6.3 which is almost neutral and lower than the whey protein powder which is 6.80±0.02, and the % yield of chicken breast powder is lost due to loss of weight of water, fat and some of the irregular coarse chicken breast powder during the process of preparation including pressure cooking, drying and sieving respectively. Thus, the moisture content (% MC) and water activity (a_w) of chicken breast powder are dependent on those changes mainly during drying. As a result, as shown in Table 3, CBL has significantly higher moisture content (5.58 ±0.01^{a}) and water activity (0.427±0.002) than both CBV (3.62±0.64), (0.280±0.002) and CBH (3.92±0.24), (0.288±0.012) respectively due to the steam continuously involved in low pressure superheated steam drying chamber. Therefore, the reason why the results compared based on different final moisture contents of product is that the humidity in the drying chamber of low pressure superheated steam dryer is possibly higher than others because of the continuous steam input in the chamber during drying. To compare with the commercial whey powder, the moisture content of chicken breast powder is higher than commercial whey powder which is 2.03±0.13, and the water activity of chicken breast powder is also higher than commercial whey powder which is 0.196±0.005. The reason behind this is that moisture content of the food products is usually measured by heating the material to dry and recording the weights of the product before and after the drying process. Nevertheless, since the moisture content of chicken breast powder is higher, this might be due to the bigger particle size of the chicken breast powder compared with the commercial whey powder, in which it could hold more moisture in the cell. For the water activity, it is a measurement of free water that is available to react with itself or another material. In addition, for the long time of storage, the food products can reuptake the moisture from the surrounding which can be referred as moisture sorption isotherm. Therefore, since the chicken breast powder was passed through the process of drying in either conventional hot air oven, vacuum oven or low pressure superheated steam oven and cooled down before packing and further analysis, so it should have more water activity compared with the commercial whey powder because it has larger particle size to rehydrate during cooled down and storage, resulting in the

absorption of moisture from the surrounding with higher humidity to the product.

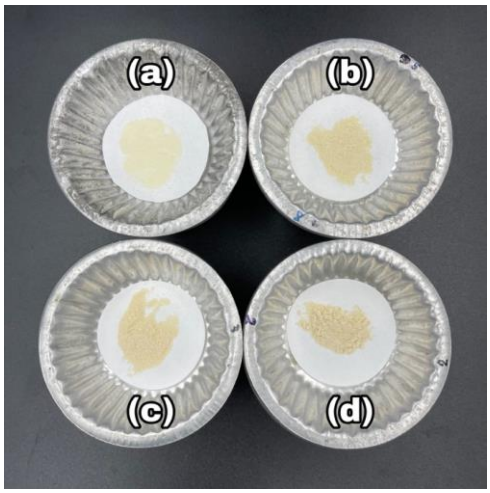

Figure 4. Colours of different types of powder: (a) Commercial whey powder, (b) Hot air-dried chicken breast powder, (c) Vacuum dried chicken breast powder, and (d) Low pressure superheated steam dried chicken breast powder

In terms of color, the color profile of both chicken breast powder was shown in Table 3. The lightness (*L**) of the chicken breast powder from conventional hot air drying (CBH) (77.60±0.24) has the darkest sample which is significantly lower *L** than CBV (78.53±0.45) and (CBL) (80.06±0.06), respectively. To clarify, the darker color of CBH could come from the longer drying process and the use of air movement with low humidity compared with vacuum and low-pressure superheated steam drying, respectively. On the other hand, in terms of redness (*a**) of the chicken breast powder, CBL (0.40±0.01^{a}) significantly redder than CBV (-0.56±0.05^{b}) and CBH (-0.89±0.05^{c}). Furthermore, for the yellowness (*b**) of the chicken breast powder, CBL is also (17.88±0.16) significantly more yellowish than CBV (16.48±0.19) and CBH (15.33±0.13). To elaborate, the more red and yellowish color of CBL could come from the browning reaction during drying since it has higher water activity, so it could increase the brownish rate. Moreover, the color profile of both chicken breast powder and commercial whey powder were shown in Table 3 and 4 respectively. The lightness (*L**) of the commercial whey powder (96.60±0.33) is higher than all types of dried chicken breast powders, whereas the yellowness (*b**) of the chicken breast powder is much higher than the commercial whey powder (1.61±1.54). To clarify, the yellowness of the chicken breast powder could come from the browning reaction during the process of pressure cooking and drying. However, in terms of redness (*a**), CBL show a higher value than others.

As a result of this, all the *L**, *a**, *b** values can be proved and calculated for the quantification of colour in several values including chroma (*c**), hue angle (*h**), whiteness index (*WI*), yellowness index (*YI*), and brownness index (*BI*), and those results were corresponded to the *L*a*b** color. To illustrate, as CBL exhibited the highest *a** and *b** values, so it gives more redness and yellowness in color, resulting in higher chroma (*c**) and hue angle (*h**) that is closer to 90° which is represent in yellow color. In addition, like chroma and hue angle, CBL also showed the highest in yellowness index (31.91±0.33^{a}) and brownness index (25.06±0.21^{a}) values since it took longer drying and steam involved, so this might extend the browning reaction during drying, resulting in generating more brownish and yellowish pigments. On the other hand, in terms of whiteness (*WI*), it exhibited contrast to the lightness of the powder in this case. To explain, whiteness index can be measured to correlate the result with the preference of the consumers for white color or the absence of color, whereas lightness correspond to the ability to reflect light of white surface, usually blue light. The higher blue color, the higher lightness in the food product. Consequently, as shown in Figure 4, CBH has significantly higher whiteness index value (28.92±0.34^{a}) than others since it has significantly lower *b** value corresponding to bluer color for higher light reflection. Likewise, even though CBL

has the highest L*, it exhibited lower whiteness index value since CBL possessed more yellowish and brownish color. Subsequently, as the consumers possibly see in Figure 4, they might see that CBL looks darker than CBH and whey powder.

Table 4. Proximate compositions of commercial whey powder

Physical properties	Whey protein powder
Moisture content (%)	2.03±0.13
Water Activity	0.196±0.005
pH	6.80±0.02
Color	
*L**	96.60±0.33
*a**	0.17±0.38
*b**	1.61±1.54
*h**	82.08±0.15
*c**	17.39±0.22
WI	27.70±0.26
YI	28.42±0.15
BI	19.54±0.10

Table 5. Functional properties of dried chicken breast powder samples

Functional properties	Conventional hot air drying	Vacuum drying	Low pressure superheated steam drying
Water absorption capacity (g H_20/ g protein)			
pH 5	2.54±0.025^{f}	2.59±0.031^{de}	2.69±0.017^{c}
pH 6	2.31±0.005^{h}	2.35±0.012^{h}	2.39±0.024^{g}
pH 7	2.57±0.021^{ef}	2.63±0.022^{d}	2.91±0.021^{b}
pH 8	2.98±0.025^{a}	2.71±0.012^{c}	2.71±0.021^{c}
Oil absorption capacity (g oil/ g protein)	1.70±0.002^{c}	1.80±0.008^{b}	1.99±0.015^{a}

*Mean values ± standard deviation (n=3) with different superscript letters (a-h) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

For the nutrients in chicken breast powder, as shown in Table 3, CBV mainly contains the highest protein as a major source which is 94.56±0.55^{a} % in dry weight basis, with lowest 7.66±0.16^{c} % of fat and highest 2.67±0.47^{a} % of ash content since it has the lowest moisture content among all samples since moisture content can further affect other compositions in dry basis.

Table 6. Functional properties of commercial whey powder

Functional properties	
Oil absorption capacity (g oil/ g protein)	1.13±0.05
Water absorption capacity (g H_20/ g protein)	
pH 5	1.04±0.041^{a}
pH 6	0.91±0.024^{b}
pH 7	0.73±0.033^{c}
pH 8	0.56±0.025^{d}

*Mean values ± standard deviation (n=3) with different superscript letters (a-d) in the same row differ significantly ($p<0.05$) analyzed by ANOVA and Duncan's test using SPSS.

Water holding capacity of chicken breast powder

Moreover, as shown in Table 5, in terms of water holding capacity, CBL could hold more water than CBV and CBH at almost all the pH since the uniform porous structure of CBL could rehydrate and absorb water into the cell. However, lower water absorption capacity (WAC) value could be observed if the adjusted pH is close to the pI or the isoelectric point of the protein. Therefore, as in all samples, at pH 6, they all showed the lowest WAC values, so this can imply that the isoelectric point chicken breast powder is around pH 6. For instance, the lowest water holding capacity of each type of chicken breast powder sample was seen for pH 6 in all CBH, CBV and CBL, while the highest water holding capacity for both CBH and CBV is at pH higher than 7. Furthermore, in case of CBL, the highest water holding capacity was seen on pH 7 since the porous uniform

structure could reuptake moisture and rehydrate well at the pH close to water; however, if the pH is adjusted to be lowered by the HCl or to be higher by NaOH, there might be slight protein denaturation to partially unfold the protein, causing in significantly lower water holding capacity of CBL at pH 8. Therefore, all in all, if we adjust the pH to be away from pH 6, the water absorption capacity of the chicken breast powder tends to increase respectively, resulting in absorbing and holding more moisture in itself. Furthermore, the degree of water absorption capacity might be affected by the drying time as well. The longer drying time like drying in CBH, the less porous structure inside the cell due to case hardening on the surface. Hence, CBV could absorb and hold more water than CBH since it is dried in shorter drying time with the assistance of vacuum pump, resulting in less denaturation and non-uniform shrinkage during drying to cause case hardening. To compare with whey protein powder, all chicken breast powders have higher water holding capacity than whey protein powder which is since whey usually has finer particle size than chicken breast powder, so it could not absorb and hold water in itself well due to the fact that whey is also soluble well in water. When comparing among whey protein samples at different pH, as shown in Table 6, it exhibited that the water holding capacity significantly increases as the pH decreases. The reason behind this is that the isoelectric point of whey protein is less than 5.5, so if the pH is lower than 5.5, the protein is more likely to precipitate at pI, resulting in less solubility in supernatant and higher in water holding capacity (17).

Oil absorption capacity of chicken breast powder

Furthermore, for oil absorption capacity, as shown in Table 5, CBL (1.99 ± 0.015^{a}) also could absorb and hold more oil than CBV (1.80 ± 0.008^{b}) and CBH (1.70 ± 0.002^{c}), respectively. Since CBL involved superheated steam in the drying process, it could lower the degree of case hardening and reduce the degree of protein denaturation on the surface. Thus, like freeze-dried protein, low-pressure superheated steam dried protein could have significantly higher oil absorption than vacuum and conventional hot air-dried proteins due to possessed possibly higher surface hydrophobicity (11). To compare with whey protein powder, all chicken breast powders have higher oil absorption capacity than whey protein powder which is 1.13±0.05 (Table 6) since whey usually has smaller particle size than chicken breast powder, so it could absorb and hold oil well due to possibly less space inside the cell.

CONCLUSION

In conclusion, vacuum drying can be considered as a better choice compared with other drying methods. It requires the lowest drying time, and gives the lowest moisture content and water activity, resulting in higher protein content in dry basis. For the color, CBL exhibited the most yellowish and brownish color due to longer drying time and steam involved, resulting in extension of browning reaction during drying process. For water absorption capacity, chicken breast powder all showed the lowest WAC values at pH 6. Low pressure superheated steam maintains uniform porous structure and exhibits higher oil absorption capacity. In the future, this study could guide the possibility of chicken breast powder to be further investigated for other potential functional properties that can help improve functions in a particular food product as an alternative protein powder.

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Comparison of extraction methods of *Ardisia elliptica* leaves on bioactive compounds and their antioxidant activities

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ABSTRACT:

Nowadays, a variety of extraction methods have been developed to increase extraction efficiency. Each method has different advantages and disadvantages; however, there is no unique extraction method for bioactive compounds from all plants. This research investigated bioactive compounds (phenolic compounds and flavonoids) and their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) assays of the extract from *Ardisia elliptica* leaves. Three extraction methods—conventional extraction (CE), ultrasound-assisted extraction (UAE) and accelerated solvent extraction (ASE)—were studied and compared to identify the optimum conditions for each method in terms of extraction efficiency. Based on these results, the best extraction method was ASE at 80°C for 10 min, which gave a total phenolic content of 49.33 mg gallic acid equivalents/g dried sample, total flavonoid content of 6.72 mg catechin equivalents/g dried sample and antioxidant activities when tested based on DPPH of 79.35 mg trolox equivalents/g dried sample and a FRAP value of 644.76 µmol Fe^{2+}/g dried sample.

Keywords: Antioxidant activities, Ardisia elliptica, Bioactive compounds, Extraction methods

INTRODUCTION

Ardisia elliptica Thunb. (commonly known in Thailand as 'Pilangkasa' or 'Ram Yai') belongs to the family Primulaceae. It is a shrub or small tree that grows in mangrove forests or coastal rainforests [1]. It is used as a traditional medicinal herb, the dried leaves are brewed as tea, and has better antioxidant efficiency than standard compounds [2, 3]. The genus *Ardisia* is a rich source of biologically potent phytochemicals, such as bergenin and ardisin and it has been reported that the polyphenol content of sample is in the range 21–72 mg gallic acid equivalents (GAE)/g dried leaves [2, 4].

Phenolic compounds are secondary metabolites, commonly found in plants, vegetables, and fruits, with the type and amount of each phenolic compounds depending on the plant species, the conditions which plants were grown, the part of the plant that is being harvested and the age of sample [5, 6]. Their structure comprises of an aromatic ring bearing one or more hydroxyl groups and they can range from simple phenolic molecules to highly polymerized compounds [7]. They are potent antioxidants and prevent damage to biomolecules within the body as well as hiving other biological activities, such as anti-inflammatory,

antidiabetic, cardioprotective, neuroprotective, antitumor, and antiaging properties [8].

Extraction is an important process for studying phenolic compounds. Several factors affect this step, such as the solvent, extraction method, temperature, time and pressure [9]. The solvent and extraction methods affect antioxidant activities [10]. Conventional extraction (CE) is a fundamental method for the extraction of bioactive compounds as it does not require complicated equipment and is cheap, but has disadvantages, such as using a large amount of solvent, consuming time, and requiring considerable energy during the extraction process [11]. However, it is commonly used as a reference method to compare the efficiency of alternative methods [12]. Currently, new extraction methods have been developed to increase the extraction efficiency, reduce the amounts and the environmental impacts of organic solvents [13]. Ultrasound-assisted extraction (UAE) uses the sound waves in the frequency range of 20 to 100 kHz, which is higher than the audible frequency range of human hearing. It involves the occurrence of cavitation and bubbles which grow in size until disrupted, with the resulting high energy producing shock waves that degrade the plant cell wall, improve solvent penetration and later the release of extracted compounds [14, 15]. Another method is accelerated solvent extraction (ASE), which uses pressure to stabilize the solvent to a liquid state at a temperature above the boiling point. In addition, the solvent can easily penetrate through the small gaps of the sample, increasing the contact surface area between the sample and solvent [16, 17]. Since there are many extraction methods, no single method is considered as a standard method for extracting bioactive compounds from all plants [18]. Typically, organic solutions with different polarities have a specific effect on the extraction of antioxidants [19].

The objectives of this research were: to investigate extraction methods (CE, UAE and ASE) in terms of phenolic compounds, flavonoids, antioxidant activities base on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) assays; to identify the optimum conditions for each method; and to compare their extraction efficiency.

MATERIAL AND METHODS

Plant material and preparation

A. elliptica leaves collected from the Bang Kachao community (Samut Prakan province, Thailand) in February 2020 were washed with chlorine solution (50 parts per million) and rinsed with clean water before being cut into small pieces about 5 mm wide. Then, they were dried in a hot-air oven model UF260 (Memmert GmbH + Co. KG; Schwabach, Germany) at 50°C for 24 hr, final moisture content of the leaves was 9.89% crushed and stored in clear polypropylene plastic bags, kept in the dark at 25°C for later investigation.

Chemicals and reagents

Folin-Ciocalteu reagent, DPPH, trolox, gallic acid, (+)-catechin hydrate and 2,4,6-tris(-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate was purchased from KemAus (Cherrybrook, NSW, Australia). Sodium hydroxide, sodium nitrite, and aluminum chloride were purchased from Ajax Finechem (Auckland, New Zealand). Sodium acetate, ferric chloride hexahydrate, glacial acetic acid and ferrous sulfate were purchased from Carlo Erba (Milan, Italy). Ethanol 95% was purchased from Liquor Distillery Organization (Chachoengsao, Thailand). Deionized water was purchased from RCI Labscan (Bangkok, Thailand).

Study of extraction conditions and selection of optimum conditions

From previous studies, it was found that 30% ethanol was the appropriate solvent for extraction of bioactive compounds from *A. elliptica* leaves. Therefore, 30% ethanol

was used as a solvent in this study. The extraction conditions of the three methods were preliminarily studied to select the optimum conditions for each method using a completely randomized design (CRD) full factorial with two factors and three levels.

Conventional extraction

Samples (each 5 g) of dried *A. elliptica* leaves were soaked in 100 mL of solvent (1:20 w/v). The studied factors were temperature (60°C, 70°C or 80°C) and extraction time (30 min, 45 min or 60 min). The samples were heated in a water bath with shaker Schutzart DIN 40050 – IP20 (Memmert GmbH + Co. KG, Schwabach, Germany) with a shaking speed of 120 rpm and passed through Whatman No. 4 filter paper. The extracts were stored in clear bottles and kept in the refrigerator at 0°C until use.

Ultrasound-assisted extraction (UAE)

Sample (each 5 g) of dried *A. elliptica* leaves were soaked in 100 mL of solvent (1:20 w/v). The studied factors were temperature (40°C, 50°C or 60°C) and extraction time (10 min, 20 min or 30 min). The samples were extracted using an ultrasound bath (Elma Elmasonic P120H; Elma-Hans Schmidbauer GmbH & Co. KG; Singen, Germany) with a frequency of 37 kHz in pulse mode. Then, they were passed through Whatman No. 4 filter paper. The extracts were stored in clear bottles and kept in the refrigerator at 0°C until use.

Accelerated solvent extraction (ASE)

The extraction was performed using a Dionex ASE 350 system (Thermo Scientific; Waltham, MA, USA). Sample (each 5 g) of dried *A. elliptica* leaves were mixed with 1.25 g diatomaceous earth (1:0.25 w/w) and placed in a 34 mL stainless steel extraction cell. A cellulose D28 filter (Dionex Corporation; Sunnyvale, CA, US) was placed at the bottom of the extraction cell to prevent sample particles accumulating in the collection vial. The samples were heated for 5 min before extraction. The studied factors were temperature (60°C, 70°C or 80°C) and extraction time (5 min, 10 min or 15 min). Later, the samples were rinsed with solvent (150% the cell size), then purged with nitrogen to clear up residual solvent in the cell for 100 s, extracted for one extraction cycle, passed through Whatman No. 4 filter paper, kept in clear bottles, and stored in the refrigerator at 0°C until use.

The extracts obtained from each method were analyzed for chemical quality values (total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities). All treatments were done in triplicate and all data were reported as mean ± SD. Analysis of variance was carried out using the SPSS Statistics software (version 12.0; SPSS Inc.; Chicago, IL, USA). Duncan's new multiple range test was used to determine the differences among means at the 95% confidence level. The optimal conditions for each extraction method were selected.

Total phenolic content (TPC)

The TPC was measured using a modified colorimetric Folin-Ciocalteu method, according to Wolfe et al. [20]. Each extract sample (125 µL) was mixed with 500 µL distilled water in a test tube; then, 125 µL Folin-Ciocalteu reagent was added, shaken and allowed to react for 6 min. Later, 7% sodium carbonate solution (1,250 µL) and distilled water (1,000 µL) were added to the test tube. The mixture was allowed to develop color for 90 min at room temperature, and the absorbance was measured at 760 nm using a Genesys 20 UV-vis spectrophotometer (Thermo Scientific Waltham, MA, USA). The absorbance was compared with standard gallic acid curves, which were expressed as milligrams of GAE per gram of dried sample.

Total flavonoid content (TFC)

The TFC was measured using a modified method of Wolfe et al. [20]. Each extract sample (250 µL) was added to distilled

water (1,250 µL) and mixed with 5% sodium nitrate solution (75 µL); then, the mixture was allowed to stand for 5 min at room temperature. Before adding 10% aluminum chloride (150 µL) and then allowed to react for 6 min. Later, 500 µL of sodium hydroxide 1 M and 275 µL of distilled water were added. The absorbance was measured at 510 nm using a UV-vis spectrophotometer. The absorbance was compared with the standard curve of the catechin solution, expressed as milligrams of catechins equivalents per gram of dried sample.

Antioxidant activities

DPPH Assay

The analysis used was modified from Maduwanthi and Marapana [21]. Samples of DPPH solution (0.05 mM) were prepared (absorbance at 517 nm was adjusted to be in the range 0.7 ± 0.02). The extract (150 µL) was mixed with DPPH solution (2,850 µL) and stored for 30 min in the dark. Later, the absorbance was measured at 517 nm using a UV-vis spectrophotometer and the %Scavenging was calculated using Equation 1 and compared with standard curve of trolox solution, which expressed as milligrams of trolox equivalents per gram of dried sample:

$$\%\text{Scavenging} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100 \qquad (1)$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

FRAP Assay

The FRAP assay was analyzed according to the method of Kubola and Siriamornpun [22]. FRAP reagent was prepared by mixing 100 mL of 300 mM acetate buffer at pH 3.6, 10 mL of 10 mM TPTZ (in 40 mM of hydrochloric acid), 10 mL of 20 mM ferric chloride hexahydrate and 12 mL of distilled water. Then, 60 µL of extract, 180 µL of distilled water and 1,800 µL of FRAP reagent were added and kept at 37°C for 4 min. The absorbance was measured at 593 nm using a UV-vis spectrophotometer. The absorbance was compared with the standard curve of the ferrous sulfate solution and reported as the FRAP value in micro moles of ferrous per gram of dried sample.

Comparison of extraction methods

The energy used and extraction efficiency of the optimal conditions for each extraction methods (CE, UAE and ASE) were calculated according to Equations 2–6, which were adapted from Piyapanrungrueang et al. [23].

Duncan's new multiple range test was

$$\text{Energy}\left(\frac{\text{kW} \times \text{h}}{\text{g}}\right) = \text{Power} \times \text{Time} \qquad (2)$$

$$\text{Efficiency of TPC}\left(\frac{\text{mg GAE}}{\text{kW} \times \text{h}}\right) = \frac{\text{TPC}}{\text{Energy}} \qquad (3)$$

$$\text{Efficiency of TFC}\left(\frac{\text{mg catechin}}{\text{kW} \times \text{h}}\right) = \frac{\text{TFC}}{\text{Energy}} \qquad (4)$$

$$\text{Efficiency of DPPH}\left(\frac{\text{mg Trolox}}{\text{kW} \times \text{h}}\right) = \frac{\text{DPPH}}{\text{Energy}} \qquad (5)$$

$$\text{Efficiency of FRAP}\left(\frac{\mu\text{mol Fe}^{2+}}{\text{kW} \times \text{h}}\right) = \frac{\text{FRAP}}{\text{Energy}} \qquad (6)$$

used to determine the differences among means at the 95% confidence level. Then, the best extraction method was selected.

RESULTS AND DISCUSSION

Extraction conditions

Conventional extraction (CE)

The extraction of *A. elliptica* leaves using the CE method, with different temperature factors (60°C, 70°C or 80°C) and extraction times (30 min, 45 min or 60 min) showed that there was not interaction between the temperature and time on the TPC and TFC (Table 1). As the temperature increased, the TPC and TFC increased, so that at 80°C, the average amounts of TPC and TFC were significantly higher than those at other temperatures. However, the extraction time did not significantly affect the TPC and TFC.

Nevertheless, there was an interaction between extraction temperature and time for antioxidant activities (Table 2). For the DPPH

assay, the antioxidant activity at 80°C for 45 min and 60 min (83.66 mg TE/g dried sample and 86.02 mg TE/g dried sample, respectively) were significantly higher than those of other treatments ($p \leq 0.05$). For the FRAP assay, the antioxidant activity was significantly the highest at 80°C for 60 min (1,005.33 µmol Fe^{2+}/g dried sample).

Extraction temperature had a significant effect on increasing the extraction rate, due to the decrease in the viscosity and surface tension of the solvent, causing the cell walls of the plants to weaken and break apart, so more bioactive compounds could dissolve. However, high temperatures can affect the degradation of some bioactive compounds [24, 25].

Considering the TPC, TFC and antioxidant activities based on the DPPH and FRAP assays, the extraction at 80°C for 60 min was selected as the optimum conditions for the CE method. The TPC, TFC and antioxidant activities (DPPH and FRAP assays) for these conditions were 60.67 mg GAE/g dried sample, 8.74 mg catechin equivalents/g dried sample, 86.02 mg TE/g dried sample and 1,005.33 µmol Fe^{2+}/g dried sample, respectively.

Ultrasound-assisted extraction (UAE)

The study of extraction temperatures (40°C, 50°C or 60°C) and time (10 min, 20 min or 30 min) from *A. elliptica* leaves using the UAE method found that there were no significant interactions between temperature and time on the TPC, TFC and antioxidant activity (DPPH assay) (Table 3). As the extraction temperature increased, the TPC, TFC and DPPH of the extracts increased, with the average amounts of the TPC, TFC and DPPH at 60°C being significantly higher than those at other temperatures. Additionally, as the extraction time increased, the TFC and DPPH increased, with the average amounts of the TFC and DPPH at 30 min being significantly higher than those of other treatments. However, the extraction time did not significantly affect the TPC.

In contrast, there was interaction between temperature and time on the FRAP values (Table 4). For a 30 min extraction time, the FRAP values were significantly

Table 1 TPC and TFC of *A. elliptica* leaves (mean ± SD) extract using conventional extraction at different conditions

Temp (°C)	Chemical qualities (per 1 g dried sample)		Time (min)	Chemical qualities (per 1 g dried sample)	
	TPC (mg GAE)	TFC (mg catechin)		TPC (mg GAE)	TFC (mg catechin)
60	49.12 ± 3.14^{b}	7.45 ± 0.32^{c}	30	52.53 ± 4.10	8.00 ± 0.57
70	51.03 ± 2.37^{b}	8.02 ± 0.44^{b}	45	51.56 ± 5.57	7.83 ± 0.56
80	58.45 ± 2.86^{a}	8.52 ± 0.29^{a}	60	54.50 ± 5.05	8.16 ± 0.58

GAE = gallic acid equivalents.
mean ± SD values with different lowercase superscript letters within each column denote significant ($p \leq 0.05$)

Table 2 Antioxidant activities (DPPH and FRAP assays) of *A. elliptica* leaves (mean ± SD) extract using conventional extraction at different conditions

Antioxidant activity (per 1 g dried sample)	Temp (°C)	Time (min)		
		30	45	60
DPPH (mg TE)	60	74.05 ± 2.69^{d}	75.34 ± 3.07^{cd}	77.31 ± 2.04^{cd}
	70	73.35 ± 1.42^{d}	75.44 ± 0.62^{cd}	80.37 ± 2.32^{bc}
	80	80.41 ± 2.63^{bc}	83.66 ± 2.71^{ab}	86.02 ± 5.11^{a}
FRAP (µmol Fe^{2+})	60	883.82 ± 13.50^{cd}	785.16 ± 46.62^{e}	854.02 ± 39.48^{cd}
	70	988.86 ± 27.69^{ab}	836.07 ± 60.47^{de}	979.38 ± 6.76^{ab}
	80	884.58 ± 31.09^{cd}	923.10 ± 21.94^{bc}	1,005.33 ± 54.67^{a}

TE = trolox equivalents.
mean ± SD values with different lowercase superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

higher than those of the other treatments. In conclusion, a temperature at 60°C for 30 min provided the optimum conditions for extraction using UAE, producing values for the TPC, TFC and antioxidant activities (DPPH and FRAP assays) of 56.05 mg GAE/g dried sample, 7.53 mg catechin equivalents/g dried sample, 100.91 mg TE/g dried sample and 814.72 µmol Fe^{2+}/g dried sample, respectively.

These optimum conditions were similar to those reported by Arteaga-Crespo et al. [26], who examined the optimal conditions for the extraction of phenolic compounds from *Ilex guayusa* leaves using the UAE method. They found that the optimum conditions involved extraction at 60°C for 29.9 min and the ethanol-to-water ratio was 76.8:23.2. These conditions yielded a phenolic compounds of 3.46 g GAE/100 g dry weight.

Chung et al. [27] compared extraction methods for phenolic compounds from soybean; they found that the UAE method yielded 54% more TPC compared to the CE and Soxhlet methods because the use of ultrasound waves increased the solvent-to-sample contact surface and cell wall permeability. When the plant cell walls are damaged by the rupture of air bubbles, the rate of mass transfer is increased [28].

Accelerated solvent extraction (ASE)

Temperature and pressure were important factors in ASE extraction. High temperatures affect the properties of the solvent, by reducing the density and surface tension of the solvent and increasing the solubility and mass transfer rate of the extract, while the use of high pressure during the extraction process causes the solvent to become liquid when the temperature is above the boiling point [29].

The analysis of the influence of temperature (60°C, 70°C or 80°C) and time (5 min, 10 min or 15 min) on the extraction of *A. elliptica* leaves using the ASE method indicated that there was an interaction between temperature and time on the TPC (Table 5). The TPC values at 80°C for 10 min and 15 min (49.33 mg GAE/g dried sample and 49.36 mg GAE/g dried sample, respectively) were not significantly different

Table 3 TPC, TFC and antioxidant activity (DPPH assay) of *A. elliptica* leaves (mean ± SD) extract using ultrasound-assisted extraction at different conditions

Temp (°C)	Chemical qualities (per 1 g dried sample)			Time (min)	Chemical qualities (per 1 g dried sample)		
	TPC (mg GAE)	TFC (mg catechin)	DPPH (mg TE)		TPC (mg GAE)	TFC (mg catechin)	DPPH (mg TE)
40	47.35 ± 1.38^{c}	6.36 ± 0.27^{b}	88.96 ± 3.40^{b}	10	49.49 ± 2.85	6.29 ± 0.48^{b}	90.00 ± 5.13^{b}
50	50.56 ± 2.51^{b}	6.30 ± 0.39^{b}	92.95 ± 5.55^{ab}	20	50.69 ± 2.44	6.47 ± 0.38^{ab}	91.28 ± 3.37^{b}
60	53.60 ± 2.50^{a}	7.03 ± 0.59^{a}	95.64 ± 5.64^{a}	30	51.33 ± 4.51	6.92 ± 0.58^{a}	96.27 ± 6.10^{a}

GAE = gallic acid equivalents; TE = trolox equivalents.
mean ± SD values with different lowercase superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

Table 4 Antioxidant activity (FRAP assay) of *A. elliptica* leaves (mean ± SD) extract using ultrasound-assisted extraction at different conditions

Antioxidant activity (per 1 g dried sample)	Temp (°C)	Time (min)		
		10	20	30
FRAP (µmol Fe^{2+})	40	689.58 ± 34.90^{e}	733.17 ± 12.20^{d}	829.32 ± 5.08^{a}
	50	635.46 ± 14.11^{f}	764.13 ± 25.01^{cd}	821.91 ± 26.45^{a}
	60	660.39 ± 19.37^{ef}	779.30 ± 10.09^{bc}	814.72 ± 26.50^{ab}

mean ± SD values with different lowercase superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

but were significantly higher than those of other treatments.

Nonetheless, there was no significant interactions between temperature and time regarding the average amounts of TFC and antioxidant activities (DPPH and FRAP assays) as shown in Table 6. As the extraction temperature increased; the TFC, DPPH and FRAP values increased. At 80°C, the average amounts of TFC, DPPH and FRAP values were significantly higher than those at other temperatures. Additionally, the extraction time did not significantly affect DPPH, but did
affect the TFC and FRAP as the extraction time increased, the TFC and FRAP tended to increase, but at 10 min and 15 min, the TFC and FRAP were not significantly different.

The optimal conditions for extraction from *A. elliptica* leaves using the ASE method were selected as 80°C for 10 min, due to lower amount of time and energy required. Extraction under these conditions produced values for the TPC, TFC and antioxidant activities (DPPH and FRAP assays) of 49.33 mg GAE/g dried sample, 6.72 mg catechin equivalents/g dried sample, 79.35 mg TE/g dried sample and 644.76 µmol Fe^{2+}/g dried sample, respectively.

These results were similar to those reported by Brachet et al. [30], who investigated the optimal conditions for extracting cocaine and benzoylecgonine from coca leaves using the ASE method. They found that the optimum conditions at 20 MPa pressure were 80°C temperature and 10 min extraction time.

Comparison of extraction methods

The optimal conditions were compared for the extraction of *A. elliptica* leaves using different extraction methods: CE (80°C, 60 min), UAE (60°C, 30 min) and ASE (80°C, 10 min) as shown in Tables 7 and 8, in terms of the TPC, TFC, antioxidant activities (DPPH and FRAP assays), power, energy required (Equation 2) and extraction efficiency (Equation 3–6). The results showed that the extraction methods affected extraction efficiency. Although the TPC, TFC and antioxidant activity (FRAP assay) values

Table 5 TPC of *A. elliptica* leaves (mean ± SD) extract using accelerated solvent extraction at different conditions

Chemical qualities (per 1 g dried sample)	Temp (°C)	Time (min)		
		5	10	15
TPC (mg GAE)	60	39.30 ± 2.47^{c}	37.98 ± 1.47^{c}	38.08 ± 1.63^{c}
	70	36.25 ± 0.75^{c}	43.80 ± 2.80^{b}	45.83 ± 1.71^{b}
	80	45.34 ± 2.26^{b}	49.33 ± 1.44^{a}	49.36 ± 1.83^{a}

GAE = gallic acid equivalents.
mean ± SD values with different lowercase superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

Table 6 TFC and antioxidant activities (DPPH and FRAP assays) of *A. elliptica* leaves (mean ± SD) extract using accelerated solvent extraction at different conditions

Temp (°C)	Chemical qualities (per 1 g dried sample)			Time (min)	Chemical qualities (per 1 g dried sample)		
	TFC (mg catechin)	DPPH (mg TE)	FRAP (µmol Fe^{2+})		TFC (mg catechin)	DPPH (mg TE)	FRAP (µmol Fe^{2+})
60	6.25 ± 0.19^{b}	77.57 ± 2.81^{b}	557.48 ± 39.57^{c}	5	6.15 ± 0.36^{b}	77.95 ± 3.76	553.94 ± 38.50^{b}
70	6.37 ± 0.33^{ab}	74.76 ± 1.60^{c}	590.26 ± 28.40^{b}	10	6.47 ± 0.26^{a}	77.39 ± 3.00	608.35 ± 37.44^{a}
80	6.61 ± 0.46^{a}	80.71 ± 2.19^{a}	637.66 ± 50.06^{a}	15	6.60 ± 0.34^{a}	77.70 ± 3.44	623.09 ± 51.66^{a}

TE = trolox equivalents.
mean ± SD values with different lowercase superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

Table 7 Compare TPC, TFC and antioxidant activities (DPPH and FRAP assays) of extraction methods (mean ± SD)

Method	Power (W/1g)	Energy (kW×hr/1g)	Chemical qualities (per 1 g dry sample)			
			TPC (mg GAE)	TFC (mg catechin)	DPPH (mg TE)	FRAP (μmol Fe^{2+})
CE[1]	2,000	2	60.67 ± 3.20[a]	8.74 ± 0.32[a]	86.02 ± 5.11[b]	1,005.33 ± 54.67[a]
UAE[2]	1,130	0.57	56.05 ± 2.27[a]	7.53 ± 0.67[b]	100.91 ± 5.05[a]	814.72 ± 26.50[b]
ASE[3]	1,000	0.17	49.33 ± 1.44[b]	6.72 ± 0.34[b]	79.35 ± 1.00[b]	644.76 ± 31.66[c]

CE = conventional extraction at 80°C and 60 min; UAE = ultrasound-assisted extraction at 60°C and 30 min; ASE = accelerated solvent extraction at 80°C and 10 min; GAE = gallic acid equivalents; TE = trolox equivalents.
mean ± SD values with different lowercase superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

Table 8 Compare the efficiencies of extraction methods in terms of TPC, TFC and antioxidant activities (DPPH and FRAP assays) (mean ± SD)

Method	Power (W/1g)	Energy (kW×hr/1g)	Extraction efficiencies			
			TPC (mg/kW×hr)	TFC (mg/kW×hr)	DPPH (mg/kW×hr)	FRAP (μmol/kW×hr)
CE[1]	2,000	2	30.33 ± 1.60[c]	4.37 ± 0.16[c]	43.01 ± 2.56[c]	502.67 ± 27.33[c]
UAE[2]	1,130	0.57	99.20 ± 4.03[b]	13.33 ± 1.94[b]	178.60 ± 8.94[b]	1,441.98 ± 46.90[b]
ASE[3]	1,000	0.17	295.96 ± 8.64[a]	40.32 ± 2.03[a]	476.12 ± 6.02[a]	3,868.56 ± 189.99[a]

CE = conventional extraction at 80°C and 60 min; UAE = ultrasound-assisted extraction at 60°C and 30 min; ASE = accelerated solvent extraction at 80°C and 10 min.
mean ± SD values with different lowercase superscript letters within each column denote significant ($p \leq 0.05$) differences between groups.

using CE were considerably higher than those of other methods ($p \leq 0.05$), ASE had the significantly highest extraction efficiencies, being 7–11 times higher than those of CE and 2–3 times higher than those of UAE. This was due to most energy used in ASE being directly applied to the solvent and not lost to the surroundings. Therefore, ASE was considered the most appropriate extraction method of bioactive compounds from leaves of *A. elliptica*.

CONCLUSIONS

Three extraction methods (CE, UAE and ASE) for bioactive compounds in *A. elliptica* leaves were investigated and compared. The result showed that the optimum condition of CE was 80°C, 60 min; UAE was 60°C, 30 min; and ASE was 80°C, 10 min. In conclusion, ASE had the significantly highest extraction efficiencies, which produced values for the TPC of 49.33 mg GAE/g dried sample, the TFC of 6.72 mg catechin/g dried sample and antioxidant activities based on DPPH assay of 79.35 mg TE/g dried sample and a FRAP value of 644.76 μmol Fe^{2+}/g dried sample. The extract from this research could be applied in various fields such as the foods, pharmaceutical, and cosmetic industries.

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Functional Properties of Marigold Flower Extract Using Ultrasound-Assisted Extraction

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ABSTRACT: The objective of this research was to determine the effect of ethanol concentration (60, 80 and 100 % v/v) and extraction time (5, 10 and 15 min) on functional properties (total phenolic content (TPC), total flavonoid content (TFC), total carotenoid content and antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays of marigold flower extract using ultrasound-assisted extraction (at constant temperature of 30 ℃ and sonication amplitude of 50%). The effects of ethanol concentration and extraction time were significant ($p < 0.05$). The results showed that ethanol concentration of 80% (v/v) and extraction time at 5 min revealed the highest values of TPC. The highest TFC was obtained at a condition of ethanol concentration of 100% (v/v) with extraction time 15 min while the condition with ethanol concentration of 60% with extraction time 5 min had the greatest carotenoid content. For antioxidant activity by DPPH and FRAP assay showed the highest condition at ethanol concentration of 80% (v/v) and extraction time at 5 min. The optimized results for TPC, TFC, DPPH and FRAP were; 660.88 ± 49.37 mg gallic acid equivalent (GAE/100g), 415.34 ± 9.78 mg quercetin equivalent (QE/100g dry basis (db)), 609.13 ± 20.04 mg/100gdb, 2474.50 ± 44.77 mM Trolox equivalent (TE)/g db and 5222.11 ± 564.28 mM TE/g db, respectively.

Keywords: *Functional properties, Marigold flower, Ultrasound-assisted extraction.*

INTRODUCTION

Marigold *(Tagetes erecta)* is an ornamental plant (Asteraceae family) that contained about 50 species of annual or perennial herbaceous plant. It contains high amounts of carotenoids, fatty acid esters and diesters of lutein. Marigold flowers, a common source of lutein pigment, contain high amounts of bioactive compounds including phenolic, flavonoid, and carotenoid compounds [7]. Several marigold species have been commercially applied in medicinal field mostly in ailments such as skin complaints, wounds, and burns and menstrual irregularities. Pigments in *Tagetes* species can be used as a coloring agent in the food industry [15]. Ultrasound is a kind of sound wave beyond human hearing which passes through the medium by generating compression and expansion and it has frequencies range between 20 kHz and 100 MHz that require an elastic medium to spread over. Ultrasound-assisted extraction (UAE) is an effective extraction method for valuable bioactive compounds extraction from plant sources because of less usage of synthetic and organic chemicals, reduced operational time, better quality of extract and higher yield within a short time [1]. To the best of our knowledge, there is no information in the literature about the effects of extraction conditions by UAE on Marigold flowers. Therefore, this research aimed to study the effects of extraction conditions (ethanol concentration and time) on the functional properties (total phenolic compound, total

flavonoid content, total carotenoid content and antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays of marigold flower using ultrasound-assisted extraction.

MATERIALS AND METHODS

Marigold flower sample preparation

Marigold *(Tagetes erecta)* flowers were collected from the local market, Bangkok, Thailand. The flowers were rinsed with water to remove dust and dirt before using. After that, the flowers were dried in a hot air oven (Memmert, DO 6062, Germany) at 60℃ for 24 hours until moisture content less than 5%. The dried marigold flower petals were ground and sieved using a 50-mesh sieve, vacuum packed in an aluminum foil-laminated bag and kept in a freezer at -20℃ for further experiments.

Ultrasound-assisted extraction (UAE)

For the extraction of marigold flower, 3 g of sample was weighed and mixed with 100 mL (60%, 80% and 100% of aqueous ethanol v/v) in a 250 mL beaker. Then, UAE was set and operated using an ultrasonic bath (GTSONIC VGT-1990QT) having frequency of 40kHz and containing timer (extraction time; 5, 10 and 15 min) and thermometer (set at 30℃). After extraction, the samples were centrifuged (Kubota, series 6000, Japan) at 10,000 rpm for 10 min at room temperature and then the supernatant were filtered by a No.1 filter paper, and evaporated under vacuum at 45℃ using a rotary evaporator (pressure: 6 bar) (Oilbath B-485, BUCHI, Switzerland). After evaporation, the final sample extract was adjusted to 10 mL with distilled water and stored in the amber glass bottles at 4℃ before further analysis.

Analysis of the functional properties of marigold flower extract

Determination of total phenolic content

The total phenolic content (TPC) of the extract was measured using the Folin–Ciocalteau method. 0.5 mL of the extract (0.5 mL) was pipetted into a test tube and mixed with 10 mL of distilled water. Folin–Ciocalteau's phenol solution (10%, 0.5 mL) was subsequently added, and the solution was then mixed well using a vortex mixer. The solution was incubated at room temperature for 5 min. sodium carbonate solution (10% w/v, 2 mL) was added into the test tube and then mixed the solution well with the vortex mixer. After that the solution was incubated at room temperature for 10 min again. Finally, the absorbance was read at 765 nm with a spectrophotometer (JEOL, JSM-IT300, Japan) using distilled water as a blank and the result was expressed as mg gallic acid equivalent (GAE)/100 g dry basis (db).

Determination of total flavonoid content

Total flavonoid content (TFC) of the extract was determined by using aluminum chloride method with slight modification. 1 mL of sample extract was added into a test tube. 1 mL of 2% aluminum chloride solution (w/v) was pipetted into a test tube and the solution was subsequently mixed well with a vortex mixer. Then, the solution was placed in a dark place at room temperature for 30 min. After that, the absorbance was determined at the wavelength of 430 nm by using distilled water as a blank and the total flavonoid content in the sample was expressed as mg quercetin equivalent (QE)/100 g.

Determination of total carotenoid content

Total carotenoid content (TCC) was done according to the method of Biswas et al 2011 [3] with some modifications. For the extraction, sample extract (1g) was weighed and placed in the glass test tube. 5 mL of acetone was then mixed with the sample and vortexed at high speed for 10 min, and centrifuged at 3339 rpm for 10 min. After that the supernatant was collected and the remained content was extracted again using 5 mL of acetone. Finally, both of the supernatant was collected together and then filtered using Whatman filter paper (No.1). The absorbance was measured at 450 nm and the results were revealed by plotting a

standard curve (mg carotenoid/100g db.) with the following equation: $y = 3.6176x-0.0101$, $R^2 = 0.99$. Where, "y" was the absorbance at 450 nm and "x" was the carotenoid concentration (mg/mL).

Determination of antioxidant activity by DPPH

The antioxidant activity of the extract was determined based on the inhibition of the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical according to the method of Brand-Williams, et al. (1995). 250 µL of sample extract was mixed with 4.75 mL of DPPH daily solution and stored in a dark place at a room temperature for 15 min. The absorbance value of the sample was performed at the wavelength of 515 nm by using methanol as a blank. The absorbance of DPPH daily solution ($A_{initial}$) was approximately 1.1 and the absorbance of sample (A_{final}) was from 0.6 to 0.7.

$$A_{difference} = A_{initial} - A_{final} \quad [5]$$

where $A_{difference}$ is the difference between DPPH and sample, $A_{initial}$ is the absorbance of DPPH and A_{final} is the absorbance of sample. $A_{difference}$ was determined from the above equation and the result was expressed in mM trolox equivalent (TE)/g db.

Determination of antioxidant activity by FRAP assay

The FRAP assay was performed according to Benzie and Strain (1996) with some modifications. 50 µL of sample extract was placed into the test tube and then mixed with 950 µL of FRAP. After that this solution was stored at room temperature for 4 min. The absorbance of sample (A_{final}) was determined at 593 nm by using distilled water as a blank.

$$A_{difference} = A_{final} - A_{initial} \quad [3]$$

where $A_{difference}$ is the difference between FRAP and sample, $A_{initial}$ is the absorbance of FRAP and A_{final} is the absorbance of sample. $A_{difference}$ was calculated in the above equation and the result was expressed in mM TE/g db.

Statistical analysis

The full factorial design was used in this study with three replicates. The significant difference among mean values was analyzed by analysis of variance (ANOVA), followed by Duncan's new multiple range tests (SPSS version 22). Statistical significance was defined at a 95 % confidence level.

RESULTS AND DISCUSSION

Total phenolic compounds

Phenolic compounds are secondary metabolites. Marigold flowers containing phenolic compounds have health benefit for human beings [15].

It was found that different extraction conditions (ethanol concentration and time) affected the TPC ($P \leq 0.05$). The results from the ethanol concentration of 60% with time 5, 10, and 15 min showed that increasing the extraction time resulted in increased TPC from 622.42 ± 46.95 mg GAE/100g to 660.88 ± 49.37 mg GAE/100g. For ethanol concentration of 100%, TPC was increased from 435.60 ± 44.70 mg GAE/100g to 631.21 ± 44.48 mg GAE/100g with increasing extraction time from 5 to 10 min. However, TPC decreased when the time was increased to 15 min. The ethanol concentration relates to degree of polarity. Ethanol is less polar than water. More polarity could increase extraction of TPC. Rostagno et al 2004 [14] reported that the binary extraction using two solvents (e.g., ethanol + distilled water) was much more effective than using only ethanol as the extraction solvent.

Total flavonoid content

Different extraction conditions (ethanol concentration and time) affected the TFC ($P \leq 0.05$). In general, increasing in ethanol concentration from 60% to 100% (v/v) resulted in the significant increase in the TFC. The use of water and ethanol as solvents can increase the extraction efficiency of flavonoids and the solubility of these compounds can be enhanced using the mixture solvent due to the fact that

ethanol is less polar than water. The increase of flavonoids is consistent with the decrease of polarity [16]. In addition, for ethanol concentration of 80% and 100%, increasing extraction time resulted in increased TFC. The interaction of ethanol concentration and time also had significant effect on total flavonoid content ($P \leq 0.05$). (Zerajić, Savić-Gajić et al. 2019) [21] revealed that increase of the extraction time led to the increase of TFC. This is due to the fact that longer extraction time can help to increase the mass transfer and then release bioactive compounds from the plant matrix. The plant cell wall and cell membrane were destroyed by ultrasound which help release of TFC.

Total carotenoid content

Carotenoids are oil-soluble terpenoid that can be found in the chloroplasts and chromoplasts of plants. Marigold flower is one of the common sources of carotenoid with biological activities such as cancer prevention, enhanced immune function, inhibition of auto-oxidation of cellular lipids, prevention of age-related molecular degeneration (Pratheesh et al. 2009) [14]. It was found that co-influences significantly affected the TCC ($P \leq 0.05$). The amount of TCC was in the range between 30.43 ± 20.00 and 609.13 ± 20.04 mg/100g. For extraction time of 5 min, 10 min ad 15 min, and increasing ethanol concentration from 60% to 100% (v/v) resulted in decreasing TCC. In 100% (v/v) ethanol concentration, TCC was increased from 39.56 ± 19.95 to 61.53 ± 20.76 mg/100g with increasing extraction time. These results are in agreement with (Gunathilake, Ranaweera et al. 2019) [8] who reported that increase in carotenoid content from 2.61 to 3.47 mg/100g was found when the extraction period increased from 30 to 90 min. This could be due to the greater disruption to cell walls at a longer extraction time by ultrasound. Therefore, more carotenoids were released from plant tissues.

Antioxidant activity by DPPH assay

DPPH is a stable free radical used in the determination of the antioxidant activity of the substances. DPPH antioxidant analysis is based on the mechanism of the reaction by providing hydrogen to the free radicals [20].

Antioxidant activity by DPPH assay of marigold flower extract had range from 2279.50 ± 49.71 to 2474.50 ± 44.77 mM trolox/g. The results showed that co-influences significantly affect the antioxidant activity by DPPH assay ($P \leq 0.05$). In general, increasing ethanol concentration from 60% to 100% resulting in a decrease in antioxidant activity by DPPH assay. Decreasing polarity resulted in decreasing of antioxidant activity. (Pradal, Vauchel et al. 2016) [13] reported that increase in ethanol content may cause decreasing in antioxidant activity and it was observed that pure ethanol was totally inefficient. Based on these results, it suggested that phenolic compounds found in the extract were moderately polar. (Paixo et al. 2007) [11] reported that DPPH assay for antioxidant activity was more favorable to react with low molecular weight phenolic compounds.

Antioxidant activity by FRAP assay

Increasing ethanol concentration resulted in reducing in antioxidant activity by FRAP method.

In the extraction at 5 min and 15 min, the antioxidant activity by FRAP assay increased with increasing ethanol concentration from 60% to 80% (v/v). However, antioxidant activity was decreased by increasing ethanol concentration to 100% (v/v). This might be due to high phenolic content found in 60% ethanol concentration and then the antioxidant activity contributed to those phenolic compounds (Jitrangsri et al) [9]. These results were in agreement with the results revealed by (d'Alessandro et al. 2012) [6]. Furthermore, increasing extraction period also enhanced the antioxidant content because of the diffusion of bioactive compounds from plant cells to solvents. However, if the extraction time took too long, bioactive compounds would breakdown and then antioxidant activity would be reduced (Gong et al. 2012) [7].

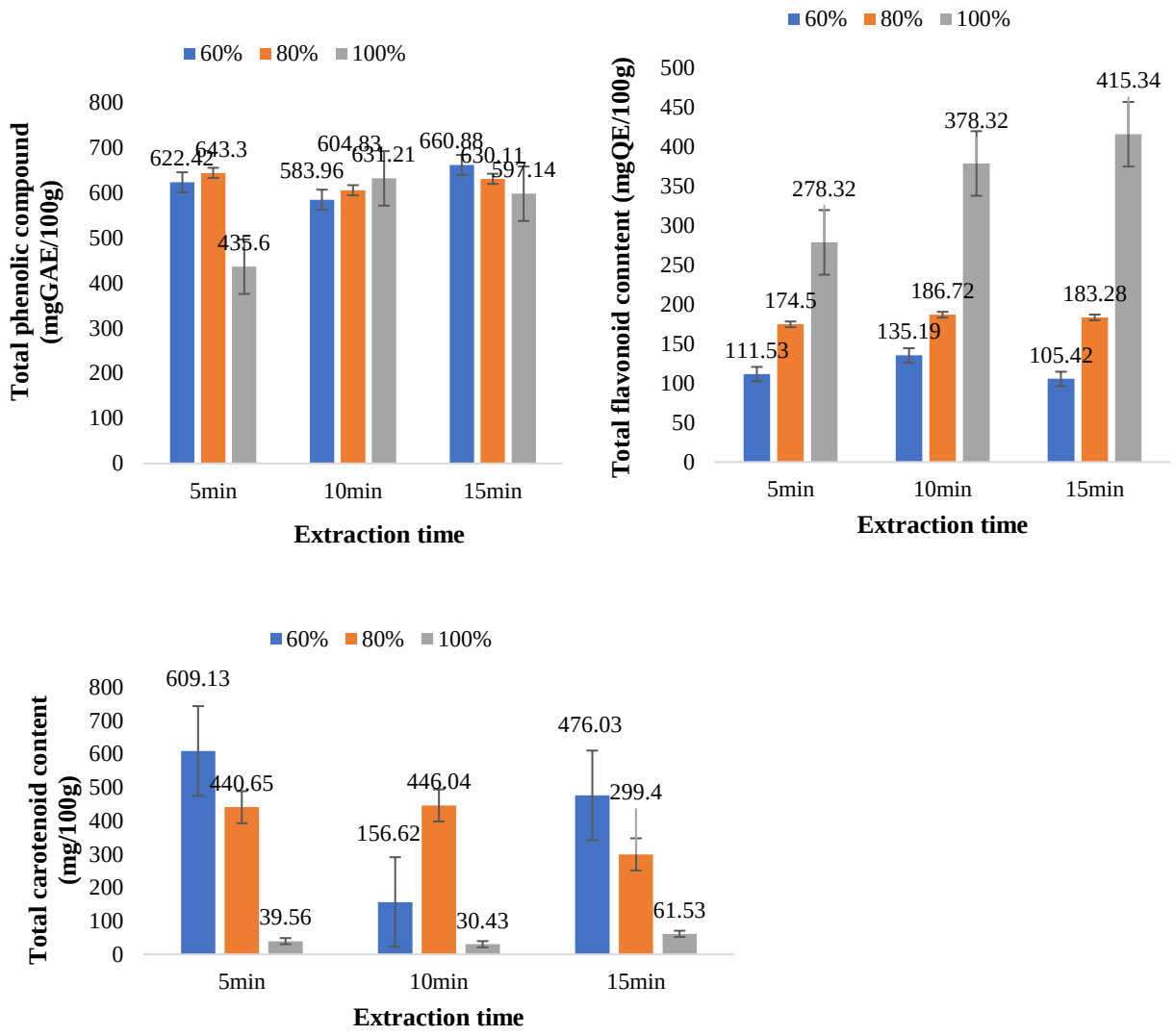

Figure 1. Total phenolic compounds, total flavonoid content and total carotenoid contents of marigold flower extract using UAE.

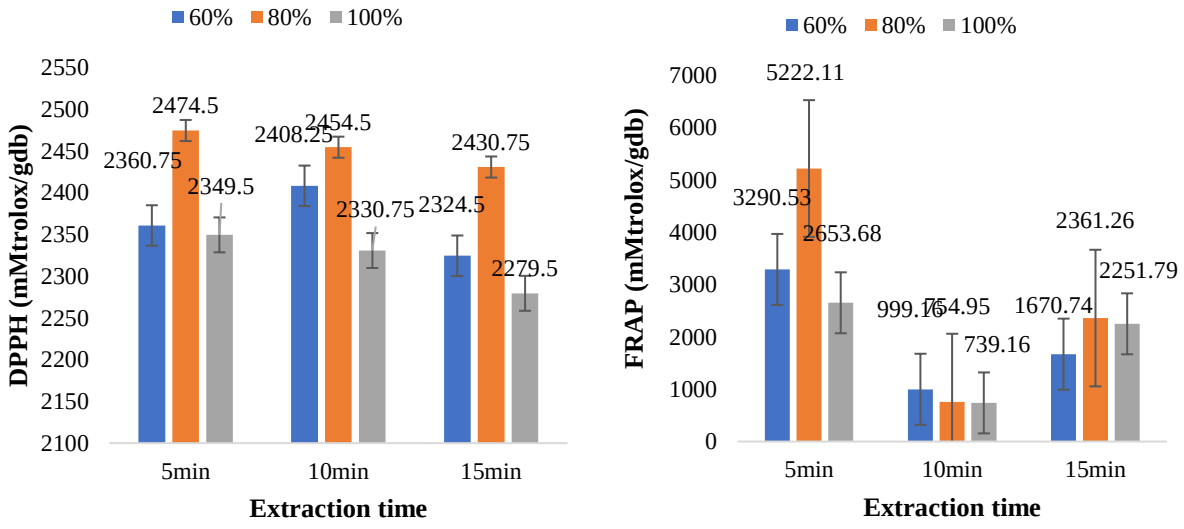

Figure 2. Antioxidant activity by DPPH and FRAP (mM TE/g) of marigold flower extract using UAE.

CONCLUSIONS

The extraction conditions (extraction time and ethanol concentration) significantly affected the amounts of bioactive compounds and antioxidant activity of the marigold flower extract. The extraction conditions of 60% ethanol and 15 min yielded highest total phenolic compounds. The extraction conditions of 100% ethanol and 15 min yielded highest total flavonoid compounds. The extraction conditions of 60 % ethanol and 5 min yielded highest total carotenoid compounds. The extraction conditions of 80 % ethanol and 5 min yielded highest DPPH activity. The extraction conditions of 80 % ethanol and 5 min yielded highest FRAP activity.

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POSTER PRESENTATION

Effects of Pre-Treatment on The Chemical Compositions of Skipjack Tuna (*Katuwonus pelamis)* Bones

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ABSTRACT:

The purpose of this study was to compare the effects of the pre-treatment methods on the chemical composition of Skipjack tuna (*Katuwonus pelamis*) bones. Untreated bones were cleaned by rinsing water. Pre-treated tuna bone was done using a pressure cooker under the condition 121°C with a controlled pressure of 0.15 MPa for 40 minutes. Alkaline-treated bones were prepared by soaking in 0.5%NaOH. All samples were analyzed for the chemical composition and color values. There were no significant differences ($p>0.05$) between the pre-treated and alkaline-treated bones in both protein (26.35% and 18.78%, respectively) and fat content (3.17% and 2.48%, respectively). The pre-treated bones have significantly ($p\leq0.05$) lower protein and fat content than the untreated bones (38.71% and 14.90%, respectively). The pre-treated bones have significantly ($p\leq0.05$) higher calcium (23.44%) and phosphorous (11.94%) contents, compared to untreated bones (15.48% and 8.55%, respectively). The Ca/P ratio of pre-treated bone was 1.51. Pre-treated bone had a significantly higher L* value (lightness) and lower a*(redness) than untreated bones ($p\leq0.05$). The pre-treated tuna bone showed a lower total difference in the color value (ΔE*) (43.19) than the untreated tuna bone (51.27). The alkaline-treated bones have significantly higher L* and lower ΔE* than others ($p\leq0.05$). Using a pressure cooker as pre-treatment has an efficiency in removing organic matters such as protein and lipid in tuna bones, thereby increasing levels of Ca and P. This result shows that alkaline treatment is the better method to improve the color of bones, while the pre-treatment can slightly improve the color of bones.

Keywords: Skipjack tuna (*Katuwonus pelamis) bone, Pre-treatment, Alkaline treatment, Pressure cooker, Chemical composition*

1. INTRODUCTION

Skipjack tuna (*Katuwonus pelamis*) is the most widely used species for producing canned tuna [1]. The canned tuna industry generates much waste from the processing of production, such as the waste of water (including tuna steamed water) and waste/residue from the tuna gutting and trimming process, such as red meat, blood, viscera, and tuna bone [2]. Tuna bone is one of the by-products that have high quantity. The remaining fishbone is considered a waste of about 10-15% of total body weight [3]. Fishbone is known to be a good source of calcium. Tuna bones are mainly composed of inorganic constituents, most of which are calcium and phosphorus in amounts up to 95% in the form of hydroxyapatite. Hydroxyapatite has the molecular formula of $Ca_{10}(PO_4)_6(OH)_2$ with the mole ratio of calcium/phosphorus being 1.67 [4]. Hydroxyapatite is a bioactive ceramic with a chemical composition similar to human bone. Fish calcium was shown to be easily absorbed into the human body [5].

There are four major steps to extract calcium from tuna bones. [1][6]. The processing to prepare hydroxyapatite includes subcritical water and alkaline hydrothermal hydrolysis [7]. The entire preparation process involves the extraction of organic matter from animal bones. First, pre-treatment is the procedure for preparing tuna bones. Fishbone is high in calcium, but it's hard and sharp. At this step, the tuna bones are prepared to be tender for easy grinding and extracting. Next, removing impurity, for the extraction of calcium to obtain pure calcium, is a procedure for removing organic matters in tuna bones such as proteins and lipids. At this step, there is much research that uses different extraction methods. In general, the method for obtaining the shortest time and maximum calcium extraction is the use of alkali treatment [1], sodium hydroxide (NaOH) was used for the extraction. Alkaline treatment has some disadvantages. The cost of waste treatment is high because the use of chemical content is quite high for this treatment. Then drying is the process of decreasing moisture in tuna bones, until the moisture content is less than 5%. Finally, milling is the final step in the process of extracting calcium, ground the tuna bones to fine particle size. It is generally ground to a size of 100-60 mesh (149-250 microns).

In a research study on the extraction of calcium from fish bones, there are different ways to prepare tuna bones, such as cutting the center of the bone longitudinally. Clean with water pressure of 120 bar, a flow rate of 360 l/h for 1-2 min [1]. Tuna bones were boiled for 2-3 minutes [6]. A research study to softening process of eel bones by using 5% acetic acid with retort at 115˚C with a controlled 0.15 MPa for 30 minutes. They found that retort had a softening effect on the fish bones and reduced protein and fat content [8].

However, there are some publication addressed the pre-treatment of tuna bone using a pressure cooker on the chemical compositions. There are studies about preparing the tuna bone powder by pre-treatment using high-pressure water or boiling water and addressed the only effect of alkaline treatment [1][6]. In this study, the objective was to present a new pre-treatment tuna bone using the pressure cooker for eliminating organic matters and to obtain the high ash content of tuna bones as it is the criterion for further calcium extraction.

2. MATERIAL AND METHODS

2.1 Preparation of bones from skipjack tuna (*Katuwonus pelamis*)

Bones with the flesh of skipjack tuna (*Katuwonus pelamis*) were utilized in this study and were kindly provided from a tuna canning factory. The bones, kept in polyethylene bags, were transported to the Department of Food Technology, Chulalongkorn University, Bangkok within 1 hr. and 30 min. They were stored at -18°C in the laboratory freezer before use.

For this study, around 3kg of tuna bones with flesh were used. First, the tuna bones were defrosted the frozen. Then, the residues flesh remained on the tuna bones were removed by rinsing them with water. Finally, the cleaned tuna bones were kept in a polyethylene bag and kept at -18°C until use.

2.2 Pre-treatment of tuna bones

Pre-treating, using a pressure cooker (Model Bigboy 18L, VIP Kitchen Hawkins Thailand, Bangkok, Thailand), the cleaned tuna bones (1000g) were boiled with a bone/water ratio of 1:4 (wt/vol) at 121°C with a controlled pressure of 0.15 MPa for 40 minutes and then cooled. While the water was boiling, look at the temperature gauge until it reaches 121°C (approximately 20 minutes). According to the method by [8]. with some modifications. The pre-treated bones were rinsed with running water.

2.3 Alkaline treatment of tuna bones

The cleaned tuna bones were immersed in 0.5% NaOH with a bone/solution ratio of 1:10 (w/v) at 95 ˚C up to 60 min. According to the method with some modifications [1]. Continuous shaking in water bath shaker at a speed of 120 rpm. Then, The alkali-treated bones were rinsed with running water until a neutral or slightly alkaline pH in the wash water was obtained.

2.4 Chemical analysis

2.4.1 Proximate analysis

Moisture, protein, fat, and ash contents were determined by following the methods of the Association of Official Analytical Chemists [9], analytical Nos. 950.46(B), 981.10, 948.15, and 920.153, respectively.

2.4.2 Determination of Calcium (Ca) and Phosphorous (P) Contents

Inductively coupled plasma optical emission spectrometer (ICP-OES) was used for the determination of Ca and P contents per the methods of Association of ISO 6869:2000 and Official Analytical Chemists [9], analytical No. 985.01, respectively.

2.5 Color analysis

Color determination of tuna bones was measured using Konica Minolta CR-400. The CR-400 was calibrated to the white calibration plate before use and recalibrated every hour during the experiment.

Obtained values were averaged and expressed in terms of CIE L* (lightness), a* (redness/greenness), and b*(yellowness /blueness). From measured L*, a*, and b* values and the total color difference (ΔE*) were calculated using the following formulas:

$$\Delta E^* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 (\Delta b*)^2}$$

where ΔL*, Δa*, and Δb* are the differences between the color parameters of the sample and the white standard (L* = 93.63, a* = -0.94, and b* = 0.40).

2.6 Statistical Analysis

The results from three replications were subjected to analysis of variance (ANOVA) and Duncan's multiple range test for significant differences at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Chemical composition of untreated, pre-treated, and Alkaline treated skipjack tuna bones.

The chemical compositions of skipjack tuna bones, the untreated tuna bones with flesh from the tuna canning factory, are shown in Table 1.

Untreated bones contained 53.64% moisture, 38.71% protein, 14.90% lipid, and 40.15% ash. The untreated bones had high protein and fat content, which closed to the reported in [10] that the skipjack tuna bones contained 56.4% moisture, 40.14% protein, 28.67%

Table 1 Chemical composition of untreated, pre-treated and alkaline treated tuna bones

Proximate compositions	Untreated bones	Pre-treated bones	Alkaline treated bones
Moisture (%)	53.64 ± 3.77[b]	37.79 ± 1.72[a]	44.75 ± 0.35[a]
Protein (%)	38.71 ± 5.20[b]	26.35 ± 0.4[a]	18.78 ± 4.80[a]
Fat (%)	14.90 ± 0.95[b]	3.17 ± 0.38[a]	2.76 ± 1.45[a]
Ash (%)	40.15 ± 4.62[a]	64.93 ± 0.03[b]	72.65 ± 0.81[c]
Calcium (%)	15.48 ± 2.67[a]	23.44 ± 1.62[b]	26.51 ± 0.73[b]
Phosphorus (%)	8.55 ± 0.72[a]	11.94 ± 0.33[b]	13.43 ± 0.80[b]
Ca/P ratio	1.40	1.51	1.53

Values are presented as mean ±SD (n=2). Different uppercase letters in the same row indicate a significant difference ($p \leq 0.05$). * Dry weight basis.

lipid, and 26.15% ash. The pre-treated bones using a pressure cooker and the alkaline treated bones had moisture contents of 37.79% and 44.75%,
respectively. There were no significant differences ($p > 0.05$) between the pre-treated and alkaline-treated bones in both protein (26.35% and 18.78%, respectively) and fat content (3.17% and 2.48%, respectively). The pre-treated bones had significantly lower protein and fat content than the untreated bones (38.71% and 14.90%, respectively)($p \leq 0.05$). The initial protein content of untreated tuna bones was reduced by 31.93% by the pre-treatment method. The high temperature and pressure that are using the pressure cooker results in the removal of proteins and fats. Some proteins are denatured and dissolve in water. To extract calcium from tuna bones, the high protein and fat content in the initial skipjack tuna bones must be reduced, to increase the ash content [6]. According to [11], the effect of high temperature and time affects the fish bones increasing the solubility of organic matters such as water-soluble proteins and fats. As a result, the pre-treatment by using a pressure cooker has an efficiency to reduce protein and fat content similar to the alkaline treatment method. Using a pressure cooker as pre-treatment has an efficient to remove organic constitutes such as protein and lipid in tuna bones. At high-pressure conditions, the pressure on the fish bone's structure has increased. As a result, fish bones become brittle faster in a shorter time, which affects some of the soluble proteins and fats that will dissolve and come out through the cracks and pores of tuna bones [12]. The pressure cooker made fish bones brittle faster compared to boiling in water.

The ash contents of the alkaline-treated bone (72.65%) were significantly higher than pre-treated bone (64.93%) ($p \leq 0.05$). The pre-treated bones have significantly higher ash content than the untreated bones (40.15% and 64.93%, respectively)($p \leq 0.05$). The results of a preliminary study showed that the amount of the ash as a major criterion in pre-treated bones and alkaline treated bones increased and the amount of the protein decreased, non-collagenous proteins and lipids were removed during this pre-treatment method, using the pressure cooker. They suggested that the pre-treatment by pressure cooker was an effective way to get rid of organic matters such as protein and fat and get the high of ash.

The results shown in Table 1 represented that calcium (Ca) and phosphorus (P) were 15.48% and 8.55%,

respectively. Both are the most important mineral of the untreated tuna bones. There were no differences between the pre-treated and alkaline treated bones in both Ca (23.44% and 26.51%, respectively) and P content (11.94% and 13.43%, respectively) ($p \leq 0.05$). Pre-treated bones and alkaline-treated bones resulted in significantly higher Ca and P content compared to untreated bone ($p \leq 0.05$). The pre-treatment by using a pressure cooker has a similar result to obtaining high Ca and P to the alkaline treatment. It's possible that protein and fat were eliminated using a pressure cooker as well as alkaline treatment. The cleaned tuna bones by water pressure [14] found the Ca and P increased by 50% and 37.03%, respectively. While The Ca and P in the pre-treated bones in this study were increased by 51.42% and 39.65%, respectively. As a result, the pre-treatment using a pressure cooker can increase the Ca and P. Extraction of calcium from tuna bones, the higher ash content in the pre-treated tuna bones coincided with higher contents of Ca and P [13]. Removing of impurity method in tuna bones is an important step to clean and remove non-collagenous proteins to obtain high Ca and P. According to Table 1, the measurement of the molar ratio of calcium to phosphorus (Ca/P) from pre-treated bone and alkaline treated bones were 1.51 and 1.54, respectively. The ratio of Ca:P should be close to 1.67 which is similar to the human bones [15]. According to [4], The Ca/P molar ratios in bones of 15 species of marine resources were in the range of 1.20–1.63. The chemical formula of hydroxyapatite is $Ca_{10}(P04)_6(OH)_2$ if the crystal structure is complete, the molar ratio of Ca/P comes to 1.67. Tricalcium phosphate was 1.50. The ratio should theoretically come to 1.50-1.67 for the mixture of hydroxyapatite and tricalcium phosphate. Therefore, the pretreated bones from this study may be a mixture of hydroxyapatite and tricalcium phosphate.

The residual organic in the untreated tuna bones causes rancidity, so pre-treatment is escential. The step will affect the shelf-life of calcium powder and consumer acceptance. As observed, pre-treated bones were softer than untreated bones. Softening the bones with a pressure cooker will make it easier for calcium extraction processes. According to [8], temperature and time affect the structure of fish bones as higher temperatures and times cause the bones to form many microscopic holes inside or bone fractures, causing the bones to be more tender. Collagen is a connective tissue that gives toughness and strength to bones when heated it turns into gelatin, the result is that bone hardness is reduced [12].

3.2 Color

The Color of untreated, pre-treated, and alkaline treated skipjack tuna bones expressed as L*, a*, and b* values are given in Table 2. The pre-treated bones have significantly higher L* (lightness) than the untreated bones ($p \leq 0.05$). However, compared with alkaline-treated bones, the pre-treated bones have significantly lower L* ($p \leq 0.05$). After pre-treatment using the pressure cooker and rinsed with running water, the pre-treated bone had significantly a lower a* (redness) than the untreated bones ($p \leq 0.05$). The pre-treated tuna bone showed a significantly ($p \leq 0.05$) lower total difference in the color value (ΔE*) (43.19) than the untreated tuna bone (51.27). The alkaline-treated bones have significantly lower ΔE* than the others ($p \leq 0.05$). It means this method can produce a better color of tuna bone powder, which is close to white standard. This result shows that alkaline treatment is the better method to improve the color of bones, while the pre-treatment can slightly improve the color. There is residual organic constituted in the untreated tuna bones, which causes it to have a dark color. The color of tuna bone from pre-treatment impacts the tuna bone powder. However, this is not the last step of the extraction of calcium from tuna bone. Other steps, such as removing impurity, or bleaching, can improve more lightness of tuna

bone. Bleaching or chemical treatment can affect the lower yield of bones powder. The chemical organic solvents are toxic solvents that will be harmful and leave toxic residue in the bones [16]. There are still several steps such as

removing impurity by NaOH or enzymatic hydrolysis, drying, and milling, which will increase the calcium and phosphorus in the final product.

Table 2 Color values of untreated, pre-treated and alkaline treated tuna bones

Color values	Untreated bones	Pre-treated bones	Alkaline treated bones
L*	49.86 ± 1.97^{a}	51.05 ± 1.17^{b}	60.0 ± 5.00^{c}
a*	4.15 ± 2.15^{b}	-3.74 ± 5.79^{a}	3.06 ± 0.62^{b}
b*	7.37 ± 4.42^{ns}	4.97 ± 0.99^{ns}	2.34 ± 0.88^{ns}
ΔE*	51.27 ± 1.97^{c}	43.19 ± 0.46^{b}	28.63 ± 0.4^{a}

Values are presented as mean ±SD (n=2). Means with (ns) superscript are not significantly different ($p>0.05$). Different uppercase letters in the same row indicate a significant difference ($p\leq0.05$).

4. CONCLUSIONS

Pre-treatment, by using a pressure cooker under the condition at 121°C with a controlled pressure of 0.15 MPa for 40 minutes, have an efficiency to remove soluble protein and lipid in tuna bones. This method can slightly improve the color of the bone. The pre-treatment method is important to reduce organic constitutes and increase calcium and phosphorus content. Moreover, the pressure cooker can help improve more tender the bone for extracting
calcium efficiently, at high temperature and pressure with no more extended time. This research suggested that using a pressure cooker can be use as a pre-treatment method of tuna bones to improve the removal of impurity method for calcium extraction from tuna bones.

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Effect of storage temperature on the change of ice crystal size in ice cream for long-term storage

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ABSTRACT:
Since commercial ice creams available in the market are too complicated to be handled in a scientific study, they have been investigated very rarely. The current study aimed to explore ice crystal formation in two types of vanilla ice cream available commercially. Kerosene was selected as a solvent to dissolve the overlapping ice crystals, without melting them, in order to observe them using an optical microscope at -26 °C. From the microscopic image for the samples, the two kinds of commercial ice cream were stored at -26 °C, -23 °C, -18 °C, and -12 °C for up to two years, and average size of the ice crystals was determined for a specific preservation period. Rate of recrystallization was analyzed by equations based on the Ostwald ripening principle; however, they were found insufficient. The Lifshitz-Slyozov-Wagner theory, which is generally used to understand ice crystal growth rate in frozen food, was also not found suitable. An alternative analytical approach using power law function was found to be suitable for analyzing the long-term process of ice crystal growth in commercial ice cream. We proposed in this study that the conventional explanation of growth rate of ice crystals in ice cream was insufficient.

Keywords: *Ice cream, Long term storage, Ostwald ripening, Recrystallization rate, Power law*

INTRODUCTION

The size of ice crystals in ice cream significantly influences its quality, especially during storage. The phenomenon of growth of ice crystals over time is called recrystallization, and it reduces commodity value of the ice cream. To control and predict this change, a lot of research on recrystallization during storage and transportation has been conducted till date [1)]. Most of the studies have used a model system of ice cream with different compositions to understand the effect of components in recrystallization.

Ben-Yoseph and Hartel[2)] had reported good agreement between experimental and simulation results for ice crystal growth in model ice cream that was prepared by a pilot plant at the University of Wisconsin. The study, however, had limitation in predicting the recrystallization of real ice cream, since the experimental data ranged over a short storage duration of only 4 weeks.

Since commercial ice cream is labeled as super-premium, premium, standard, or economy, and

categorized into various types as in the USA[3], more factors, such as the effect of minor components like flavor or ingredients, might influence ice crystal growth. Therefore, both ice crystal structure and size distribution are complicated.

Using ice cream purchased from several local grocery stores, MacDonald had studied the correspondence of ice crystal size with sensory evaluation[4]. However, since he used microscope images from low temperature scanning electron microscopy to measure the ice crystals size, it was difficult to distinguish most of the air bubbles and other ingredients from ice crystals. As an established experimental technique, studies on ice crystals in ice cream, in general, are conducted under sub-zero temperatures, with the optical microscope set in a glove box. Before the observation, the ice cream was diluted with a suitable organic solvent on the glass slide kept at sub-zero temperature to dissolve any overlapping ice crystal and to eliminate bubbles from the sample. Although Donhowe et al.[5], had reported that ice crystals can be observed clearly in a kind of model ice cream prepared originally, there was no report in actual commercial ice cream.

In a previous study, ethyl acetate, kerosene, or 1-pentanol had been used as a solute to dissolve overlapping ice crystals[6-7]. While some researchers recommend the use of ethyl acetate for clear imaging[8], there are opposite views as well[9]. Therefore, the best method is yet to be decided. Since commercial products have more complicated composition, as described above, than a model system of ice cream, the suitable solvent may differ in the former from that in the latter. In this study, two different types of commercial ice cream were used for comparison; they were classified according to the milk fat content as per Japan's classification. In this study, first, we searched for an appropriate condition of observing ice crystals in commercial ice cream using an optical microscope; we compared three kinds of solvents and three ambient temperatures at which the ice cream was dissolved in solvent on the slide glass. After determining the kind of solute and temperature optimal for observing the size of ice crystals in the sample most clearly, change in ice crystal size was investigated over time, i.e., 2 years, which corresponded to the preservation term in practical level. For analyzing the growth of the obtained ice crystal, not only the formula based on Ostwald ripening was attempted but also alternative general methods were tried.

MATERIAL AND METHODS

1. Samples

Two types of vanilla ice cream, A and B, were used in the study; ice cream is classified in Japan according to the amount of milk fat[10]. The samples were purchased at the local market. Sample A had high price and included more than 15% milk fat, whereas sample B was cheaper with at least 3% milk fat. Both samples were stored in deep freezers (SC-DF25, Twinbird Industry Co., Ltd., Niigata, Japan) at different temperatures (-12 °C, -18 °C, -23 °C, and -26 °C) and for different durations (3 days, one week, two weeks, one month, two months, three months, five months, eight months, one year, and two years). Temperature inside the deep freezer was approximately the specified temperature ± 1 °C. Temperature fluctuation of the sample during the storage period was minimal.

2. Microscopy of ice crystals

An optical microscope (NECROS D21-4166-10, NaRiKa Co., Ltd., Tokyo, Japan) was set up in the freezer (RIO-100SS2, DAIREI Co., Ltd., Tokyo, Japan), wherein the temperature could be controlled. Thereafter, the sample was prepared and ice crystal size observed at sub-zero temperatures (Figure 1). A heater was set on the microscope stage to control the ambient temperature of the sample at -12 °C, -18 °C, and -26 °C within the fluctuation of ± 1 °C (Figure 2). A small amount of ice cream was taken from the central part of its cup and placed on a cooled object slide. Ethyl acetate (FUJIFILM Wako Pure Chemical Co., Ltd., Osaka, Japan),

kerosene (FUJIFILM Wako Pure Chemical Co., Ltd., Osaka, Japan), or 1-pentanol (FUJIFILM Wako Pure Chemical Co., Ltd., Osaka, Japan), was added dropwise to the sample, a cover glass placed on it, and it was then spread with a cotton swab. After determining the solvent and temperature most suitable for observing ice crystals in ice cream, the two types of vanilla ice cream (samples A and B) were stored for a predetermined period at each temperature.

3. Measurement of ice crystal size

Two images were considered for each observation condition to determine the ice crystal size. In an image, ice crystal was selected using a pen tablet (CTL-672, Wacom Co., Ltd., Saitama, Japan) and WinROOF 2018 image analysis software (MITANI Co., Ltd., Fukui, Japan) was used to calculate the ice crystal area accurately. Figure 3 shows the processed image of an ice crystal. In this study, the equivalent diameter (diameter of a circle having an area equal to the size of ice crystals) was used for evaluation.

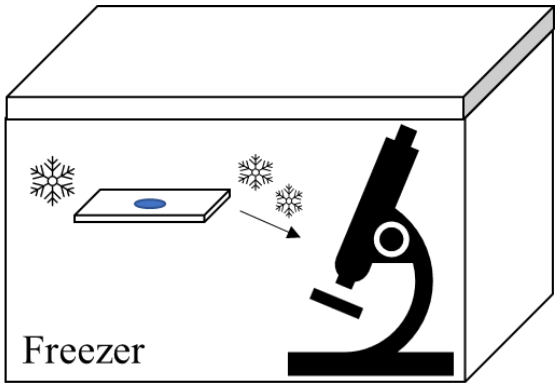

Figure 1 Schematic of observation of ice crystal by an optical microscope in a freezer

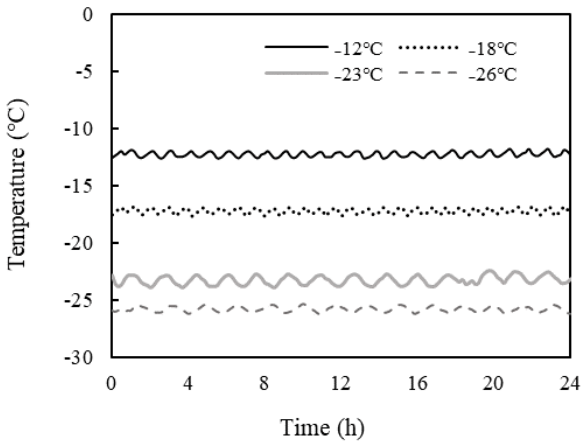

Figure 2 Temperature profiles of ice cream during storage

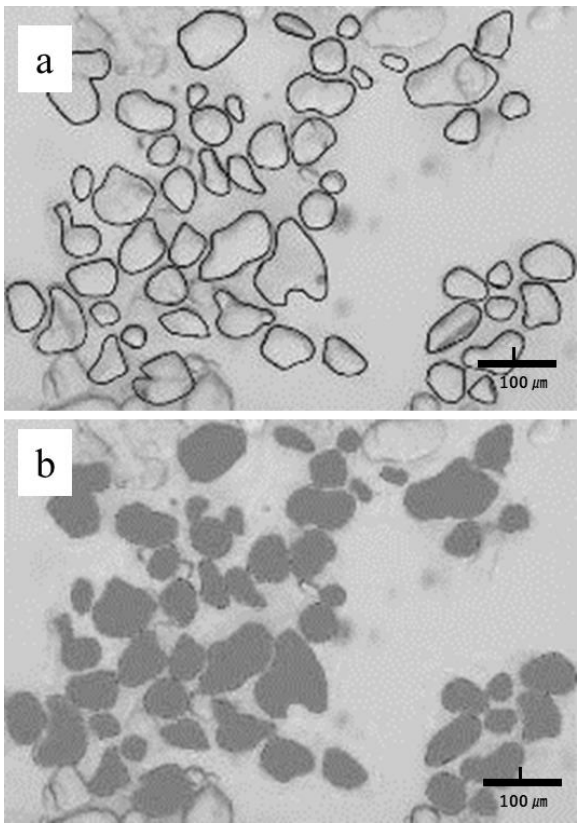

Figure 3 Microscopic images of ice crystal (a: selection of ice crystals, b: area of ice crystals)

RESULTS AND DISCUSSION

1. Observation of ice crystal with different solvents at different temperatures

Figure 4 shows the ice crystals of Sample A, observed using three kinds of solvent with the temperature controlled at -12 °C, -18 °C, and -26 °C. In any of the solvents, some ice crystals melted and mixed with the solvent at -18 °C or higher, and the number of ice crystals observed tended to decrease. On the other hand, many air bubbles were recognized in ethyl acetate even at -26 °C, and the contrast between the ice crystal part and other components was ambiguous. The observed air bubbles decreased when 1-pentanol or kerosene was used, and kerosene made the ice crystal boundaries clearer and easier to observe. Ethyl acetate has 2 times higher solubility in water than the other two solvents; it easily dissolves in the non-freezing part, i.e., freeze-concentrated phase, of ice cream. In an aqueous solution with organic solvent, the surface tension decreases as the organic solvent concentration increases[11]. Therefore, ethyl acetate was considered to be soluble easily into an unfrozen concentrated phase around the ice crystals such that the surface tension became higher with large air bubbles remaining, as indicated with arrows in Figure 4. As a result, the appearance of large air bubbles while using ethyl acetate as solute made it difficult to determine the ice crystal size. Since 1-pentanol and kerosene show lower solubility in water, it can be useful for dissolving air bubbles. However, between 1-pentanol and kerosene, the better one could not be decided based on the physicochemical properties. From the results in Figure 4, kerosene appeared to be effective as a solvent that allows the dissolution of ice crystals and air bubbles at -26 °C. Therefore, kerosene was used as the solvent throughout this study.

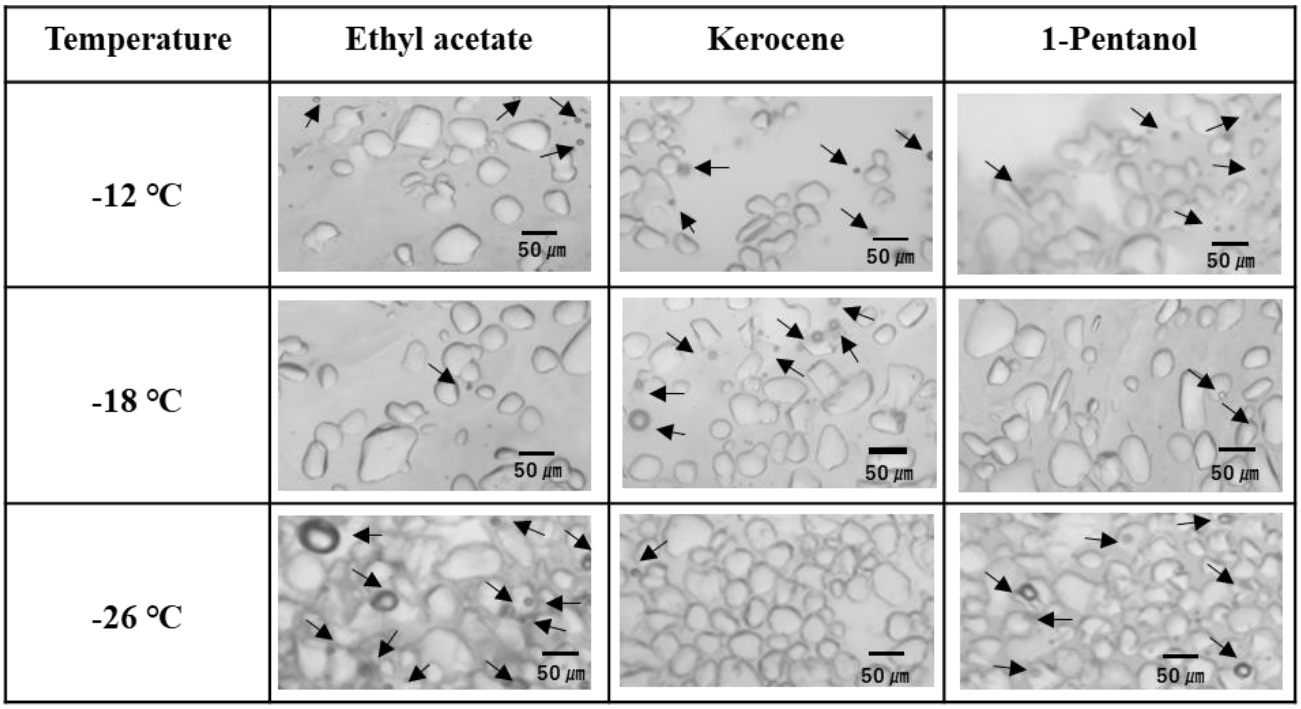

Figure 4 Microscopic image of ice crystals in ice cream using different solvents at different temperatures (arrows indicate air bubbles)

2. Ice crystal size changes in ice cream during long-term storage at different temperatures

Figure 5 shows the change in ice crystal size depending on the storage period of each sample, the maximum period being 2 years. Overall, only when the storage temperature was set at -12 °C, the ice crystal size in both ice creams, A and B, increased dramatically, up to 10 times, even after one year of storage.

When stored at -18 °C, however, the ice crystal size for sample A increased clearly, up to 6 to 7 times, after 2 years. In contrast, for sample B, the growth was only within three times. The increase in ice crystal size below -23 °C was too small to be recognized, if at all, in the normal plot shown in Figure 5.

Although the enlarged views of short-term growth are shown in Figure 5, there was no difference across the data below -18 °C, (irrespective of the temperatures or the kind of sample). Compared to previous studies, till date, the storage period in this study was longer, being over 1 year. Change in ice crystal size in real ice creams over such a long storage has been reported for the first time here, providing valuable information.

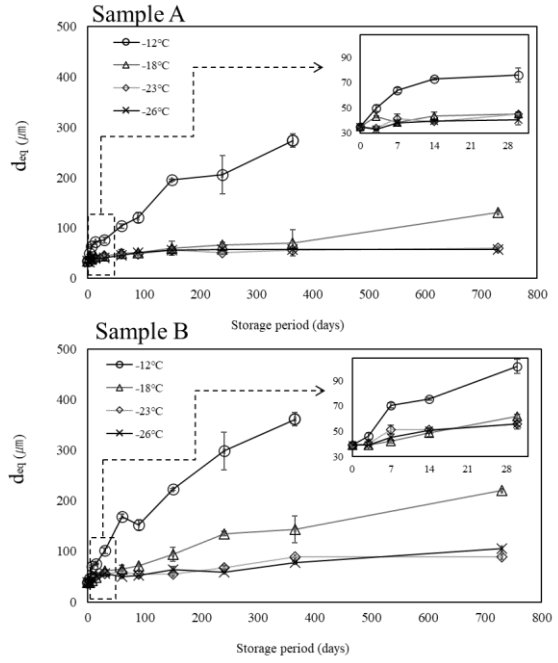

Figure 5 Changes of the ice crystal size in two types of ice cream stored at various storage temperatures

Herein, in order to discuss the rate of recrystallization, i.e. growth of ice crystals in ice cream, from the obtained data, at first, conventional analytical approaches were attempted. Conventionally, some equations derived from the Ostwald ripening principle[12] have been used to quantify the ice crystal size in frozen food. For the analysis of ice crystal size in protein-rich food materials, such as meat, equation 1 derived by Zaritky et al.[13], was considered to describe the increase in ice crystal size, the latter being proportional to the square of time[14]. For ice crystal growth in frozen food, such as a model of ice cream consisting of sugar solution, equation 2 proportional to the cube of time, according to the Lifshitz-Slyozov-Wagner (LSW) theory[15], was used[16-17].

$$d^2 = c + k\,t. \qquad (1)$$

$$d^3 = k\,t\,. \qquad (2)$$

Hartel et al.[18] had suggested equation 3 as the general formula, including both the above equations.

$$d = R\,t^{\frac{1}{n}}\,. \quad (3)$$

Therefore, at first, according to equation 2, we tried to analyze the change in ice crystal size for all storage temperatures over a period of 1 year. Cube of ice crystal diameter and storage time were plotted as shown in Figure 6. Although the plot, overall, seemed to be a straight line, there remained ambiguity and large deviation. In the enlarged figures for small ranges of vertical axis, the plots at each storage temperature seemed to deviate from the straight lines that corresponded to equation 2, especially for sample A, although not so obvious for sample B.

This indicated that the mathematical formula used for expressing the increase in ice crystal size was inappropriate. Therefore, the order of ice crystal diameter in the equation could not be a cube. Based on equation 3, the relationship

between crystal size and time was plotted in a log-logarithm to determine the order n.

$$\log(r - r_0) = \log R + \frac{1}{n} \log t \,. \quad (4)$$

According to the following equation 4, obtained by rearranging equation 3, the relationship between crystal size and time was plotted as a log-logarithm to determine the order n. Results are shown in Figure 7. Ice crystal size at each temperature during the storage period increased linearly in both logarithms without large deviation. Moreover, the slope of linear-fitted lines varied, depending on the storage temperature and kind of the sample. The order n of ice crystal diameter in equation 3 changed not only with the composition of sample but also with the temperature.

Table 1 summarizes the values derived from the logarithmic plots of each sample. Samples A and B at -12 °C were relatively close to the 1/3rd power, but moved far from the 1/3rd power when the temperature decreased. In other words, n changed significantly depending on the storage temperature, but log k had a slight fluctuation. Comparing samples A and B, a significant difference was found in the value of n at -18 °C. However, the interpretation for this interesting finding is still under consideration. In brief, recrystallization of ice particles in ice cream should be expressed by the general power law, as in equation 3, rather than the Lifshitz-Slyozov-Wagner equation (equation 2) with 3rd power or Zaritky et al.[13] equation (equation 1) with 2nd. At this stage, the power n is available to characterize the recrystallization phenomenon depending on the kind of ice cream and the storage temperature.

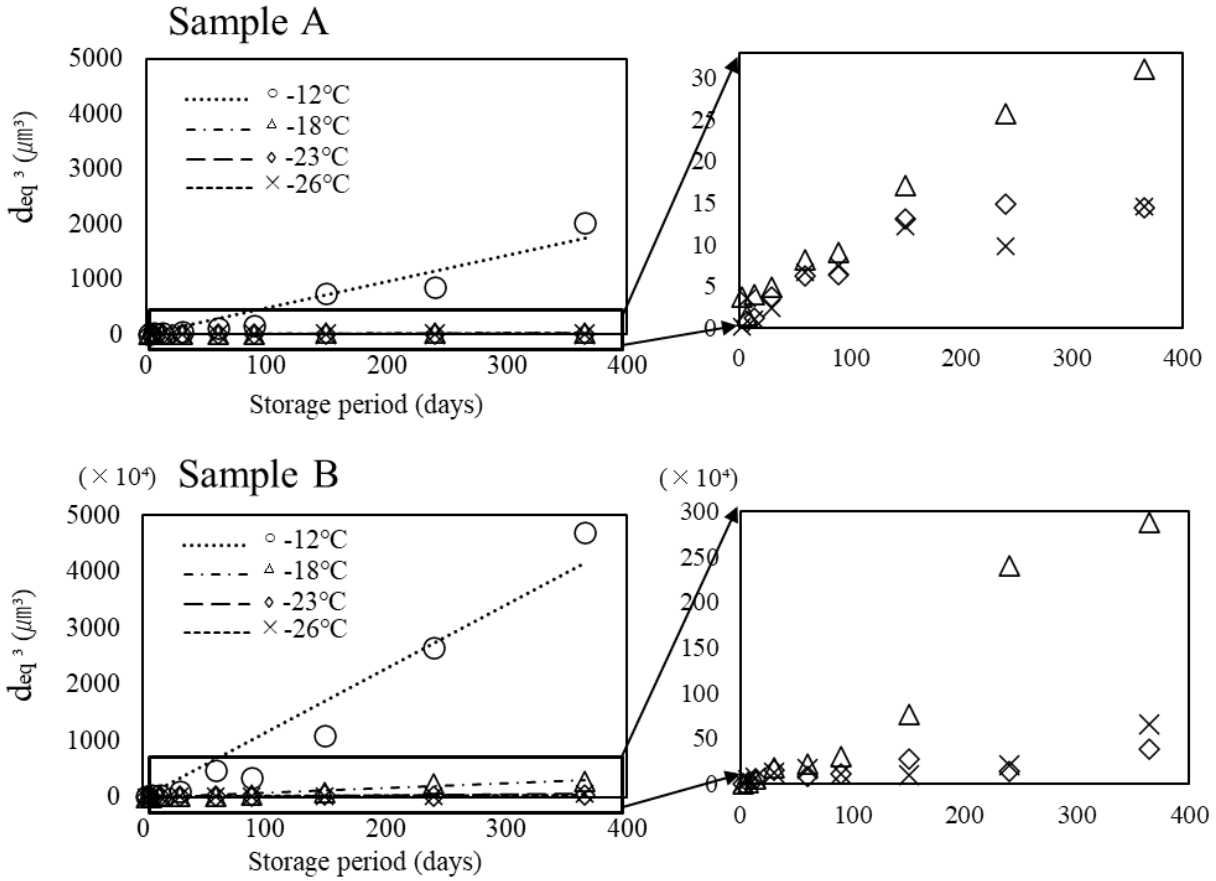

Figure 6 Relationship between the cube of ice crystal diameter and the storage period. Plots show the experimental results, the lines being calculated from LSW theory

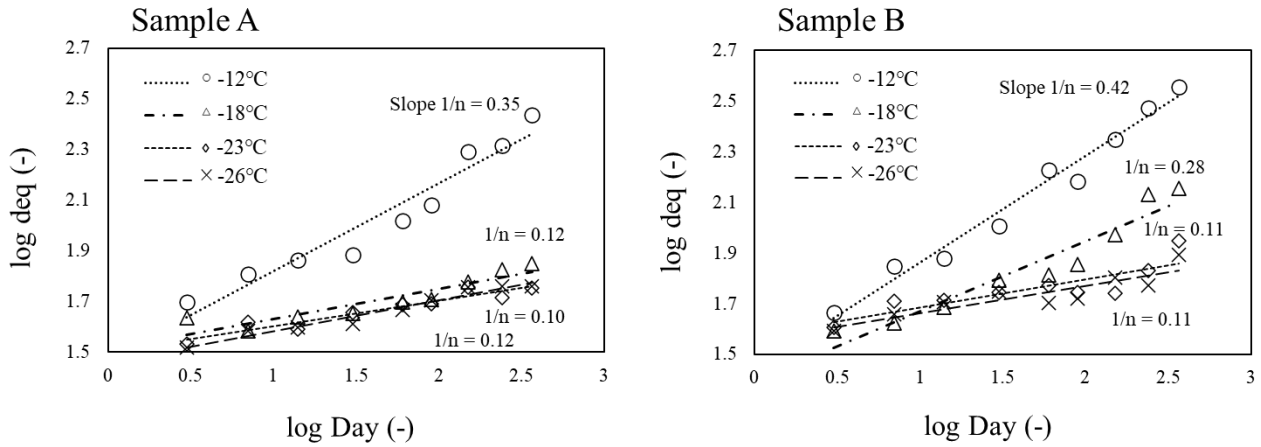

Figure 7 Log-log plot of storage period and change in ice crystal size

Table 1 The values derived from Fig. 6 in each commercial ice cream

Sample	Temperature (℃)	n (-)	log k (-)	R^2 (-)
A	-12	2.87	1.47	0.93
	-18	8.40	1.51	0.85
	-23	10.04	1.50	0.91
	-26	8.16	1.46	0.96
B	-12	2.38	1.44	0.97
	-18	3.62	1.39	0.92
	-23	8.89	1.57	0.73
	-26	9.23	1.55	0.80

CONCLUSIONS

Using commercially available ice cream, changes in ice crystal size during prolonged storage of up to 2 years were investigated; the data provided new valuable information. Results showed that ice crystal growth could not be predicted by an equation based on the conventional Ostwald ripening theory.

Since all results of ice crystal diameter and storage time were plotted on logarithmic coordinates, the experimental relationship between ice crystal diameter and storage time was well expressed by an alternative power law equation, such as equation 3.

In near future, the results could serve as the origin of a new theoretical tool to predict and control the recrystallization phenomenon, not only in various ice creams but also in frozen food.

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DIVISION C
Food Product Development, Sensory, and Consumer Research

ORAL PRESENTATION

Optimization of maltitol syrup content as glucose syrup replacer in gummy candies by using mixture design method

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ABSTRACT: Gummy confectionery is one of the foods that have widespread consumption among different age groups. As in all confectionery products, calorie reduction has gained importance in this product group. The aim of this study was to determine moisture content, water activity, soluble solid content (SSC), and also color, texture and sensory properties of gummy candies in whch 42 DE glucose syrup (0.0-54.17 g/100 g) was substituted by maltitol syrup (0.0-54.17 g/100 g) in the formulation. Experimental points (n= 5) were determined with simplex lattice mixture design. Significant linear, cubic, quartic and quadratic models with R^2 values between 0.895-1.000 were determined for moisture content (20.7-29.6 g/100 g), SSC (73.1-79.7°Bx), L* (32.40-39.81), b* (2.96-4.77), texture [hardness (577.1-1122 g), cohesiveness (0.97-0.99 s), gumminess (568.4-1101 g), chewiness (745.3-1759 g), resilience(0.765-0.825%)] and the main sensory properties according to independent variables ($P < 0.05$). According to the results of this study, maltitol syrup has the potential to replace glucose syrup in gummy candies to inhibit sucrose crystallization and modify texture properties.

Keywords: Confectionery, Sugar, Sugar alcohol, Glucose syrup, Optimization, Maltitol

INTRODUCTION

In recent years, the reformulation of foodstuffs with high caloric value or acute glycemic response has been among the priorities of international health authorities [1]. Thus, the substitution of sucrose and glucose syrup with healthier bulking agents in confectionery gains importance [2]. Glucose syrup in the production of gummy confectionery has effects on improving texture and microbial stability as well as stabilization of other components such as sucrose or gelatin [3]. In addition, it has been determined that presence and different concentrations of sugar affect functional, physicochemical and structural properties [4]. However, the increasing interest and demand for low sugar or sugar-free products in recent years has accelerated the search for alternative

ingredients to sucrose and glucose syrup in gummy confectionery [5]. Non-digestible carbohydrates, certain oligosaccharides (eg, inulin and fructooligosaccharides) and particularly sugar alcohols can be regarded as the main bulking agents used in the food industry [6, 7].

There are generally three main reasons for the use of artificial sweeteners or sugar substitutes; (i) reduction of calorie intake, (ii) protection of dental health, (iii) alternative confectionery for diabetic consumers [8]. Maltitol and maltitol syrups have been used for a long time in Europe, USA, and Japan, especially in bakery and other foods, and have a low glycemic index and calorie values [9, 10]. They are produced by catalytic hydrogenation of suitable sugars, and have following properties; (i) do not participate in Maillard browning reactions [10], (ii) dissolution temperature close to sucrose [11], (iii) low hygroscopicity [11] and calorie [10].

In this study, it was aimed to replace glucose syrup with maltitol syrup in gummy confectionery composition. For this purpose, some physicochemical, texture and sensory properties were accepted as a response and an optimization study was performed by using the mixture design.

MATERIAL AND METHODS

Materials

In the preparation of candy samples; water, sucrose (Konya Sugar, Konya, Turkey), 42-DE glucose syrup (Cargill, Bursa, Turkey), maltitol syrup (Lycasin® 80/55, in Roqouette, France), 250 bloom gelatin (Burjel, Bursa, Turkey), citric acid (Tayaş food, Kocaeli, Turkey), apple aroma (Etol Frutarom, Kocaeli, Turkey), colorant (Endemix, Kocaeli, Turkey) were used.

Study model

There are multiple mixture designs including simplex-lattice, simplex-centroid, axial design, and D-optimal design [12]. In this study, an experimental simplex-lattice mixture design was used to investigate the effect of two independent variables on the 5-point model system. Two independent variables are glucose syrup (X_1) and maltitol syrup (X_2). Table 1 showed the coded levels and experimental values of the independent variables according to the simplex-lattice design and the results obtained.

Sample preparation

Gummy candy samples were prepared using water, sucrose, glucose syrup, maltitol syrup, citric acid solution (50%), gelatin solution (33%), aroma and colorant (Table 1). For this purpose, in the thermal mixer (Thermomix TM5, Vorwerk, Wuppertal, Germany), water, sucrose-glucose syrup-maltitol syrup combination and citric acid solution were heated with stirring until it reached boiling point and heat treatment continued (200-300 rpm) with stirring for 10 minutes. Then, samples were cooled rapidly to 60ºC and ºBrix measurements were performed. The colorant and flavor agent were then added and mixed (1min, 200-300 rpm). 250 bloom gelatin: water solution (1: 3) was added and stirred at 60ºC/200-300 rpm for 5 minutes. At the end of the period, the final mixture was transferred to silicone molds and kept at 20ºC for 24 h. Samples taken from the mold were coated with starch to prevent them from sticking to each other and kept in polyethylene bags at 20.0 ± 2.00 ºC.

Physico-chemical analysis

Moisture determination was carried out using gravimetric technique in an incubator (Pol Eko Apatura, Slajski, Poland) at 60°C for a constant weight [2]. The water activity of the samples was measured using a Lab- Master a_w (Novasina, Switzerland). Water activity (a_w) value of each sample was measured after a follow-up day of sample preparation. Brix measurements by using refractometer (Abba, Atago, Tokyo, Japan) was used to determine the amount of soluble solid content. All analysis performed at least in triplicate.

Color analysis

L* (brightness) a*: (± red-green), and b* (± yellow-blue) values of samples were

determined using a colorimeter (Chroma Meter CR-400, Konica Minolta, Japan). Chroma (C*), and hue (h°) values were calculated using the following equations;

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^* = \arctan\ (b^*/a^*) \quad (2)$$

Sensory analysis

Nine-point hedonic scale sensory analyses were conducted to identify significant differences between samples. The sensory evaluation test was carried out by untrained panellists. The panel (n= 34) was composed of staff and graduate students from Food Engineering department at Siirt University. The age composition of the panelists was 79.4% (20-25) and 20.66% (30-46) whereas the gender composition was 38.3% male and 61.7% female. Panellists evaluated the effects of maltitol syrup (MS) and glucose syrup (GS) on the sensory characteristics of samples, as appearance, color, aroma, texture, hardness, gumminess, sweetness, springiness, intense of buying and general acceptance, and consumed water and crackers between each assessment to cleanse palate [13].

Texture analysis

Texture profile analysis (TPA) was performed using a TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, UK) equipped with a load cell of 5 kg and a 2 mm diameter cylindrical probe. The test conditions involved two consecutive cycles of 50% compression with 15 s between cycles. The test speed was 1 mm/s. From the resulting force-time curve, the following parameters were quantified; hardness (g), gumminess, springiness, cohesiveness, resilence, and chewiness (hardness x springiness x cohesiveness) (g) [13].

Statistical analysis

Design Expert (Stat-Ease Inc. version 12.0, Minneapolis, US) program was used for data analyses. Regression coefficients of linear, quatric, quadratic, cubic and interaction terms were determined ($P<0.05$).

RESULTS AND DISCUSSION

Physico-chemical (moisture content, water activity and soluble solid content) properties of samples were given in Table 2. The moisture content, water activity (a_w) and soluble solid content (SSC) values of the samples were determined as 20.7-29.6 g/100 g, 0.764-0.791 and 73.1-79.7 ºBx, respectively. Fitted models according to usage levels of Maltitol syrup (MS) and glucose syrup (GS) were determined for moisture content, a_w and SSC as linear, quadratic and cubic, respectively. R^2 values of these models were 0.923, 0.591 and 0.999, respectively, and the effects of GS and MS on moisture content and soluble solids ratios were successfully explained by the model (Figure 1). The model and lack of fit expressing the effect of these components on the relevant parameters were found to be significant ($P<0.05$). In gummy candies, Equations 3 and 4 were found to predict moisture content and SSC values from GS (X_1) and MS (X_2) levels in the product composition. Both variables were found significant.

$$\text{Moisture Content}=20.39.X_1+29.70.X_2 \quad \text{(Eq. 3)}$$

$$\text{SSC}=77.79.X_1+73.09.X_2+9.13.X_1.X_2+15.18.(X_1.X_2).(X_1-X_2) \quad \text{(Eq. 4)}$$

The main textural properties of gummy candy samples determined by Texture Profile Analysis (TPA) and the relationship between these properties and GS and MS ratios used in the composition were investigated (Table 3). The fitted models for hardness, cohesiveness, springiness, chewiness, gumminess and resilienceproperties depending on MS and GS usage rates were determined as quartic, linear, quadratic, quartic, quartic and quadratic, respectively. R^2 values of these models were 1.00, 0.895, 0.247, 1.00, 1.00 and 0.968, respectively. The effects of GS and MS on texture characteristics other than springiness were successfully explained by models. Figure 1 showed the effect of GS and MS ratios, on the hardness, springiness, cohesiveness, gumminess, chewiness and resilienceproperties

of samples. The model and lack of fit expressing the effect of these components on the relevant parameters were found to be significant ($P < 0.05$). Equations 5, 6, 7, 8 and 9 were established for their relationship with these parameters, in which the levels of use of GS (X_1) and MS (X_2) used in the composition of the gummy candies were determined to be significant.

$$\text{Hardness}=861.4X_1+813.2X_2+1139.6.X_1.X_2+466.13.X_1.X_2(X_1-X_2)-8920.X_1.X_2.(X_1-X_2)^2 \quad \text{(Eq. 5)}$$

$$\text{Cohesiveness}=0.9692X_1+0.9704X_2+0.0578.X_1.X_2 \quad \text{(Eq. 6)}$$

$$\text{Gumminess}=834.6X_1+787.9X_2+1160.6X_1.X_2+443.47.X_1.X_2.(X_1-X_2)-8687.2.X_1.X_2.(X_1-X_2)^2 \quad \text{(Eq. 7)}$$

$$\text{Chewiness}=745.33X_1+969.81X_2+762.49.X_1.X_2-3564.16X_1.X_2.(X_1-X_2)-7865.38.X_1.X_2.(X_1-X_2)^2 \quad \text{(Eq 8)}$$

$$\text{Resilience}=0.7670X_1+0.8061X_2+0.1668X_1.X_2 \quad \text{(Eq. 9)}$$

Various sensory properties of samples were evaluated by untrained panellists using 9-point scale (Table 4). The fitted models for the sensory properties were determined as quadratic for appearance, color, aroma, gumminess, sweetness, chewiness, and quatric for texture, springiness, buying intense and general acceptance (Table 4). Of the sensory parameters, only the cubic model for the hardness parameter was formed. R^2 values of these models showed changes in the range of 0.511-1.000 (Table 2). The effects of GS and MS on color (Eq. 10), texture (Eq. 11), springiness (Eq. 12), buying intense (Eq. 13) and general acceptance (Eq. 14) were significantly explained with the models ($P<0.05$). For the other sensory parameters, the level of compatibility was not significant. Figure 2 shows the effect of GS and MS ratios on sensory properties of samples.

$$\text{Color}=7.38.X_1+8.06.X_2-5.02.X_1.X_2 \quad \text{(Eq. 10)}$$

$$\text{Texture}=7.50.X_1+7.70.X_2+0.8.X_1.X_2+3.20.X_1.X_2.(X_1-X_2)-21.33.X_1.X_2.(X_1-X_2)^2 \quad \text{(Eq. 11)}$$

$$\text{Springiness}=7.50^*X_1+8.0^*X_2-0.6.X_1.X_2+4.53.X_1.X_2.(X_1-X_2)-13.6.X_1.X_2.(X_1-X_2)^2 \quad \text{(Eq. 12)}$$

$$\text{Buying Intense}=6.7.X_1+7.8.X_2-0.6.X_1.X_2+6.13.X_1.X_2.(X_1-X_2)-26.4.X_1.X_2.(X_1-X_2)^2 \quad \text{(Eq. 13)}$$

$$\text{General Acceptance}=7.2.X_1+8.1.X_2-1.0.X_1.X_2+7.73.X_1X_2.(X_1-X_2)-22.67.X_1.X_2.(X_1-X_2)^2. \quad \text{(Eq.14)}$$

The main color properties of the confectionery samples (*L**, *a**, *b**, chroma (C*) and hue angle (h^o) and the relationship between these properties and GS and MS ratios used in the composition were examined in the study (Table 2). One of the most important parameters affecting the sensory perception of candies is their color and visual characteristics. The parameters that provide information about the visual characteristics of the objects include color, brightness and transparency, which generally determine the perception of appearance. In this study, color parameters of the samples were determined by CIE-Lab technique. The fitted models *for L**, *a**, *b**, C*, and h^o properties depending on MS and GS concentration were determined as quartic, cubic, cubic, quadratic and cubic, respectively. The R^2 values of these models were determined as 1.00, 0.875, 0.990, 0.750 and 0.942, respectively. The effects of GS and MS on L* (Eq. 15) and b* (Eq. 16) parameters were modelled significantly. However, this was not possible for *a**, C* and h^o values (Table 2). The model and lack of fit expressing the effect of these components on the relevant parameters were found to be significant ($P < 0.05$). Figure 1 shows the effect of GS and MS ratios on the *L**, *a**, *b**, C* and h^o of the samples.

$$L^*=34.54.X_1+33.53.X_2+23.10.X_1.X_2+13.68.X_1.X_2.(X_1-X_2)-94.53.X_1.X_2.(X_1-X_2)^2 \quad \text{(Eq. 15)}$$

$$b^*=2.95.X_1+3.00.X_2+6.62.X_1.X_2+6.64.X_1.X_2.(X_1-X_2) \quad \text{(Eq. 16)}$$

Moisture can lead to remarkable changes on textural properties for all food materials, and even low levels of change in some products, such as hard candies [12, 14] can lead to quality problems such as crystallization, stickiness, development and acceleration of rancidity, structural incompatibilities, chewiness differences, hardness, shaping problems and textural problems [3, 14]. This is because high moisture content can cause internal molecule mobility and polyol recrystallization in confectionery [15]. Also water acts as a plasticizer. The formation of new hydrogen bonds between gelatin and water molecules, can be observed at low moisture content [16]. The amount of moisture is also effective on the release of

aroma components which is of great importance for gummy candies. The presence of soluble substances, such as low-molecular saccharides, is influenced by the change in the amount of free water [17]. The results of this study indicated that the amount of moisture increased with the increase of MS level in the formulation. Since GS is obtained by hydrolysis of starch, it contains molecules larger than glucose and sucrose. Therefore, the binding sites for water molecules may be different from the mixture of sucrose and MS at the same concentration. As a result, it can be stated that gummy candies with acceptable moisture levels can be obtained by substituting GS with MS. However, in determining GS and MS levels, the recommended moisture value for gummy candies should be below 24% [2].

Water activity can be defined as usable water in the food composition. It is used to evaluate microbial stability and shelf life of foods [18]. Sweeteners, especially small molecular weight saccharides, have a significant effect on the sensory properties as well as the textural properties of the hydrocolloid matrix, viscosity of the aqueous phase and water activity [17]. However, a significant model for water activity prediction could not be determined by MS and GS levels.

To prevent microbial growth in confectionery products at ambient temperature, the soluble solids content of the products should be above 76 g/100 g. At these levels, some sucrose is substituted with other sugars (eg glucose syrup or invert sugar) to increase solubility in order to prevent crystallization [19]. When sugar substitutes are used, if the total solids content changes, the texture of the gelatin-containing products is weakened. Therefore, the total amount of solids should be adjusted using bulking agents along with sugar substitution [5]. Molina-Rubio et al. [20] found that the type of hydrocolloid in the structure of sugar syrups and the Brix value of this syrup affect the hardness property. In this study, the use of MS for GS substitution was modeled with a high R2 value (0.999) on the SSC. The increase in MS usage and decrease in GS caused an increase in SSC significantly. Therefore, GS substitution with MS may provide advantage for inhibition of microbial growth and crystallization.

Crystallization of sugars in confectionery products is critical for proper texture, appearance and storage stability. The presence of sugar crystals is desired in some confectionery products (eg, fondant, toffee and fudge), but not in gummy confection [21]. One of the important functions of GS is that it inhibits the crystallization of sucrose [8]. Sucrose is generally used in the composition of foods in granular form and as a sweetener. It is used in accordance with GS. GS improves the solubility of sucrose and delays or prevents crystallization in the food structure. Sucrose also has an effect on the texture and sensory profiles of gel structures, as well as increasing the product density, affecting the structure and mouth feel [4]. Different types of glucose syrups and sucrose affect the physical properties of confectionery in different ways [12]. It is therefore important for an alternative component that it exhibits the same effect as glucose syrup. According to results of this study, confectionery samples with similar hardness, cohesiveness and gumminess properties were obtained in the use of GS or MS alone. Hardness may increase in confectionery due to crystallization and glass transition or gelatin gel network formation [18]. Therefore, high-glucose corn syrups are important for the control of sucrose crystallization in confectionery products [22]. Thus, it can be stated that MS has a similar effect as GS on sucrose crystallization. However, the effect of GS-MS combination (50:50) on hardness is remarkable. This effect is thought to be due to another mechanism that causes increased hardness other than crystallization; strengthening of the gel network.

Hardness can be increased using methods such as increasing the concentration of the gelling agent, lowering the pH, using glucose syrup with low DE, or reducing the sucrose/glucose syrup ratio when using low DE glucose syrup [23]. 40 DE corn syrup has a high

level of low molecular weight sugars (eg. glucose), which can lead to a low T_g value of the confectionery and a tendency to stickiness [12]. The use of high-maltose corn syrups can also result in products that are hard and also have increased stickiness [24]. Low molecular weight carbohydrates have lower T_g values [12]. If T_g is too high, the hardness of the candy increases and shows fragility [15]. The use of MS in conjunction with GS can indirectly lead to a decrease in the DE value of GS as a result of the dilution effect, leading to significant increases in confectionery hardness. The results obtained by this study confirm this possible effect.

The cohesiveness properties of gummy confection samples have varied within a narrow range. However, the model related to this property according to the use of MS and GS has a lower R^2 (0.895) value compared to other TPA properties but could be defined by an appropriate model. Cohesiveness is a property which is felt during compression of a material between the teeth before it breaks. It is a parameter that does not correlate with other TPA parameters and is an indicator of the strength of internal bonds [18]. The use of MS to obtain gummy confection with cohesiveness similar to GS-containing samples was considered to indicate that both sweeteners had similar effects on the formation of gelatine bonds. It may be useful to examine other factors affecting this parameter in gummy candies prepared using MS. For example, it has been reported that Brix value is effective on cohesive work in semi-solid matrices obtained as a result of gelling of sugar syrups [20].

Gumminess is a TPA parameter investigated in semi-solid foods [25]. Periche et al. [2] found that the changes in the gelatin in the product formulation had the most important effect on textural parameters such as hardness and gumminess, and also stated that sugars and combinations affect these mechanical properties.

Delgado and Banon [26] stated that chewiness value is more useful in defining gummy candies compared to gumminess value since it is faced with a solid matrix. However, in this study, the change of gumminess property depending on MS and GS concentration could be significantly modeled.

The main technological functions of gelatin can be specified as gelling, thickening, stabilization, texture modification and emulsification [27]. However, the presence and concentration of sugars in the composition have an effect on these functions. Because the gelatin gel configuration of sugars has the effect of stabilization. Thus sucrose supports gel solubility and stability in the final product whereas sucrose/GS mixtures form a continuous liquid phase with gelatin [3]. Sugars affect the functional properties of proteins such as adsorption and gelation [19]. Therefore, alternative sweeteners used for GS substitution are expected to have a similar effect on gelation. The chewiness values obtained in TPA analysis of gummy candies are related to compression force and energy required for mastication. Altan Kamer et al. [4] stated that the addition of sorbitol and sucrose positively affected the gelling, texture, thermal and rheological properties, and that the addition of sugar or polyol increased the binding zone between proteins and the formation of triple helix structure. As a result of this study, extreme changes on the chewiness of MS use were observed. In the presence of sucrose, protein-protein interaction is enhanced. This may lead to the observation of protein aggregations [19]. It can be stated that the presence of maltitol in the aqueous phase may not cause a similar effect. However, it can be noted that, although there are components of different molecular weights in the use of GS, it is advantageous for MS to increase the proportion of components having a sucrose-like molecular size, resulting in the association of gelatin chains and increase for the binding sites.

Color is one of the most important quality indicators of foods. Its impact on consumers is not only visual, but also associated with its variety, quality and freshness [29]. In general, an increase in gelatin levels causes an increase in L*, a* and b*

values in gummy candies [2]. The increase in gelatin level also results in increased hardness. In this study, it has been determined that the product compositions which result in increased hardness have a brighter surface and therefore a high L* value (Table 2 and 4).

CONCLUSIONS

Glucose and corn syrups are important ingredients in confectionery technology. However, consumers may have negative attitudes toward them which have high calorie values. There is a need to develop alternative products to address these consumers' concerns. According to the results of this study, the effect of maltitol syrup usage as a glucose syrup replacer at different amounts on quality parameters of gummy confectionery was modelled. It was found that maltitol syrup can fulfil functional properties, particularly inhibition of sucrose crystallization, moisture retention and sweetness. It was demonstrated that this component has the potential to be used in different confectionery products.

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Table 1 Study design and sample preparation

Sample	Coded values		Real Values*		Sucrose*	Gelatine Solution*	Citric Acid*	Coloring Agent*	Aroma*	Water*	Total**
	X_1	X_2	Glucose Syrup	Maltitol Syrup							
1.1	1.00	0.00	54.17	0.00	42.27	8.13	2.43	0.01	0.25	22.51	129.77
1.2	0.75	0.25	40.6275	13.5425	42.27	8.13	2.43	0.01	0.25	22.51	129.77
1.3	0.25	0.75	13.5425	40.6275	42.27	8.13	2.43	0.01	0.25	22.51	129.77
1.4	0	1	0.00	54.17	42.27	8.13	2.43	0.01	0.25	22.51	129.77
1.5	0.50	0.50	27.085	27.085	42.27	8.13	2.43	0.01	0.25	22.51	129.77

* g/100 g, ** g. X_1: Glucose Syrup, X_2: Maltitol Syrup

Table 2 Moisture content, soluble solid content and water activity and colour properties and fitted models

Sample	Moisture (g/100 g)	Water Activity (a_w)	Soluble Solid Content(°Bx)	L*	a*	b*	C*	hº
1.1	20.7±0.20	0.766±0.005	77.8±0.06	34.54±2.31	3.77±0.99	2.96±0.23	4.83±0.71	0.68±0.15
1.2	22.7±0.13	0.784±0.005	79.7±0.67	35.47±1.13	3.68±0.19	4.73±0.17	6.00±0.25	0.91±0.01
1.3	28.8±0.28	0.791±0.019	74.5±2.02	32.40±1.08	7.55±1.08	3.51±0.28	8.33±1.08	0.44±0.03
1.4	29.6±0.39	0.764±0.038	73.1±1.76	33.53±1.72	5.20±0.75	3.02±0.13	6.03±0.58	0.53±0.08
1.5	23.1±0.02	0.774±0.002	77.8±1.55	39.81±0.54	4.34±0.12	4.77±0.27	6.45±0.26	0.83±0.02
Model	Linear	Quadratic	Cubic	Quartic	Cubic	Cubic	Quadratic	Cubic
R^2	0.9230	0.5908	0.9996	1.00	0.8746	0.9898	0.7496	0.9422
Adjusted- R^2	0.9038	0.3180	0.9990	1.00	0.6866	0.9745	0.5827	0.8556
Predicted- R^2	0.8688	-1.5275	0.9693	nd	-9.0487	0.1814	-1.4947	-3.6288
AdeqPrecision	13.141	2.8835	106.93	nd	5.6388	16.5191	4.1908	8.5906

Mean ± SD. L*; brightness, h°; hue angle, C*; chroma. [a] P<0.05. [b] nd: not determined (In case of R^2 and prediceted R^2 equal to 1.00). Three replicates of soluble solid content, water activity (a_w) and moisture content and five replicates of the colour analysis were performed.

Table 3 Texture and fitted models

Sample	Hardness (g)	Springiness (%)	Cohesiveness (s)	Gumminess (g)	Chewiness (g)	Resilence (%)
1.1	861.4±192.4	0.89±0.01	0.97±0.01	834.6±183.3	745.3±160.4	0.765±0.013
1.2	688.6±50.2	1.46±0.66	0.98±0.00	674.9±50.7	979.0±40.7	0.817±0.010
1.3	577.1±23.6	3.093±0.22	0.99±0.01	568.4±24.0	1759±191.9	0.823±0.012
1.4	813.2±94.5	1.267±0.70	0.97±0.00	787.9±92.3	969.8±436.7	0.809±0.008
1.5	1122±214.7	0.952±0.03	0.98±0.00	1101±212.8	1048±202.3	0.825±0.005
Model	Quartic	Quadratic	Linear	Quartic	Quartic	Quadratic
R^2	1.00	0.2473	0.8951	1.00	1.00	0.9677
Adjusted- R^2	1.00	0.0592	0.8252	1.00	1.00	0.9462
Predicted- R^2	nd[b]	-1.0485	0.0507	nd[b]	nd[b]	0.7771
Adeq Precision	nd[b]	2.1753	7.0292	nd[b]	nd[b]	13.2566

Mean ± SD. [a] P<0.05. [b] nd: not determined (In case of R^2 and prediceted R^2 equal to 1.00). Five replicates of analysis were performed.

Table 4 Sensory properties and fitted models

Sample	App	Color	Aroma	Texture	Hardness	Gms	Sws	Cws	Sprs	BI	GA
1.1	7.70±1.25	7.40±1.27	6.80±1.40	7.50±1.43	6.60±1.78	6.90±2.13	6.80±1.81	7.30±1.25	7.50±1.18	6.70±1.83	7.20±1.23
1.2	6.50±1.18	6.50±0.71	6.40±1.27	7.00±0.67	6.60±2.32	6.20±2.04	5.40±1.71	6.90±1.66	7.30±1.16	6.20±1.14	6.90±1.52
1.3	6.80±1.14	6.80±1.03	5.90±1.73	6.50±1.35	5.90±1.60	6.50±1.96	5.00±2.16	6.50±1.27	6.70±1.34	5.60±1.27	5.90±1.52
1.4	8.10±0.88	8.00±1.05	7.80±1.32	7.70±1.06	6.60±1.96	7.00±2.11	7.60±1.96	8.00±0.82	8.00±0.82	7.80±1.40	8.10±1.10
1.5	7.00±1.49	6.80±0.92	6.80±1.14	7.80±1.03	6.70±1.83	6.70±2.11	6.60±1.71	7.30±1.16	7.60±0.97	7.10±1.37	7.40±0.97
Model	Q	Q	Q	Qt	C	Q	Q	Q	Qt	Qt	Qt
R^2	0.8401	0.9145	0.5111	1.00	0.6220	0.5966	0.4840	0.4714	1.00	1.00	1.00
Ad-R^2	0.7335	0.8575	0.18.52	1.00	0.0549	0.3276	0.1400	0.1190	1.00	1.00	1.00
Pre-R^2	0.4487	0.6667	-3.4304	nd[b]	-29.297	-0.2021	-2.2067	-3.4968	nd[a]	nd[a]	nd[a]
AP	6.4053	9.6515	3.0887	nd[b]	2.9728	3.3806	2.7015	2.8236	nd[a]	nd[a]	nd[a]

Q: Quadratic, C: Cubic, Q; Quatratic, Adj-R^2: Adjusted-R:^{2}, Pre-R^2: Predicted-R:^{2}, AP: Adeq Precision, App: Appearance, Gms: Gumminess, Sws: Sweetness, Cws: Chewiness, Sprs: Springiness, BI: Buying Intense, GA: General Acceptance. Number of panelists was 34. Mean ± SD. [a] P<0.05. [b] nd: not determined (In case of R^2 and prediceted R^2 equal to 1.00).

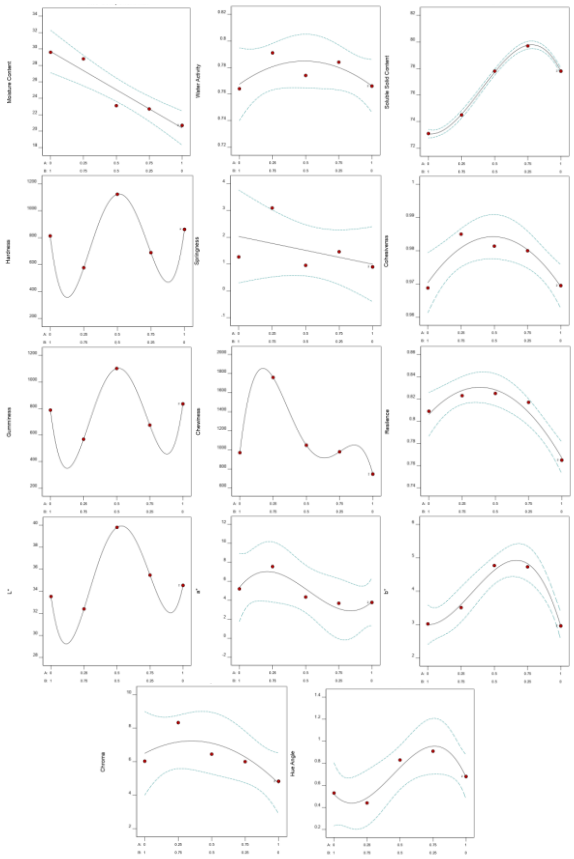

Figure 1 Modelling of physico-chemical, color and textural properties (A: Glucose Syrup; B: Maltitol Syrup)

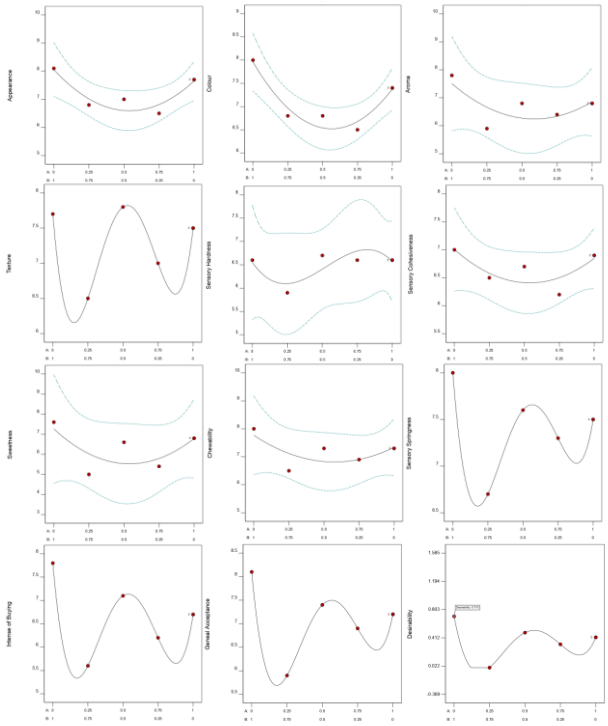

Figure 2 Modelling of sensory properties and optimization (desirability) according general acceptance results (A: Glucose Syrup; B: Maltitol Syrup

Study of the relationship between meat consumption behavior and the potential to consume cricket protein

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ABSTRACT:
The objective of this study was to investigate the relationship between meat consumption behavior, including meat-only and flexitarian diet consumptions, and the potential to consume cricket protein. Consumer lifestyles, attitudes toward crickets and attitudes toward environment and sustainability were compared between two age groups (generation Y and generation Z). Survey data using questionnaires were conducted from 260 respondents and analyzed by descriptive statistics, inferential statistics (Independent sample t-test) and hypothesis testing using Pearson Chi-square statistic. Findings found that meat consumption behavior was significantly related to potential to consume cricket protein. Generation Z had significantly different potential to consume cricket protein between meat consumption groups, while generation Y had the similar potential to consume cricket protein between meat-only and flexitarian diet groups. The results of the analysis of both generations revealed that there were differences in lifestyles. Generation Y often focus on body shape and weight, shopped by following trends and reviews, and liked to try new food products. Generation Y group has more awareness of environmental and sustainability impact than generation Z, including solving environmental problems must start with myself, environmentally friendly products can reduce environmental problems, and consuming environmentally friendly foods giving good mental health. For attitude toward cricket insects, both generations were statistically significant difference in consuming protein products from crickets is better than eating meat where generation Z agreed with this attitude higher than generation Y. These results can contribute to stakeholders or businesses related to edible insects with additional information on communication strategies and targeting the potential segments.

Keywords: Edible insect, Protein alternative, Cricket insect, Generation, Flexitarian diet

INTRODUCTION

Global warming and sustainable food supply has become challenges facing all countries. In 2050, the world population is estimated to reach 10 billion people [1]. The amount of food supply is expected to be not enough for future demand [2]. In 2013, the Food and Agriculture Organization of the United Nations (FAO) announced edible insects as a new alternative food source (novel food) to secure the growth of the world population. Edible insects are highly nutritious food, especially rich in protein [3, 4]. In addition, edible insect farming is more environmental sustainability than other livestock productions, including use less land and water, emit lower greenhouse gas, require lower production cost, and get better feed conversion efficiencies [1]. Therefore, edible insects have gained global attentions as a potential alternative source of protein in the future.

In Thailand, the government has the policy to promote Bio Circular Green Economy, known as BCG models [1] and drives Thailand to be the center of insect production or the world's insect hub to serve the future food sources. Cricket is one of the most potential markets on edible insects [4, 5]. Even though insect consumption has been among Thai people for more than a century, especially in the North and Northeast, many Thais, located in urban area are not familiar with eating insect [2]. Part of reasons are less appealing image, inconvenient to access at the store, disgust and fear, and unconvinced of insect safety. Study by Sriwaranun (2017) [4] suggested that attitude toward the behavior (i.e. safety and taste) and perceived behavioral control (i.e. disgust and fear) have effect on intention to consume edible insects. The acceptance on edible insects is still limited in specific segments and regions. Hartmann (2015) [6] indicated that Chinese people are more likely to accept edible insect than German. Orsi *et al.* (2019) [7] and Liu *et al.* (2019) [8] found that young adults have positive attitude toward edible insects compared with seniors, while gender, education, and attitude toward environment are insignificant with respect to the acceptance of insects.

Growing flexitarian trend in Thailand leads to consumers reducing their consumption of animal meat and shift to alternative sources of protein likes plant-based diets [9]. In addition, younger people tend to aware of environment, animal welfare, and sustainability than the older generation, which may affect the acceptance of edible insects as protein alternative. Thus, research is needed to understand the causes of this trend and seek for opportunities to promote edible insects as a future food market. This research aims to investigate the relationship between meat consumption behavior and the potential to consume cricket protein by comparing two age groups (generation Y and Z). The differences on lifestyles, attitude toward cricket insects, and environment concerns between two age groups were compared.

MATERIAL AND METHODS

1. Define samples and collect data

Data were conducted through purposive sampling approach by allocating online questionnaires among consumers at generation Y (1981-1996) and generation Z (1997-2012) [10]. The respondents aged 18 years or older were selected for the analysis.

2. Develop questionnaire

The questionnaire consisted of 4 parts: part 1 demographic characteristics (age, gender, education, income, and social media), part 2 meat consumption behavior (meat and plant consumption pattern and concern about meat consumption), part 3 lifestyle, part 4 attitude toward cricket insect and attitude toward environment and sustainability. The statements

on attitudes have two directions: positive (+) and negative (-). The questions in parts 3 to 4 uses 5-point Likert scale to measure the degree of agreement (1 = completely disagree to 5 = completely agree).

The questionnaire was pre-tested with 30 respondents to obtain the final questionnaire. The reliability of the questionnaire was tested with Cronbach's Alpha Coefficient of the 5-point Likert scale. The coefficient must be equal 0.7 or greater [11].

3. Statistical data analysis

Data on demographic characteristics, and meat consumption behavior were analyzed with descriptive statistics. Part 3 and 4 were analyzed using m*ean* ($\bar{x}$) and *standard Deviation* (SD) and compared the difference between means from two groups (generation Y and Z) using independent sample t-test. The relationship between meat consumption behavior (with generation Y and Z) and potential to consume cricket protein was analyzed by using Pearson Chi-square test.

RESULTS AND DISCUSSION

1. Reliability test of questionnaires

Results of the reliability test of questionnaires on part 3 lifestyle and part 4 attitude toward cricket insect and attitude toward environment and sustainability were 0.89, 0.81, and 0.91, respectively. Therefore, the questionnaire was reliable.

2. Demographic data analysis

A sample of 260 respondents were divided into two generations as shown in Table 1. The survey sample reported that 43.1% of respondents are generation Y (Gen Y) and the rest belongs to generation Z (Gen Z). Most respondents of both generations are female and have level of education at bachelor's degree. Most generation Y earned monthly income between 15,001-30,000 baht, while generation Z earned less than 15,000 baht per month. The top 3 of social media were used by generation Y including *Line*, *Facebook*, *Instagram*, respectively. For generation Z, they tend to use *Instagram* more than *Line* and *Facebook*.

Table 1 Demographic characteristics of sample

Characteristics	Percentage (%)	
	Gen Y (n=112)	Gen Z (n=148)
Gender		
Male	24.1	18.2
Female	75.9	81.8
Education		
below bachelor's degree	1.8	5.4
bachelor's degree	62.5	81.8
postgraduate	35.7	12.8
Income (baht/month)		
less than 15,000	3.6	74.3
15,001-30,000	43.8	20.3
30,001-45,000	23.2	3.4
more than 45,000	29.4	2.0

3. Meat consumption behavior

Meat and plant consumption pattern were divided into four groups: meat-only diet (or normal meat i.e. pork, chicken, beef), more meat than plant-based diet, less meat than plant-based diet, and vegetarian (Table 2). Findings found that 57.1% of generation Y consumed only meat, followed by consumption pattern on more meat than plant-based diet (39.3%). The majority meat consumption pattern of generation Z were only meat (52.7%) followed by more meat than plant-based diet (41.9%). Generation Z tend to be flexitarian more than generation Y. Flexitarian diet consumptions include reducing meat consumption and choosing to consume alternative sources of protein; for example, consuming less red meat, consuming more plant-based diet with the occasional meat or dairy.

Table 2 Meat consumption behavior

Meat consumption behavior	Percentage (%)	
	Gen Y (n=112)	Gen Z (n=148)
Meat-only diet	57.1	52.7
More meat than plant-based diet	39.3	41.9
Less meat than plant-based diet	2.7	4.1
Vegetarian	0.9	1.4

Concerns about normal meat consumption showed that generation Y had concerns about growth promoters (20.9%), carcinogens (20.9%), fat contents (19.1%), digestion system (13.8%), and environmental impacts (4.0%). Generation Z had concerns about fat contents (20.1%), carcinogens (18.8%), digestion system (15.8%), growth promoters (15.6%), and environmental impacts (5.2%). Generation Z tended to aware of normal meat consumption relating to environmental impact more than generation Y. Report by Creative Economy Agency (2020) [12] also indicated that generation Z have faced environmental problems throughout their lifetimes, thus they are more likely to interested in environmentally friendly products and support green businesses.

4. Consumer lifestyle

Results of consumer lifestyles between two generations as presented in Table 3. In general, generation Y have stronger agree on lifestyles than generation Z where statistically *significant mean differences between two generations* were focus on body shape and weight, like trying new food products, and shop following trend and review. Both generation Y and Z tend to use technology as part of their daily lives (4.06, 4.03), consume good quality foods (3.92, 3.83), and always read the label/nutritions before purchasing (3.76, 3.68), respectively.

Table 3 Lifestyle

Lifestyle	$\bar{x}$ ± SD		*t-test*
	Gen Y (n=112)	Gen Z (n=148)	
Use technology as part of daily lives	4.06±0.84	4.03±1.14	0.29
Tend to eat good quality food	3.92±0.71	3.83±1.05	0.81
Always read the label/nutritions before purchasing	3.76±0.95	3.68±1.07	0.60
Focus on body shape and weight	3.73±0.91	3.47±1.15	2.09**
Observe body abnormalities	3.63±0.90	3.43±1.17	1.50
Like trying new food products	3.63±0.88	3.12±1.29	3.74***
Shop following trend and review	3.18±0.89	2.92±1.23	1.97**
Update health news from online media	3.46±0.90	3.22±1.12	1.98
Exercise regularly	2.88±1.02	2.82±1.18	0.42

Note: * p-value = 0.1, ** p-value = 0.05, *** p-value = 0.01

5. Attitudes toward cricket insects and environmental and sustainability

Findings showed that a statistically significant mean difference between two generations was that generation Z agreed with consuming protein products from crickets is better than eating meat more than generation Y. Both generation Y and Z agreed that cricket insect consumption is not bizarre and cricket insects have high nutritional value (Table 4). Since edible insects have been with Thai people for long period and perceived as local foods, especially in rural area [2], many Thais are familiar with eating insects, but they may not treat edible insects as common diet.

Table 4 Attitudes toward cricket insects

Attitudes	$\bar{x}$ ± SD		*t-test*
	Gen Y (n=112)	Gen Z (n=148)	
Cricket insect consumption is not bizarre (+)	3.67±0.97	3.83±0.99	-1.32
Cricket insects have high nutritional value (+)	3.59±0.82	3.68±0.99	-0.75
The consumption of protein products from crickets was better than the consumption of meat (+)	2.83±0.86	3.03±1.03	-1.70*

Note: * p-value = 0.1, ** p-value = 0.05, *** p-value = 0.01

Table 5 presented the mean differences on attitudes toward environment and sustainability between generation Y and Z. Results revealed that the *difference between two means* are statistically *significant, giving that* generation Y have more awareness of environment and sustainability than generation Z. Report by Creative Economy Agency (2020) [12] revealed that generation Y were more caring for the wellness and the environment than other generations. However, level of green consumption was varied among mixed generation Y and Z. Generation Y believed that solving environmental problems must start with myself (4.19±0.84), environmentally friendly products can reduce or prevent environmental problems (4.02±0.82), and consuming environmentally friendly foods giving good mental health (3.71±0.87). Study by Chaihanchanchai *et al.* (2021) [13] found that environmental considerations were not a statistically significant indicator of green shopping behavior. Although consumers were highly concerned about the environment impact, they may not be willing to purchase environmentally friendly products.

Table 5 Attitudes toward environment and sustainability

Attitudes	$\bar{x}$ ± SD		*t-test*
	Gen Y (n=112)	Gen Z (n=148)	
Solving environmental problems must start with myself (+)	4.19±0.84	3.97±0.98	1.85*
Environmentally friendly products can reduce or prevent environmental problems (+)	4.02±0.82	3.81±0.92	1.92*
Consuming environmentally friendly foods giving good mental health (+)	3.71±0.87	3.51±0.94	1.75*
Purchasing a product with carbon labelling reduces the environmental impact (+)	3.74±0.87	3.58±0.90	1.44

Note: * p-value = 0.1, ** p-value = 0.05, *** p-value = 0.01

6. Analysis on the relationship between meat consumption behavior and potential to consume cricket protein

This study focused on potential to consume cricket protein, which asked respondents to choose among three choices: consume cricket protein in the future, try it first and then decide, and no plan. Findings on Pearson Chi-square test between generations and potential to consume cricket protein found that there was statistically significant correlation at *p-value* < 0.05 (Table 6). This is consistent with research by Liu *et al.* (2019) [8] who found that younger adults had more positive attitudes to eating insects than older adults and indicated that age affects insect acceptance.

Table 6 The relationship between generations and the potential to consume cricket protein

Generation	Consume cricket protein in the future	Try it first and decide	No plan
Y	17	59	36
	15.2%	52.7%	32.1%
Z	45	59	44
	30.4%	39.9%	29.7%
Pearson Chi-square	8.626**		

Note: * p-value = 0.1, ** p-value = 0.05, *** p-value = 0.01

In this study, two meat consumption behaviors included meat-only diet and flexitarian diet consumptions. Analysis on the relationship between meat consumption behavior and potential to consume cricket protein using Pearson Chi-square test was presented on Table 7. Findings indicated that meat-only diet and flexitarian diet groups had a statistically significant difference in potential to consume cricket protein at *p-value* < 0.05. Flexitarian diet group were more likely to consume cricket protein than meat-only diet group. This is consistent with Jakobs (2018) [14], who indicated that flexitarians were more open-minded to insect consumption than meat consumers.

The relationship between meat consumption behavior within each generation and the potential to consume cricket protein found that Generation Y had no statistically significant relationship between meat consumption behavior and potential to consume cricket protein. Generation Y would rather try cricket protein products first and make decision than decide to consume cricket products in the future. On the other hand, for generation Z, meat consumption behavior was associated with a statistically significant difference in potential to consume cricket protein (*p-value* < 0.05), indicating that generation Z with flexitarian diet behavior were more likely to consume cricket protein than generation Z with meat-only diet. Overall, flexitarian consumers and generation Z tend to be more accepting of insects than generation Y.

Table 7 The relationship between meat consumption behavior and potential to consume cricket protein

Meat consumption behavior	Consume cricket protein in the future	Try it first and decide	No plan
Generation Y			
Flexitarian	18.8%	54.2%	27.1%
Meat-only	12.5%	51.6%	35.9%
Pearson Chi-square	1.410		
Generation Z			
Flexitarian	37.1%	44.3%	18.6%
Meat-only	24.4%	35.9%	39.7%
Pearson Chi-square	8.197**		
Total			
Flexitarian	29.7%	48.3%	22.0%
Meat-only	19.0%	43.0%	38.0%
Pearson Chi-square	8.828**		

Note: * p-value = 0.1, ** p-value = 0.05, *** p-value = 0.01

CONCLUSIONS

Nowadays meat consumption behavior has changed to response various drivers such as health concerns, global warming, and sustainable food supply. Flexitarian diet has become emerging trend, which is combination of meat and plant consumptions, and seeking for alternative sources of protein. Edible insect sector has been expected to be one of future foods in Thailand. This study explored the relationship between meat consumption behavior and the potential to consume cricket protein. In general, both generations (Gen Y and Z) are not much different in lifestyle, excepting concern about body shape and weight, like trying new food products, and shop following trend and review. Generation Y have different attitudes toward cricket insects form generation Z,

generation Z tented to prefer consuming protein products from crickets than eating meat when compared with generation Y. For attitudes toward environment and sustainability, generation Y have more awareness of environment and sustainability than generation Z.

Findings revealed that generation (Gen Y and Z) was related to the potential to consume cricket protein. When meat consumption behaviors were divided into two groups: meat-only diet and flexitarian diet, flexitarian consumers and generation Z tend to be potential customers of cricket insects than generation Y with meat-only diet. Generation Z with flexitarian diet behavior were more likely to consume cricket protein than generation Z with meat-only diet. To promote consumption of cricket insects, generation Z, especially flexitarian diet group could be major segment by focusing on alternative protein diets and communicating additional environmental impacts related to livestock production via *Instagram*. Generation Y should consider the campaign on promote wellness aligned with health and environmental concerns. In addition, various application uses of cricket insects on new food products and free samples should be promoted via *Line* and *Facebook* to increase the acceptance level of cricket consumptions.

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POSTER PRESENTATION

Optimization of gelatin and sorbitol for sugar-infused dried 'Nam Dok Mai Si Thong' mango (*Mangifera indica* Linn.) production

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ABSTRACT: The objective of this work was to study the relationship between sorbitol and gelatin on the characteristics of sugar-infused dried mango (SIDM). The various sorbitol powder and gelatin powder was used in sugar-infusion to optimize variations. The relationship among physicochemical properties, sorbitol content and gelatin content was also evaluated by using Multivariate analysis. The results showed that water activity (a_w), total soluble solids (TSS), pH and acidity (as malic acid) values of SIDM were 0.497-0.571, 74.00-84.10 ºBrix, 3.26-3.96, and 0.23-0.62 g per 100 g, respectively. The firmness, total sugar, and reducing sugar values of SIDM were in the range of 15.15-29.83 N, 58.05-75.97 g per 100 g, and 35.83-51.80 g per 100 g, respectively. This investigation highlighted that sorbitol content in the range of 12.50-25.00 g per 100 g of fresh mango significantly reduced the firmness and the browning reaction of SIDM. In contrast, gelatin content significantly affected on a_w, brightness (L^* value), and a small effect on TSS or sugar loading of SIDF. The minimum redness value (a^* *value* of 4.66) and firmness (firmness of 17.54 N) was attained using the sorbitol content of 23.79 g/100g and the gelatin content of 0.20 g/100 g of mango. The principal component analysis indicated that sorbitol is associated with a firmness of texture, pH, and redness (a^* value), and gelatin content is related to a_w.

Keywords: Dried mango, Sugar infusion, Gelatin, Sorbitol, Firmness

INTRODUCTION

Mango is recognized as a good source of vitamins, minerals, and fiber, including carotenoids. As the climacteric fruit, mango can possess physical, chemical, and microbial deterioration because of their postharvest physiology [1].

Sugar-infusion and drying is the process that can preserve the fruit by using a high concentration of sucrose solution ca. 65-70% infusion to the fruit pulp [2] [3]. The high sugar content in combination with low water activity prevents the growth of microorganisms, thus prolonging the shelf-life [4]. Likewise, the sugar-infused dried fruit is candied dried fruit which applies to various fruits, including plums, dates, cashews, apples, pineapple, mango, and papaya [5]. The high amounts of sugar in the thermal process and final product can cause a significant change of physicochemical property during the processing and storage [3]. High-intensity sugar may be considered sweeteners for dried fruit where browning reactions are not desired. The non sulphitation treatment of sugar-infused dried fruits guarantees food safety, but the product can occur browning reaction during process and storage. The Maillard reactions are mainly formed by the heating dried fruits which significantly affects the quality of products.

Sugar alcohols or polyols are commercially available, such as sorbitol, maltitol, and mannitol. Polyols have a similar structure with sugar but differ in hydroxyl

instead of a carbonyl. Some fruits and vegetables, including mushrooms, contain polyols naturally. The sweetness of polyols is lower than sucrose but similarly in physical and chemical properties, such as water-binding capacity and ability to exist in crystalline and amorphous states. Polyols provide low caloric value and low glycemic index [6]. Some dried fruits, such as prunes and apricots, have high sorbitol levels, which can cause laxatives [4]. Sorbitol is in the list of Generally Recognized as Safe (GRAS). However, the warning must declare in the food labeling that ‘‘excess consumption may have a laxative effect’’ in case that consumed sorbitol higher than 50 g a day [7]. Reduced hardness in mango jam with increasing sorbitol substitution is based on the hydrogen bonding pattern of sorbitol with water was more pronounced than sucrose [8]. Lin et al. [9] reported that sugar alcohols could not cause the Maillard reaction. Naknean et al. [10] reported that sorbitol could reduce the brown color in Osmo-dried cantaloupe.This study investigated the relationships between sorbitol and gelatin content on the characteristics of sugar-infused dried mango (SIDM). The present study was aimed to optimize sorbitol and gelatin content for producing SIDM and evaluated the physiochemical property of the final product.

MATERIALS AND METHODS

Materials

Fresh hard-ripe ‘Nam Dok Mai Si Thong’ mangoes (Firmness approximately 6.17 N) were obtained from a regional superstore in Chiang Mai province (Thailand) and stored at 4ºC before use. No physical damage and similar fruit size were selected. Granulated sugar (Mitr Phol brand, Thailand) and gelatin (food grade, McGarrett brand, Thailand) were obtained from a regional supermarket, and powdered sorbitol was purchased from UnionScience (Chiang Mai, Thailand). All chemicals used were of analytical grade.

Methods

Sample preparation

The mango pieces at a hard-ripe stage were prepared in-cylinder ca. 53 mm (diameter) and 12 mm (thickness) and controlled the total weight of 100 g. Each mango fruit could be cut into four pieces. Immerse four fresh mango pieces (weighing approximately 100 g) in the mixture of 50 g granulated sugar, 0.1 g of NaCl, powdered sorbitol (S), and gelatin (G) at a certain amount (Table 1), and heat it directly on a Teflon-coated flat pan for 30 min. The excess syrup was drained from the mango pieces prior to put into a hot air oven at a temperature of 70 ºC for 24 h or until the final weight was constant.

Physicochemical property

Water activity

The a_w of SIDM was measured at 25 °C (AquaLab Pre, Decagon Devices, Pullman, WA, USA).

pH

Five grams of sample was blended with 95 g of distilled water and used the suspension for pH measurement using pH meter (Metrohm-827 pH Lab Meter, Switzerland), calibrated with buffer standard at pH 4.0 and 7.0.

Total soluble solids

The total soluble solids (TSS) of SIDM was determined by blending the sample with distilled water at a dilution ratio of 5:15 (w/w), previously filtered by filter paper, and measured utilizing a hand refractometer (ATAGO; Atago Instruments, Tokyo, Japan). The dilution factor for TSS calculation is 4.

Titratable acidity

Blending a SIDM with distilled water at a ratio of 1: 10 and titrated the supernatant obtained with 0.1 N NaOH to the endpoint (pH 8.0). The titratable acidity (TA) was calculated as the malic acid content. The milliequivalent of anhydrous malic acid is 0.067 [11].

Total sugar and reducing sugar

The Lane and Eynon Volumetric method quantified the TS and RS content. The RS content was determined by titration with Fehling's reagents, while TS was analyzed after hydrolysis by HCl and then titrated with Fehling's reagents. The results were presented in the unit of g glucose per 100 g of sample [12].

Firmness test

The firmness of SIDM was measured using a texture analyzer (a TA.XT Plus Texture Analyzer, Stable Microsystems, Surrey, UK). The measurement was performed for 2-3 samples for each treatment. A 50 kg load cell and cylindrical probe (p/6) were equipped. The measurement conditions were set as follows: trigger load of 5 g, a target distance of 15 mm, pre-test, test, and post-test speed of 1 mm/s.

Color measurements

Color measurements were performed at least 3 points to cover one sample's surface area using a colorimeter (CR-400, Chromameter, Minolta Co. Ltd., Osaka, Japan). A white color standard calibration plate (CR-A43 Calibration plate) was used for calibration. The values were averaged to obtain the L^* (lightness), a^* (positive value of redness), b^* (positive value of yellowness), H^o (appearance color), and C^* (color intensity) of the particular trials.

Statistical analysis

The central composite design (CCD) with four replications at a center point has been carried out on 11 randomized runs using Minitab 14.0. The following equation was used to fit the quadratic regression model [13].

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

Principal component analysis (PCA) was performed to display the inter-correlation of all variables using SPSS 14.0.

Table 1 Levels of sorbitol and gelatin in the CCD

Variables	Levels		
	-1	0	1
Sorbitol (g/100 g mango)	0.00	12.50	25.00
Gelatin (g/100 g mango)	0.00	0.25	0.50

RESULTS AND DISCUSSION

The effects of sorbitol and gelatin levels on physiochemical properties of SIDM are presented in Tables 2, 3, and Fig. 1. As presented in Table 2, the a_w, TSS, pH, and acidity values were in the range of 0.497-0.571, 74.00-84.10 ºBrix, 3.26-3.96, and 0.23-0.62 g/100 g, respectively. The sugar-infused dried fruits have a high concentration of sucrose solution ca. 65-70% which can prevent the growth of microorganisms [2] [4]. The firmness, TS, and RS values were in the range of 15.15-29.83 N, 58.05-75.97 g per 100 g, 35.83-51.80 g per 100 g, respectively. As for the effects of sorbitol on the firmness, the high firmness values were observed at Run 1, 3, and 5, which no adding sorbitol. Whereas the low firmness values were observed at Run 2 (SG ratio of 25.00:0.00), Run 6 (SG ratio of 25.00:0.25), and Run 7 (SG ratio of 12.50:0.00) (Table 2). Naknean et al. [10] reported that hardness was significantly lower in sorbitol-treated dried cantaloupe samples, while it was maximum in sucrose-treated samples. The most significant amount of sugars was absorbed when produces SIDM with heat. The lowest TS was observed in Run 3 (SG ratio of 0.00:0.50), Run 6 (SG ratio of 25.00:0.25), and Run 7 (SG ratio of 12.50:0.00). The use of sorbitol could reduce a_w, RS, and TS content in the final product compared to sucrose [10].

Table 2 Water activity and physiochemical properties of sugar-infused dried mango (SIDM)

Run	Sorbitol	gelatin	a_w	TSS (°Brix)	pH	Acidity (g/100 g)	Firmness (N)	TS (g/100 g)
1	0.00	0.00	0.568	74.00	3.72	0.62	28.77	71.31
2	25.00	0.00	0.571	77.30	3.26	0.35	15.44	68.30
3	0.00	0.50	0.546	76.70	3.62	0.24	24.90	58.95
4	25.00	0.50	0.508	77.73	3.51	0.38	17.67	65.50
5	0.00	0.25	0.497	84.10	3.90	0.27	29.83	65.55
6	25.00	0.25	0.522	78.10	3.30	0.36	15.31	58.16
7	12.50	0.00	0.534	79.80	3.52	0.35	15.15	58.05
8	12.50	0.25	0.550	78.20	3.88	0.23	19.98	71.40
9	12.50	0.25	0.544	76.60	3.42	0.44	23.04	75.97
10	12.50	0.25	0.547	80.40	3.96	0.55	21.86	63.90
11	12.50	0.25	0.550	81.00	3.51	0.34	21.08	67.02
Run	Sorbitol	gelatin	RS (g/100 g)	L^*	a^*	b^*	H^o	C^*
1	0.00	0.00	43.30	51.77	4.38	20.46	78.04	20.93
2	25.00	0.00	43.15	53.67	1.39	44.00	88.14	44.02
3	0.00	0.50	44.83	50.46	7.98	28.83	73.10	30.04
4	25.00	0.50	51.80	46.63	2.10	30.73	86.10	30.80
5	0.00	0.25	45.19	49.63	8.44	27.64	72.80	28.90
6	25.00	0.25	24.77	49.12	6.52	37.65	80.16	38.22
7	12.50	0.00	49.64	52.96	4.69	35.82	82.52	36.14
8	12.50	0.25	39.27	50.56	6.87	38.48	79.66	39.10
9	12.50	0.25	40.22	50.08	3.73	28.02	82.52	28.27
10	12.50	0.25	44.70	48.84	4.18	20.48	78.46	20.90
11	12.50	0.25	35.83	48.34	5.67	18.62	77.24	22.37

a_w = water activity; TSS= total soluble solid; TS=Total sugar; RS=reducing sugar.

The L^*, a^*, b^*, H^o, and C^* values were in the range of 46.63-53.67, 1.39-8.44, 18.62-44.00, 72.80-88.14, 20.90-44.02, respectively. Maenhiran et al. [14] reported that dried 'Nam Dok Mai Si Thong' mango had L^* value of 53.00-56.34,

a^* value of 5.19-7.85, b^* value of 29.02-35.17, and H^o value of 74.60-79.49, and C^* value of 30.14-35.83. The H^o values that have values in the range of 60-90 present yellow to lime color [15].

The coefficients of quadratic model in Table 3 revealed that sorbitol content had a significant adverse effect on the firmness of the SIDM ($P < 0.05$). At the same time, gelatin had a significant negative impact on the L^* (brightness) and H^o values (appearance color) of SIDM. It was increasing in sorbitol content and decreasing in firmness of SIDM. Gelatin content increased and decreased in the brightness and appearance color of SIDM.

The contour plot of firmness, RS, a^* value, and H^o value of SIDM as a function of sorbitol and gelatin content is shown in figure 1 . The lowest firmness and a^* value are presented in the high content of sorbitol (dark blue area of contour plot). Naknean et al. [10] indicated that invert sugar could enable the Maillard reaction during the drying process of dried cantaloupe samples, resulting in a high intensity of browning formation and increased redness (positive a^* value). The Maillard reaction begins with condensation between an amino protein group and an a-hydroxyl carbonyl moiety of RS in foods. In contrast, sugar alcohols cannot cause the Maillard reaction [9]. Naknean et al. [10] reported that sorbitol could reduce the brown color in Osmo-dried cantaloupe.

Table 3 Estimated Regression Coefficients

Term	Coefficient					
	a_w	TSS	pH	Acidity	Firmness	TS
Constant	0.545	76.739	3.758	0.548	28.171	68.509
sorbitol	0.003	0.026	-0.010	-0.008	-1.076*	-0.046
gelatin	-0.125	23.444	0.552	-0.767	17.538	2.581
sorbitol*sorbitol	-0.000	-0.000	-0.000	-0.000	0.019	-0.008
gelatin*gelatin	0.223	-41.600	-1.47	0.200	-45.333	-37.293
sorbitol*gelatin	-0.003	-0.181	0.028	0.033	0.488	0.764
R^2	0.423	0.285	0.562	0.433	0.902	0.183
Lack of fit	0.001	0.117	0.796	0.549	0.082	0.162
Term	Coefficient					
	RS	L^*	a^*	b^*	H^o	C^*
Constant	47.581	51.727	4.883	23.099	77.944	23.488
sorbitol	0.554	0.103	-0.242	0.431	0.636	0.455
gelatin	-87.584	-13.134*	22.391	-0.748	-37.698*	5.384
sorbitol*sorbitol	-0.035	-0.001	0.006	0.019	-0.012	0.017
gelatin*gelatin	180.053	20.877	-34.093	26.223	58.810	17.203
sorbitol*gelatin	0.570	-0.458*	-0.232	-1.731	0.232	-1.787
R^2	0.564	0.921	0.686	0.576	0.883	0.590
Lack of fit	0.073	0.964	0.239	0.774	0.432	0.752

* Significant ($P < 0.05$) a_w = water activity; TSS= total soluble solid; TS=Total sugar; RS=reducing sugar.

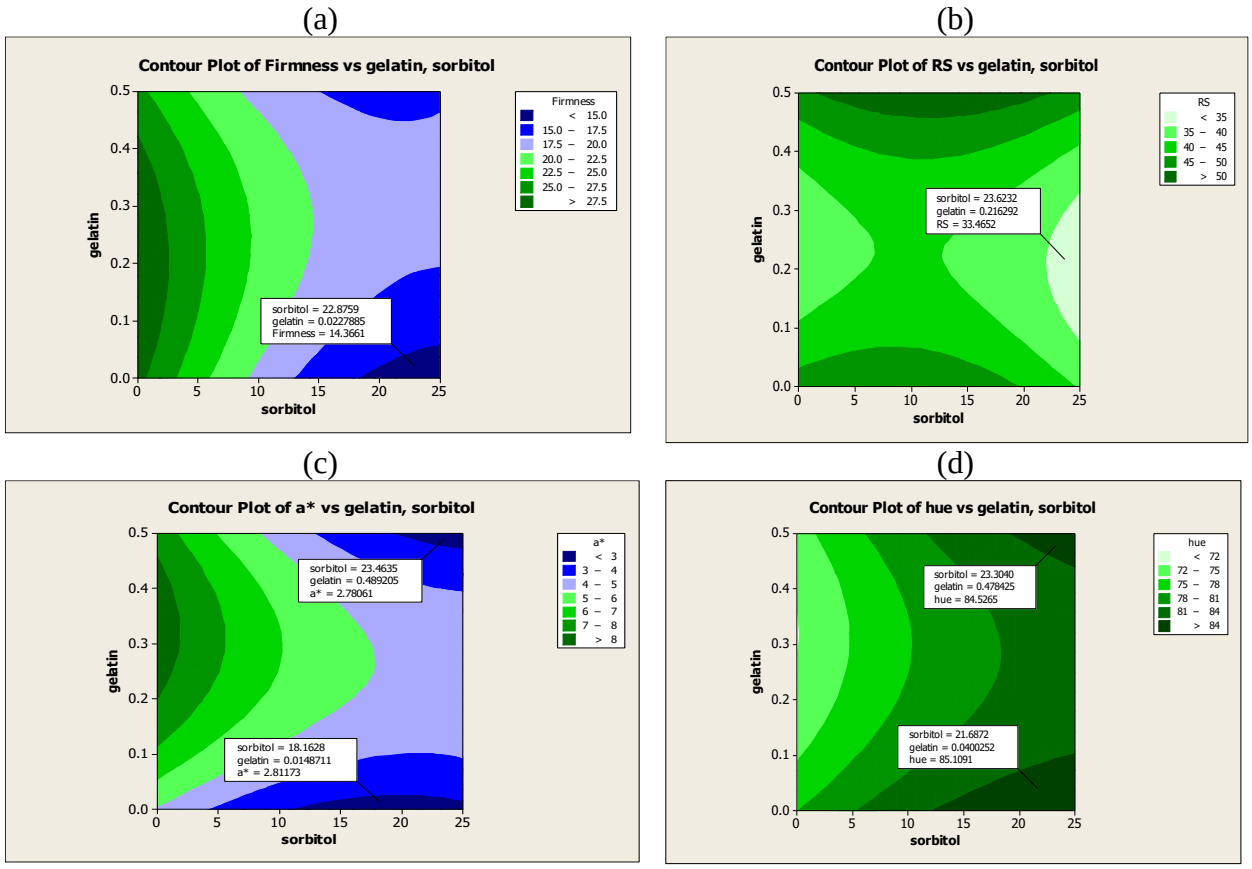


Figure 1 Contour plot of (a) firmness, (b) reducing sugar, (c) a* value, and H^o value of sugar-infused dried mango (SIDM) as a function of sorbitol and gelatin content

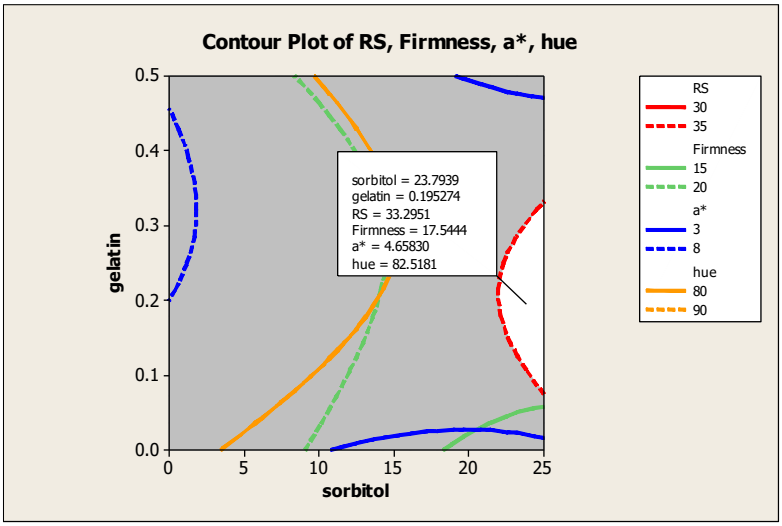
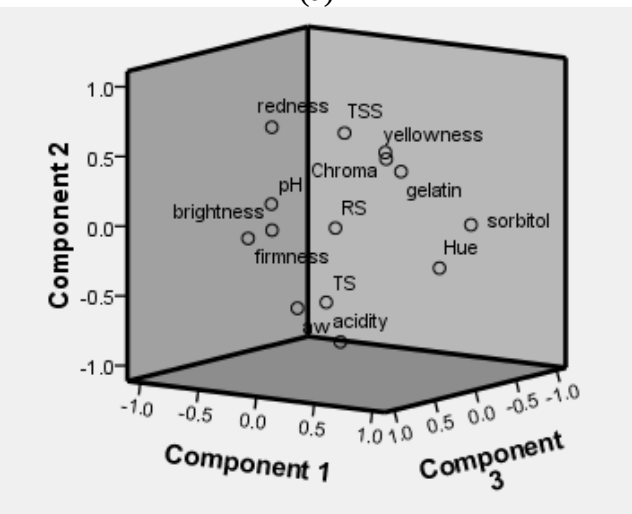


Figure 2 Overlaid contour plot of reducing sugar (RS), firmness, a* value, and H^o value of sugar-infused dried mango (SIDM)

(a)



(b)

	Component			
	1	2	3	4
sorbitol	0.949			
gelatin			-0.786	
aw		-0.539	0.652	
TSS		0.546	-0.338	
TS		-0.575		
RS				0.937
pH	-0.734			0.383
acidity		-0.881		
firmness	-0.884			
brightness			0.979	
redness	-0.722	0.572		-0.349
yellowness	0.642	0.560	0.429	
Hue	0.924			
Chroma	0.627	0.605	0.418	

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 7 iterations.

Figure 3 Principal component analysis (a) Component plot in rotated space and (b) Loading of components and physicochemical variables of 11 sugar-infused dried mango (SIDM) samples.

The study uses CCD to optimize the sorbitol and gelatin content to enhance the quality of SIDM. The overlay plot displayed optimal process variable settings (blank area). The low redness value (a* value of 4.66) and firmness (firmness of 17.54 N) was obtained using the process variables such as the sorbitol content of 23.79 g/100g and the gelatin content of 0.20 g/100 g of mango.

This investigation highlighted that sorbitol content might significantly affect firmness and anti-browning reaction. In contrast, gelatin content may have a significant effect on a_w, brightness, and a small positive effect on TSS (sugar loading) of SIDF.

A PCA was performed (Figure 3). The correlation matrix shows various levels of correlation, with coefficients above magnitude 0.615 being significant. The component matrix in the table gives the loading of each variable on the components. A high magnitude (near to +1 or -1) for loading means that the variable is loads negatively on component 3. RS loads strongly and positively on component 4. components are the factors extracted by analysis, and component 1 explains almost 35%, and by four components, almost 81% is explained. The component plot shows a spread of loadings with firmness, pH, and a* value separated from H° value, b* value, C* value, and sorbitol content on component 1. Component 2 shows a separation linked to high acidity and moderate TS and aw from a*, b*, C* values, and TSS. Component 3 shows a separation linked to high gelatin content and low TSS from L*, b*, C* values, and aw. Component 4 shows a separation linked to a* value from RS and pH. highly correlated with that factor, but higher than 0.3 can be enough for importance [16]. Thus sorbitol content, Hue (H^o value), yellowness (b^* value), Chroma (C^* value) load firmly on component 1, whereas firmness, pH, and redness (a^* value) load negatively on component 1. Acidity loads strongly and negatively on component 2, whereas gelatin

In terms of concealed information, sorbitol is associated with a firmness of texture, pH, and redness (a^* value), possibly linked to a sorbitol content loaded in SIDM and reduction of browning reaction of sorbitol. Gelatin content is related to a_w, which may reflect formulation contents in terms of the free water-binding capacity of gelling agent. Water activity is an important property that allows predicting product quality changes during storage [3]. Gelatin content is also related to brightness (L^* value). The sorbitol-treated dried cantaloupe samples had a higher L^* value and lower a^* value and browning intensity than sucrose and invert sugar-treated samples, respectively [10].

CONCLUSION

This result presents the potential of the effect of sorbitol and gelatin content on the firmness and anti-browning of SIDM. The combining process of sugar infusion with heat, followed by hot air drying, has been proposed to obtain SIDM without sulfur dioxide. This modified process provides the final product with natural color and soft texture. The high sorbitol content is associated with a soft texture of SIDM and a low positive a^* value, possibly linked to a reduction of the browning reaction of sorbitol. Gelatin content is related to a_w and brightness (L^* value) of SIDM.

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Development of high-fiber and gluten-free pasta from cassava flour

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ABSTRACT: The main objectives of this research were to develop a high-fiber and gluten-free pasta from cassava flour (55%) with the addition of rice flour (30%, 20%, 10%, and 0%), corn starch (0%, 10%, 20%, and 30%) and hydrocolloid (guar gum 1% and 2% w/w of the composite flour), and to compare the developed pasta with a control pasta (cassava flour: cassava fiber ratio 85:15) and commercial gluten-free cassava pasta. These starch and flour pasta blends were evaluated in terms of their chemical composition, swelling power (SP) and water solubility index (WSI), and pasting properties. Among the various blends studied, the cassava flour, rice flour, and cassava fiber at a ratio of 55:30:15 with a combination of 2% w/w guar gum (RF30GG2) significantly increased peak (PV), trough (TV), setback (SB), and final viscosity (FV), but decreased breakdown (BD) of pasting properties ($p \leq 0.05$) compared to the control flour. In the sheeting-cutting process, the addition of rice flour and guar gum significantly improved the cooking properties and firmness of cooked pasta. RF30GG2 exhibited a dietary fiber content of 11.51±0.84% db, which is considered a high-fiber food. This pasta formula had the best cooking properties and firmness, comparable with commercial gluten-free cassava pasta with no significant difference ($p > 0.05$). Moreover, sensory evaluation using a 9-point hedonic scale showed that RF30GG2 had the most desirable texture and overall acceptability.

Keywords: *Gluten-free pasta, Cassava flour, Cassava fiber, High-fiber, Guar gum*

INTRODUCTION

The number of celiac disease sufferers is rising significantly every day, leading to increased demand for gluten-free products [1]. Avoiding the consumption of gluten sources (wheat, barley, rye, and malt) is considered the only treatment for patients with celiac disease [2]. Globally, pasta is one of the most popular staple foods due to its simple preparation, deliciousness, variety, and richness in carbohydrates [3]. Unfortunately, celiac disease patients cannot consume pasta mainly made of wheat semolina containing high gluten. Hence, more gluten-free pasta varieties made of other cereal or tuber flours have been developed for these patients as alternatives.

Pasta with good cooking resistance, firm texture, and low cooking loss is considered a high-quality cooked pasta [4, 5]. Disulfide bonding among gluten molecules creates a three-dimensional network contributing to the excellent textural and cooking qualities of pasta. The key factor for the quality of gluten-free pasta is the presence of an organized structure composed of retrograded starch formed during the different steps of the pasta-making process [6]. At the same time, the amylose content of starch is an important parameter for the behavior of composite flour because amylose can form a gel network through strong linkage and promote good cooking properties in gluten-free pasta products [6, 7]. In general, the use of starches with high amylose content, gums, and/or

hydrocolloids is the best means to improve the cooking and texture qualities of gluten-free products.

Cassava (*Manihot esculenta*, Euphorbiaceae), also called manioc, tapioca or yucca, is widely used in the food industry because of its high production capacity and low cost [8]. It has mainly been employed as a thickener and stabilizer in food products [9]. Cassava starches contain lower amylose content (23.5%) than rice and corn starch (25% and 29.4%, respectively) [10], as well as exhibiting relatively low gelatinization temperature, high swelling power and solubility, and a low degree of retrogradation, resulting in a clear cohesive soft gel compared to cereal starches [11]. Therefore, they cannot be solely used for any set-gel-like structure foods such as noodles or pasta without blending with other high amylose starches. Cassava fiber (cassava pulp or cassava bagasse) is a byproduct of cassava flour production, consisting mainly of starch and fiber [12]. In recent years, dietary fiber has been used to fortify many foods, including pasta, as functional foods to support digestive health [13].

One of the main objectives of this research was to develop a high-fiber and gluten-free pasta made from cassava flour with the addition of rice flour, corn starch, and guar gum. The pasta was made through the sheeting-cutting process.

MATERIALS AND METHODS

Materials

The pasta blends were developed using the following food-grade ingredients: Cassava flour with 7.42±0.10% db total dietary fiber (Sanguan Wongse Industries Co., Ltd., Nakhon Ratchasima, Thailand), rice flour (General Food Products Co., Ltd., Nakhon Ratchasima, Thailand), corn starch (Vijayanagar Biotech Pvt. Ltd., India), cassava fiber with 54.75±1.01% db total dietary fiber (T-Fiber Innovation Co., Ltd., Nakhon Ratchasima, Thailand), guar gum (F.A. International Co., Ltd., Bangkok, Thailand), and commercial gluten-free cassava pasta (Jovial Foods, Inc., North Stonington, USA). Cassava and rice starches were isolated from cassava and rice flours using the alkali method [14].

Chemical composition

Moisture, crude protein, crude fat, ash, carbohydrate, and total dietary fiber contents of starches and flours were determined according to the methods approved by the AOAC [15]. The apparent amylose content of starch was determined by amperometric titration with potassium iodate solution according to Gibson et al. [16].

Swelling power and water solubility index

Swelling power (SP) and water solubility index (WSI) of starches and flours at 40, 50, 60, 70, 80, and 90°C were determined according to the method described by Leach et al. [17].

Pasta making by sheeting-cutting process

The pasta was formulated based on 100-g dry ingredients consisting of cassava flour mixed with rice flour, corn starch, and cassava fiber (Table 1). Dry ingredients (100 g) and water (90 g) were roughly mixed to form a dough using a KitchenAid stand mixer (Heavy Duty Bowl-Lift 5Quart, Whirlpool Corporation, Ohio, USA) at speed 2 (low) for 5 min. Afterward, the dough was heated by kneading with a spatula on a non-stick pan until the internal temperature of the dough was between 50-55°C. The dough was again mixed with the KitchenAid stand mixer to form a smoother dough at speed 4 (medium-low) for 5 min. The dough was covered with plastic wrap and rested at room temperature (25±2°C) for 10 min. Then, the dough was cut into 20 cm long, 5 mm wide, and 1 mm thick using a pasta maker machine (Atlas150, Marcato s.r.l, Campodarsego, Italy). The pasta was dried for 4 hrs at 50±5°C in a hot-air oven to achieve a moisture content of less than 12%. The pasta formula that obtained the best characteristics in terms of pasting, cooking, and texture properties was selected for further enrichment with guar gum at 1 and 2% w/w.

Table 1 High-fiber and gluten-free pasta formulations

Pasta formula	Cassava flour (g)	Rice flour (g)	Corn starch (g)	Cassava fiber (g)
Control	85	0	0	15
RF30	55	30	0	15
RF20CS10	55	20	10	15
RF10CS20	55	10	20	15
CS30	55	0	30	15

Pasting properties

The pasting properties of starch, flour, and composite flour formulated were evaluated by a Rapid Visco Analyzer (RVA-4, Newport Scientific Pty. Ltd., Warriewood, Australia) according to AACC method 61-02.01 [18].

Pasta cooking quality and cooked pasta firmness

Cooking quality and pasta firmness were determined according to the AACC method 66-50.01 [18]. For the optimal cooking time (OCT), 25 grams of pasta were added to 300 ml of boiling distilled water. Every 30 seconds, a piece of pasta was pressed between two glass plates until the starchy central core was no longer visible. After boiling, the pasta was rinsed in cold water and drained in a sieve for 15 min at room temperature, and then weighed to determine cooking yield % (CY). Thereafter, the cooking water was collected and evaporated at 115 °C for 24 hrs to determine cooking loss % (CL). The firmness of the cooked pasta was determined by a compression test using a texture analyzer (Model TA Prime, Tech Quality Co., Ltd., Bangkok, Thailand) with a thin knife blade. Pasta strands were aligned on the base plate (4 cm wide and 10 cm long) with no space between them and cut with the blade across the strands, with the test speed set at 0.5 mm/s and the post-test speed set at 10.0 mm/s.

Sensory evaluation

Sensory evaluation was conducted with 30 panelists who were employees of Sanguan Wongse Industries Co., Ltd., Thailand. COA No.190/2564 from the Office for Human Research Protections (OHRP). Pasta samples were cooked in boiling water to their optimal cooking time and served to panelists with random three-digit codes. The sensory attributes: appearance, color, aroma, taste, texture, and overall acceptability of the cooked pasta were evaluated using a 9-point hedonic scale (1= Dislike the most, 5 = Neither like nor dislike, 9 = Like the most).

Statistical analysis

All experiments were performed in three replications, except for sensory evaluation (n = 30). The results were expressed as mean ± standard deviation and analyzed using one-way analysis of variance. The significance of differences was analyzed with Duncan's new multiple range test (MRT) at a level of 0.05 by using IBM SPSS version 25 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Chemical compositions

The chemical compositions of starches and flours are shown in Table 2. Cassava flour contains a low amount of crude protein. Normally, tubers such as potatoes, sweet potatoes, and cassava are low in storage protein. Rice flour contains a much higher amount of crude protein than cassava flour. This protein was a storage protein surrounding starch granules within rice endosperm [19]. The contents of crude fat and ash were less than 1% in all samples (Table 2). The ash content could be caused by the number of mineral components such as sodium, magnesium, calcium, and potassium in the starch and flour. Cassava flour contained significantly ($p \leq 0.05$) higher in crude fiber than other flour and starch, which were undetectable in crude fiber. Therefore, only cassava flour and starch were analyzed for their dietary fiber contents. Accordingly, cassava flour is a good fiber source for making high-fiber pasta. Rice and corn starch contained significantly ($p \leq 0.05$) higher amylose than cassava starch. Therefore, they could contribute to a three-dimensional network within a gluten-free cassava pasta having good cooking properties [6, 7].

Table 2 Chemical composition of starch and flour[A]

Sample[B]	Chemical composition (% db)						Amylose g/100g defatted sample
	Crude protein	Crude fat	Ash	Crude fiber	Carbohydrate	Dietary fiber	
CAS	0.10^{e}±0.00	0.16^{c}±0.01	0.20^{c}±0.03	0.00^{b}±0.00	99.54^{a}±0.04	0.61^{b}±0.01	16.31^{c}±0.16
CAF	0.91^{c}±0.04	0.35^{b}±0.09	0.43^{b}±0.02	1.18^{a}±0.06	97.14^{c}±0.06	7.42^{a}±0.10	NA[C]
RS	1.06^{b}±0.00	NA	NA	NA	NA	NA	21.35^{b}±0.15
RF	6.93^{a}±0.10	0.47^{a}±0.04	0.58^{a}±0.03	0.00^{b}±0.00	92.01^{d}±0.13	NA	NA
CS	0.26^{d}±0.01	0.27^{b}±0.05	0.12^{d}±0.01	0.00^{b}±0.00	99.35^{b}±0.04	NA	23.15^{a}±0.49

[A] Different superscript letters in the same column indicate significant differences ($p \leq 0.05$).
[B] CAS: Cassava starch; CAF: Cassava flour; RS: Rice starch; RF: Rice flour; CS: Corn starch.
[C] NA: data not available.

Swelling power and water solubility index

SP and WSI indicate the gelatinization and dispersion of starch granules when heated in water. The determining factors for SP and WSI include the type of starch, the ratio of amylose to amylopectin, temperature, and the presence of other components (such as lipid, protein, fiber, phosphorus) [20, 21]. The SP and WSI of starches and flours increased with a temperature rise of 50-90°C (Figs. 1a and 1b, respectively).

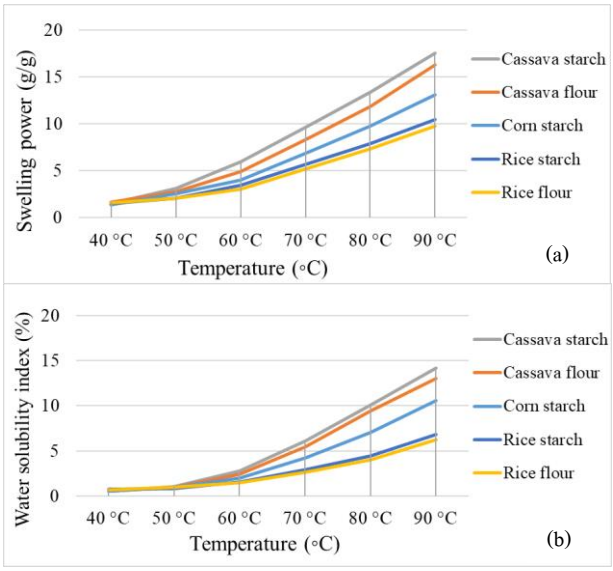

Fig. 1 Swelling power (a), and water solubility index (b) of starches and flours

Both rice and cassava flours had lower SP and WSI than their starch counterparts. Flours contain dietary fiber, fat, and protein (Table 2), which could retard water absorption of starch granules [22]. Rice flour exhibited much lower SP and WSI than cassava flour. In rice flour,
proteins and starches could form a matrix, causing uneven water distribution and hindering starch swelling [23]. Cassava starch exhibited higher SP and WSI than corn and rice starches at all temperatures (Fig. 1). This could be due to the relatively high phosphate group (0.001-0.008% db) in cassava starch [24]. The negatively charged phosphate groups are linked with amylopectin, which increases the hydration of cassava starch [20, 25]. Normally, cereal amylose is partly complex with lipids, and this amylose-lipid complex is mostly located on the surface of starch granules [26]. The hydrophobicity of the amylose-lipid complex could prevent water absorption of starch granules, resulting in low SP and WSI [27].

Pasting properties of starch, flour, and composite flour

The pasting properties of starch, flour, and composite flours are summarized in Table 3. Peak viscosity (PV) and pasting temperature (PTemp) of flours and starches were well relevant to their SP (Fig. 1a). Cassava and rice flours had significantly ($p \leq 0.05$) lower PV and higher PTemp than their starch counterparts.

The non-starch components of flour could delay starch swelling. Therefore, starch granules require a higher temperature to swell. Corn starch had the lowest PV but the highest AM content (Table 2).

Table 3 Pasting properties of starch, flour, and composite flour[A]

Sample[B]	Viscosity (RVU)[C]					PT (min)	PTemp (°C)
	PV	TV	BD	FV	SB		
Starch and flour							
CAS	533.4^{a}±4.2	186.6^{c}±3.3	346.8^{a}±0.9	278.8^{c}±0.2	92.2^{bc}±3.1	3.9^{h}±0.0	68.4^{g}±0.0
CAF	324.8^{c}±5.4	192.3^{c}±0.4	132.5^{b}±5.9	278.5^{c}±2.3	86.2^{cd}±2.8	4.3^{g}±0.0	70.4^{f}±0.6
RS	372.8^{b}±2.6	264.6^{a}±6.5	108.7^{c}±1.8	438.5^{a}±4.8	174.4^{a}±8.1	5.8^{b}±0.1	77.7^{b}±0.6
RF	272.6^{d}±2.5	207.1^{b}±1.6	65.5^{f}±4.1	382.4^{b}±3.2	175.3^{a}±4.8	6.5^{a}±0.1	90.5^{a}±0.6
CS	236.7^{g}±3.1	151.6^{f}±1.1	85.2^{d}±2.1	252.2^{d}±2.1	100.6^{b}±1.1	4.7^{f}±0.0	73.2^{c}±0.0
Composite flour							
Control	244.8^{f}±0.6	135.9^{hi}±1.2	108.8^{c}±0.6	208.3^{gh}±0.1	72.4^{e}±1.1	4.4^{g}±0.0	71.5^{e}±0.1
RF30	207.5^{i}±1.8	143.9^{g}±0.6	63.6^{f}±2.5	224.0^{f}±0.9	80.2^{de}±1.5	5.2^{d}±0.0	72.8^{cd}±0.6
RF20CS10	208.3^{i}±2.4	139.5^{gh}±2.2	68.9^{ef}±0.2	217.8^{fg}±1.2	78.3^{de}±1.0	5.0^{e}±0.0	72.4^{cde} ±0.1
RF10CS20	216.1^{h}±0.1	143.4^{g}±2.1	72.8^{e}±2.0	220.0^{f}±0.3	76.7^{de}±1.8	4.9^{e}±0.1	72.7^{cd}±0.6
CS30	217.7^{h}±2.7	130.7^{i}±3.9	87.0^{d}±1.2	203.7^{h}±3.9	73.0^{e}±0.0	4.6^{f}±0.0	72.0^{de}±0.5
RF30GG1	234.0^{g}±1.8	160.4^{e}±0.8	73.7^{e}±1.1	238.3^{e}±1.6	77.9^{de}±0.8	5.3^{c}±0.1	73.2^{c}±0.1
RF30GG2	257.3^{e}±5.5	169.5^{d}±4.4	87.8^{d}±1.1	253.7^{d}±3.2	84.1^{cd}±1.2	5.2^{d}±0.0	73.2^{c}±0.0

[A] Different superscript letters in the same column indicate significant differences ($p \leq 0.05$).
[B] CAS: Cassava starch; CAF: Cassava flour; RS: Rice starch; RF: Rice flour; CS: Corn starch; GG: Guar gum. Control, RF30, RF20CS10, RF10CS20, and CS30 denoted the pasta formulas as listed in Table 1. The addition of 1% and 2% guar gum in RF30 showed in RF30GG1 and RF30GG2, respectively.
[C] PV: Peak viscosity; TV: Trough; BD: Breakdown; FV: Final viscosity; SB: Setback; PT: Peak time; PTemp: Peak temperature

Amylose content showed a negative correlation with PV because amylose inhibits swelling and paste viscosity upon heating, while amylopectin readily absorbs water and swells [28, 29]. Breakdown (BD) indicates starch granule stability; a low BD value indicates high starch granule integrity [30]. Cassava starch exhibited much higher BD than rice and corn starches, which could indicate that cassava starch granules are weaker and more soluble than cereal starches. Therefore, cassava starch had a higher WSI than other starches (Fig. 1b). Setback (SB) indicates a tendency for starch retrogradation [30]. Rice flour and starch showed the highest SB, indicating a high rate of starch retrogradation, followed by corn starch, cassava starch, and cassava flour, respectively. Cassava flour had the lowest SB because it was low in amylose and high in fiber, which reduces the degree of starch retrogradation [31].

The pasting properties of the control flour were changed to a certain extent according to the pasting properties of the additional rice flour and corn starch (Table 3). The control flour contained only cassava flour and cassava fiber, and therefore exhibited relatively high PV and BD, and low SB. Flour with these pasting characteristics might not be suitable for making pasta in the sheeting-cutting process due to lacking a firm network structure. Substitution with rice flour and corn starch, especially RF30, was able to decrease PV and BD, and increase SB of the control flour more than RF20CS10, RF10CS20, and CS30. Therefore, the RF30 formula was selected for further addition with guar gum at 1% and 2% w/w (RF30GG1 and RF30GG2). Guar gum increased all the pasting parameters of the composite flour (RF30) because it enhances the ability of starch granules to swell and enclose other components in the composite flour during gelatinization according to Kaur et al. [32], who also observed this similar effect of guar gum on the viscosity of potato, corn, and mung bean starch pastes. With the highest SB, RF30GG2 flour is probably the most suitable for making high-fiber and gluten-free pasta from cassava flour.

Table 4 Cooking properties and firmness of pasta[A]

Pasta[B]	Optimal cooking time (min)	Cooking yield (%)	Cooking loss (%)	Firmness (gf)
Control	7.5	107.29^{d}±2.67	10.66^{a}±0.44	25.90^{e}±1.49
RF30	7.5	118.65^{b}±0.88	6.53^{cd}±0.52	38.64^{ab}±0.61
RF20CS10	7.5	115.09^{bc}±1.43	6.75^{cd}±0.89	36.97^{b}±2.04
RF10CS20	7.5	113.04^{c}±0.76	7.15^{c}±0.41	32.00^{c}±1.16
CS30	7.5	111.11^{cd}±1.91	8.80^{b}±0.98	30.43^{cd}±1.58
RF30GG1	7.5	124.93^{a}±5.42	6.39^{cd}±0.14	39.30^{ab}±3.28
RF30GG2	7.5	128.30^{a}±1.82	6.01^{d}±0.08	39.66^{ab}±2.57
Commercial	12	129.76^{a}±1.42	5.91^{d}±0.11	40.77^{a}±2.92

[A] Different superscript letters in the same column indicate significant differences ($p \leq 0.05$).
[B] RF: Rice flour; CS: Corn starch; GG: Guar gum. Control, RF30, RF20CS10, RF10CS20, and CS30 denoted the pasta formulas as listed in Table 1. The addition of 1% and 2% guar gum in RF30 showed in RF30GG1 and RF30GG2, respectively.

Cooking properties of pasta and cooked pasta firmness

The cooking properties of pasta and the firmness of cooked pasta are summarized in Table 4. All pasta samples had the same optimal cooking time (OCT) at 7.5 min. The cooking yield (CY) of the control pasta was significantly ($p \leq 0.05$) lower than that of the other gluten-free pasta with rice flour and corn starch substitution (RF30, RF20CS10, and RF10CS20). The high CY in the pasta could be attributed to the high amylose content in rice and corn starch (Table 2), which can form a three-dimensional network through strong linkage during retrogradation. This 3D network can promote water retention during cooking and increase the CY of gluten-free pasta [6, 7].

The cooking loss (CL) of the control pasta was the highest, followed by the pasta containing rice flour and corn starch. This could be due to the solubility of each ingredient (Fig. 1b), with the solubility of cassava flour being higher than that of corn starch and rice flour, leading to an increase in CL of the control pasta. Moreover, the high fiber content of cassava fiber and flour, which is responsible for weakening the gluten-free starch network, might increase the CL of pasta [33, 34]. Replacing cassava flour with rice flour and corn starch in gluten-free pasta (RF30, RF20CS10, RF10CS20, and CS30) can reduce CL.

The firmness of cooked pasta ($p \leq 0.05$) increased significantly with the substitution of rice flour and corn starch. This could be due to the low SP of rice and corn starches (Fig. 1a). During cooking, starch granules in pasta structure absorb water and swell by gelatinization. If the starch granules inside the pasta swell at a low degree and keep their integrity, these intact swollen granules will help the pasta to resist the applied force. In contrast, the pasta will not have any large solid particles to resist the applied force if the starch granules swell at a high degree until complete collapse. RF30 showed a firmer texture than the control pasta and the other pasta containing corn starch. Therefore, RF30 was selected for further fortification with guar gum at a concentration of 1% and 2% (w/w).

Gluten-free pasta with the addition of 1% and 2% guar gum (RF30GG1 and RF30GG2) did not differ significantly ($p > 0.05$) from commercial gluten-free cassava pasta in terms of CY, CL, and firmness. Similarly, Sabbatini et al. [35] reported that the addition of 2% guar gum can improve cooking qualities and provide firmer gluten-free noodles. This is due to the solubility of guar gum, which encapsulates the starch matrix and other components of gluten-free pasta during cooking to reduce CL [23]. Guar gum could form a complex with amylose and increase water binding capacity to increase the CY of pasta [34, 36]. Moreover, the hydrophilic component of guar gum hydrocolloid interacts with proteins in rice flour (6.93% db in Table 2)

Table 5 Sensory evaluation of high-fiber and gluten-free pasta and commercial gluten-free cassava pasta using the 9-point hedonic scale (n = 30)

Pasta[C]	Appearance [A]	Color [A]	Aroma[B]	Taste[B]	Texture [A]	Overall acceptability [A]
Control	6.33^{b}±0.92	6.17^{b}±1.29	7.17±1.39	6.63±1.25	5.20^{d}±0.96	5.10^{d}±0.55
RF30	6.47^{b}±1.80	6.30^{ab}±1.66	7.20±1.27	6.87±1.33	6.47^{bc}±1.31	6.83^{b}±1.12
RF20CS10	6.43^{b}±1.65	6.40^{ab}±1.48	7.37±1.19	6.66±0.89	6.07^{c}±0.69	6.13^{c}±0.68
RF10CS20	6.35^{b}±0.92	6.39^{ab}±0.86	7.21±1.73	6.61±1.80	5.89^{c}±1.65	5.99^{c}±1.22
CS30	6.42^{b}±1.29	6.41^{ab}±1.43	7.25±1.49	6.67±1.66	5.23^{d}±1.48	5.43^{cd}±1.19
RF30GG1	6.60^{b}±1.73	6.20^{b}±1.49	7.23±1.36	6.93±1.46	7.00^{ab}±1.20	7.03^{ab}±1.16
RF30GG2	6.83^{ab}±0.86	6.50^{ab}±1.43	7.37±1.19	6.99±1.03	7.47^{a}±1.20	7.53^{a}±1.07
Commercial	7.13^{a}±0.86	7.03^{a}±1.19	7.23±1.04	6.87±1.20	6.87^{ab}±1.35	7.37^{a}±1.10

[A] Different superscript letters in the same column indicate significant differences (p≤0.05).
[B] Means within the same column were not significantly different (p>0.05).
[C] RF: Rice flour; CS: Corn starch; GG: Guar gum. Control, RF30, RF20CS10, RF10CS20, and CS30 denoted the pasta formulas as listed in Table 1. The addition of 1% and 2% guar gum in RF30 showed in RF30GG1 and RF30GG2, respectively.

due to ionic charges, thus maintaining a suitable level of swollen starch granules and indirectly strengthening the firmness of pasta [37].

Sensory evaluation of different formulated pasta

Sensory evaluation of high-fiber and gluten-free pasta and commercial gluten-free cassava pasta revealed that samples exhibited no significant difference ($p>0.05$) in terms of aroma and taste, as shown in Table 5. The texture and overall acceptability scores of the control pasta and CS30 were significantly lower than those of the commercial gluten-free cassava pasta and the others containing rice flour and guar gum. The sensory attributes of RF30GG2 containing cassava flour, rice flour, and cassava fiber at a ratio of 55:30:15 with a combination of 2% (w/w) guar gum were not significantly different ($p>0.05$) compared to commercial gluten-free cassava pasta. In agreement with Sabbatini et al. [35], the addition of 2% guar gum to gluten-free noodles resulted in the highest sensory score.

CONCLUSIONS

This research developed high-fiber and gluten-free pasta made from cassava flour combined with rice flour and guar gum. The pasta formula RF30GG2 (cassava flour: rice flour: cassava fiber in the ratio of 55:30:15 and 2% w/w guar gum) contained a dietary fiber content of 11.51±0.84% db (or 25% of Thai RDI), which could be considered a high-fiber food according to the notification by the Ministry of Public Health concerning nutrition labeling. This RF30GG2 pasta exhibited cooking properties, firmness, and sensory attributes which were not significantly different ($p>0.05$) from those of commercial gluten-free cassava pasta.

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Cereal bar flavored with curried Thai river sprat fish

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ABSTRACT: The objective of this study was to develop and evaluate chemical composition, sensory properties and microbiological of cereal bars flavored with curried Thai river sprat fish. The main ingredients were puffed brown rice, dried Thai river sprat fish, pumpkin seeds, sunflower seeds, and dried Thai herbs (lemon grass, dill, basil, shallot, and chili) .Cereal bars were developed with Thai river sprat fish in three different levels: 0%,12% and 24% of the total weight, respectively. The adding of Thai river sprat fish did not influence the amount of moisture, lipids, carbohydrates, and energy values. Whereas it significantly affected the greater levels of protein (6.05 to 13.24%) and ash content (1.18 to 9.22%). Aw value (0.36 to 0.39) and moisture content of all samples (4.86 to 4.98%) were relatively low, they could be classified as low-moisture foods. The total aerobic plate count, yeast and molds were within the recommended guidelines for the human consumption. The addition of Thai river sprat fish resulted in lower score in terms of texture, flavour and overall liking. However, all formulas were accepted in all sensory attributes with overall liking scores ranged 7.8-6.6 (like very much to like moderately). These results demonstrate that the adding of 12 to 24% Thai river sprat fish is effective for increasing the protein and mineral matter of cereal bars with being accepted by consumers.

Keywords: Chemical composition, Cereal bars, Product development, Thai river sprat fish

INTRODUCTION

Cereal bars are a type of snack that is eaten between main meals. They are often made of a base of processed cereal grains and may have various ingredients such as nuts, seeds, fruit, raisins, and chocolate [1]. They can be added with vitamins, minerals, and dietary fiber to make them into a healthy food [2]. Many processes have been used to improve the nutritional composition of foods; such as, the partial or total substitution of some traditional ingredients by alternative products, and the development of products that add nutritional quality with low cost [3].

Due to brown rice is an important agricultural crop in Thailand with high fiber content. Moreover Thailand is rich source of freshwater fish; Thai river sprat fish that is commonly found in standing waters and large

rivers, such as Sirindhorn Dam Reservoir, Ubon Ratchathani Province and Lampao Dam Reservoir Kalasin Province. It is low cost fish but highly nutritious in protein and calcium. Therefore, brown rice and Thai river sprat fish can be a good alternative to improve the nutritional quality of cereal bars. Further, in addition to improving their nutritional potential, the addition of Thai river sprat fish could promote, even indirectly, fish consumption. Thus, the objective of this study is to develop and evaluate the microbiological, chemical composition, and sensory quality of cereal bars flavored with curried Thai river sprat fish.

MATERIAL AND METHODS

Cereal bar preparation

The main ingredients were puffed brown rice, dried Thai river sprat fish, pumpkin seeds, sunflower seeds, and dried Thai herbs (lemon grass, dill, basil, shallot, and chili). Dried Thai River sprat river fish was included in the cereal bar formulation based on the weight. The cereal bar preparation was conducted using a completely randomized design, with three replicates per treatment, resulting in a total of three treatments: Treatment 1 = without dried Thai river sprat fish addition (control), Treatment 2 = 12% dried Thai river sprat fish addition and Treatment 3 = 24% Thai river sprat fish with three replicates per treatment. After the mixture of basic ingredients, the cereal bar was formulated by adding mixture hot honey and glucose syrup. All ingredients were placed in an aluminum shape and pressed (for better compression) and then baked for 10 minutes at 150°C. After baking and cooling at ambient temperature, the product was cut into bars and packed in aluminum foil for posterior analyses.

Chemical composition and Energy value analysis

The chemical composition was performed following the official methodology [4] for moisture, protein, lipid and ash content. The carbohydrate was accomplished by the difference between components [100 – (moisture content + protein content + lipid content + ash content)]. The caloric value was calculated using Atwater factors [CV = (9 × Lipid content) + (4 × Protein content) + (4 × Carbohydrate content), and the result was expressed in kilocalories [5].

Sensory evaluation

Samples of cereal bars were cut into pieces of 25 g, wrapped in aluminum foil, identified with three random numbers, and offered to 30 untrained panelists. The sensorial characteristics evaluated (following the hedonic scale of 9 points, from 1 – dislike very much to 9 – liked very much) were: appearance, color, odor, texture, flavor and overall liking.

Microbiological analysis

The cereal bars were analyzed microbiologically by standard plate count aerobic standard plate count, yeast and molds according to the method suggested by AOAC [4].

Statistical analysis

All analyses were performed in triplicate. Statistical analyses were carried out with Duncan's multiple range test ($P<0.05$) using SPSS statistical software (SPSS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Chemical composition

According to the cereal bar chemical composition presented in Table 1, the moisture, lipids, carbohydrate content, and energy value showed no significant differences between treatments. Whereas increasing of dried Thai river sprat fish addition significantly affected the greater levels of protein (6.05 to 13.24%) and ash content (1.18 to 9.22%).

With respect to the protein content of the cereal bars, Bureau of nutrition affirmed that the dried Thai river sprat fish contain hight amount protein with 36% (Bureau of nutrition, 2018).

In this work, the cereal bars with the addition of Thai river sprat fish had higher protein levels than control treatment. The increase in ash content may have beneficially increased the minerals contained in cereal bars. Minerals commonly found in fish are sodium, potassium, calcium, magnesium, phosphorus, sulphur, iron, manganese, zinc, copper, and iodine [6]

Table 1 Chemical composition of cereal bar flavored with curried Thai river sprat fish

Chemical Composition	Dried Thai river sprat fish (%)		
	0	12	24
Aw^{ns}	0.36 ± 0.02	0.38± 0.01	0.39 ± 0.01
Moisture (%)^{ns}	4.86 ± 0.16	4.59 ± 0.31	4.98 ± 0.14
Energy (Kcal)^{ns}	385.37 ± 18.23	391.40 ± 7.10	403.67 ± 11.21
Carbohydrate (g)^{ns}	77.04 ± 6.05	69.66 ± 10.26	61.36 ± 7.23
Protein (g)	6.05 ± 0.23^{c}	9.55 ± 0.19^{b}	13.24 ± 0.12^{a}
Lipid (g)^{ns}	6.13 ± 1.94	8.31 ± 2.01	10.03 ± 3.21
Ash (g)	1.88 ± 0.23^{c}	8.81 ± 0.45^{b}	9.22 ± 0.11^{a}

Mean ± sd with different superscript in a row differ significantly; ns: non-significant

Sensory properties

The effect of adding dried Thai river sprat fish on the sensory characteristics were shown in Table 3. The results of the Duncan's showed that the appearance, color and odor were statistically similar. Due to the dried Thai river sprat fish had a larger size than other ingredients, the addition of greater portion of fish resulted in reduced adhesion of ingredients in the sample and a distinct fishy smell. This study shows that addition of 12-24% dried Thairiver sprat fish in cereal bars tend to reduce score of texture, flavor and overall liking. However, all formulas were accepted in all sensory attributes with overall liking scores ranged 7.8-6.6 (like very much to like moderately)

Table 2 Sensory score (9-point hedonic scale) of cereal bar flavored with curried Thai river sprat fish

Sensory characteristics	Thai river sprat fish (%)		
	0	12	24
Appearance^{ns}	7.0 ± 1.7	7.3±0.7	6.9±0.9
Color^{ns}	7.4±1.3	7.4±0.9	7.0 ± 1.3
Odor^{ns}	7.2 ± 1.3^{a}	6.5 ± 1.7^{b}	6.1 ± 1.3^{c}
Texture	7.5 ± 1.1^{a}	6.5 ± 1.8^{b}	6.5 ± 1.7^{b}
Flavour	7.2± 0.8^{b}	7.6 ±1.1^{a}	6.8± 0.8^{b}
Overall liking	7.5 ± 1.1^{a}	6.9 ± 0.9^{a}	6.6 ± 1.3^{b}

Mean ± sd with different superscript in a row differ significantly; ns: non-significant

Microbiological analysis

The results of microbiological analysis of cereal bars flavored with curried Thai river sprat fish (Table 3) showed good quality and were consistent with the microbiological standards set by Thai law [7] and are within guideline for human consumption.

Table 3 Microbial analysis of cereal bars flavored with curried Thai river sprat fish (24% died Thai river sprat fish)

Microbial analysis	CFU/g
Total aerobic plate count	2.4×10^{4} CFU/g
Yeast and molds	Not detected

CONCLUSIONS

The addition of 12 to 24% of dried Thai river sprat fish influenced the protein and ash content and resulted in no change for the other nutrients composition. All formulas were accepted in all sensory attributes. These results demonstrate that the addition of dried Thai river sprat fish is effective for increasing the protein and mineral matter of cereal bars with being accepted by consumers.

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Development of reduced fat coconut milk ice cream using pea protein isolate

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ABSTRACT: This study aimed to formulate reduced fat coconut milk ice cream using pea protein isolate (PPI). For this propose, PPI was used to replace coconut milk powder at four different ratios (100:0 (control), 37.5:62.5 (A), 50:50 (B) and 62.5:37.5 (C)). Physical, chemical and sensory analysis of coconut milk ice cream formulations were determined. The results showed that the color of coconut ice cream with PPI addition was darker than the control formula. The overrun and melting rate decreased while first dripping time, complete melting time and hardness increased with PPI addition. The increase of PPI could improve the amount of protein while fat content was reduced. For sensory evaluation, it found that higher amount of PPI resulted in lower liking scores. However, no significant difference was found on the liking scores of appearance, odor, taste, texture and overall in coconut milk ice cream with 0 (control), 32.5 and 50% PPI. The proximate composition indicated that coconut milk ice creams with 32.5 and 50% PPI contain 5.23 and 6.56% protein respectively which could be claimed as good source of protein. Moreover, the fat content was reduced by 47.98 and 55.64%, respectively.

Keywords: *Ice cream, Coconut, Pea protein isolate, Fat, Sensory evaluation*

INTRODUCTION

Ice cream is a delicious, wholesome, and nutritious frozen dairy dessert, which is widely consumed globally, and it is very popular among people of all ages because of its taste and cool sensation property [1]. The main ingredients of ice cream are milk, cream, sweeteners, natural flavourings, and other optional ingredients such as eggs, nuts, fruits, chocolate, and candy. There are various types of ice creams available in the market including a wide range of flavours, colours, textures, and ingredients. Coconut milk ice cream is one of the most popular frozen dessert especially in tropical countries. Coconut milk is a major ingredient and contains about 32.2% fat [2]. It seems that fat plays an important role in response to sensory and the texture of the ice cream [3]. However, in this recent decade, the consumers are becoming increasingly concerned about healthy products due to the increased risk of chronic disease such as diabetes and obesity [4]. For this reason, the food industry tries to manufacture the product by reducing fat and sugar.

Pea (*Pisum sativum*) is a type of pulse, which become a popular alternative protein source with its high protein content as well as versatility as a sustainable, nutritious, and low allergen food source. Dried Peas are generally composed of carbohydrates (35%), proteins (27%), fiber (27%) and a very small amount of lipids. Many studies suggested that pea protein (most times, pea protein hydrolysates and specific peptide fractions) has antioxidant, antihypertensive, anti-inflammatory, lowering cholesterol, and modulating intestinal bacteria activities [5,6]. In food processing, pea protein also shows the contributing to the structure and

texture of food products, including its solubility, water holding capacity (WHC) and oil-holding capacity (OHC), emulsifying properties, foaming properties, and gelling properties [5]. There were many studied on pea protein isolate utilized in bakery, process food, noodle and beverage [7].

Therefore, this study aimed to formulate reduced fat coconut milk ice cream using pea protein isolate (PPI), which is a new alternative to animal proteins with health benefits. For this propose, four different coconut milk ice cream formulations were produced by replacing coconut milk powder with PPI (100:0 (control), 37.5:62.5 (A), 50:50 (B) and 62.5:37.5 (C)). Physical, chemical and sensory analysis of coconut ice creams were determined. The results of this study could provide a prototype of new coconut milk ice cream formulation with better nutritional values.

MATERIAL AND METHODS

The ingredients used for producing manufacture the coconut milk ice cream was coconut milk powder (One Thai Foods Co., Ltd, Thailand), pea protein isolate (Argi-Mark Co., USA) sugar (Mitr Phol Co., Ltd, Thailand) salt (Thai Refined Salt Co., Ltd, Thailand). Stabilizer and emulsifier were purchased from bakery shop in Bangkok, Thailand.

Preparation of coconut milk ice cream

Coconut milk ice cream consists of 100 g coconut milk powder, 52 g sugar, 16 g stabilizer, 0.4 g emulsifier, 0.75 g salt and water. In preliminary trials, the upper limit of PPI addition was done using sensory evaluation and found that it could not higher than 62.5% of coconut milk powder. Thus, coconut milk ice cream was formulated to contain 0% (control), 62.5% (A), 50% (B) and 37.5% (C) PPI of coconut milk powder. Coconut milk ice creams were prepared by mixing all ingredients together and heat at 80°C for 1 min. The mixtures were homogenized, and cooled down at 4°C for 18-24 h. Then, the mixes were fed into the ice cream making machine (Perfect Scoop, 1.5 L/h, N2ice Co., Ltd, Nonthaburi, Thailand). Coconut milk ice cream was filled into a clean container and stored in a freezer at -20°C prior to analyze.

Physical measurements

The overrun of the coconut milk ice creams were determined using the formula 1 [8] .

Overrun (%) = (weight of unit mix – weight of an equal volume of ice cream)/weight of equal volume of ice cream *100 (1)

First dripping and complete melting times were measured according to Guven Karac [9]. Twenty-five grams of tempered samples were left to melt (at room temperature, 25±2°C) on a 0.2 cm wire mesh screen above a beaker. First dripping and complete melting times of samples were determined as seconds. The melting rate was determined according to Cottrell *et al.* [10]. The ice creams were taken from the plastic container they were in, and their weight was determined (25 g) and placed on beakers with a 2.5 mm mesh wire strainer. The melted fractions accumulated under the beakers, and then at 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min. The weight of the solutes collected in the beaker was measured. The results were calculated by the formula 2 and expressed in %

Melting rate (%) = weight of melted part/weight of ice cream *100 (2)

A texture analysis was evaluated on frozen ice cream samples stored at −20°C using a texture analyser (TA.XT2, Stable Micro Systems Ltd, YL, UK) equipped with a P/36R cylindrical probe. The ice cream samples were placed at room temperature (25±2°C) for 5 min before the analysis. The texture analysis circumstances were as follows: penetration depth, 20 mm; trigger force, 5 g; probe speed during penetration, 2 mm s-1. Hardness was considered as the peak pressure force (g) during penetration [11]. Color of samples were

measured L* (brightness or whiteness), a* (redness to green-ness), and b* (yellowness to blueness) value by using colorimeter (Colorflex EZ, HunterLab, Spectrophotometer, USA).

Chemical analysis

The pH of melted coconut milk ice cream was measured using a digital pH meter and titratable acidity was measured by titrating 10 g of sample with 0.1 N NaOH using phenolphthalein as an indicator [12]. The calculation of the titratable acidity was based on malic acid. The total soluble solids of samples were determined using a refractometer meter.

Protein and fat content of coconut milk ice cream

Protein and fat content of coconut milk ice creams were obtained from the calculation using INMUCAL Nutrients Software (version 4.0).

Sensory evaluation

Untrained panellists (n=50) were recruited from staff and student of Instituted of nutrition, Mahidol University with age more than 20 years old. They are regular ice cream consumers and no history of allergy to coconut milk and pea protein isolate. Panellists received a sample served in a plastic cup coded with a 3-digit random number to avoid bias. The panellists were educated on testing terminologies and requested to evaluate the various coconut milk ice cream samples for appearance, colour, odour, taste, texture, and overall liking using a 9-point hedonic scale (1 = disliked extremely, 5 = neither like nor dislike, 9= like extremely) [13]. Panellists were provided with a glass of water and, instructed to rinse and swallow water between samples. The protocol was approved by Mahidol University Ethical Board (MU-CIRB) (No. MU-CIRB 2022/023.0802).

Statistical analysis

The experiment design was completely randomized design (CRD) for physical and chemical properties and Randomized Complete Block Design (RCBD) for the sensory analysis. All measurement except sensory evaluation was performed in triplicates. Experimental data was analysed using computer software (IBM SPSS Statistics 19.0 IBM, Chicago, Illinois, USA). One-way Analysis of Variance (ANOVA) and Duncan's multiple range tests was performed to compare mean value. Average values are considered significantly different when $p < 0.05$.

RESULTS AND DISCUSSION

Physical properties

The physical properties of coconut milk ice creams were determined and shown in Table 1. The color measurement of coconut milk ice creams presented that L*, a* and b* values were varied between 83.56 to 70.54, -0.29 to 4.35 and 22.01 to 5.42, respectively (Figure 1). Coconut milk ice cream without PPI was lighter than coconut milk ice creams with PPI. This was due to the color of PPI. Moreover, it found that a* and b* values was increased with increasing of PPI. Similar results obtained by Sivasankari *et al.* who reported that the a* and b* values of ice creams increased with the increase in pulses protein concentration, (14). Overrun values of coconut milk ice creams change between 32.57 and 24.62%. It seemed that the overrun decreased with increase of PPI. This could be due to the very high viscosity gel matrix which might has a negatively effect on the air incorporation during the freezing process [14]. The result was similar with Mahdian *et al.* [15]. The addition of PPI prolonged the first dripping and complete melting time. However, there was no significant difference on first dripping time and complete melting time in coconut ice creams with different amount of PPI. Moreover, hardness of coconut ice cream samples also was increased with increase of PPI. This was due to its function as a stabilizer.

Table 1 Physical properties of coconut ice creams

Physical properties	Coconut milk ice cream formulas			
	Control	A	B	C
Color				
L*	83.56±2.42^{a}	70.54±2.17^{c}	71.77±0.48^{bc}	74.16±0.31^{b}
a*	-0.29±0.03^{b}	4.35±0.65^{a}	3.50±0.47^{a}	3.24±0.21^{a}
b*	5.42±1.20^{c}	22.01±1.44^{a}	20.63±0.82^{ab}	18.83±1.28^{b}
Overrun (%)	32.57±0.83^{a}	24.62±0.36^{b}	25.47±0.48^{b}	25.58±0.44^{b}
Melting rate (%)	43.93±5.22^{a}	17.77±1.64^{b}	18.96±1.68^{b}	21.75±2.14^{b}
First dripping time (s)	1180±91.65^{b}	1653±0.00^{a}	1487±150.61^{a}	1548±135.77^{a}
Complete melting time (s)	3440±277.13^{b}	4710±636.40^{a}	4200±169.71^{a}	4440±254.56^{a}
Hardness (g)	2416.22±127.64^{d}	5533.36±134.95^{a}	4537.61±34.28^{b}	3450.49±33.35^{c}

*Each value represents as mean ± SD. $^{a-d}$ Different letters in the same row indicate significant difference between means (DMRT, $p<0.05$). Control: 0%, A: 65%, B: 50%, C: 37.5% PPI

Control A. B. C.

Figure 1 Coconut ice creams (Control: 0%, A: 65%, B: 50%, C: 37.5% PPI)

Chemical properties

Table 2 presented the gross composition of coconut milk ice creams. The addition of PPI had affected pH, titratable acidity, total soluble solids, protein and fat contents of coconut milk ice creams. The pH values of coconut milk ice cream mixes decreased while titratable acidity increased by replacing coconut milk powder with PPI in the control formula. The differences in pH and titratable acidity values among coconut milk ice cream formulations might be due to the differences in chemical composition and mainly the protein content. The obtained results were in harmony with those obtained of Patel *et al.* [16] and Pandiyan *et al.* [17]. Moreover, the increasing of PPI also resulted in the decrease of total soluble solids. This decrease could be due to the different in total sugar content and a small portion of soluble proteins, amino acid and other organic materials. For protein and fat contents, it found that coconut milk ice cream with 32.5, 50 and 62.5% PPI contained 5.23, 6.56 and 7.88% protein which was increased almost 3, 4 and 5 times from the control formula, respectively. It seemed that coconut milk ice cream with 50 and 62.5% PPI could be claimed as a source of protein. The fat content of coconut ice cream with 0, 62.5, 50 and 37.5% PPI were 11.61, 6.04, 5.15 and 4.16%, respectively. This indicated that fat content was reduced by 47.98, 55.64 and 64.17% from the control formula, respectively. The results are related to the protein and fat contents of PPI and coconut milk powder which are main ingredients of the coconut milk ice cream. These coconut milk ice cream with PPI could be claimed as fat reduced product as according to Thai FDA regulation on nutrition labeling report [18].

Sensory evaluation

The sensory evaluation of coconut ice creams is shown in Table 3. There was no significant difference on liking score of appearance, odor, texture and overall in all coconut ice cream formulations ($P\geq0.05$). However, the liking score of color decreased after PPI addition. This result could be explained by the dark yellow color of coconut milk ice cream with PPI addition. Moreover,

liking score of taste in control formula showed no significant difference with coconut ice cream with 37.5 and 50% PPI while a significant difference was found in the coconut ice cream with 62.5% PPI. This result might be attributed to the specific taste and odor of the pea protein. Similar results were reported by Graças Pereira *et al.* [19] and Ahanian *et al.* [20].

Table 2 Gross composition of coconut milk ice creams

	Coconut milk ice cream formulas			
	Control	A	B	C
pH	7.45±0.04^{a}	6.99±0.04^{b}	7.17±0.25^{ab}	7.19±0.25^{ab}
Titratable acidity (%)	0.14±0.01^{b}	0.35±0.01^{a}	0.35±0.04^{a}	0.31±0.02^{a}
Total soluble solids (°Brix)	21.33±0.88^{a}	18.25±0.35^{b}	18.83±0.29^{b}	19.33±0.58^{b}
Protein (%)	1.61±0.01^{d}	7.88±0.61^{a}	6.56±0.51^{b}	5.23±0.40^{c}
Fat (%)	11.61±0.08^{a}	4.16±0.32^{d}	5.15±0.40^{c}	6.04±0.47^{b}

*Each value represents as mean ± SD. $^{a-d}$ Different letters in the same row indicate significant difference between means (DMRT, $p<0.05$). Control: 0%, A: 65%, B: 50%, C: 37.5% PPI

Table 3 Sensory evaluation of coconut ice creams

Sensory attributes	Coconut milk ice cream formulas			
	Control	A	B	C
Appearance^{ns}	7.54±0.87	7.04±1.44	7.02±1.30	7.26±1.35
Color	7.82±0.90^{a}	7.00±1.43^{b}	7.00±1.54^{b}	7.06±1.32^{b}
Odor^{ns}	6.82±0.84	6.54±1.55	6.72±1.46	6.94±1.28
Taste	7.02±0.95^{a}	6.14±2.07^{b}	7.00±1.50^{a}	7.14±1.57^{a}
Texture^{ns}	7.07±0.76	6.66±1.71	7.04±1.29	7.22±1.25
Overall liking^{ns}	7.02±1.19	6.62±1.83	6.94±1.45	7.26±1.47

*Each value represents as mean ± SD. $^{a-b}$ Different letters in the same row indicate significant difference between means (DMRT, $p<0.05$). ^{ns} no significant difference. Control: 0%, A: 65%, B: 50%, C: 37.5% PPI

CONCLUSIONS

Results showed that reduced fat coconut milk ice cream could be formulated using pea protein isolate with increasing of protein content. Higher amount of PPI addition might result in changing physical properties and reducing the liking scores of coconut milk ice cream. However, the liking scores of reduced fat coconut milk ice cream with 32.5 and 50% PPI was found to close to the control. Moreover, protein content of these formulas was increased by 3 and 4 times, respectively

and this amount of protein could be considered as a good source of protein product. Moreover, fat content was reduced by 47.98 and 55.64%, respectively. Therefore, this finding may have an important application potential in the food industry to consider a functional new product.

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Effect of chicken protein precipitate and mechanically deboned chicken meat as partial chicken meat substitute on the physicochemical properties and texture of chicken nugget

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ABSTRACT: Poultry is one of the fastest growing segments of the food industry sector and chicken slaughterhouses produce the oversupply of chicken carcasses. Therefore, increasing the utilization of chicken carcass would overcome this problem. The objective of this study was to investigate the results of replacement chicken meat in nugget formulation with mechanically deboned chicken meat or chicken protein precipitate on physicochemical properties and texture of cooked chicken nugget. Three different chicken nugget formulations were studied. They are 1) a partial substitution of the meat by mechanically deboned chicken meat (T1), 2) a partial replacement with chicken protein precipitate (T2), and 3) The control, without partial replacement. The products were characterized for their process yield, color value, proximate composition, pH, water holding capacity and texture profile analysis. The percentage of process yield for samples formulated with T1 or T2 was higher than the control ($p<0.05$). The chroma and hue angle value of all treatments were not significantly affected by mechanically deboned chicken meat and chicken protein precipitate ($p\geq0.05$). A chicken nugget formulation with partial substitution of the meat by mechanically deboned chicken meat and chicken protein precipitate displayed higher moisture content than the control sample($p<0.05$). However, fat and carbohydrate content of all samples were lower than the control($p<0.05$). Protein content showed no significant differences ($p\geq0.05$). Results showed that all nugget formulas containing mechanically deboned chicken meat or chicken protein precipitate had significantly higher water holding capacity, in comparison with the control ($p<0.05$). The hardness of the control sample was also lower than the samples containing T1 and T2($p<0.05$). Therefore, it was possible to partially replace chicken meat with mechanically deboned chicken meat or chicken protein precipitate in chicken nugget product to obtain the product with similar quality but higher process yield.

Keywords: Chicken, Nugget, Mechanically deboned chicken meat, Chicken protein precipitate

INTRODUCTION

Nowadays there is an increase in the consumption of poultry product. Poultry meat, with the largest production, was expected to grow by 1.5 million tons in 2021 or 1.1 percent increase from 2020 [1]. In the chicken slaughterhouses process, the increased demand for poultry necessitates affect the oversupply of chicken carcasses.

Carcasses weight are 18-22% of their chicken body weight. Carcasses can be used to produce mechanically deboned chicken meat. Mechanically deboned chicken meat is produced from edible tissue on chicken bones by deboning or separation techniques [2]. The proximate compositions of mechanically deboned chicken meat ranged from 13 to 15% protein content, 13 to 15% fat content and 69 to 70% moisture content. Normally, mechanically deboned

chicken meat is high in lipid content and includes more free heme groups due to a blend primarily consisting of tissues which is not generally contained meat such as bone, cartilages, skin and the scraps of meat remaining on the bones [3]. In addition, carcasses have high protein content from scraps of meat remaining on the bones, approximately 18%. The high protein content makes its a good binder to stabilize emulsions of meat products, increase the water-holding capacity, decrease shrinkage during cooking, increase the nutritional value and reduce formulation costs [4]. Due to high protein content and the inexpensive cost, carcasses are a potential source of meat protein for food applications and functional ingredient such as binder to improve texture and functional properties in food industry. Frozen chicken nuggets are ready to eat product with easy and simply preparation. Chicken nugget products are a food product consisting of a small piece of meat product with breaded or battered coatings and then frozen for preservation. The main composition of nugget is chicken or protein from vegetarian source and gum [5]. The nugget processing requires protein to act as an emulsifying agent for binding fat and water to create an emulsion system [6]. Mechanically deboned chicken meat and chicken protein precipitate from chicken carcasses have been investigated whether they could be added to chicken nuggets as a substitute for chicken meat or not.

This research therefore aims to develop chicken nuggets added with mechanically deboned chicken meat or chicken protein precipitate from chicken carcasses using alkaline extraction as a binder. Physicochemical properties and texture of chicken nugget were also studied.

MATERIAL AND METHODS

Preparation and extraction of chicken protein precipitate

Mechanically deboned chicken meat was obtained from B. Foods Product International Co., Ltd (Lopburi province, Thailand).

Chicken protein precipitate was prepared according to alkaline extraction method with modification [7]. Chicken carcass was mixed with solution containing NaCl, pH 10.15, at the ratio of 1:3 (w/v) in vacuum jacket pot for 185 mins. The slurry was further adjusted to pH 5.3 using HCl 1 M. The precipitated protein was separated by 100-micron and 25-micron filter nylon cloth. Mechanically deboned chicken meat and chicken protein precipitate were packed in high-density polyethylene and stored at -20ºC.

Formulation of chicken nugget

Three chicken nugget formulations were developed (Table1), as follows: 1) Control treatment (Control): a commercial formulation, 2) Partial replacement chicken meat with mechanically deboned chicken meat formulation (T1) and 3) Partial replacement chicken meat with chicken protein precipitate formulation (T2).

Chicken nugget preparation

Chicken boneless leg meat and breast without skin were minced using a meat grinder with a 5 mm opening disc. Mechanically deboned chicken meat or chicken protein precipitate and salt were added into a mixer and mixed about 1.30 minutes. Subsequently, sodium tripolyphosphate, isolate soy protein and other ingredients were added into the premixed chicken meat and mixed for 10 minutes until homogeneous mixture was obtained (A). The dough nuggets were separated and moulded into small portions (B) and then breaded using the following three steps: 1) milk wash (C) 2) predust (D) 3) batter (E). The battered nuggets were fried in soybean oil at 175ºC for approximately 1.30 minutes (F) and then steamed until an internal temperature of 73 ºC was reached (G). After steamed, the products were frozen (H) and stored at -18ºC.

Table 1 Chicken nugget formulations

Ingredients	Control[1] (%)	T1[2] (%)	T2[3] (%)
Boneless leg meat	39.00	31.39	38.50
Breast without skin	40.00	40.00	33.00
Mechanically deboned chicken meat	-	4.65	-
Chicken protein precipitate	-	-	12.50
Sodium Tripolyphosphate	0.40	0.40	0.40
Salt	0.50	0.50	0.50
White pepper	0.30	0.30	0.30
Sugar	1.00	1.00	1.00
Garlic powder	0.40	0.40	0.40
Nugget seasoning	1.00	1.00	1.00
Flavoring	0.40	0.40	0.40
Isolate soy protein	2.25	2.25	2.25
Native wheat flour	3.00	3.00	3.00
Tapioca starch	2.00	2.00	2.00
Ice	9.75	12.71	4.75

[1]Commercial formulation
[2]Replace chicken meat by mechanically deboned chicken meat formulation
[3]Replace chicken meat by chicken protein precipitate formulation

Measurement of yield and color value in chicken nugget.

The percentage of yield was measured using the following formula:

Process yield (%) =

$$\frac{A}{100} x \frac{B}{100} x \frac{C}{100} x \frac{D}{100} x \frac{E}{100} x \frac{F}{100} x \frac{G}{100} x \frac{H}{100} x100 \quad (1)$$

Where,

$$\text{A:Mixing yield(\%)} = \frac{\text{weight after mixing}}{\text{weight before mixing}} \text{x100} \quad (2)$$

$$\text{B:Forming yield(\%)} = \frac{\text{weight after forming}}{\text{weight before forming}} \text{x100} \quad (3)$$

$$\text{C:Milkwash yield(\%)} = \frac{\text{weight after milkwash}}{\text{weight before milkwash}} \text{x100} \quad (4)$$

$$\text{D:Predust yield(\%)} = \frac{\text{weight after predust}}{\text{weight before predust}} \text{x100} \quad (5)$$

$$\text{E:Batter yield(\%)} = \frac{\text{weight after batter}}{\text{weight before batter}} \text{x100} \quad (6)$$

$$\text{F:Frying yield(\%)} = \frac{\text{weight after frying}}{\text{weight before frying}} \text{x100} \quad (7)$$

$$\text{G:Cooking yield(\%)} = \frac{\text{weight after cooking}}{\text{weight before cooking}} \text{x100} \quad (8)$$

$$\text{H:Freezing yield(\%)} = \frac{\text{weight after freezing}}{\text{weight before freezing}} \text{x100} \quad (9)$$

The color value of the nuggets was measured using Image J. According to the method of Duangmal et al. [8], the values were expressed as L*, a* and b* values. L* represents lightness, a* = red (+) to green (-) axis, b*=yellow (+) to blue (-) axis. Chroma, representing color intensity, was then calculated by $(a^*2+b^*2)^{1/2}$, which is the distance of a color from the origin(a*=b*=0) in the a* and b* plane. Hue angle was calculated by (arctan b*/a*), which expresses in degrees from 0°C to 360°C, where 0°C (red) being a location on the +a*, 270°(blue) for –b*, and back to 360° =0°.

Determination of proximate composition and pH analysis

The AOAC Official Methods of Analysis [9] were used to determine the moisture content (950.46), protein content (981.10) with conversion factor of 6.25 and fat content (991.36). The percentage of total carbohydrates was calculated by difference (AOAC method). The pH values were determined using a pH meter.

Water holding capacity (WHC)

The centrifuge tubes were filled with 20g of thoroughly raw batter nugget and 32 mL solution with one of the three salt solutions (0.0, 0.3 or 0.6 mol/L). The experiments were conducted using a centrifuge with 1000 g and 2000 g for 20 minutes at 4 C°. After the centrifugation, the free solution in the test tubes was removed and weighed. WHC (%) was calculated using the following equation: [10]

$$WHC(\%) = \frac{\text{solution added into sample - solution removed after(g)}}{\text{meat sample mass (g)}} \text{ x100 (10)}$$

Texture Profile Analysis (TPA)

The Texture Profile Analysis (TPA) of the nuggets was carried out using a Texture Profile Analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK.) previously calibrated with a standard weight of 1 kg. The samples were then compressed to 75% of the original height using an aluminium probe (SMS P/75) with speed of 5 mm/s and time between two compressions of 3.0 s. The texture analyses were carried out with five samples (per treatment) and the parameters analysed included hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness [11].

Statistical analysis

Experiments were based on a complete randomized design (CRD). All analyzes in the present study were performed with three replications excluding Texture Profile Analysis. The analysis of variance (ANOVA) was used to compare the means. The means comparison was tested using Turkey's multiple comparison test at 95% ($p<0.05$). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

Percentage of yield

The process yield of the nugget is depicted in Table 2 indicating the significant increase in process yield from 130.95% (control sample) to 133.36% and 135.48% in T1 and T2 chicken nugget samples, respectively ($p<0.05$). Since the functional ingredients can affect process yields due to changing WHC and protein binding ability [12]. Mechanically deboned chicken meat and chicken protein precipitate affect the process yield of the nugget production. There was significantly increase in process yield of chicken nuggets because water holding capacity of raw batter nugget added with mechanically deboned chicken meat and with chicken protein precipitate were higher than those of the control. Relatively high water holding capacity is attributed to high level of protein solubility [13]. Higher water holding capacity positively causes higher moisture content in food due to macromolecules such as fat or protein holds water in the product system [14].

Color values

Color value (L^*, a^*, b^*) of the chicken nuggets was measured in this study because of the importance of the meat color in the role of consumer acceptance. L^* is the most important value as it is considered as eye-catching to the consumers [12]. Based on the results of color values (Table 3), significant differences were found in L* and a* values ($p<0.05$). Results showed that a formulation with substitution of the meat by mechanically deboned chicken meat significantly increased the lightness and redness value in comparison with the control. Hue angle and Chroma of all samples were no significant differences, in comparison with the control.

Proximate composition and pH analysis

The proximate composition and pH analysis are presented in Table 4. The results showed that the moisture content of chicken nugget, which was added with mechanically deboned chicken meat or chicken protein precipitate, showed significantly differences ($p<0.05$) higher than the control nugget. This means that the use of mechanically deboned chicken meat and chicken protein precipitate affect the moisture content of nuggets. The higher moisture content may be related to the higher water holding capacity of raw batter nugget added with mechanically deboned chicken meat and chicken protein precipitate compared to commercial formulation. In cooked

Table 2 Process yield of chicken nuggets as affected by mechanically deboned chicken meat and chicken protein precipitate

Formulations*	Yield (%)								
	Process								
	A ns	B ns	C	D	E ns	F ns	G ns	H ns	Process
Control	99.08 ± 0.62	99.05 ± 0.25	105.2 ± 0.23^{b}	107.0 ± 0.39^{a}	129.8 ± 0.69	92.77 ± 0.19	99.01 ± 0.92	99.33 ± 0.19	130.95 ± 2.34^{c}
T1	99.02 ± 0.18	99.18 ± 0.33	106.0 ± 0.23^{a}	106.2 ± 0.09^{b}	130.1 ± 0.11	93.52 ± 0.69	99.77 ± 0.60	99.17 ± 0.26	133.36 ± 0.37^{b}
T2	99.52 ± 0.45	99.85 ± 0.09	106.1 ± 0.58^{a}	105.9 ± 1.19^{b}	130.4 ± 0.50	92.98 ± 0.53	100.45 ± 1.09	99.58 ± 0.29	135.48 ± 0.51^{a}

* Nugget formulation was explained in Table 1
$^{a-c}$ Differences letters in the same column indicate a significant difference ($p<0.05$)
^{ns} Means within the same column are not significantly difference ($p\geq0.05$)
Mean ± SD (from 3 replicates)

Table 3 Color values (L*, a* and b*) of chicken nuggets as affected by mechanically deboned chicken meat and chicken protein precipitate

Formulations*	L*	a*	b*^{ns}	Chroma ^{ns}	hue angle ^{ns}	ΔE
Control	66.50 ± 2.49^{b}	1.41±0.10^{b}	47.78 ±1.68	47.80±1.68	88.31±0.07	-
T1	68.97 ±0.21^{a}	1.45±0.03^{a}	47.43±1.41	47.46±1.41	88.25±0.02	2.49
T2	67.82 ±2.44^{ab}	1.41±0.03^{b}	47.53±1.99	47.55±1.99	88.30±0.10	1.34

* Nugget formulation was explained in Table 1
$^{a-c}$ Differences letters in the same column indicate a significant difference ($p<0.05$)
^{ns} Means within the same column are not significantly difference ($p\geq0.05$)
Mean ± SD (from 3 replicates

chicken nuggets, the partially replace chicken meat by mechanically deboned chicken meat or chicken protein precipitate affect to decrease fat content ($p<0.05$). This could be attributed to water loss and oil absorption. It has been acceptable that oil absorption happen as moisture is removed from the food during frying and the amount of oil absorption has been shown that it's directly proportional to the amount of moisture loss [16]. However, the substitution of chicken meat by mechanically deboned chicken meat and chicken protein precipitate did not significantly affect protein content as compared to the control ($p\geq0.05$). Moreover, carbohydrate content of all sample were lower than those of the control depends on three step coating processes in chicken nugget manufacturing.

pH is a major criterion for functional properties of the meat products. The pH value of nugget ranged from 6.03 to 6.38. Addition of product as more water loss because of the shrinkage of the product [17]. The result of mechanically deboned chicken meat and chicken protein precipitate gave significant increase in pH ($p<0.05$). The mean value of pH of raw batter nuggets with mechanically deboned chicken meat was 6.38 that was higher than control. Addition of chicken protein precipitate showed decrease in pH. Therefore, mechanically deboned chicken meat and chicken protein precipitate had a different pH value from chicken meat. The results also indicated that the addition of mechanically deboned chicken meat increased the pH value of the product, due to high pH value of mechanically deboned chicken meat compared to chicken meat.

Water holding capacity (WHC)

Water holding capacity (WHC) of meat product is an important quality property for meat processors as it relates to the process yield. It also has large effect on the quality of water holding capacity is presented in the Table 5. Mechanically deboned chicken meat

and chicken protein precipitate had significantly effect on the WHC of the chicken nugget product. The results of overall WHC averages show that WHC decreased with the increase of centrifugal force. Since higher force could compress more water from the muscle fibre structure [10]. The result displayed that water binding of chicken nugget increased with increasing salt solution concentration. Chicken nugget with added mechanically deboned chicken meat and chicken protein precipitate exhibited higher WHC values, in comparison with the control. Addition of mechanically deboned chicken meat gave WHC value range between 4.70% and 11.63% while chicken nugget added with chicken protein precipitate gave WHC value range from 4.98% to 11.80%. These data show the higher water-retain ability of T1 and T2 batters resulting in the higher yield than the control.

Table 4 pH and proximate composition (moisture, fat, protein, crude fiber, ash, carbohydrate)) of chicken nuggets with different formulations

Formulations*	pH	Proximate composition (%)			
		Moisture	Fat	Protein ^{ns}	Carbohydrate
Control	6.20 ± 0.10^{b}	55.35 ± 0.10^{c}	8.38± 0.11^{a}	12.13 ± 0.35	20.89 ± 0.58^{a}
T1	6.38 ± 0.08^{a}	60.43 ± 0.36^{b}	7.91 ± 0.14^{b}	12.68 ± 0.10	15.55± 0.06^{b}
T2	6.03± 0.01^{c}	62.53± 0.07^{a}	7.64± 0.12^{b}	12.45± 0.20	15.25± 0.19^{b}

* Nugget formulation was explained in Table 1
$^{a-c}$ Differences letters in the same column indicate a significant difference ($p<0.05$)
^{ns}Means within the same column are not significantly difference ($p\geq0.05$)
Mean ± SD (from 3 replicate)

Table 5 Water holding capacity of raw batter nuggets with different formulations

Water holding capacity (%)				
Centrifugal force (g)	Formulations*	Salt solution concentration		
		0.00 mol/L	0.30 mol/L	0.60 mol/L
1000	Control	7.66 ± 0.55^{a}	9.08 ± 0.35^{b}	9.11 ± 1.14^{b}
	T1	6.43 ± 1.63^{b}	8.85 ± 0.97^{b}	11.63 ± 1.83^{a}
	T2	8.23 ± 0.45^{a}	11.67 ± 3.73^{a}	11.80 ± 3.23^{a}
2000	Control	2.71 ± 1.15^{b}	6.02 ± 1.00^{b}	6.43 ± 1.19^{b}
	T1	4.70 ± 0.66^{a}	8.41 ± 1.42^{a}	11.21 ± 0.68^{a}
	T2	4.98 ± 0.51^{a}	8.76 ± 1.04^{a}	11.10 ± 1.56^{a}

* Nugget formulation was explained in Table 1
$^{a-c}$ Differences letters in the same column indicate a significant difference ($p<0.05$)
Mean ± SD (from 3 replicates)

Texture

Texture is an important characteristic of cooked chicken nugget products due to its appearance desirability and palatability. Hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness values of fried nuggets are presented in Table 6. The results revealed that Springiness of all samples were not significantly different ($p \geq 0.05$). In contrast, hardness, adhesiveness, cohesiveness, gumminess and chewiness of fried nuggets showed significant difference ($p < 0.05$). Chicken nuggets added with mechanically deboned chicken meat and chicken protein precipitate were harder compared to control samples ($p < 0.05$).

Table 6 Texture Profile Analysis of chicken nuggets with different formulations

Formulations*	Hardness (g-force)	Adhesiveness	Springiness ^{ns}	Cohesiveness	Gumminess (g-force)	Chewiness (g-force)
Control	31462.92 ± 802.04^{b}	-0.34± 0.10^{b}	0.84± 0.01	0.61± 0.03^{b}	19172.07 ± 1194.83^{b}	16163.68 ± 846.89^{b}
T1	33654.31 ± 260.17^{a}	-0.48± 0.19^{c}	0.83± 0.02	0.64± 0.01^{a}	21486.75 ± 557.66^{ab}	17932.58 ± 1026.39^{ab}
T2	33664.61 ± 2164.76^{a}	-0.26± 0.11^{a}	0.83± 0.02	0.65± 0.01^{a}	22001.23 ± 969.78^{a}	18263.69 ± 1350.96^{a}

* Nugget formulation was explained in Table 1
$^{a-c}$ Differences letters in the same column indicate a significant difference ($p < 0.05$)
^{ns}Means within the same column are not significantly difference ($p \geq 0.05$)
Mean ± SD (from 5 replicates)

CONCLUSIONS

The resulted showed that it is possible to develop chicken nuggets added with mechanically deboned chicken meat or chicken protein precipitate from chicken carcasses using alkaline extraction to obtain the chicken nugget with similar quality but higher process yield. The substitute chicken meat replacement in nugget formulation improve the characteristics of chicken nugget in term of increasing water holding capacity. The results also showed that this chicken meat substitute could add value to chicken carcass.

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Study of consumer behavior of tofu from jackfruit seeds and chemical analysis of jackfruit seeds flour from different preparation methods

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ABSTRACT: The purpose of this research was to study consumer demand for the use of jackfruit seed flour in tofu products using a questionnaire of 211 participants and investigate chemical composition of jackfruit seeds flour from different preparation methods that is essential for the development of tofu. The result of tofu product purchasing behavior and marketing factors influencing consumer purchasing decisions was that chose to eat tube tofu (86.7%), bought it for themselves (55.9%), ate it as a main dish (92.4%), and ate it 1-2 times a month (40.8%). In terms of product development, consumers were interested in alternative protein products (67.3%), used to eat jackfruit seeds in boiled form (76.8%), unaware that jackfruit seeds were high in protein (46.2%). Consumers are interested if there is an alternative product "Jackfruit Seed Tofu," which is high in protein, good for nutrition, easy to digest, has no bean smell and can be eaten by health-conscious people, as well as soft tube tofu (49.3%). The results of the laddering technique was found that soft tofu eating behaviors in tube form, boiled cooking style. Consumers like smooth and soft texture, mellow taste, cook a variety, packaging is convenient to use, easy to chew, and protein-rich, whereas consumers dislike products that are not very diverse, the smell of beans, too messy texture, and the packaging that is difficult to remove. Smooth and soft texture, delicious and mellow taste, packing size 120 g, flavor and color from jackfruit seeds all influence consumer purchasing decisions. Three types of jackfruit seed flour were studied for their chemical composition, which is required for the development of tofu. The yield of Jackfruit Seed Flour (JSF), Thermal Jackfruit Seed Flour (TJSF) and Jackfruit Seed Protein Isolate (JSPI) were 32.66, 24.06 and 0.67 (% w/w), respectively. The protein content was 11.52, 11.31 and 68.24% respectively. The study revealed that there is interest in developing tofu products from jackfruit seeds as a health-conscious alternative. In addition, jackfruit seed flour has a high yield and protein content, making it suitable for use in food products.

Keywords: Purchasing decision, Jackfruit seed, Jackfruit seed flour, Protein, Tofu

INTRODUCTION

At present, there is an increasing market of health lovers. Since the outbreak of the coronavirus in late 2019, it has become a market catalyst for more fruit snacks such as baked jackfruit and crispy jackfruit. Including export markets to China and other countries as a result of the COVID-19 situation, fresh jackfruit is unable to be exported in sufficient

quantities, resulting in more jackfruit processing. The amount of jackfruit produced in Thailand in 2020 from January to September amounts to 98,173,495 kilograms, with an export volume of 29,506,076 kilograms, or possibly more than 50,000,000 kilograms of jackfruit processed, yielding 4,000,000 - 7,500,000 kilograms of seeds as a by-product per year [1], which are leftovers that are not being used. Jackfruit seeds are high in nutrients. One hundred grams of jackfruit seeds (wet weight basis) contain 5.5 grams of protein, 105 milligrams of phosphorus, and 1.74 milligrams of vitamin B1 [2]. There have also been studies that show the protein content of jackfruit seeds to be as high as 10 - 15% [3]. However, jackfruit seeds are being used less commonly at the moment. It is only consumed in the form of boiling. Therefore, the researcher has an idea to investigate various methods of jackfruit seed starch preparation.

With the current COVID-19 outbreak, consumers around the world are increasingly focusing on health and wellness, animal welfare awareness and resource utilization. Therefore, consumers try to reduce the consumption of processed meat that increases the risk of disease, and instead switch to plant-based protein. Thailand's plant-based food market is expected to expand rapidly. The market value is approximately 834 million US dollars in 2019 and is expected to grow at a rate of about 10% per year from 2020 to 2024. [4]

Based on the lockdown situation due to the coronavirus outbreak last year, researcher has reported a shortage of tofu in the market. According to the Nielsen report, tofu sales increased by 40% in the first half of 2020 compared to the previous year. As a result, the tofu market's growth rate has accelerated significantly. The global tofu market is estimated to be worth $2.31 billion US dollars in 2018 and is expected to grow at a Compound Annual Growth Rate (CAGR) of 5.2 % from 2019 to 2025. [5]

Tofu is a curd that is made directly from soybeans. Tofu is water-extracted and salt- or acid-coagulated soy protein gel with water, soy lipids, and other constituents trapped in its network. It is inexpensive, nutritious, and versatile. There are various types of tofu, hard and soft, deep-fried and non-fried, coarse and fine texture, fluffy and not fluffy, etc. Thai people like to eat tofu in general food. There are many dishes that can be divided into both Chinese and Japanese tofu to cook. It as a supporting ingredient with other seasonings such as sweet and sour stir-fry, stir-fried mixed vegetables, etc., and is the main ingredient such as fried tofu, mushroom tofu, salted tofu, etc. with this Thai food, food cultures of many nations can be harmoniously integrated [10].
The objectives of this study were to study consumer behavior on tofu made from jackfruit seed and to investigate various methods of jackfruit seed flour preparation on chemical composition that is essential for the development of tofu.

MATERIAL AND METHODS

The study of consumer demand for jackfruit seeds in tofu products

Two parts of consumer questionnaires were conducted. The first part surveyed 211 consumers to determine the frequency of consumption, type of tofu, method of consumption, age range, and consumer interest in jackfruit seed tofu products. Multiple response questions or checklist questions were used to obtain ideas from consumers to develop products.

The second part was an in-depth interview. This was a laddering technique [11] interview with 10 participants who recently ate tofu products more than four times a month to determine their interest in using jackfruit seeds in tofu products, product characteristics, packaging. To obtain the form of a product that meets the needs of consumers, ask questions and use consumer answers to create new questions until the final answer is available.

The study of various methods for preparing jackfruit seed flour

Material

Jackfruit seeds (*Artocarpus Heterophyllus Lamk*) were purchased from TalaadThai, Pathum Thani, Thailand.

Preparation of samples

Preparation of Jackfruit Seed Flour (JSF) were adapted from the research of Zuwariah et al. [6]. The seed was manually separated from the pulp, and the seeds were washed with filtered tap water. After that, the seeds were sliced to 3 mm and dried at 60°C 24 hrs. in a tray dryer (PG 9-4, PROGRESS, Thailand). The dried samples were ground into flour using a grinder (HR-08B, Microcomputer timer-switch Grinder, China) and sieved (mesh no 50). The grounded mash was packed into an aluminium-plastic laminated bag and stored in a refrigerator for further laboratory testing.

Preparation of Thermal Jackfruit Seed Flour (TJSF) were adapted from the research of Zuwariah et al. [6]. About 150 g of JSF were homogenized with water and then heated to 60-80°C for 30 to 60 mins. Then the solution was separated by gravity filtration in a muslin cloth and allowed to settle for 2 hrs, and then dried at 60ºC in a tray dryer 24 hrs. (PG 9-4, PROGRESS, Thailand). The dried samples were ground into flour using a grinder (HR-08B, Microcomputer timer-switch Grinder, China) and sieved (mesh no 50). The ground mash was packed into an aluminium-plastic laminated bag for further laboratory testing.

Preparation of Jackfruit Seed Protein Isolate (JSPI) were adapted from the research of Akter et al. [7]. JSF was suspended in distilled water (1:10 w/v). The pH of the slurry was adjusted to 9.0 using 1 M NaOH solution and kept in rest for one hour. The slurry was then centrifuged (SIGMA 6-16KS, SIGMA, Germany) at 12,600 g, for 15 min. The supernatant protein solution was separated and adjusted to pH 4.5 using 1 M HCl. The solution was then stirred for 30 min and left undisturbed for cold precipitation overnight (4°C). The supernatant was carefully siphoned off, and the obtained protein precipitate was collected by centrifugation at 2217 g for 15 min. The final protein was poured into a clean tray and dried in vacuum dryer (WTC binder, Germany) at 60°C for 48 h. The dried protein was collected and subsequently pulverized into a fine powder (JSPI) by using a mortar and sieved (mesh no 50). The ground mash was packed into an aluminium-plastic laminated bag for further laboratory evaluation.

Analysis of the yield

Extraction yield was analyzed by weighing jackfruit seed flour initially used. The weight of the extracts powder was calculated by the following equation.

$$Yield(\%\, w/w) = \frac{\text{Extracted weight of flour}}{Initial\ weight\ of\ flour} \quad (1)$$

Proximate composition

JFS and TJSF were sent for proximate analysis at Asia Medical and Agricultural Laboratory and Research Center, Bangkok, Thailand. (In house method TM-CH-017 based on AOAC (2019) 992.23).

Determination of protein content

The protein content of JSPI was determined by the Kjeldahl method (AOAC, 2005). The average values from the replicated experiments were accepted using a nitrogen conversion factor of 6.25.

Statistical method

The data was analyzed using SPSS software version 25 One-way analysis of variance (ANOVA) was used to determine significant difference between samples ($p < 0.05$). Duncan's new multiple range test was used to determine the difference of means value.

RESULTS AND DISCUSSION

The results of a survey of 211 consumers, demographic characteristics included female (78.2%), age range 23 - 39 years (69.2%), bachelor's degree (76.3%), company employee (33.2%), income 10,001-20,000 baht (44.6%) (Table 1).

Table 1 General data on demographic characteristics (N=211)

Variables	Frequency	Percentage
Gender		
Male	41	19.4
Female	165	78.2
Not specified	4	1.9
LGBTQI+	1	0.5
Age		
< 22	45	21.3
23 - 39	146	69.2
40 - 54	16	7.6
> 55	4	1.9
Education level		
Primary school	1	0.5
Secondary school	12	5.7
Diploma	10	4.7
Bachelor's degree	161	76.3
Master's degree/higher	27	12.8
Occupation		
Student	62	29.4
Civil servants	24	11.2
Company employee	70	33.2
Personal business	22	10.4
General employee	5	2.4
Freelance	24	11.4
University staff	1	0.5
Maid	1	0.5
Unemployed	2	1
Income (baht)		
< 5,000	26	12.3
5,000 - 10,000	31	14.7
10,001 - 20,000	94	44.6
20,001 - 30,000	28	13.3
30,001 - 40,000	14	6.6
> 40,000	18	8.5

The result in terms of tofu product purchasing behaviour and marketing factors influencing consumer purchasing decision (Table 2) was tube tofu, it was tofu that most consumers chose to eat (86.7%), bought it for themselves (55.9%), and ate it as a main dish (92.4%). Consumers eat tofu because it is a

Table 2 Buying behavior of Tofu products and marketing factors affecting the purchasing decision of consumers (N=211)

Variables	Frequency	Percentage
Types of tofu that consumers usually to eat		
Tube tofu	183	86.7
Tofu pudding	92	43.6
Medium firm tofu	75	35.5
Firm tofu	56	26.5
Extra firm tofu	24	11.5
Others (tofu skin)	1	0.5
Consumer decision to buy tofu		
Buy and eat by yourself	118	55.9
Buy for others to eat	51	24.2
Someone else bought it to eat.	42	19.9
Tofu consumption		
Eat as main dishes	195	92.4
Eat as a dessert	64	30.3
Others (fried tofu)	1	0.5
People Influencing Tofu Purchase Decisions		
Self	131	62.09
Parents	49	23.22
Relatives	11	5.21
Friend	10	4.74
Various media	10	4.74
The reason for eating tofu		
Habits	136	64.5
Easy to chew	79	37.4
Can be used to cook a variety of foods	79	37.4
High protein	68	32.2
Easy to digest	52	24.6
Low calorie	50	23.7
Meat substitute	35	16.6
Health-loving group	24	11.4
Vegetarian	22	10.4
High energy	17	8.1
Others (like, useful)	12	5.9
The frequency of eating tofu by consumers		
< 1 time per month	38	18
1-2 times per month	86	40.8
3-4 times a month	43	20.4
> 4 times a month	44	20.9

Table 3 Information about product development (N=211)

Variables	Frequency	Percentage
Attention about alternative protein products		
Interested	142	67.3
uncertain	55	26.1
Not interested	14	6.6
Consumer perception that jackfruit seeds are edible		
Know	170	80.6
Uncertain	11	5.2
Unknown	30	14.2
Consumers who used to eat jackfruit seeds		
Used to eat	162	76.8
Uncertain	7	3.3
Never eat	42	19.9
Characteristics of eating jackfruit seeds		
Boiled	159	92.4
Types of curry	35	20.3
Eat as a dessert	14	8.1
Fried	5	2.9
Jackfruit seed milk	1	0.6
Never eat	1	0.6
Consumer perception that jackfruit seeds are high in protein.		
Know	67	31.9
Uncertain	46	21.9
Unknown	97	46.2
Attention of consumers if there is an alternative product "tofu from jackfruit seeds, high protein, good nutrition, easy to digest, easy to eat, no bean smell, and health lovers can eat.		
Interested	147	69.7
Uncertain	53	25.1
Disinterested	10	4.7
Indifferent	1	0.5
Types of tofu from jackfruit seeds that consumers are interested in.		
Tube tofu (egg tofu, soft white bean curd)	103	492
Medium firm tofu	46	22
Tofu pudding	30	14.4
Firm tofu	20	9.6
Extra firm tofu	10	4.8

Table 4 Consumer behaviour of soft tofu in tube form (N=10)

Consumer behaviour	Number of mentions
Characteristics of cooking	
Boiled	10
Stir	6
Fry	5
Microwave	1
Purchased quantity	
4 or more tubes (tube:120 g)	6
1-3 tubes (tube:120 g)	4
Amount eaten per time	
1 (tube:120 g)/time	8
2 (tube:120 g)/time	2
Place to buy tofu	
Department stores	6
Market	4
Convenience stores	3
The reason for eating tofu	
Delicious, mellow taste	9
Can be used to cook a variety	8
Accustomed	6
Healthy eating	5
Easy to chew	5
Favourite	3
No smell	3
Meat substitute	2
Easy to digest	1
What consumers want from eating tofu	
The texture is smooth and soft	10
protein	8
Purchase decision influencers	
Self	9
Family	4
Friends	1
Product factors that influence the purchase decision	
Texture	8
Taste	7
Nutritional value	4
Smell	3
Packaging	2
Food standards (FDA etc.)	2
Marketing Factors Influencing Purchasing Decisions	
Price	9
Promotion	9
Distribution locations	6
Brands	4

habit, they feel they must have when eating food such as boiled soup, suki (64.5%), and the frequency of eating tofu 1-2 times a month (40.8%).

In terms of product development information (Table 3), consumers were interested in alternative protein products (67.3%), recognized that jackfruit seeds are edible (80.6%) and eaten jackfruit seeds (76.8%). Consumers were unaware that jackfruit seeds were high in protein (46.2%). If there is an alternative product "Jackfruit Seed Tofu," high protein, good nutrition, easy to digest, easy to eat, no bean smell, and health lovers can eat, consumers are interested (69.7%). Customers are most interested in soft tube tofu made from jackfruit seed (49.2%).

The laddering technique was used to determine the results of the in-depth questionnaire. It gathers the attributes mentioned by consumers and then groups responses that are similarly meaningful. It was discovered that consumers' soft tofu eating habits in tube form were boiled cooking style, with buying quantity of four or more tubes per time (Table 4).

The reason for eating tofu was because it has a delicious and mellow taste. They want a smooth and soft texture from tofu, and themselves are influencing their purchasing decision. Texture influence purchasing decision and price and promotion are marketing factor that influence purchasing decision.

Table 5 shows the result of the laddering technique. It can be concluded that the characteristics that consumers prefer that influence their decision to purchase soft tofu in tubes are smooth texture (9 persons), mellow taste (9 persons), the ability to cook a variety of dishes (7 persons), and the packaging is convenient to use (7 persons) and unique (6 persons).

Table 6 shows the results of the laddering technique. It can be concluded that the feature that consumers dislike that affects their decision to purchase soft tofu is that tube tofu products are not very diverse, that each brand looks the same, that smell of soy bean curds is too strong, and that texture feels too messy when cooking. A sharp knife is needed to maintain the desired shape of the tofu, and those who did not buy tofu from soybeans because they thought it was difficult to find must go shopping at the department store.

Table 5 Consumer preferences that influence their decision to purchase soft tofu in tube form (N=10)

Characteristics	Number of mentions
The texture is smooth and soft.	9
Mellow taste	9
Can be used to cook a variety	7
Packaging is convenient for use.	7
The packaging is unique.	6
Contains protein	6
Easy to chew	6
Characteristics	Number of mentions
Easy to buy	4
Egg flavor	4
Bean flavor	3
Bounce texture	2

Table 6 Characteristics that consumers dislike affecting the decision to buy soft tofu in tubes (N=10)

Disliked characteristics	Number of mentions
Bean smell	6
Not much variety	6
The texture is too messy.	5
Difficult to remove packaging	5
Unsalted soy tofu	5
Soybean tofu is hard to find.	4
Egg smell	4
Some brands are salty.	2
Water coming out of the tofu	1

Table 7 shows the result of data collecting on characteristics influencing consumers' purchasing decisions of jackfruit seed tofu. There were smooth and soft texture, delicious and mellow taste, tofu flavor from jackfruit seeds, natural color from jackfruit seeds, and packing size 120 g.

Table 7 Characteristics influencing consumer's decision to buy soft tofu from jackfruit seeds (N=10).

Variables	Number of mentions
The texture is smooth and soft.	10
Delicious, mellow taste.	10
Tofu taste from jackfruit seeds.	10
Color from jackfruit seeds	10
Packing size 120 g	10
Packaging is convenient for use.	5
Protein equivalent on the other brand	7
Tube packaging	6
Higher protein than the market	3

Yield and chemical compositions of jackfruit seed flour from various preparation methods.

The protein content of jackfruit seeds reported in this study (11.31-11.52%) was higher than that reported by Amadi et al. [8], Zuwariah et al. [6] who reported 10.09% and 9.78%, respectively and lower than reported by Ulloa et al. [9] who reported 14.04% protein content in jackfruit seeds (Table 8). These might be due to the seeds coming from different jackfruit varieties or due to the jackfruit flour studied by Ulloa et al. [9] was defatted. The JSPI contained the highest protein content (68.24 %).

Table 8 Chemical composition of JSF and TJSF

Chemical composition	JSF	TJSF
Protein (g/100g)	11.52±0.58	11.31±0.68
Carbohydrate (g/100g)	76.94±5.39	78.84±4.73
Total Fat (g/100g)	1.15±0.07	0.96±0.07
Ash (g/100g)	3.64±0.29	2.43±0.12
Moisture (g/100g)	6.75±0.47	6.46±0.52
Total energy (kcal/100g)	364.19±25.49	369.24±29.54

The chemical composition of JSF and TJSF are quite similar. The authors expected to see differences in proteins, but this was not the case. This could be because the thermal process has no effect on the chemical composition or loss.

Yield quantity of JSF, TJSF and JSPI were 32.66, 24.06, and 0.67 (%w/w) respectively (Table 9).

Table 9 Yield of various types of jackfruit seed flour

Treatment	Yield (% w/w)
JSF	32.66 ± 9.61^{b}
TJSF	24.06 ± 1.07^{b}
JSPI	0.67 ± 0.03^{a}

^{a,b}Values followed by the same letter within the same row are not significantly different from each other ($p<0.05$).

CONCLUSIONS

Consumers are interested in health products. Most consumers have eaten boiled jackfruit seeds but are unaware of the benefits. There is interest in tofu product from jackfruit seeds with high protein, good nutrition, easy to digest, easy to eat, and no bean smell. In addition, the chemical composition of JSF were rich in protein and high yield content making it suitable for use in food products.

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Drinkable fermented RD43 rice jelly with soymilk protein and inulin

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ABSTRACT: This research aimed to study consumer needs for fermented RD43 rice with soymilk jelly and the effects of various concentration of inulin (1, 2%) and gelatin (3, 4%) having on textural and color parameters. Treatments were divided into 3% gelatin with 1% Inulin (G3I1), 3% gelatin with 2% inulin (G3I2), 4% gelatin with 1% inulin (G4I2), and 4% gelatin with 2% inulin (G4I2). The quality of product was investigated by the measurement of color and texture analysis using forward extrusion method. The sensory properties were evaluated using 9-point hedonic scale and 5-point scale of Just-About-Right (JAR) using 30 untrained panellists. The results of consumer behaviour study showed that the most important purchase intent factors of drinkable jelly product were an absence of preservative factor, followed by nutritional values, and flavor. For the quality measurement, the higher concentration of inulin and gelatin statistically resulted in a significant decrease in a^* value (redness) and b^* value (yellowness) ($p \leq 0.05$); however, no significant difference in L^* value (lightness) ($p > 0.05$) was found. In sensory evaluation, the sample of G3I2 received the highest score in firmness and overall liking. According the result, other attributes such as appearance, color, flavor and sweetness were not significantly different ($p > 0.05$). From JAR test, G3I2 received the most net score on flavor and sweetness; while G3I1 received highest score in appearance. All samples have received net score of 20.0 and above on firmness; however, from 9-point hedonic score on firmness preference, consumers had the tendency to choose G3I2 over other samples.

Keywords: Khao-Mak, Rice Jelly, Inulin, Soymilk, Gelatin

INTRODUCTION

Historically, fermented foods have played a significant role in many countries of Asia including Thailand. There are countries such as Japan and Korea, which created products made from fermenting rice. Among fermented rice dishes, the use of sweet glutinous rice in Japanese “Amazake”, Korean “Makgeolli” and Thai “Khao-Mak” are popularly consumed by local population for their sweet acidic taste and enticing aroma of alcohol. Khao-Mak is known as a Thai fermented sweet rice dessert, which uses sticky rice as a base. The fermentation involves Look Pang or a traditional fermentation starter lasts for several days, which produces low amount of alcohol and gives a sweet taste. Khao-Mak is a probiotic product containing mold and yeast, which offer various health benefits such as

maintaining a healthy gut flora and enhancing digestive system [10]. Nowadays, Khao-Mak products have become unpopular among younger generations who live around the city area due to western culture influence. However, plant-based foods have been gaining popularity presently as society has become more aware of health and sustainability especially during COVID-19 pandemic; therefore, there is a further potential growth in the market [8].

Soymilk has received much attention in beverages and dessert industries as it is often used for milk substitution especially for lactose intolerance patients. Soymilk contains high protein, Vitamin B, iron, unsaturated fatty acids, lecithin, and isoflavones, which is why it is considered to be an excellent alternative for dairy products [1].

Inulin is a prebiotic found in fruits and vegetable, which is a highly fermentable fiber that increases activity of gastrointestinal bacterial in guts. Inulin is often used as sweetener, fat substitute, and texture modifier in food industry [11].

Jelly drink is a ready to drink dessert that was created as a snack to delay hunger. It contains water, sweetener, artificial flavors and gelling agent. Nowadays consumers are preferring this sort of snack to help their busy schedule in order to ease their hunger during the day. However, many are concerned of its high sugar level and the nutritional value this product can offer [7].

Hence, the idea of this research is to create value added Khao-Mak by creating a ready-to-eat drinkable dessert product such as jelly mixed with Soymilk and Inulin. This will also provide an alternative choice for both business and consumer sectors to try a newly developed Khao-Mak dessert mixed with healthy benefits of soymilk and Inulin to help enhance digestibility and excretory system. Therefore, the objectives of this research are to study consumers' needs for drinkable jelly products and the effect of different concentration of gelatin and inulin of Khao-Mak rice jelly with soymilk on textural and sensory properties.

MATERIAL AND METHODS

Materials

RD43 rice used in this study was supplied by Ecovist Intertrade (Thailand) Co., Ltd. Gelatin (GEL; Food grade, Food Additive INS No. 428, 100% purity, made from cow skin) was purchased from Krungthepchemi (Thailand) Co., Ltd. Glutinous rice and Look-Pang were all purchased from a local farmers market at Ratchaburi, Thailand. All other chemicals used in this study were of analytical grade and purchased from chemical suppliers.

Methods

1. Study of consumer needs for fermented rice jelly

1.1 Study of consumer needs fermented rice jelly through questionnaire

Questionnaire was developed for studying the consumer needs and main factors for purchase. The questionnaire was divided into 3 parts;

Part 1: General information of respondents; demographic, gender, age, education, occupation and income.

Part 2: Information on consumer behaviours and activities associated with purchase of healthy goods

Part 3: Information of consumer needs and preferred characteristics of fermented rice jelly.

The 100 participants of age 20-70 who have consumed jelly products within the past 3 months and live around Bangkok area were chosen for as this questionnaire's targets.

Data analyzed by using SPSS for Windows. The statistics used are frequency, percentage, and mean. The conclusion resulted from consumer purchase factors and intentions were further used for in-depth interview.

1.2 Study of consumer needs fermented rice jelly through in-depth interview

2 age groups (21-37 and 38-53) were selected from questionnaires according to

purchase intention and product interest. Questions were divided into 7 parts;

Part 1: General information of interviewee's background; age, occupation, and city of residence.

Part 2: Experience questions relating to past experiences on jelly consumption

Part 3: Behaviour questions in relation to purchasing habits, influences, inconveniences found.

Part 4: Feeling questions in relation to health, concerns, and current solutions.

Part 5: Perspective/opinion questions regarding available jelly products in the market, interest for future products, and preferred packaging.

Part 6: Trade-off questions regarding whether reviews, word-of-mouth, samples would help making purchase decision for new products in the market and the acceptability on market's price differences.

Part 7: Knowledge questions regarding awareness and acknowledgement of materials and health benefits.

2. Effect of different concentration of gelatin and Inulin on textural and sensory properties of Khao-Mak rice and soymilk jelly

2.1 Khao-Mak Preparation

3 kg of raw RD43 rice was cooked in a water-to-rice ratio of 1:2 and left to cool down at 25°C for 30 minutes. The cool rice was mixed with minced Look Pang (a traditional starter culture) at ratio of 1:1 by weight (1 Look Pang to 1 kg of cooked rice) and was fermented for 3 days at 35°C. Fermented rice was then filtered through filter cloth. The sample liquid received from filtration was taken to pasteurization at 75°C for 10 minutes. The 25 g of stevia, 100 g of lychee liquid food flavor, and 32 g of citric acid were added to the Khao-Mak sample. During Khao-Mak preparation, soymilk was also heated at 75°C for 10 minutes. Heated Khao-Mak and soymilk at the ratio of 30:70 by weight were mixed.

2.2 Jelly Samples Preparation

The mixed liquid was added with inulin and gelatin. Samples were divided into 4 treatments as this follows: 3% gelatin with 1% Inulin (G3I1), 3% gelatin with 2% inulin (G3I2), 4% gelatin with 1% inulin (G4I1), and 4% gelatin with 2% inulin (G4I2). All samples were pasteurized at 75°C for 30 seconds and distributed into Ziploc bags. Samples are cooled down at 25°C for 30 minutes and stored at 4°C in the refrigerator. 3 replications were done for the experiment.

2.3 Color measurements

The color of drinkable Khao-Mak rice and soymilk jelly product was measured as lightness (L*), redness (a*), and yellowness (b*) values, using a colorimeter (Color Flex 45/0, Hunter Lab, USA), which was calibrated with a white standard plate. 100g of each sample was mixed well and transferred into a glass. The surface color was measured in triplicate. Mean and standard deviation values were calculated for each sample.

2.4 Texture analysis

Analysis of the texture was done by forward extrusion method (model TA-HDi, UK) with forward extrusion rig (TA-93). The sample was prepared by mixing the jelly into a container that has 5 mm. opening on the bottom. The test setting was at test speed of 1 mm/s, and distance 20.0 mm. The samples were measured in triplicate. Mean and standard deviation values (S.D.) were calculated for each sample. The samples' firmness was recorded for further discussion.

2.6 Sensory evaluation

Thirty untrained panellists who have purchased drinkable jelly within 1 month prior to the evaluation were recruited to participate in sensory acceptance evaluation of the products. The samples were prepared by loading 5x7cm Ziploc bags. The sample code was assigned using a 3-digit random number and served at room temperature.

Panellists were asked to evaluate their preferences in appearance, color, odor, flavor, taste, texture, and overall preference using the 9-point hedonic scale (9 - liked extremely, 1 - disliked extremely) and the 5-point Just About Right (JAR) to score from 5 (too strong) to 1 (too weak) on the same attributes. When JAR % is above 70.0 there is no need to make adjustment; however, if JAR % is below than 70, Net score is necessary to determine the direction of the adjustment. Net scores or net effect can be determined by the percentage of "too strong" responses minus the percentage of "too weak" responses. The penalty is considered significant when the Net score is greater than 20.0. When Net score results below the threshold of 20.0 demonstrates that there is no need for further adjustment (Rothman and Parker, 2009). Positive value net score indicates that reduction needed and negative net score value indicates that an increase in intensity is needed for adjustment.

2.7 Statistical analysis

Completely randomized design (CRD) was used for analysis of color and texture profile. Randomized complete block design (RCBD) was used for sensory evaluation. Analysis of variance (ANOVA) with Duncan's new multiple range test (DMRT) was applied. The level of significance used was 95% ($p \leq 0.05$). Net score or Net effect was used to demonstrate Just-About-Right, Net scores or net effect can be determined by the percentage of "too strong" responses minus the percentage of "too weak" responses (Rothman and Parker, 2009).

RESULTS AND DISCUSSION

1. Study of consumer needs for drinkable Khao-Mak rice and soymilk jelly product

1.1 Questionnaire

Part 1: Demographic data

The Demographic characteristics of 100 respondents, who are regular drinkable jelly consumers were shown in Table 1. 11.0% of male and 89.0% of female responded in questionnaires. Most of the respondents had age range between 31-40 years old (35.0%), and 41-50 years old (31.0%), beyond 24 years old (24.0%) and 20-30 years old (10.0%). Most respondents (53.0 %) have the highest degree of study in Bachelor's degree, followed by master's degree (43.0%), and below Bachelor's degree (4%) respectively. Most of the respondents were private company employees (49.0%), followed by business owners/contractors (26.0%), 17.0% have selected other for occupations as they are private/public school teachers. 5.0% are in government service, 2% are students, and 1% is a housewife. 58% of the respondents has income per month of more than 30,001 baht, followed by 10,001-20,000 baht (29.0%), 20,001-30,000 baht (11.0%) and below 10,000 baht (2.0%).

Part 2: Consumer behaviour, preference and purchase intent factors of drinkable jelly products

Respondents were to rate importance of each factor from 1-5, 1 being the least critical and 5 being the most critical factor. Mean was calculated for purchase intent factors of drinkable jelly products in Table 2. No preservatives were the most critical factor affecting consumer buying behaviour (4.6±0.5), followed by nutritional values (4.5±0.7), convenience (4.3±0.7), price (3.9±0.8) and advertisement (3.5±0.8).
Moreover, the most notable motives for purchasing drinkable jelly products are in Table 3, which respondents rate on a scale of 1-5 (1 = least important and 5 = most important). Health improvement was the crucial among consumers (4.5±0.7). Followed by flavor (4.4±0.7), skin improvement (4.0±0.9), hormone stabilization (3.4±1.1), and entertainment (2.8±0.8).

1.2 Study of consumer needs for drinkable Khao-Mak rice and soymilk jelly product through in-depth interview

16 drinkable jelly products regular consumers were interviewed (8 of Gen Y age 21-37 and 8 of Gen X age 38-53).

The responses showed that 100% of the interviewees prefer retort pouch packaging for drinkable jelly in both age groups as find it convenient and can be kept for multiple consumption. 100% of Gen Y prefer to purchase drinkable jelly products in convenient store such as 7-11; while, 80% of Gen X purchase these products at 7-11 and 20% at supermarket. Additionally preferable price for both age group range from 10-15 baht per bag. 90% of Gen Y, prefer a dominant flavor being sourness than sweetness while 100% of Gen X prefer sourness to sweetness as they are more health conscious and some are concerned regarding to their congenital diseases.

Regarding to an important factor that could intrigue purchase intention, 80% of Gen Y prefer to taste food samples prior to making purchase decision while, 60% of Gen X require certain conditions such as nutrition label, recommendations from advertisement or friends to be able to make purchase decision.

In knowledge questions, it was found that 50% of Gen Y consumers have basic knowledge of probiotics while 37.5% of Gen X have basic knowledge and 62.5% have awareness of the terminology but no knowledge of probiotics. Most of the consumers in both age groups (90%) do not have knowledge of Inulin.

Table 1 Demographic characteristics of respondents

Demographic characteristics	Frequency	Percentage
Gender		
Male	11	11.0
Female	89	89.0
Age		
20 –30 years old	10	10.0
31 – 40 years old	35	35.0
41 – 50 years old	31	31.0
Above 50 years old	24	24
Marital Status		
Single	57	57.0
Married	40	40.0
Divorce	3	3.0
Education		
Below bachelor's degree	4	4.0
Bachelor	53	53.0
Above bachelor's degree	43	43.0
Occupation		
Student	2	2.0
Government/civil servant	5	5.0
Private business employee	49	49.0
Owned business/contractor	26	26.0
Housewife/husband	1	1.0
Other	17	17.0
Income		
Below 10,000 Baht	2	2.0
Demographic characteristics	Frequency	Percentage
10,001 – 20,000 Baht	29	29
20,001 – 30,000 Bath	11	11.0
Above 30,001 Baht	58	58.0

Table 2 Purchase intent factors of drinkable jelly products

Factors of purchasing decision	Mean	S.D.
Price	3.90	0.8
Flavor	4.34	0.6
No preservatives	4.60	0.6
Nutritional value	4.50	0.7
Convenience	4.28	0.7
Advertisement/reviews	3.46	0.8

Remark: 5-point categorial scale (1 = Extremely unimportant, 3 = Somewhat important and 5 = Extremely important).

Table 3 Purchasing motives for drinkable jelly products

Motives	Mean	S.D.
Flavor	4.41	0.7
Health improvement	4.46	0.7
Skin improvement	4.02	0.9
Hormone stabilization	3.41	1.1
Entertainment	2.80	1.1

Remark: 5-point categorial scale (1 = Extremely unimportant, 3 = Somewhat important and 5 = Extremely important).

2. Effect of different concentration of gelatin and inulin on textural and sensory properties of drinkable Khao-Mak rice and soymilk jelly product

2.1 Color

The effect of color for different concentration of gelatin and inulin on Khao-Mak rice and soymilk jelly product were shown in Table 4. The result shows that L* values (Lightness) for all samples are not significantly different ($p > 0.05$). However, jelly sample of G4I2 and G3I2 formulas have the tendency to show higher L* value among all samples (77.4± 0.2 and 77.3±0.2 respectively). Delgado and Bañón (2017), also reported that the higher concentration of inulin results in higher L* value. This depends on the inherent optical properties of each gelling agent. A colorless inulin can increase jelly lightness. Additionally, another previous study of Serdaroglu et al. (2016) reported a similar result for sample that consisted of inulin and gelatin that it increases lightness; while reduces redness as the concentration of gelatin and inulin increase.

The sample with highest b* value being G3I1, which has the lowest concentration of both gelatin and inulin. However, in comparison to G4I1 that has the same concentration, the result is relatively low, which, means that inulin may not be the major factor that effects the yellow tonality.

The results also demonstrated that a* value tends to decrease as the concentration of gelatin increases similarly to b* value. The sample that has the lowest a* value is G4I2 (1.9±0.1), followed by G4I1 (2.1±0.0). The sample that has highest b* value is G3I1 (16.9±0.1), followed by G3I2 (15.7 ± 0.1), which shows that the lower concentration of gelatin, the more yellow tonality presents. The result of a* and b* values align with a previous study that showed both values decreased with an increase in gelatin concentration [6].

Table 4 Effect of different concentration of gelatin and inulin on color parameters

Sample	Mean ± S.D.		
	Lightness (L*) [ns]	Redness (a*)	Yellowness (b*)
G3I1	77.2 ± 0.1	2.12 ± 0.0[a]	16.92 ± 0.1[a]
G3I2	77.3 ± 0.2	2.05 ± 0.0[b]	15.70 ± 0.1[b]
G4I1	77.1 ± 0.5	2.04 ± 0.0[b]	15.57 ± 0.1[c]
G4I2	77.4 ± 0.2	1.92 ± 0.0[c]	15.56 ± 0.01[c]

[a,b,c] Mean±Std.deviation (SD) with different superscript letters indicating significant differences ($p \leq 0.05$) [ns] Not significantly different ($p > 0.05$).

2.2 Texture analysis

The effect of different concentration of gelatin and inulin of drinkable Khao-Mak rice and soymilk jelly product on texture properties were shown in Table 5**.** The result shows that G4I1 has the highest firmness among all samples at 3174.0 ± 34.1 g_f. Although inulin effected in formulas with 3% gelatin as firmness increase as inulin concentration increases. The sample of G4I2 which had 4% of gelatin and 2% of inulin presented a lower firmness than G4I1 sample, which contains 4% of gelatin and 1% of inulin. The result is aligned with a previous study on gellan gels and inulin's effect on texture (Evageliou et al., 2010) where the presence of inulin initially affects gellan gel strength and firmness. As gellan gels are formed in presence of cations; therefore, the concentration of inulin effects mechanical property in forming gel network and increases its gel strength; however, a high concentration of inulin decreases the gel strength. Another previous study of Serdaroglu et al. (2016) reported high concentration of inulin and gelatin, shows lower values in texture properties such as hardness, gumminess and chewiness as the presence of inulin and gelatin can pose fat and water binding capacity, which can reduce emulsion stability. Hence, this shows that there is an interaction between high concentration of gelatin and inulin; which, affects gel network and causes firmness to reduce. The fiber in inulin may interrupt the gelation process in high gelatin concentrated sample.

Table 5 Effect of different concentration of gelatin and inulin on texture property

Sample	Firmness (g_f)
	Mean ± S.D.
G3I1	1323.5 ± 6.4[d]
G3I2	1608.5 ± 26.6[c]
G4I1	3174.0 ± 34.1[a]
G4I2	2141.3 ± 45.7[b]

[a,b,c,d] Mean±Std.deviation (SD) with different superscript letters indicating significant differences ($p \leq 0.05$).

2.4 Sensory evaluation

Effect of different concentration of gelatin and inulin of drinkable Khao-Mak rice and soymilk jelly product on sensory properties was shown in Table 6. Statistically, attributes on appearance, color, flavor, and sweetness are not significantly different. However, there is a tendency for the sample of G4I2 to receive a higher sensory score on appearance and color (6.9±1.5 and 6.9±1.5) in comparison to other samples. Whereas, G3I2 tends to receive higher score among other attributes. The sample of G3I2 received a highest score firmness (7.3±1.7) and overall liking (7.3±1.4), which shows a significant difference statistically in comparison to other samples. The sample that received the lowest score in firmness and overall liking being G4I1 and G4I2. This shows that the incorporation of 3% gelatin contributes positively to consumers' liking.

Samples that have received highest JAR (%JAR≥70) being G3I2 (70%) and C4I1 (76.7%). All samples have received Net score below 20.0 on appearance; hence, there is no need to make further adjustment. All samples

have received Net score on firmness over 20.0; which means that there needs to a further adjustment by making them less firm. Sample that has received highest JAR% among all samples on color, flavor, sweetness, and firmness being G3I2 (Range 60-67%); however, these attributes are still below 70%. When investigated Net score for sample G3I2, attributes such as flavor, sweetness and firmness are above 20.0, which means that there needs to be further adjustment in these attributes. Considering the result from 9-point hedonic scale, panellists have the tendency to prefer G3I2 over other samples on color, flavor, and firmness; therefore, on future experiment, a slight reduction on these attributes can help increase Net score for sample G3I2.

Table 6 Effect of different concentration of gelatin and inulin on sensory properties

Sample	Mean ± S.D.					
	Appearance^{ns}	Color^{ns}	Flavor^{ns}	Sweetness^{ns}	Firmness	Overall liking
G3I1	6.6±1.3	6.60±1.3	6.7±1.5	6.7±1.5	7.1±1.8^{ab}	6.9±1.4^{ab}
G3I2	6.7±1.4	6.90±0.9	7.0±1.2	7.1±1.4	7.3±1.6^{a}	7.3±1.4^{a}
G4I1	6.7±1.2	6.67±1.3	6.6±1.2	6.3±1.4	6.3±1.6^{b}	6.4±1.1^{b}
G4I2	6.9±1.5	6.97±1.5	6.9±1.5	6.9±1.5	6.4±1.9^{ab}	6.7±1.5^{ab}

^{a,b} Mean±Std.deviation (SD) with different superscript letters indicating significant differences ($p \leq 0.05$). ^{ns} Not significantly different ($p > 0.05$).

Table 7 Percentage of consumers considering Appearance, Color, Flavor and Firmness as just about right (JAR) and deviation (positive and negative values) for each drinkable Khao-Mak rice and soymilk jelly product sample

Sample	Net Effect				
	Appearance	Color	Flavor	Sweetness	Firmness
G3I1	20.0	10.0	30.0	6.7	33.4
G3I2	16.6	6.7	36.7	26.7	26.7
G4I1	16.7	10.0	6.6	-6.7	36.7
G4I2	10.0	3.3	6.6	6.7	46.6

Remark: Just-about-right (JAR) scale (0 = Too weak, 3 = JAR and 5 = Too strong).
Net score<20.0 = Just right, no need further adjustment. Net score>20.0 =Adjustment needed

CONCLUSIONS

According to the consumer behaviour, the most important purchase intent factor of drinkable Khao-Mak rice and soymilk jelly product was the absence of preservative preference factor. For color measurement, L* values for all samples are not significantly different ($p > 0.05$); however, there shows a tendency for higher L* value in samples with higher concentration of gelatin and inulin. On the other hand, higher concentration of gelatin and inulin resulted in lower a* value and b* value. According to texture analysis, firmness is highest in sample of 4% gelatin and 1% inulin; while the firmness of sample with 4% gelatin and 2% inulin decreases. The topmost sample chosen by most participants was the sample of 3% gelatin and 2% inulin formula in terms of firmness and overall liking scores. JAR test reveals that sample with 3% gelatin and 2% inulin and 4% gelatin and 1% inulin formulas have received highest JAR% among all samples. Considering 9-point hedonic scale result, sample of 3% gelatin and 2% inulin is most preferred by the panellists; which means that for future product development of this formula, there needs to be a further adjustment in slight reduction on flavor, sweetness and firmness in order to match consumer preference.

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Observation on consumer behavior and market trends for health promoting products with fiber fortification

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ABSTRACT: The urban lifestyle may affect to increase risk factors for noncommunicable diseases (NCDs) which are the important health issue for Thai people. Due to the pandemic of Covid-19, self-isolating of the consumers, especially for the ones having work outside home lifestyle, resulted in increasing of stress and weight control problem due to inactivity. NCDs and their behavioral risk factors, moreover, enhance susceptibility for infection and severe symptoms of Covid-19. These causes led to increasing of consumers' needs for wellness products to support both ~~of~~ physical and mental conditions. The present work aimed to elucidate consumers' behavior and needs considering nutritional products. The market trends were also explored in order to provide the interesting way for nutritive product development. The survey was conducted with the target consumers who had rush lifestyle of work outside home pattern. The survey indicated unhealthy lifestyle of the consumers which related to increasing risk factors for NCDs involving less vegetable consumption and no exercise. These behaviors also led to promote occurrence of constipation problem. To solve constipation problem, the consumers tended to increase their water, fruits, and vegetable intake, as well as purchase the products with constipation relief effect. Requirement of the target consumers for the constipation relief effect had been enlighten for the refreshing beverages, and additional claims were also important, especially for the claims of less sugar, improve immune system, and antioxidant effect. Considering the market trends on nutritive products, the "value" trend indicating clear benefits of the products, especially bioactivity claim was emphasized. This suggested the importance of scientific supporting data on functional properties of the used ingredients, which was in accordance with needs of the present target consumers on selection of the product with constipation relief effect. For the domestic market trends, the products fortified with fiber were interesting, owing to functional effects for supporting excretion, weigh control, and gut microbiome.

Keywords: Covid-19, Consumer behavior, Nutritional product, Fiber fortification, Market trends

INTRODUCTION

Nowadays, importance of noncommunicable diseases (NCDs) is growing continuously, regarded to their high fatal rate and economic burden relating to treatment and lost productive capacity of the pateients. The important risk factors of NCDs are related to behavior in daily life of consumers, which are obesity, physical inactivity, alcohol drinking, and smoking [1]. These behavioral risk factors are relevant to urban lifestyle which is always rush, less excersizing time, and high stress. Moreover, inactive behavior may be enhanced due to a presence of some facilities such as elevator and escalator.

Since late2019, the World population has been threatened by the Covid-19 pandemic. Considering Thailand, the first infection case was reported on 13 January 2020, and the infected cases have been increased continuously. Outbreak of the Covid-19 leads

to self-isolating and spending more time at home of consumers, thereby affecting their behavior, especially for the people who have work outside home lifestyle. NCDs and their behavioral risk factors, moreover, affect to promote susceptibility for infection and severe symptoms of Covid-19 [1].

As driven by NCDs epidermic and Covid-19 pandemic, the interest of consumers for well-being products that can enhance both physical and mental conditions has been growing. The present study aimed to elucidate the interesting trend for development of food products with beneficial health effect. To accomplish this purpose, behavior of the consumers who have urban lifestyle was explicited. The effects of the Covid-19 pandemic on market trends were also determined in both international and domestic markets.

MATERIAL AND METHODS

Consumer survey

The study on consumer behavior considering health relating behaviors and health problems was conducted with 130 consumers during the period of February to March 2021. The target consumers were selected based on the rush lifestyle of work outside home pattern with the age of ≥ 20 years old, because of their consideration for health care as well as own purchasing power. The data of demographic, health relating behavior, and health problem were collected. Then, the major health problem was focused, and the consumers with the problems were selected as the representatives for deep interview. The data considering ways to solve problem, pain points and factors affecting functional food products choosing were further gathered.

Market trends for functional products

Market trends for functional food and beverage products, especially for the ones with fiber fortification was explored using the Global new products database [2]. To accomplish this goal, both report analysis from GNPD's experts and product databases were collected and discussed. The used keywords were "functional claim" and fiber ingredient", and the products were selected based on their availability in the market since the worldwide emergence of Covid-19 outbreak, *i.e.*, January 2020 to February 2022.

RESULTS AND DISCUSSION

Survey on consumer behavior

Firstly, the questionnaire was used to observe the target group who had the rush lifestyle of work outside home pattern. The demographic of the consumer was described in Fig. 1. Most of them were female (69.2 %) with the age of 20–30 years old (66.9 %). The main occupation of the consumers was company employee (59.2 %) with the average income of 10,000–30,000 THB/month (67.69%).

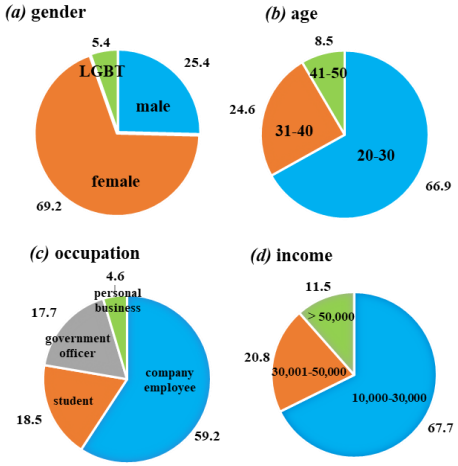

Figure 1 Demographic data of the consumers: *(a)* gender, *(b)* age (years old), *(c)* occupation, and *(d)* income (THB/month)

Next, behaviors relating personal health were observed. Most of the consumers took vegetables for 1/3 (41.5 %) and less than 1/4 (40.0 %) portion of their plate/meal. The highest average amount of drinking water was 1.0–1.5 L/day (43.8 %), and most of consumers took less than 5 cups of coffee or tea per week (41.5 %). Most of the consumers (54.6 %) had no exercise. As recommended by the Thai

Health Promotion Foundation for healthy lifestyle, Thai people should have daily intake for fruits and vegetables not less than 1/2 of plate per meals and 8 cups (*ca.* 1.9 L) of water, and exercise is recommended [3]. The present survey results, therefore, suggested the unhealthy lifestyle of the consumers. This may increase risk for occurrence of some physical disorders, especially for the NCDs. NCDs consisting of four main diseases *i.e.*, cancer, cardiovascular diseases, diabetes, and chronic obstructive pulmonary disease, are major causes of Thai people death [1]. Large economic burden relating to treatment and lost productive capacity of the pateients is also affected by the NCDs, in which 1.6 trillion THB equivalent to 9.7 % of 2019 Thai GDP has been reported [1].

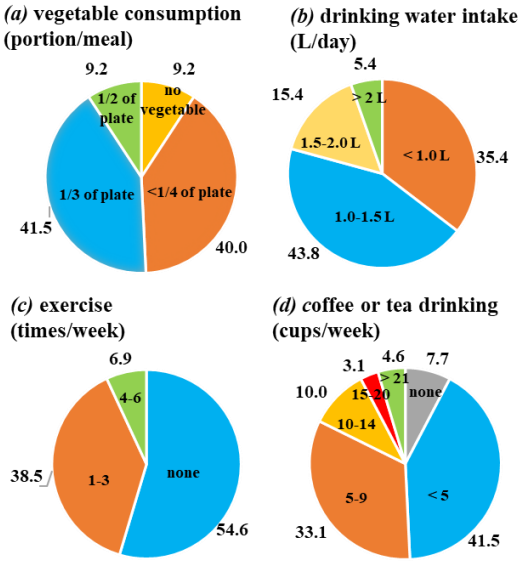

Figure 2 Health relating behavior of the consumers: *(a)* vegetable consumption, *(b)* drinking water intake, *(c)* exercise, and *(d)* coffee or tea drinking

To more elucidate behavior of the consumers, the survey relating on health and mental problems was performed. Considering health condition, most of the consumers used to have constipation problem with the percentages of 87.5 %, 94.12 %, 85.71 %, and 92.86 % for the consumers with > 35 years old, 31-35 years old, 26-30 years old, and 20-25 years old, respectively. Constipation defined as a usually abnormal conditions of bowel movements with less than 3 times/week of excretion and having difficulty for excretion with harder or smaller size of stools [4]. As classified by gender, about 78.8 %, 90.9 %, and 100 % of the male, female, and LGBT had been suffered from constipation, respectively. Fifty percent of the consumers said that their constipation occurrence was always related to the behaviors of no exercise, less fruits and vegetable consumption, and stress condition. This result was in accordance with the study of [5] reporting the positive correlation between constipation frequency and stress of the medical students. It should be noted that apart from health status, the target consumers also faced to stress condition.

The behavior of the consumers when facing the constipation problem was further explored, and the result was shown in Figure 3. Upon constipation problem, changes in the consumers' behavior were found by increasing intake amounts of fruits and vegetables (27.3 %), having milk products with probiotics (26.6 %), and drinking more water (23.7 %). Almost 40 % of consumers were interested in the products with constipation relief effect including dairy products and other foods and beverages. The factors affecting consumers' choosing for constipation relief products were also revealed in Figure 3. Selection of consumers for the products with constipation relief effect was mainly based on recommendation from close people (27.6 %). Scientifically support data (26.4 %) were also important for consumers' product selection. This supported the market trends on "value" of the selected products, especially for the bioactive claim which was discussed thereafter. Interestingly, the deep interview provided the data that the consumers with constipation problem also suffered from other abnormally physical conditions including stomachache, bloating, and increase urinary frequency, especially for the ones taking laxative and/or more amount of water intake.

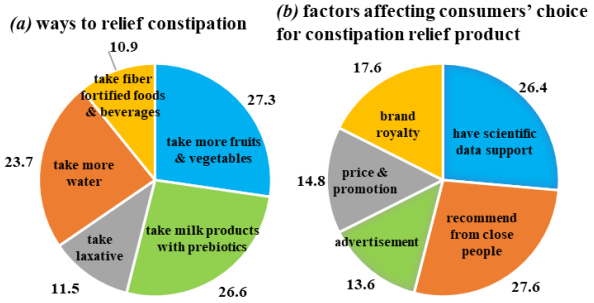

Figure 3 Behaviors of the consumers with constipation problem: *(a)* ways to relief and *(b)* factors affecting anti-constipation product selection

Based on the survey, requirement of the target consumers for the products with constipation relief effect was focused for beverage and drinks. According to the work outside home lifestyle and hot climate of Thailand, the target consumers preferred beverages with convenience to purchase and eating way, *i.e.*, the product should provide the effective constipation relief effect with reasonable serving size. Requirement for the product detail was shown in Figure 4.

From Figure 4, one can see that the beverages with clear appearance (60.8 %) were the most required, and the products should provide refreshing flavor. The other health claims attracting consumers were the claims of less sugar (28.4 %), improve immune system (24.5 %), and antioxidant effect (21.3 %). The survey implied the use of indigenous Thai fruits as a key ingredient for product development, owing to their refreshing flavor and natural presence of phytochemicals with bioactivities supported by scientific data. From the interview, the consumers preferred local ingredients regarded to freshness and lowering energy consumption due to material transportation.

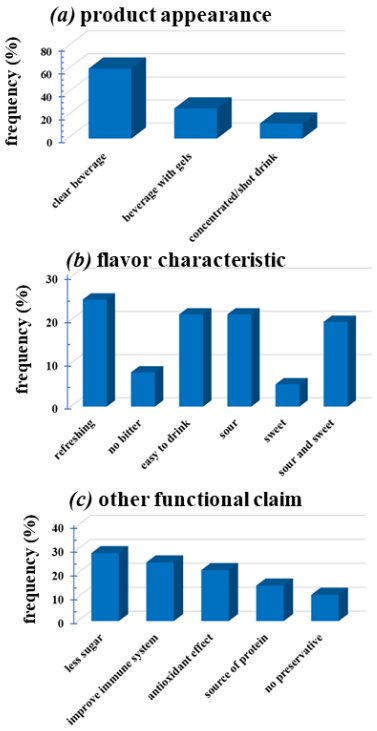

Figure 4 Requirement of the target consumers for the products with constipation relief effect

Market survey for nutritional products

Due to the pandemic of Covid-19, product trend in "well-being" for both physical and mental conditions had been growing, resulting in the worldwide market trend for nutritive food products [6]. Consumers' need for "value" was also mentioned, in which the tangible and measurable benefits from what they pay in terms of quality, budget, convenience, and premium attributes were important [6.]. This value trend corresponded to the behaviors of the consumers reported in the present work, in which scientific support data was important for their product selection (see Figure 3). Considering the outspread of Covid-19, moreover, consumers had to self-isolate and spend more time at home, resulting in stress increasing and struggling with weight gaining due to inactivity [7]. Top three of mental concerns of the consumers related to future life (48.0 %), financial status (41.0 %), and health

condition (40.0 %) [8]. Several countries have proceeded the self-quarantine and lockdown measures including Thailand, resulting in gaining stress, rising living expenses, and unproductive work under pressure due to fear of unemployment [9]. This affected to market trends, in which seeking for joyful products that boost the flavor, color, textures, and aromas of foods and drinks have been increased [10].

The top three categories and claims for foods and beverages launched during January 2020 to February 2022 were determined to overview market trend, and the result was revealed in Table 1. In all surveyed markets, most of the products were claimed for functional properties, which was in accordance with consumers' well-being needs for healthy. The claims of "suitable for" suggested to importance of product optimization for each consumer group such as Halal, vegetarian, and diabetic persons. The "natural" claim highlighted the importance of clean ingredients, *i.e.*, the materials derived from natural sources such as herbs, and no use of artificial additives and/or flavorings in the products. The term of "plus" represented the addition of nutritive value, *i.e.*, fiber and proteins, and bioactive compounds, *i.e.*, vitamins and minerals, to the products.

Enlighten of consumers' requirement on well-being foods could be confirmed by increasing in fiber fortified products, especially for the ones with prebiotic effect. It had been scientifically supported that consumption of foods with high fiber could enhance excretion, and decrease risk of obesity, diabetes type II and cardiovascular diseases [11, 12]. Considering the fiber with prebiotic efficacy, moreover, recent studies had been supported the importance of healthy gut microbiome on weight loss [13,14]. It should be noted that important risk factors for NCDs were suggested for obesity, physical inactivity, alcohol drinking, and smoking [1].

Table 1 Top three categories and claims of functional foods and beverages launched during January 2020 to February 2022 in different regions

	all regions	Asia Pacific	Thailand
categories	1. dairy	1. dairy	1. dairy
	2. snacks	2. nutritional drinks & other beverages	2. baby food
	3. nutritional drinks & other beverages	3. snacks	3. nutritional drinks & other beverages
claims	1. functional	1. functional	1. functional
	2. suitable for	2. suitable for	2. suitable for
	3. natural	3. plus	3. plus

Jointing to the present consumer survey, the fiber fortified beverages available in Thailand market were focused, and the result for market survey was shown in Figure 5.

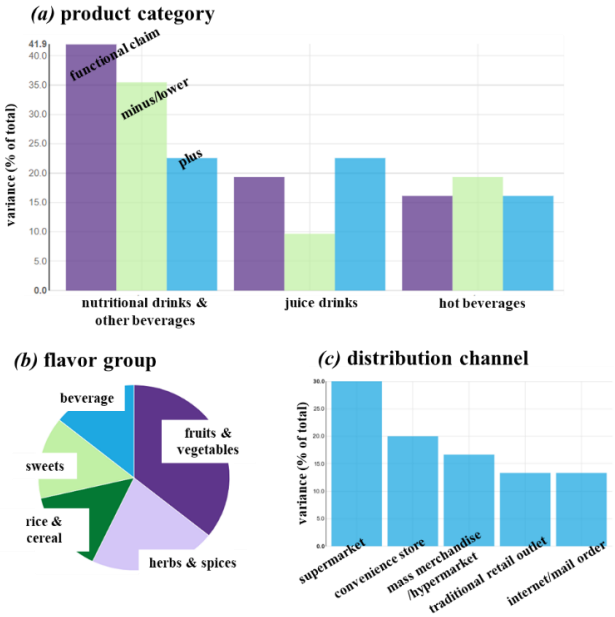

Figure 5 Market survey for the beverages fortified with fiber available in Thailand during January 2020 to February 2022

The most available product categories of the fiber fortified beverages were nutritional products, juices, and hot beverages, in which the top three subcategories of the launched products were nutritional & meal replacement drinks, juices, and malt & other hot drinks. Popularity on physical and mental well-being

trend was suggested from the market survey as indicated by most of the products were claimed for functional properties involving physical (*e.g.*, eye health, digestive, and immune system) and mental (e.g., stress & sleep); minus/lower (*e.g.*, levels of calorie and sugar); and plus (*e.g.*, contents of fiber, vitamins, and proteins). The most available flavor was found for the flavor tones of fruits & vegetables. The convenience to buy was also important as suggested by the highest used distribution channels were supermarket and convenience store.

Considering product ingredients, the most employed raw materials as a fiber source for food and beverage products were explored and represent in Table 2. Inulin possessed the highest used in all regions, followed with fructo-oligosaccharide (FOS) and polydextrose /dextrin. Popularity for usage of these ingredients might be accounted for their health promoting benefit as dietary fiber and prebiotic effect as well as the effective functional property to enhance product characteristic such as acting as fat replacer, sugar replacer, and texture modifier. These agents are also safety as GRAS status and generally produced from natural sources, making them interesting as a clean ingredient.

Table 2 The most employed ingredients as a fiber source for food and beverage products launched during January 2020 to February 2022 in different regions

all regions	Asia Pacific	Thailand
1. inulin (23.9 %)	1. inulin (21.1 %)	1. inulin (26.1 %)
2. FOS (13.1 %)	2. FOS (19.9 %)	2. dextrin (25.5 %)
3. polydextrose (11.2 %)	3. polydextrose (13.7 %)	3. FOS (17.6 %)

Numbers in parenthesis indicated percentages of products with the ingredients compared to all products

The market trend was further explored by investigating the fastest growing ingredients used for nutritional drinks and other beverages, and the result was shown in Table 3. The worldwide market clearly showed interesting in functional products relating to weight controlling effect as indicated by the fastest growing use of dietary fiber and manganese gluconate with potential weight loss effect [15]. Interestingly, magnesium supplementation might provide supportive effect for Covid-19 remedy, owing to its prevention effect for disorders and diseases related to respiratory system [16]. This trend was also observed for the market of Thailand, in which non-caloric sweetener is popularly used. Employment of vitamin C might be supposed due to its effective antioxidative property as well as capacity to minimize occurrence of some chronic diseases such as cancer and heart disorders [17]. Moreover, vitamin C could enhance immune system [18] and exhibited antiviral capacity [19], thereby possibly corresponding to prevent Covid-19 infection [20]. The clinical studies showing the remedy effect of ascorbic acid against pneumonia of the Covid-19 patients had been reported [21]. The present result suggested the market trends of the nutritive products driven by the Covid-19 situation.

Table 3 the fastest growing ingredients for nutritional drinks and other beverages for the worldwide and Thailand markets

all regions	Thailand
soluble corn fiber (+ 353.1 %)	vitamin C (+ 57.5 %)
Lactobacillus paracasei (+ 251.5 %)	trisodium citrate (+ 43.4 %)
manganese gluconate (+ 193.6 %)	acesulfame potassium (+25.3 %)

Numbers in parenthesis showed the percentage changes in market penetration of the ingredients in last 12 months compared to the previous period

Within the last year, moreover, there is emergence of new ingredients employed for the nutritional drinks and other beverages. In Thailand, the most used new ingredients were subjected in natural substances including gymnema and colorant astaxanthin. The gymnema is derived from *Gymnema sylvestre* which is the herb traditionally used as a folk medicine. Broad therapeutic effects of

gymnema have been reported such as asthma, hypercholesterolemia, diabetes, cardiopathy, microbial infection, indigestion, as well as constipation remedy effects [22]. As relevant to the fastest growing ingredients, the interesting trend for new product launched in Thailand might be regarded to weight controlling effect, which was in agreed with the international market trend [10]. For the Worldwide market, moreover, the most used new ingredient was Fava bean protein isolate. This emphasized the increasing in utilization of plant-based protein in the global food product.

CONCLUSIONS

The present study suggested the importance of NCDs epidemic and Covid-19 pandemic on consumer behavior and market trends of food and beverage products. Interest in the products providing "well-being" with supporting effect both physical and mental health has been growing. Moreover, the "value" of the products, especially for the claimed bioactivity, was emphasized implying the importance of scientific supporting data on functional properties of the food ingredients. For the domestic market, the products with fiber fortification were interested regarded to functional properties to promote excretion, weigh control, and gut microbiome. Moreover, the products should provide convenience and other functional claims were also important, especially for calorie lowering and immune enhancing effects.

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Effect of egg white hydrolysate on the quality characteristics of gluten-free bread

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ABSTRACT: Gluten-free products have been created for celiac disease patients. However, developing a good quality gluten-free bread (GFB) is considered a challenge. Many proteins are used to enhance the physicochemical properties of foods. Moreover, enzymatic hydrolysis is an effective way to improve protein functionalities**.** The aim of this research was to evaluate the effect of egg white hydrolysate (EWH) and freeze-dried egg white hydrolysate (FD-EWH) by Thermoase PC10F on the quality characteristics of GFB. The results showed that EWH had higher the surface hydrophobicity ($p \leq 0.05$) compared with the egg white powder (EW). EWH increased ($p \leq 0.05$) both of the surface hydrophobicity and the foaming capacity but these were decreased ($p \leq 0.05$) after freeze drying. Foam stability of EWH and FD-EWH at 60 min decrease ($p \leq 0.05$). Substitution of gluten-free flours with 0.5% EW, 0.5% EWH, and 0.5-1.0% FD-EWH increased ($p \leq 0.05$) the specific volume of the GFBs. An increase in specific volume corresponded to a decrease in hardness and chewiness in GFB with 0.5% EWH and 0.5–1.0% FD-EWH. In addition, the springiness and cohesiveness of GFB with 0.5% of EWH and FD-EWH had significantly higher ($p \leq 0.05$) than that of the GFB with EW at the same level. The cross-section images revealed that GFB with 0.5% EWH, and 0.5-1.0% FD-EWH had a well-aerated crumb structure, while the crumb structure of the control GFB was incredibly dense. As a result, adding 0.5% EWH and 0.5–1.0% FD-EWH to the GFB formulation enhanced the softness of the bread crumb. Our results suggested that FD-EWH could be used instead of EWH for ease of use.

Keywords: Gluten-free bread, Freeze-dried egg white hydrolysate, Egg white hydrolysate, Thermoase PC10F

INTRODUCTION

Wheat flour plays a crucial role in the bread making process because it contains two important proteins, namely gliadin and glutenin [1]. After hydration and kneading, both proteins form a gluten network, which is responsible for the dough's elasticity and ability of dough to retain CO_2 gas produced by yeast, resulting in the characteristic foam structure of bread [1]. However, gluten can lead to health problems such as celiac disease [2]. Gluten-contaminated food is a trigger of an immunological reaction in the small intestine of celiac patient causing a range of symptoms, including diarrhea, weight loss, anemia, and other serious complications [2]. Thus, gluten-free breads (GFB) have been designed for people who suffer from celiac

disease. A bread without gluten often has low specific volume, rigid texture, and low nutritional value [3]. GFB with added or substituted protein is an interesting because protein not only improves functional properties but also enriches the nutritional values of GFB [4]. Previous studies indicated that egg white protein improved the specific volume and texture of GFB. However, the quality of GFB is still lower than that of the bread with wheat flour [4]. A few researchers found that bread with protein hydrolysates using enzymatic hydrolysis increased the specific volume and decreased the crumb hardness compared to bread with native proteins [5,6,7]. Enzymatic modification may improve the protein's functionalities by changing the molecular size and hydrophobicity [8,9]. In addition, the functional properties of proteins have key influence on the physical characteristics of food products, such as textural and sensory properties [10]. Several studies indicated that enzymatic hydrolysis at an optimum degree improved the foaming properties of proteins [7,9,11,12], due to potentially enhancing their ability to retain gas during the proofing and the baking processes. Consequently, better quality GFB could be produced. Many researchers studied and used egg white protein to improve the quality of GFB, but none of them studied the effect of egg white hydrolysate (EWH) on the quality of GFB. The objective of this research was to evaluate the foaming properties of EWH by Thermoase PC10F and the effect of EWH on the quality characteristics of GFB. Moreover, the study was extended to produce freeze-dried EWH to enhance the ease of use in GFB. Since the freeze-drying process could affect the structure and functional properties of proteins [13]. Therefore, the freeze-dried egg white hydrolysate (FD-EWH) was also used in this research.

MATERIAL AND METHODS

Materials

Rice flour, tapioca starch, job's tears flour, instant dry yeast, sugar, salt, honey, apple sauce, cider vinegar, vegetable (soybean) oil were purchased from local supermarkets (Pathum Thani, Thailand). Xanthan gum, guar gum, disodium phosphate, sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium alginate, and calcium chloride ($CaCl_2$) were obtained from Chemipan Corporation Co., Ltd. (Bangkok, Thailand). Egg white powder was obtained from Thai food and chemical Co., Ltd. (Bangkok, Thailand). Thermoase PC10F from *Bacillus stearothermophilus* was donated by Amano Enzyme Asia Pacific Office (Thailand Science Park, Pathum Thani, Thailand).

Enzyme immobilization

Thermoase PC10F immobilization was prepared according to Adinarayana and coworking [14] with slight modification. A ratio of enzyme:substrate (E/S) was fixed at buffer (20mM, pH8.0) at concentration of 1.8% w/w. Enzyme solution was mixed with 2.4% w/w sodium alginate in a ratio of 3:5 w/w.

Table 1 Composition of batters (in percentage of total weight).

Sample	Rice flour	Tapioca starch	Job's tears flour	Various egg white hydrolysate types	Other ingredients[a]
0.0% EW (Control)	16.00	15.00	8.00	0.00	61.00
0.5% EW	15.79	14.81	7.90	0.50	61.00
0.5% EWH	15.79	14.81	7.90	0.50	61.00
0.5% FD-EWH	15.79	14.81	7.90	0.50	61.00
1.0% FD-EWH	15.59	14.62	7.79	1.00	61.00

[a] Composed of 30% water, 0.3% xanthan gum, 0.25% guar gum, 0.7% salt, 1.5% sugar, 1.2% instant dry yeast, 13% honey, 11% apple sauce, 0.5% cider vinegar and 2.55% vegetable oil.

Twenty milliliters of the mixture was extruded

dropwise from a 10 mL syringe into 100 mL of 0.3M $CaCl_2$ solution. Beads were collected by filtration then washed with distilled water and finally washed with phosphate buffer (20mM, pH8.0).

Egg white hydrolysate preparation

Egg white powder (EW) was dispersed in phosphate buffer (20mM, pH8) at a concentration of 3% w/v. After stirring for 60 min at room temperature, the pH of the solution was adjusted to 8.0±0.1 with 1.0N NaOH or 1.0N HCl, then pre-heated at 65°C for 10 min in a shaking water bath. Immobilized Thermoase PC10F was added into the egg white solution, then incubated at 65°C for 45 min. During hydrolysis, the pH of the solution was maintained at 8.0±0.1 with 1.0N NaOH. After the reaction end, the immobilized enzyme was removed from the egg white hydrolysate solution (EWH) by filtration. The EWH solution was incubated in an ice bath at 0°C for 10 min. The EWH solution was kept at 4°C in a refrigerator then freeze-dried. The freeze-dried egg white hydrolysate (FD-EWH) was stored in an aluminum foil bag at room temperature until further use.

Egg white hydrolysate solution preparation for determination of foaming properties and surface hydrophobicity

The EW and FD-EWH were dispersed in phosphate at a concentration of 3% w/v, then stirred for 60 min at room temperature. The pH of the solution was adjusted to 8.0±0.1 with 1.0N NaOH or 1.0N HCl.

Degree of hydrolysis

Degree of hydrolysis (DH, %) of the EWH sample was modified from Adler-Nissen [15] using the pH-stat method, which was estimated from the amount of base (1.0N NaOH) added every 15 min to maintain the pH at 8.0±0.1 during the hydrolysis. The DH was calculated according to the following equation:

$$DH\ (\%) = \frac{N_b \times B \times 100}{\alpha \times m_p \times h_{tot}}$$

where N_b is the normality of the base, B is the consumption of the base in mL, α is the degree of dissociation of α-amino groups ($1/\alpha = 1.06$ at 65°C and pH 8.0±0.1), m_p is the mass of protein in grams and h_{tot} is the total amount of peptide bonds per weight unit of a protein. The h_{tot} can be calculated from its amino acid composition (for EWPs h_{tot} is 7.67 mmol/g protein)

Foaming properties

Foaming properties of protein solutions were determined as described by Uzun et al., [16] with slight modification. Briefly, 20 ml of the EW, EWH, and FD-EWH solutions were transferred to a 250 ml-graduated glass cylinder equipped with an air-feeder. The air was applied into the bottom of the glass cylinder by air pump (SC-7500 BOYU air pump, China) at a constant flow rate of 10.5 mL/s for 20 s. Immediately after turning off the air pump, the initial foam volume was measured. Foaming capacity (%FC) was expressed as an increasing percentage after foam formation at 0 min, which was calculated according to the following equation:

$$\%FC = [V_2\text{-}V_1]/V_1 \times 100$$

where V_1 is the volume before foam formation and V_2 is the volume after foam formation. Foam stability (%FS) at 30 and 60 min were estimated as the percentage of foam remaining after 30 and 60 min, respectively. The foam stability was calculated according to the following equation:

$$\%FS = [V_t/V_0] \times 100$$

where V_0 is the volume after foam formation at 0 min and V_t is the volume after foam stays at a time interval.

Surface hydrophobicity

Hydrophobicity on the surface of protein solutions were measured by a fluorescence probe method as described by Alizadeh-Pasdar and Li-Chan [17] with slight modification. 1-anilinonapthalene-8-sulfonic acid (ANS^-) was

used as a hydrophobic fluorescence probe. Briefly, 8 mM solution of ANS^- (in 0.1M phosphate buffer, pH7.0) was prepared. EW, EWH, and FD-EWH solutions were serially diluted with phosphate buffer (20mM, pH8.0) to a proper concentration (0.01–0.45 mg/mL). Then 5 µL of ANS^- solution was added to 1 mL of diluted samples. The fluorescence intensity of the samples was measured using a microplate reader (Infinite M200 PRO, TECAN Austria GmbH, Austria) under excitation and emission wavelength at 390 nm and 470 nm, respectively. The slit was set to 5 nm. The slope of the relative fluorescence intensity versus the protein concentration (mg/ml) was calculated and used as a hydrophobicity (H_0) for protein surface hydrophobicity.

Bread making procedure

The formulation of GFB was slightly modified from Threewiriyanukoon [18]. Composition of batters in percentage of total weight was shown in Table 1. The bread-making process was as follows: Instant dry yeast was added into 40°C sucrose solution followed by gentle stirring then waiting for 5 min. Next, the yeast mixture and all dry ingredients were added into the bowl of a BioloMix mixer with a flat beater (BM6178, China) and mixed for 4 min at medium speed (speed 3 from 6). After that, honey, apple sauce, and cider vinegar were added and mixed at the medium speed for 0.5 min, then at the high speed (speed 6 from 6) for 2.5 min. The vegetable oil was added to the batter and continued beating at the medium speed and high speed for 0.5 and 5.5 min, respectively. Seventy grams of the homogeneous batter were placed into an aluminum pan (70x54x35 mm), proofed at 35°C for 30 min, mixed one more at medium speed (speed 2 from 6) for 20 s., and baked at 180°C for 20 min in the oven (HÄFELE, HBO-9FUN70L, German). After baking, the GFB was taken from the pan, cooled for 1 h at room temperature, and then stored in a low density-polyethylene (LDPE) bag for 24 h before being measured for specific volume and texture.

Specific volume measurement

The specific volume of the GFB was determined by rapeseed displacement method according to AACC [19]. Three independent batches were performed for each treatment.

Texture profile analysis

Texture profile analysis of the GFB was measured using the texture analyser TA-XTPlus (Stable Micro Systems, UK) after 24 h of storage at room temperature. Sample was cut from the centre of the loaf to a cubic shape (20x20x20 mm) then compressed to reach 50% deformation by a P/50 aluminium cylinder probe. The test condition for TPA was: 2 cycles with 5 s delay, pre-test speed 1.0 mm/s, test speed 1.7 mm/s and post-test speed 10 mm/s. Hardness, cohesiveness, springiness and chewiness were recorded [18].

Statistical analysis

Completely randomized design (CRD) with three replicates was used for all measurement. Data were compared using analysis of variance (ANOVA) and Duncan's multiple range tests with a significance level of 95%. All statistics was performed using the SPSS for windows version 26.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

Degree of hydrolysis

Degree of hydrolysis (DH) of egg white protein by immobilized thermoase PC10F (4.5:100 E/S ratio) for 45 min was 6.07 ± 0.23%. A previous study showed that the %DH value of the egg white hydrolyzed with papain for 45 min using a 3:100 E/S ratio was approximately 6.25% [20]. The degree of hydrolysis can affect the number of peptides produced, peptide size, and peptide conformation, which can be used to classify potential applications in food products based on the functional properties of peptides [21].

Surface hydrophobicity

Hydrophobicity on the surface of the protein represents a number of non-polar amino acid groups of protein in contact with the surrounding aqueous phase, which is

considered to play an important role in its adsorption efficiency at the air-water interface [22,23]. The formation of an elastic interfacial membrane that helps stabilize air bubbles could be encouraged by highly hydrophobic interaction, thereby improving the foaming properties of protein [22,23]. Figure 1 shows the surface hydrophobicity of EW, EWH, and FD-EWH. EWH had a higher ($p \leq 0.05$) surface hydrophobicity than that of EW and FD-EWH. The structural conformation of the protein could probably change by enzymatic hydrolysis and heat treatment, the exposed hydrophobic regions previously buried inside the protein molecule, results in a higher surface hydrophobicity than that of the native protein [23]. However, the result showed that the surface hydrophobicity of egg white hydrolysate was decreased ($p \leq 0.05$) after freeze-drying. This could be explained as the co-influence between hydrolysis and freeze-drying, which probably occurs by excessive protein unfolding [13]. Consequently, the hydrophobic side chains of amino group could interact with each other by hydrophobic interaction. Thus, the decrease in surface hydrophobicity was found [24].

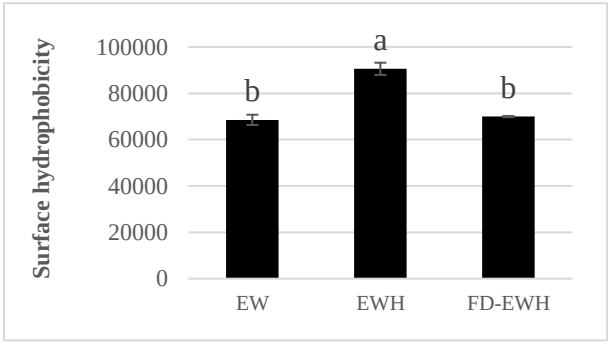

Figure 1 Surface hydrophobicity of egg white powder (EW), egg white hydrolysate (EWH) and freeze-dried egg white hydrolysate (FD-EWH).

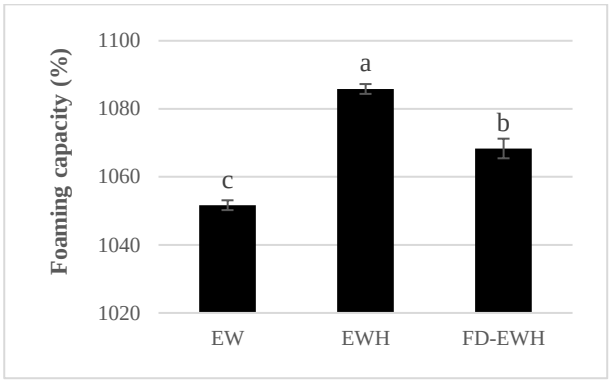

Figure 2 Foaming capacity of egg white powder (EW), egg white hydrolysate (EWH) and freeze-dried egg white hydrolysate (FD-EWH).

Foaming properties

Foaming capacity and foam stability of EW, EWH and FD-EWH are shown in Figure 2 and 3, respectively. EWH had the highest foaming capacity, followed by FD-EWH. The results could be explained as the better rearrangement of the protein hydrolysate molecule at the interfacial layer as a result of the decreasing in molecular weight, thus enhancing the molecular diffusion rate and flexibility [25]. Moreover, as seen in Figure 1 and 2, the surface hydrophobicity of EW, EWH, and FD-EWH relates with its foaming capacity.

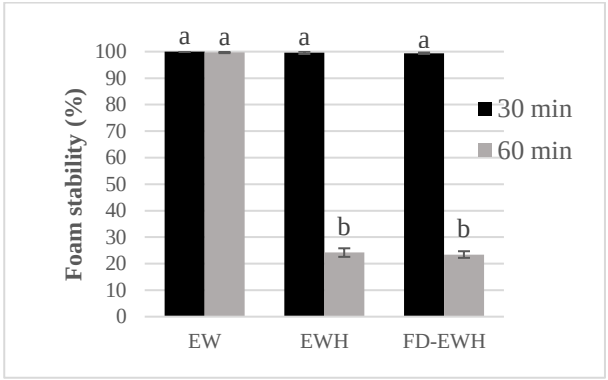

Figure 3 Foam stability after 30 and 60 min of egg white powder (EW), egg white hydrolysate (EWH) and freeze-dried egg white hydrolysate (FD-EWH).

This could be explained as the migrating of the hydrophobic amino acid groups of intra-molecular to the protein surface, resulting in a

reduction in the surface tension at the air-water interface [12]. As a result, the initial stability to absorb at the interface increases, improving the foaming capacity [25]. However, Foam stability of EWH and FD-EWH at 60 min decrease ($p \leq 0.05$). The reduced size of the polypeptide after being hydrolyzed could negatively affect the strength of the interfacial layer, thus also decreased foam stability over time [12].

Specific volume of GFB

Specific volume of GFB is presented in Table2. Substitution of rice flour, tapioca starch and Job's tears flour with 0.5% EW, 0.5%EWH, 0.5% FD-EWH, and 1.0% FD-EWH increased ($p \leq 0.05$) the specific volume of the GFB compared with the control. In addition, GFB with EW, EWH, and FD-EWH had no significant difference in specific volume ($p > 0.05$). These results linked to their foaming capacity, as shown in Figure 2. Even though the foaming capacity of EWH was higher ($p \leq 0.05$) than that of FD-EWH, the addition of EWH and FD-EWH had no effect ($p > 0.05$) on the specific volume of GFB.

Texture profile analysis

The texture profile analysis of GFB is presented in Table3. The GFB formulations with added 0.5% EW, 0.5% EWH and 0.5-1.0% FD-EWH had a lower ($p \leq 0.05$) hardness than that of the control. The softening effect of bread crumb corresponded to the specific volume (Table 2).

Increasing the specific volume of bread relates to its ability to retain more CO_2 gas during the proofing and baking process, resulting in a lower crumb hardness [26]. All GFB with egg white hydrolysate added were not significantly different ($p > 0.05$) from the control bread in parameters of springiness and cohesiveness. However, the springiness and cohesiveness of GFB with EWH and FD-EWH at a level of 0.5% were significantly higher ($p \leq 0.05$) than that of GFB with EW at the same concentration. A high springiness value could be attributed to ability of material to recover to its initial form after stressing, which positively related to crumb elasticity [27]. Crumb cohesiveness reflects the internal cohesion of the material [27]. Therefore, breads with slightly higher springiness and cohesiveness could have high consumer acceptance. [27]. The chewiness of GFBs showed a similar trend to the hardness. Chewiness is defined as the amount of energy required to chew a solid food until it is ready for swallowing [28]. The control GFB and GFB with 0.5%EW had more ($p \leq 0.05$) rigid crumb texture than that of GFB with 0.5% EWH and 0.5-1.0% FD-EWH.

Image analysis

The images of the top and cross-section views of GFB are shown in Figure 4. GFB with 0.5% EW, 0.5% EWH, and 0.5-1.0% FD-EWH had more well-aerated crumb structure compared to control GFB with a corresponding high volume and soft texture (Table 2 and 3). This is due to the presence of egg white protein, which acts as an excellent foaming agent [4]. In addition, the size of porous in GFB with 0.5%EWH and 0.5-1.0% FD-EWH had larger than that of GFB with EW.

This result could be attributed to the decrease in foam stability of EWH compared to EW (Figure3). Therefore, the size of porous (gas cells) merged and increased during heating [29]. Larger porosity results in a decrease in the density of crumb hardness, which helps the texture of bread to soften [5]. Thus, the GFB with 0.5% EWH and 0.5-1.0% FD-EWH had lower crumb hardness than that of GFB with 0.5% EW (Table3). However, GFB with large porosity could have weak structure [29].

Table 2 Specific volume of gluten-free bread with various egg white hydrolysate types and of percentages.

Sample	Specific volume (cm^3/g)
0.0% EW (control)	$3.16^{b} \pm 0.04$
0.5% EW	$3.36^{a} \pm 0.01$
0.5% EWH	$3.44^{a} \pm 0.06$
0.5% FD-EWH	$3.43^{a} \pm 0.03$

a,b Different letters in the same column indicate significant differences between means ($p \leq 0.05$)

Table 3 Texture profile of gluten-free bread with various egg white hydrolysate types and of percentages.

Sample	Hardness (g)	Springiness	Cohesiveness	Chewiness
0.0% EW (Control)	$177.80^{a} \pm 6.54$	$0.98^{a} \pm 0.00$	$0.78^{ab} \pm 0.00$	$136.38^{a} \pm 4.61$
0.5% EW	$167.45^{b} \pm 3.48$	$0.96^{b} \pm 0.02$	$0.75^{b} \pm 0.04$	$128.10^{b} \pm 7.35$
0.5% EWH	$147.87^{c} \pm 5.55$	$0.99^{a} \pm 0.00$	$0.80^{a} \pm 0.01$	$116.37^{c} \pm 3.84$
0.5% FD-EWH	$138.27^{d} \pm 4.39$	$0.99^{a} \pm 0.01$	$0.79^{a} \pm 0.01$	$108.14^{c} \pm 2.17$
1.0% FD-EWH	$149.05^{c} \pm 3.54$	$0.98^{ab} \pm 0.01$	$0.80^{a} \pm 0.02$	$115.56^{c} \pm 1.13$

a,d Different letters in the same column indicate significant differences between means ($p \leq 0.05$)

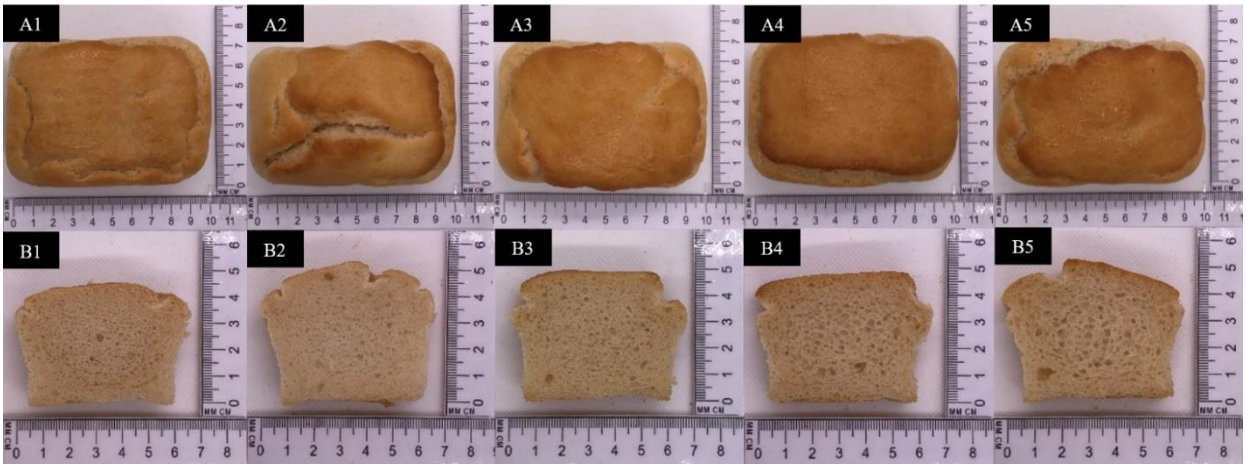

Figure 4 Images of top (A) and cross-section views (B) of gluten free bread with various egg white hydrolysate types and of percentages (1: 0.0% EW (Control), 2: 0.5% EW, 3: 0.5% EWH, 4: 0.5% FD-EWH, 5: 1.0% FD-EWH)

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CONCLUSIONS

After hydrolysis, EWH had a higher surface hydrophobicity than that of EW, which enhanced foaming capability. Even though the surface hydrophobicity and the foaming capacity of FD-EWH were lower than that of EWH. These could be attributed to the freeze-drying process. The addition of 0.5% EWH and 0.5-1.0% FD-EWH had no impact on the specific volume of GFBs. The GFB with 0.5% EWH and 0.5-1.0% FD-EWH had higher specific volume compared with the control. However, there was no significant difference in the specific volume of GFB with EW, EWH and FD-EWH. In addition, adding 0.5% EWH and 0.5-1.0% FD-EWH to GFB increased the softness of the bread crumb. Therefore, FD-

EWH could be used instead of EWH for more convenience.

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Using Kano's model for understanding of customer requirements in plant-based drinking yogurt for Generation Y.

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ABSTRACT: Generation Y consumers are more concerned about their health in recent years. Plant-based yogurt is one of the healthier products that are widely consumed with several reasons. Therefore, the understanding of generation Y consumers' attitudes and consumption behavior is more important for developing novel plant-based drinking yogurt. The objective of this research was to investigate of consumption behavior and analyze the attributes that are important to developing of plant-based drinking yogurt for generation Y. This study was based on qualitative and quantitative research method. The qualitative research method was used to conduct an in-depth interview via Zoom online with two groups of dairy drinking yogurt consumers. The qualitative research method uses an online survey and Kano's model to classify attributes that influence consumer satisfaction with novel plant-based drinking yogurt. Kano's model was used for a consumer survey and was conducted with 100 consumers across 15 attributes. The theme of each attribute was developed from the results of in-depth interview. The online survey studies behavior and experience consumption of drinking yogurt. The result of the survey suggested the characteristics of the target group and the appropriate price for the product. Based on Kano's model, the 15 attributes were categorized into attractive and one-dimension. The result indicated that high protein added, omega-3 added, fiber added, synbiotic added, vitamin B12 added, made from organic grains, free from synthetic additives and pourable were attractive attributes in plant-based drinking yogurt. The development of plant-based drinking yogurt with desirable consumer attributes, health benefits and good nutrition has become a focus of research for the products in the future.

Keywords: Generation Y, In-depth interview, Kano's model, Plant-Based Drinking Yogurt

INTRODUCTION

In recent years, many consumers have had an increased interest in plant-based foods because of several reasons. Firstly, the lifestyle of consumers is changed. The second reason is more attention on alternative diets. Thirdly, the awareness about sustainable food products is increased [1]. Plant-based yogurt or non-dairy yogurt is one of plant-based foods. The yogurt is suitable for consumers who are tolerant to lactose and allergic of dairy products. Therefore, there has recently been an increase in consumer interest in plant-based yogurt, especially since generation Y accounts for the majority of consumers in the plant-based yogurt market. This is an opportunity for plant-based market to target generation Y. The global market of plant-based yogurt is growing up. The market was worth USD 1.6 billion in 2019 and is expected to grow at a CAGR of 20% from 2020 to 2027 [2].

People in Generation Y, also known as the millennial generation, were born between 1981 and 1995 and will be between the ages of 27 and 41 in 2022 [3]. This generation is the largest generation of consumers. When they are compared with other generations, generation Y are more conscious of the benefits of healthy food consumption. However, the majority of generation Y consumers prefer organic, natural weight-control foods, protein-rich foods, and comfort foods [4]. Therefore, this generation was chosen to conduct an interview in this study.

Consumer research has several methods for study. Kano's model is one of the methods that is gaining more acceptance. In a broad range of industries, the understanding of the voice of consumers and consumer satisfaction is studied by kano's model. It is used to identify functional and dysfunctional attributes. In addition, it is integrated with quantitative measures to generate the novel food product for consumers [5]. The consumer satisfaction on kano's model can be classified into six attributes. 1. Must-be attribute is a competitive factor in the product. The consumer will be dissatisfied if they are not fulfilled with this attribute. 2. One-dimension attribute is the proportion of consumer satisfaction and need of products. Consumer satisfaction will be increase, If this attribute is enhanced in the product. 3. Attractive attribute is an attribute that has the most influence on products. This attribute is considered to exceed consumer expectations. If they are not met in the product, consumer will not be dissatisfaction. 4. Indifference attribute is the attribute that is not interested by consumers, so its presence or absence in this product does not affect consumer satisfaction or dissatisfaction. 5. Reverse attribute has an adverse effect on consumer satisfaction. This attribute does not want and should be improved in this product. 6. Questionable attribute is an essential attribute that should be mostly awareness in the product [6]. Previous studies applied kano's model to health food development [7].

The objective of this study was to investigate consumption behavior and analyze the attributes that are important to the development of plant-based drinking yogurt for generation Y.

MATERIAL AND METHODS

This study used both qualitative and quantitative methods of consumer research to comply with study objectives. In-depth interviews and survey results were contacted in January 2022. This research adopts qualitative and quantitative research method to analyze the consumer needs for plant-based drinking yogurt. For this research, the target consumer is generation Y consumers aged between 25-39 years old were recruited.

A qualitative study: in-depth interviews

Qualitative research aims to understand and gather detailed opinions about the topic from selected participants [8]. An in-depth interview is an important qualitative method that involves an intensive interview of individuals through the videoconferencing platform (zoom) with a total of 16 participants (12 women, 4 men) using purposive sampling. There are two groups of selected participants who consumes drinking yogurt at least three times/week. The first group includes eight healthy people who exercise and consume healthy food at least three times/week. The second group includes eight general-interest people. As in-depth interviews could be used to study the behavior and emotion of the participant, so try to analyze the facial expressions, gestures, body movements of the respondent. The most frequently asked questions follow from previous responses. For example, if an interviewee responds that "Yogurt may not be for everyone," an appropriate question should be, "Why do you think yogurt is not appropriate for everyone?" The structure of the conversation guide was based around five major themes. (Table 1)

A quantitative study: Online survey

Quantitative study is applied to estimate numerical data and statistical inference from a large population. A questionnaire is used in sample surveys to elicit information from respondents. In this research, the structured questionnaire was generated by the data collected from in-depth interviews. The survey was conducted with 100 consumers who had consumed diary drinking yogurt. The questionnaire was organized into sections with information on: 1) Demographic data; 2) Behavior and experience consumption of drinking yogurt; and 3) Kano's model (Table 2) Demographic data was collected on age, gender, education, and income. Data on frequency, volume of consumption, and purpose of consumption were used to collect information about the behavior and experience of people who drink yogurt. The last part of the questionnaire is about Kano's model that has 15 attributes, including "High protein added", "Probiotic added", "Omega-3 added", "Fiber added", "Prebiotic added", "Synbiotic added", "Vitamin B12 added", "Made from organic grains", "Free from synthetic additives", "Free from beany flavor", "Ability to be poured", "Homogeneous texture", "Low sugar content", "Low energy content" and "Fat free product" Each attribute was evaluated by two questions (functional/dysfunctional). For example, the questions that ask whether you are interested in a product that increase protein benefits (functional) and whether you are interested in a product that does not increase protein benefits (dysfunctional). The answer has five options included like, must be, neutral, live with it and dislike [9]. The measurement of Kano's model was based on Kano's Evaluation Matrix. (Table 3)

The calculation of the consumer satisfaction coefficient as calculated in equation 1 and 2.

$$\text{Extent of satisfaction} = \frac{A+O}{A+O+M+I} \quad (1)$$

$$\text{Extent of dissatisfaction} = \frac{O+M}{(A+O+M+I)\times(-1)} \quad (2)$$

Table 1 Themes and subthemes of in-depth interview through the videoconferencing platform, zoom.

Questions for target consumers
Theme 1: Behavior on plant-based beverage
Theme 2: Understanding and interesting about plant-based
Theme 3: Attitude towards plant-based drinking yogurt
Theme 4: New product concept for plant-based drinking yogurt

Table 2 Themes and subthemes of online survey questionnaire.

Questions for target consumers
Theme 1: Demographic
Theme 2: Behavior and experience of consumption of Drinking yogurt
Theme 3: Kano's model (15 attributes)

RESULTS AND DISCUSSION

A qualitative study: in-depth interviews

As illustrated in Table 4, the results of the in-depth interview about plant-based drinking yogurt are elaborated. This table showed whether each group of consumers has each attribute or not. The behavior of each group based on the attributes are described as follows.

Table 3 Kano's Evaluation Matrix.

Consumer requirements	Dysfunctional				
Functional	Like	Must-be	Neutral	Can live with it	Dislike
Like	Q	A	A	A	O
Must-be	R	I	I	I	M
Neutral	R	I	I	I	M
Can live with it	R	I	I	I	M
Dislike	R	R	R	R	Q

Note: A=attractive attributes, O=one dimensional attributes, M=must be attributes, I=indifferent attributes,

In the first theme, they said that they understand what the plant-based is. However, only healthy consumers have experienced plant-based product apart from plant-based milk (100%), such as plant-based minced meat, and plant-based nuggets. Some of them choose to have a flexitarian diet. Although general consumers have not tried any plant-based products apart from milk, some of them are interested in plant-based products. They said that if they had the opportunity, they might try plant-based products.

In the part of behavior consumption, the result shows that all consumers have consumed plant-based milk at least once per two weeks (100%). They said that they chose to drink soy

Table 4 The results of in-depth interview about plant-based drinking yogurt.

Themes	Target consumer (%)	
	Healthy consumer	General consumer
1. Understanding and interesting in plant-based products		
• Have basis knowledge about plant-based	100	75
• Have consumed other plant-based products apart from plant-based milk, such as plant-based minced meat, and plant-based nugget, etc.	50	25
2. Behavior on plant-based beverage		
• Drink plant-based beverage more than two times per week	100	100
• Substitute plant-based milk for a meal	75	75
• Prefer a low sugar formula for plant-based milk	100	50
• Consider promotion as a factor in a buying decision	87.5	87.5
3. Attitude towards drinking yogurt		
• Consider a sugar content included in drinking yogurt	100	50
• Prefer 0% fat of drinking yogurt	100	50
• Consider promotion as a factor in a buying decision	87.5	87.5
4. New product concept for plant-based drinking yogurt		
• Be interested in plant-based drinking yogurt	100	100
• Prefer a Low sugar formula for plant-based drinking yogurt	100	50
• Prefer a product that is drinkable and pourable	100	100
• Prefer a plant-based drinking yogurt produce by high protein material	100	100

milk or almond milk due to its accessibility. Meanwhile, some of them have tried dairy drinking yogurt. In buying decision, Healthy consumers prefer and expect low sugar products that have a sugar content below 10 g/100 ml rather than normal products, while general consumers buy any product with little regard to the sugar level. Moreover, if there is a novel plant-based drinking yogurt, both groups would like to try it. Both of them prefer the product that is drinkable and pourable, because it is easy to consume. Moreover, high protein material is also the factor of both buying decisions because they expect to have the same amount of nutrition as the dairy products.

Our results from Table 4 are aligned with the previous study [10] that utilized semi-structured in-depth interviews. The result of the previous study shows the same important features of yogurt consumption in young consumers (aged 20 to 25): healthy, natural ingredients, free from artificial coloring agents, free from preservatives, low fat content, low calorie content, low carbohydrate content and organic.

A quantitative study: Online survey

An online survey is used in this study to collect answers from each participant. The demographic profile of the survey participants can be shown in Table 5. The online survey was completed by 100 Generation Y participants. All of participants were female (79%) and male (21%) of total consumers. An even distribution of education level was reported: 77% of the participants had bachelor's degree, 17% had master's degree and 6% had high school or College degree. This means that the bachelor's degree consumer's education will be to buy dairy drinking yogurt. Overall, a rage of annual incomes was identified as 20,000-30,000 baht (48%), followed by 30,000-40,000 baht (39%) and > 40,000 baht (13%). The majority of consumers have an income of 20,000-30,000 baht.

Table 3 Respondent demographic characteristics. (N=100)

Variables	Percentage (%)
Gender	
Female	79
Male	21
Education level	
High school degree	6
Bachelor's degree	77
Master's degree	17
Income per month	
20,000-30,000 baht	48
30,000-40,000 baht	39
> 40,000 baht	13

Table 4 Respondent Behavior and experience of consumption of drinking yogurt.

Variables	Percentage (%)
Price of the purchase	
≤ 20 baht	68
21-30 baht	14
31-40 baht	9
41-50 baht	3
> 50 baht	6
Purchase demand	
1 bottle	59
2 bottles	24
3 bottles	4
> 3 bottles	13
Frequency of consumes	
2-3 times per week	24
> 3 times per week	3
1-2 times per month	59
1-2 times per year	14
Purpose of consumption	
Benefit digestive health	58
It tastes good	31
Source of protein on weight-loss diet	7
Meal replacement	4

Table 5 The quality attributes of plant-based drinking yogurt by Kano's model.

Rank	Attribute	A	O	M	I	R	Q		Sat	Dissat
A1	High protein added	49	12	3	27	3	6		0.67	-0.1648
A2	Probiotic added	28	24	12	24	3	9		0.59	-0.41
A3	Omega-3 added	44	9	3	32	1	11		0.60	-0.14
A4	Fiber added	39	19	9	21	2	10		0.66	-0.32
A5	Prebiotic added	33	22	9	27	1	8		0.60	-0.34
A6	Synbiotic added	37	21	7	26	0	9		0.64	-0.31
A7	Vitamin B12 added	57	7	2	24	3	7		0.71	-0.10
A8	Made from organic grains	50	12	2	28	2	6		0.67	-0.15
A9	Free from synthetic additives	31	16	3	40	1	9		0.52	-0.21
A10	Free from beany flavor	19	23	9	36	3	10		0.48	-0.37
A11	Ability to be poured	29	9	3	28	7	24		0.55	-0.17
A12	Homogeneous texture	29	23	20	15	5	8		0.60	-0.49
A13	Low sugar content	13	45	14	18	2	8		0.64	-0.66
A14	Low energy content	20	23	5	30	16	6		0.55	-0.36
A15	Fat free product	48	18	5	22	2	5		0.71	-0.25

Note: A=attractive attributes, O=one dimensional attributes, M=must be attributes, I=indifferent attributes,

Table 6 presents the behavior and experience of consumption of drinking yogurt. A price of the purchase was reported: 68% of participants purchased drinking yogurt at price (≤ 20 baht), 14% were 21-30 baht, 3% were 31-40 baht and 6% were > 50 baht. The majority of purchase demand was for 1 bottle (59%) followed by 2 bottles (24%), more than three bottles (13%) and 3 bottles (4%).

The frequency of consumption in drinking yogurt is reported. The majority of the frequency of consumption was 1-2 times per month (59%), 2-3 times per week (24%), 1-2 times per year (14%) and more than 3 times per week (3%), respectively. More than half the participants (58%) consume drinking yogurt to improve digestive health. 31% of the

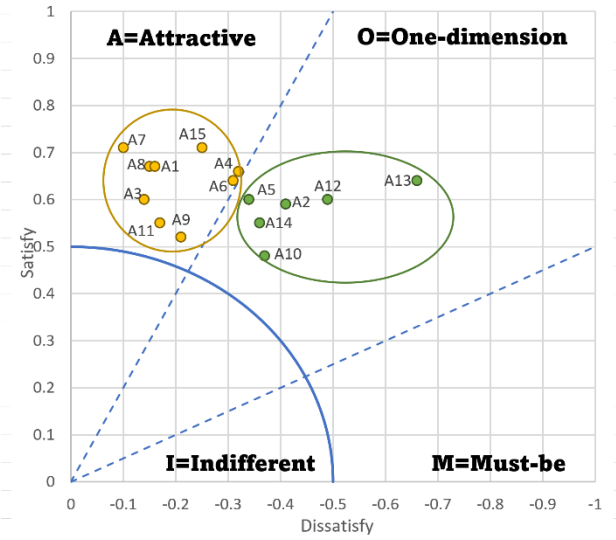

Figure 1 Classification of quality attributes by A-kano model.

respondents liked the taste. 7% and 4% of the participants have purpose to consume drinking yogurt for weight-loss diet and meal replacement.

The consumer satisfaction on the plant-based drinking yogurt was analyzed by kano's model. Table 7 shows the description of the 15 attributes and the results of the evaluation of attributes. The results of the evaluation of each attribute according to frequencies. The perception of participants is represented in the columns by number. We applied the method of A-Kano's model to identify mixed class distribution. Figure 1 showed the results that remaining nine attributes (high protein added, omega-3 added, fiber added, synbiotic added, vitamin B12 added, made from organic grains, free from synthetic additives and ability to be poured) are in the attractive attributes zone. This classification indicates that plant-based drinking yogurt is performed those attributes. It would increase the attractiveness to consumers. The consumers showed the hidden demand for plant-based drinking yogurt. The majority of dairy product fortified with synbiotic continues to increase as consumer desire for functional foods. The addition of synbiotic to yogurt can promote the intestinal health and improve the absorption of calcium and other minerals [11]. The other six attributes can be categories in the zone of one-dimensional attributes, which are probiotic added, prebiotic added, free from beany flavor, homogeneous texture, low sugar content and low energy content. The one-dimensional attributes can attract and retain consumers earlier than the attractive attributes according to the M>O>A>I rule. If the product has these attribute, the consumer may be more satisfied. The results of low sugar content and low energy content that demonstrated the consumers' demand for healthier products because they are concerned about the relationship between diet and health. The generation Y consumers are increasingly aware of healthy foods and are most likely to consume healthy foods [12].

CONCLUSIONS

In this study, consumer satisfaction is investigated for the development of a novel plant-based drinking yogurt. To understand consumers, qualitative and quantitative research methods are performed along with kano's model. According to the findings of in-depth interviews, both groups of generation Y consumers have limited knowledge of plant-based products and are most interested in the nutrition of plant-based products. In addition, interviewees are interested in a novel plant-based drinking yogurt. The consumption habits of 100 generation Y consumers revealed in an online survey that the benefit of digestive health is the most important factor in deciding to buy. As a result, the target generation y people for plant-based drinking yogurt should have two characteristics. First, the target people should have graduated in bachelor's degree. Second, the salary of the target people should be between 20,000 - 30,000 baht. Moreover, the appropriate price, which the drinking yogurt should be approximately 20 baht. The outcome of kano's survey indicates plant-based drinking yogurt should be produced by organic raw materials that have high protein, fiber, vitamin B12 and omega 3. Following the results, plant-based drinking yogurt can attract the generation Y consumers.

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Effect of rice flour and coconut oil on the physical properties of Thai custard (Khanom Mo Kaeng)

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ABSTRACT: Thai custard is a traditional popular food that has a short shelf-life. The effects of the addition of rice flour (0-1.5%) and coconut oil (0-5%) on the physical properties of Thai custard were investigated. The results showed that the appearance, color and texture characteristics were significantly ($p \leq 0.05$) affected. Increasing the amount of rice flour in the custard significantly ($p \leq 0.05$) increased its firmness and total color difference (ΔE*) and reduced its syneresis and Chroma (C*). However, increasing the level of coconut oil resulted in significantly decreased ($p \leq 0.05$) hardness and gumminess as well as changing the surface from glossy to oily. Also, the hardness of the custard decreased and cohesiveness increased both significantly ($p \leq 0.05$). Response surface methodology showed that a more intensive effect of coconut oil on the texture related to the rice flour concentration. The sensory evaluation showed that the texture of custard is very important for product acceptability. Overall, adding the rice flour between 0.5-1.5% could improve the storage stability and adding 3% could enhance the product appearance.

Keywords: Thai custard, Rice flour, Coconut oil

INTRODUCTION

Thai custard (kihanom mo kaeng) is a traditional Thai dessert, similar to egg custard, made from coconut milk, eggs, sugar, salt, and oil. It has a soft and smooth texture, and a sweet creamy and caramel flavor. Coconut milk differentiates Thai custard from other custards giving it its unique characteristics. Some other ingredients can be added to modify its texture and flavors including taro, mung bean, pumpkin and sweet potato. However, after baking, the shape can be mushy and its texture flabby, which can affect consumer acceptance, therefore it is generally marketed in baking trays or cups. Also, the shelf-life of Thai custard is only about 3 days at room temperature and 7 days in a refrigerator (Malee, 1999).

Because a major ingredient of Thai custard are eggs, their protein can be denatured since the baking temperature is above 150°C, Which results in the protein being denatured causing the liquid egg to harden, forming a solid gel and losing their water-holding properties (Pornchalermpong and Rattanapanon, n.d.) resulting in syneresis (the contraction of a gel accompanied by the separating out of liquids (Grachev *et al*., 2008).

Some formula for Thai custard include rice flour to give it a firmer texture and stability and lower the liquid leakage, but this can result in a harder texture, drier surface and loss of characteristic flavor. The purpose of this research was therefore, to test the effect of rice

flour and coconut oil on the syneresis and physical properties of Thai custard.

MATERIAL AND METHODS

Preparation of Thai custard samples

Thai custard samples comprise of whole eggs (33%), coconut milk (37%), coconut palm sugar (17%), water (13%), rice flour (the 0, 0.5, 1, and 1.5 %) coconut oil (0, 3 and 5 %) and sodium benzoate (1,000 ppm). The preparation process involved beating whole egg, coconut palm sugar, coconut milk, water, rice flour, coconut oil and benzoate until it was blended, but not foamy. After that, the mixture was filtered through a cheesecloth and poured into aluminum foil cups. Each cup was placed in a deep baking pan and water was added to a depth of 0.5-1.0 cm and then baked at 180°C, using the upper and bottom heat, for 30 min and then only top heat for 10 min for surface browning. The cups were removed and cooled on wire racks and stored at room temperature (about 25ºC) for 2 days before being analysed.

Determination of color

The samples were cut horizontally into two pieces. The inside color was determined by a Hunter Lab Colorimeter (Color Quest XE, USA) using illuminant D-65, 10º of observation angle, mode of reflectance specular included (RSIN), nominal UV filter and 1 in of port size. Color determinations were expressed as CIE L*a*b*. The color difference (ΔE*) and chroma (C*) were calculated according to following equations;

$$\Delta E^* = \sqrt{(\Delta L)^2+(\Delta a)^2+(\Delta b)^2} \quad \text{and}$$

$$C^* = \sqrt{(a^2+b^2)}$$

Determination of texture

The hardness and adhesiveness of the samples were measured using a TA.XT plus Texture Analyzer (Stable Micro Systems, UK). Each sample was transferred onto a stage with a fixed height of 30 mm. A 30 mm diameter, Perspex probe (P/50) was used to compress each sample twice at a defined rate for 1 min to a depth of 15 mm. Data collection and calculations were performed using the texture exponent software of the instrument.

Sensory evaluation

Three samples were selected based on their appearance and texture analysis. The organoleptic evaluation was performed by 40 panelists. Samples were evaluated for color, flavor, texture, and overall linking sing a 9-points Hedonic scale ranging from 1 (Dislike Extremely) to 9 (Like Extremely).

Statistical analysis

The experiment was designed as a 3 x 3 factorial in a completely randomized design and statistical analysis of all data was performed using SPSS Version 20 (SPSS Inc., USA). A Duncan's New Multiple Range Test was used for comparisons to show level of significant differences ($p \leq 0.05$). Texture analysis was by using Response Surface Methodology (RSM) using design expert software (Design Expert 7.0 trial).

RESULTS AND DISCUSSION

The appearance of Thai custard is important for consumer satisfaction and acceptance. When baking, the egg protein denatures and coagulates forming a thick gel, which is similar to other custards. However, the formula used in the controls for this experiment (no flour and oil added) had the softest gel and still had a slight mushy and flabby texture after storage for 48 hours, with some liquid leakage (Table 1) known as syneresis (Grachev *et al.*, 2008), which is unacceptable for the product characteristics.

Table 1 The appearance of the Thai custards made with various concentration of rice flour and coconut oil.

Rice flour (%)	Coconut oil (%)	Product appearance
0	0	Softer gel, moist, some syneresis, dried matte surface, little mushy and flabby texture.
	3	Softest, much syneresis, oily surface, little mushy and flabby texture.
	5	Softest, most syneresis, oily surface, much mushy and flabby texture.
0.5	0	Soft and elastic gel, a littler syneresis, dried matte surface, smooth and firm texture.
	3	Softer and slightly elastic gel, a little syneresis, glossy surface, smoother and firm texture, thick mouth feel.
	5	Softest and slightly elastic gel, some syneresis, glossy surface, smoother and firm texture, thick mouth feel.
1.0	0	Soft and more elastic gel, a littler syneresis, smoother and firmer texture.
	3	Soft and more elastic gel, a littler syneresis, glossy surface, rather smoother and firmer texture.
	5	Soft and slightly elastic gel, a little syneresis, glossy surface, smoother and firmer texture.
1.5	0	Soft and slightly elastic gel, no syneresis, a little starchy flavor, smooth and firmest texture.
	3	Soft and more elastic gel, no syneresis, glossy surface, a little starchy flavor, smooth and firm texture.
	5	Soft and more elastic gel, a littler syneresis, glossy surface, a little starchy flavor, firm texture.

Adding rice flour over the range of 0.5-1.5% increased the firmness but decreased syneresis of the custards indicating that rice flour supported the protein gel structure and added more body that gave the custard a firmer texture and prevented the liquid leaking during storage. However, at 1.5% flour content, a starchy flavour was identified by the panel.

The addition of coconut oil resulted in the custard surface becoming glossier, which was a positive effect, but at 5% the custard surface became oily and appeared to be softer. This result indicated that oil increased the ratio of oil in water and weakened gel structure of the custard.

Color Analysis

The brown color development, caused by the Maillard reaction, was unequally distributed in the custard. The upper surface color of the custard samples ranged from light brown to caramel color while the inside color ranged from cream to light yellow. Also, the intensity of color on the surface depended on the position of dessert cup in the oven.

Table 2 Effect of rice flour and coconut oil content on color characteristics of Thai custard.

Rice flour (%)	Coconut oil (%)	ΔE*	C*
0	0	0.00	$25.59^{a} \pm 0.10$
	3	$1.86^{de} \pm 0.75$	$25.75^{a} \pm 0.22$
	5	$0.27^{g} \pm 0.05$	$25.62^{a} \pm 0.10$
0.5	0	$1.24^{ef} \pm 1.00$	$25.06^{b} \pm 0.05$
	3	$2.18^{d} \pm 0.50$	$23.58^{f} \pm 0.34$
	5	$2.64^{c} \pm 0.43$	$24.42^{d} \pm 0.36$
1.0	0	$2.60^{c} \pm 0.75$	$24.12^{e} \pm 0.45$
	3	$2.74^{c} \pm 0.04$	$22.89^{g} \pm 0.04$
	5	$1.38^{f} \pm 0.20$	$24.69^{c} \pm 0.22$
1.5	0	$3.29^{b} \pm 0.06$	$22.65^{h} \pm 0.05$
	3	$3.84^{a} \pm 0.04$	$22.13^{i} \pm 0.26$
	5	$4.22^{a} \pm 0.03$	$21.47^{j} \pm 0.03$

* ΔE* was calculated by comparing to custard with 0% rice flour and 0% coconut oil.

For this experiment, the inside color of custard were measured and calculated in total color difference (ΔE*) and Chroma (C*) (Table 2).

Increasing rice flour from 0 to 1.5%, ΔE* value tended to increase while C* significantly ($p \leq 0.05$) decreased. The result may be due to cooked rice flour turning to an opaque white gel resulting in lower in a* and b* values and lower C* value in the product with higher amount of flour.

Texture Profile Analysis (TPA)

Preparation of Thai custard is notoriously finicky. Heat causes egg proteins to coagulate, forming a main thick solid structure. Cornstarch or flour may be added to prevent curdling, but this can also detract from its flavor (Master Class, 2021). The effect of rice flour and coconut oil content on texture characteristics of Thai custard indicated that increasing rice flour and coconut oil significantly ($p \leq 0.05$) affected all texture measurement including hardness, adhesiveness, cohesiveness and gumminess (Figure 1).

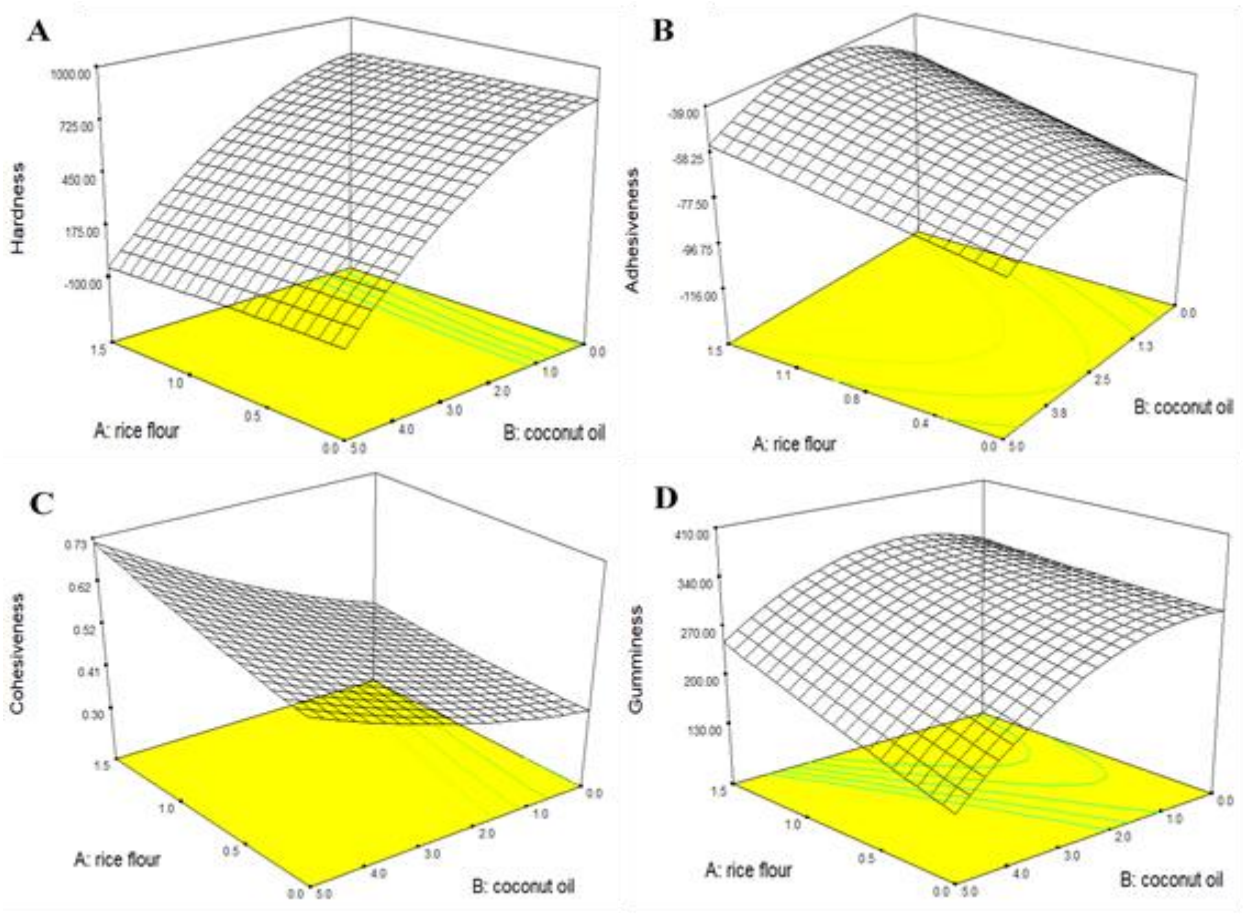

Figure 1 The response surface plot on the effects of the content of rice flour and coconut oil on hardness (A), adhesiveness (B), cohesiveness (C) and gumminess (D) values of Thai custard.

Hardness of food products is usually considered a value that refers to the force used to compress it until the sample is deformed (Sirilert, 2007). Therefore, this parameter indicates the firmness rather than syneresis. In this study, hardness value were ranged from 486-1,424 N. It was noted that as coconut oil increased, hardness decreased. For hardness, a response model was fitted to a quadratic equation with correlation coefficient (R^2) of 0.9539 ($p < 0.0001$). In addition, the lack of fit value was insignificantly (p-lack of fit = 0.7981) indicating that model was suitable. The hardness regression equation is shown in equation (1). Where A and B represent rice flour content and coconut oil concentration, respectively.

$$\text{Hardness} = 895.87 + 144.40A + 212.37B + 422.94AB - 78.05A^2 - 73.33B^2 \quad (1)$$

Adhesiveness represents the force used to move a sample in the mouth (usually the roof of the mouth) during the chewing process (Sirilert, 2007). The range of adhesiveness values varied between -19 to -144. The adhesiveness data showed a good fit to a quadratic model with R^2 of 0.8407, which means that the model effectively explained upto 84.07% of the result. Moreover, the lack of fit value 0.2888 was not significantly ($p > 0.05$), which means no other model fitted. The adhesiveness regression equation is shown in equation (2).

$$\text{Adhesiveness} = -54.46 + 22.90A + 30.97B + 26.18AB - 40.78A^2 + 2.40B^2 \quad (2)$$

Cohesiveness is the strength of the internal bonds that form in a sample and cause the sample to withstand the forces acting before the sample breaks or splits (Sirilert, 2007). In this experiment, the cohesiveness values were between 0.27 and 0.46 and the cohesiveness was not a good fit to the quadratic model with the lowest R^2 (0.6549) and lack of fit of 0.7670. The cohesiveness regression equation is shown in equation (3).

$$\text{Cohesiveness} = 0.34 - 0.038A - 0.013B + 0.094AB + 0.040A^2 - 0.071B^2 \quad (3)$$

Gumminess was defined as the force required to separate a semi-solid sample until it deforms (Sirilert, 2007). Response model was fitted to a quadratic equation with R^2 of 0.9150 (p <0.0001) presenting a suitable model for gumminess. The gumminess regression equation is presented in equation (4).

$$\text{Gumminess} = 332.12 + 29.44A + 142.32B + 287.05AB - 67.13A^2 - 137.85B^2 \quad (4)$$

The surface response of effect of rice flour and coconut oil content on texture characteristics of Thai custard were plotted show that both parameters affected on all variables (Figure 1). Hardness, adhesiveness, cohesiveness and gumminess increased as increasing rice flour and coconut oil (Figure1 A-D). However, increasing the amount of coconut oil over the range of 0 to 5% resulted in higher effect due to higher concentration compared to rice flour over the range of 0 to 1.5%. Increasing coconut oil resulted in the custard having a lower hardness but higher cohesiveness.

After a few day of production, three formulas products were selected for sensory evaluations based on the product appearances; soft smooth and firm gel, less or no syneresis and glossy surface which were (1) 0.5% rice flour and 3% coconut oil, (2) 0.5% rice flour 5% coconut oil and 1.5% rice flour.

Sensory evaluation

Generally, Thai custard has particular characteristics of flavor and texture due to the coconut sugar and coconut milk in the ingredient. In this research, sensory evaluation showed that sample with 0.5% flour and 5% oil had significantly ($p \leq 0.05$) lower liking scores in all attributes (Table 3). Both formulae were not statistical difference ($p > 0.05$) with the higher scores between 7.25 (like moderately) - 8.00 (like very much). This meant a positive liking score of 7 or higher on 9 points, which indicated the high sensory liking in their product characteristics (Everitt, 2009). The sensory evaluation showed that custard with 0.5% rice flour 5% coconut oil had lower liking score than others in all attributes and only taste were indifferent ($p \leq 0.05$) compared to that with 1.5% rice flour. However, two formula; (1) 0.5% rice flour and 3% coconut oil and (2) 1.5% rice flour were not statistically difference ($p > 0.05$), therefore, both formula were chosen.

Table 3 Sensory liking scores of selected Thai custard formulas.

%Rice flour	%Coconut oil	Appearance	Color	Odor	Taste	Texture	Overall liking
0.5	3	7.93^{a}±0.92	7.82^{a}±1.11	7.68^{a}±1.35	7.25^{ab}±1.51	7.68^{a}±1.27	7.48^{a}±1.30
0.5	5	6.30^{b}±1.64	7.13^{b}±1.32	6.50^{b}±1.28	6.80^{b}±1.38	6.45^{b}±1.66	6.65^{b}±1.37
1.5	0	8.00^{a}±0.99	7.80^{a}±1.16	7.50^{a}±1.20	7.60^{a}±1.19	7.88^{a}±1.20	7.70^{a}±1.11

* 9-point Hedonic scale ranging from 1 = dislike extremely to 9 = like extremely.

CONCLUSIONS

The addition of rice flour and coconut oil in the production of Thai custard affected its appearance, color and texture. Increasing the amount of rice flour resulted in the custard being firmer and less syneresis. Also, increasing the coconut oil concentration resulted in the custard having a glossy to oily surface and more syneresis. The sensory evaluation showed that the Thai custard with 0.5% flour and 3% coconut and that with 1.5% flour had the highest liking score.

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Market survey and a study of consumer behavior, attitudes and needs towards plant-based nuggets.

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ABSTRACT: The objective of this research was to survey markets of plant-based food and study behavior, attitudes, and needs of consumers towards plant-based nuggets. The majority of consumers are flexitarian, a flexible vegetarian diet using vegetables and grains as a meat substitute. The research consisted of 3 parts. Part 1, survey and collect information of commercial plant-based food products sold globally from Global New Products Database (GNPD) in the plant protein group. There were 16,481 products that were claimed as plant-based, vegan, and vegetarian during 2016-2020. The highest number of products belonged to the products that categorized as bakery (3,547 products; 21.5%), followed by products in processed meat category (3,448 products; 20.9%). Considering by region, it was found that Europe had the highest number of plant-based foods (9,233 products; 56.0%) compared to Asia Pacific which had 3,410 products (20.7%). Whereas, Thailand had product numbers of plant-based foods of 113 products, which most them were claimed as vegetarian (96 products; 85.0%). The highest product numbers were in main dish category (38 products; 36.6%), followed by processed meat (33 products; 29.2%). Part 2, survey plant-based food products sold in Thai markets in September 2020, it was found that there were 94 products which could be divided into 5 types based on product form. The types with the highest product numbers were Block/Cubed/Ball (38.3%). Part 3, survey behavior, attitudes, and needs of consumers towards plant-based food products of 150 consumers was investigated using a developed questionnaire. The results showed that most consumers were flexitarian (71.7%), 70.8% of them had plant-based foods for good health, as a main dish (63.7%), and 72.6% of them did not have a problem consuming plant-based food. While 27.4% of them suggested that they usually had dry and tough texture, high price, beany flavor, and weak taste. Consumers were interested plant-based nugget (97.3%) with a square shaped (41.8%). The top-three protein sources they want in the nugget were soybeans, corn, and potato (62.7, 57.3, 52.7% respectively). Moreover, the extremely important factors that affected buying decision of plant-based nuggets were taste, flavor, price, nutrition value and appearance.

Keywords: Market survey, Consumer behavior, Plant-based food, Plant protein, Nuggets.

INTRODUCTION

Nowadays, consumers pay more attention to health as well as the environment. They choose to have foods that are good for health with high nutritional value, and at the same time try to reduce meat consumption by eating mainly plants, vegetables, and grains. Plant-based foods are becoming more diverse according to different consumer preferences. The way of eating plant-based is not about restrictive. Consumers who eat mainly plant-based diet may still choose to eat small amounts of meat, poultry, fish, seafood, and dairy (also known as semi-vegetarian, flexitarian or pescatarian). There are many styles of plant-based eating for example vegetarian, vegan, and flexitarian diets.[1] Plant-based eating are

gaining more popularity, especially flexitarian consumers[2], causing the global food market in Thailand to grow at an average rate of 10% per year.[3] As a result, many plant-based food products are developed to respond consumer behavior and demands.

Nuggets are foods that are very popular to both children and adults, they respond to the changing lifestyle of consumers, which increasingly turning to fast food and processed food. Meat in nuggets could be mixed with other plant-based ingredients then coating with flour and breadcrumbs.[4]

The objective of this study was to survey markets and study behaviors, attitudes and needs of consumers towards plant-based nuggets. Data from market surveys was used to develop a questionnaire for attitude, behavior and need assessment of target consumers. The results of this study can be used to develop product and product concept, which will be useful for development of plant-based nuggets product.

MATERIAL AND METHODS

The study of consumer behavior, attitudes and need of consumers towards plant-based foods consisted of 3 parts.

1. Survey plant-based food products sold globally from Global New Products Database (GNPD).

Information of plant-based food products in plant protein group that sold globally during 2016-2020 was collected from GNPD database using keywords “plant-based, vegan and vegetarian”.

2. Survey plant-based food products sold in Thai markets.

In September 2020, plant-based food products in processed meat category were surveyed from 5 markets in Bangkok. (Gourmet Market at the Mall Ngamwongwan, Villa Market at La Villa Ari, Tops Market at Central Ladprao, Big C Extra at Ladprao and Max Value at Lak Si) in Bangkok.

3. Consumer survey with the developed questionnaire.

Develop a questionnaire based on data from market survey and test its completeness online with 30 representatives of target consumers (health-concerned and consumed foods from plants and cereals). The questionnaire reliability was tested using Cronbach’s alpha coefficient, which must be greater than or equal to 0.71, indicating high reliability of the questionnaire. Therefore, can be used for testing. Determine the sample size by calculating the population proportion from the equation[5] (1)

$$n = p(1-p)\, Z^2/E^2 \qquad (1)$$

where n = sample size, p = the proportion of consumers who interested in buying a developed plant-based nuggets, q = 1-p, Z = confidence value at 95%, E = accepted tolerance (5%).

An online survey of 150 consumers were conducted using a develop questionnaire.

RESULTS AND DISCUSSION

1. Survey plant-based food products sold globally from Global New Products Database (GNPD).

From GNPD database, in a plant protein group with the keywords “plant-based, vegan and vegetarian”, it is found that there were 16,481 products. The group with highest product numbers was vegetarian (10,240 products), followed by “vegan” (7,797 products) and “plant-based” (3,168 products). This suggested that consumers think of plant-based products as vegetarian products. These products can be divided into 12 category (Figure 1). The category that had the highest product numbers was bakery (3,547 products; 21.5%), followed by processed meat category (3,448 products; 20.9%).

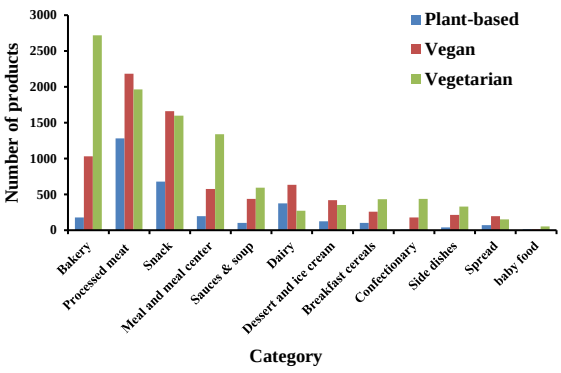

Figure 1 Category of plant-based, vegan, and vegetarian food products from GNPD database.

Figure 2 showed products categorized according to region, it is found that Europe continent had the highest product numbers (9,233 products; 56.0%), followed by Asia Pacific (3,410 products; 20.7%).

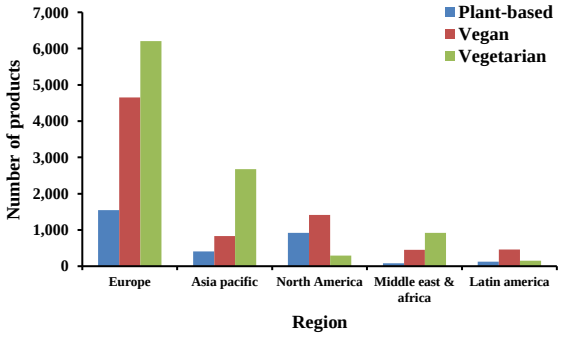

Figure 2 Plant-based, vegan, and vegetarian food products categorized by region.

Among all countries in the Asia Pacific, it is noticed that most products (99.8%) claimed in this region as "vegetarian" (Figure 3). India had the highest product numbers of "vegetarian" (1,390 products; 40.8%), followed by Australia (658 products; 19.3%) which had similar numbers of products claimed as vegan and vegetarian (55.2% and 47.3% respectively). Thailand was in the 8th rank, with 113 products and most products were also claimed as vegetarian (96 products; 85.0%).

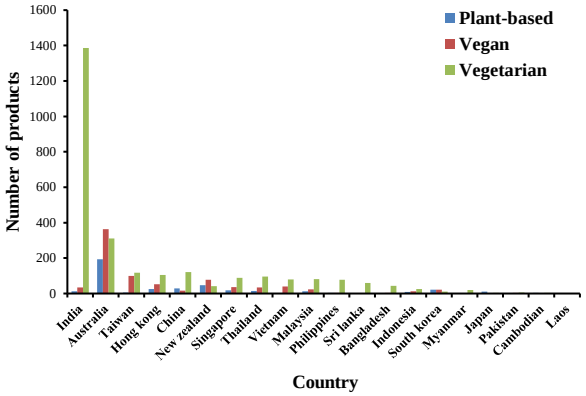

Figure 3 Plant-based, vegan, and vegetarian food products of Asia Pacific categorized by country.

Figure 4 showed product categories in Thailand, it is found that main dish category had the highest product numbers (38 products; 33.6%), followed by processed meat (33 products; 29.2%). In both categories, "vegetarian" was the highest claimed (90-94%) and "plant-based" was highly claimed in processed meat, dairy and snack category.

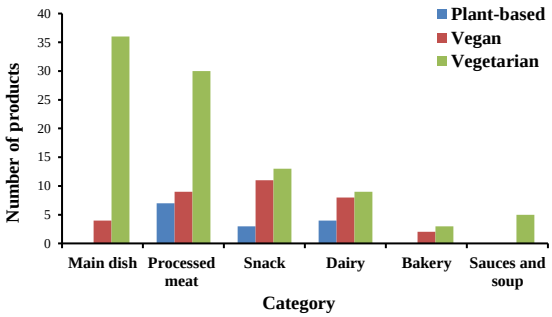

Figure 4 Category of plant-based, vegan, and vegetarian food products in Thailand.

2. Survey plant-based food products sold in Thai markets

Plant-based food products in processed meat category sold in Bangkok were surveyed from 5 markets during September 2021. The results showed that there were 94 products, which could be divided into 5 types based on product form which were Block/ Cubed/ Ball, Burger, Sausage, Sliced/ Fillet and Shredded/ Minced. The types with the highest product numbers were Block/ Cubed/ Ball (36

products; 38.3%), followed by Burger, Sausage, Sliced/ Fillet and Shredded/ Minced equalled to 19, 16, 15, 8 products, respective (Table 1). Considering ingredients, it was found that most of main ingredient used in the product were the protein source from soy protein, wheat protein and pea protein. In addition, there were other ingredients such as wheat flour, yeast extract and some food additives such as methylcellulose, carrageenan and xanthan gum as binders in the products.

Table 1 Form of plant-based food products in processed meat category sold in Bangkok.

Types	Numbers	%
Block/ Cubed/ Ball	36	38.3
Burger	19	20.2
Sausage	16	17.0
Sliced/ Fillet	15	16.0
Shredded/ Minced	8	8.5

3. Consumer survey with the developed questionnaire.

The questionnaire was developed based on market survey data and was online tested the completeness with 30 respondents. The reliability Cronbach's alpha coefficient was 0.81. The proportion of consumers who interested in buying a developed plant-based nuggets was 0.93. Then, the calculated sample size was at least 100. Therefore, the developed questionnaire was used with 150 target consumers. They were 80% female and 20% male. Table 2 showed that most of them were in the age of 25-39 years old (59.3%), had bachelor degree (79.3%), worked as company employees (45.3%) and had income in a range of 15,001-25,000 baht (38.0%).

The results of consumer behaviour and attitudes towards plant-based foods (Table 3) showed that 75.3% of consumers had plant-based foods before. Among those consumers, most of them are flexitarians (71.7%), 70.8% of them had plant-based foods for good health and often had them during vegetarian festivals (32.7%), followed by 1-2 times/week (28.3%). Most consumers had plant-based foods as a main dish (63.7%), and 72.6% of them did not have a problem consuming plant-based foods. While 27.4% of them suggested that plant-based foods usually had dry and tough texture (67.7%), high price (58.1%), beany flavour (54.8%) and weak taste (48.4%).

Table 2 The demographic data of consumers. (n=150)

Demographic data	Freq.	%
Gender		
- Female	120	80.0
- Male	30	20.0
Age		
- 15 - 24	49	32.7
- 25 – 39	89	59.3
- 40 - 60	9	6.0
- > 60 years	3	2.0
Educations		
- Middle school/Vocational certificate	2	1.3
- High school/High vocational certificate	7	4.7
- Bachelor degree	119	79.3
- Higher than bachelor degree	22	14.7
Occupations		
- Company employee	68	45.3
- Student	38	25.3
- Government officer/State enterprises	22	14.7
- Trade/Personal business	17	11.3
- Housewife, nurse, unemployed	5	3.4
Income per month (baht)		
- < 5,000	4	2.7
- 5,001 – 15,000	24	16.0
- 15,001 – 25,000	57	38.0
- 25,001 – 35,000	44	29.3
- 35,001 – 50,000	14	9.3
- > 50,000	7	4.7

Table 4 revealed that 97.3% of consumers were interested in a square shaped (41.8%) plant-based nugget. The top-three protein sources they want in the nugget were soybeans, corn, and potato (62.7, 57.3, 52.7% respectively).

Additionally, the extremely important factors that affected buying decision of plant-based nuggets were taste, flavor, price, nutrition value and appearance (Table 5). The result was in agreement with the research of Hirisatcha (2018), she reported that factors affecting consumers decision in buying vegetarian food

Table 3 Consumer behavior and attitude towards plant-based food.

Questions	Freq.	%
Have you ever had plant-based foods? (n=150)		
- Yes	113	75.3
- No	37	24.7
What kind of plant-based consumer are you? (n=113)		
- Flexitarian	81	71.7
- Vegetarian	21	18.6
- Vegan	11	9.7
Why do you consume plant-based foods?* (n=113)		
- Good healthy	80	70.8
- Weight loss, food control	44	38.9
- High nutritional value	43	38.1
- Plant-based foods have good taste	31	27.4
- Concern of animal welfare and environment	20	17.7
- Make merit/Do good things	18	15.9
- Consume as close people do	17	15.0
- Want to try	2	1.8
- Need high-protein, low-fat foods	1	0.9
How often do you consume plant-based foods? (n=113)		
- During vegetarian festivals	37	32.7
- 1-2 times/week	32	28.3
- 2-3 times/month	20	17.7
- 3-4 times/week	10	8.8
- Every day	5	4.4
- Buddhist holy day	5	4.4
- When opportunity provided	2	1.8
- 3 times/year	1	0.9
- Some meals	1	0.9
What is your plant-based diet pattern? (n=113)		
- Main dish	72	63.7
- Snack, appetizer	40	35.4
- When opportunity provided	1	0.9
Do you have a problem consuming plant-based foods? (n=113)		
- No	82	72.6
- Yes	31	27.6
What is your problem consuming plant-based foods?* (n=31)		
- Dry and tough texture	21	67.7
- High price	18	58.1
- Beany flavour	17	54.8
- Weak taste	15	48.4
- Less distribution locations	14	45.2
- Green smell of vegetables	8	25.8

* means a question that the respondents can choose more than one answers

are product factors in terms of taste affecting the deliciousness.[6] Moreover, Bryant et al. (2019) reported the majority of Chinese consumers considered taste and health benefits as important factors that affecting their buying decision of plant-based foods.[7]

Table 4 Consumer needs towards plant-based nuggets.

Consumption needs	Freq.	%
If a plant-based nugget was developed, would you be interested in? (n=113)		
- Interested	110	97.3
- Not interested	3	2.7
What shape of plant-based nuggets would you like? (n=110)		
- Square	46	41.8
- Stick	36	32.7
- Sliced	20	18.2
- Fancy	8	7.3
What kind of grain or plant protein source do you want to use in a product?* (n=110)		
- Soy protein	69	62.7
- Corn	63	57.3
- Potato	58	52.7
- Quinoa	37	33.6
- Carrot	37	33.6
- Green pea	37	33.6
- Wheat protein	36	32.7
- Cockle mushroom	30	27.3
- Lentils	12	10.9
- Young jackfruit	8	7.3

* means a question that the respondents can choose more than one item

Table 5 Importance of factors affecting the buying decision of plant-based nuggets. (n=110)

Factors	Mean±SD	Level of importance
Taste	4.6 ± 0.8	Extremely important
Flavor	4.5 ± 0.8	Extremely important
Reasonable price	4.4 ± 0.8	Extremely important
Nutritional value	4.3 ± 0.8	Extremely important
Appearance/ appetizing	4.3 ± 0.8	Extremely important
Convenient to buy	4.2 ± 0.8	Very important
Shelf life	4.2 ± 0.8	Very important
Promotions	4.1 ± 0.8	Very important
Environmentally-friendly packaging	4.1 ± 0.8	Very important
Brand	3.8 ± 0.8	Very important

* The score is rated from 1 to 5 (1 = less important and 5 = extremely important)

CONCLUSIONS

The results of the survey of plant-based foods during 2016-2020 from GNPD database, in a plant protein group with the keywords "plant-based, vegan and vegetarian" showed that there were 16,481 products. Bakery is the category with the highest product numbers of 3,547 products. Europe continent had the highest product numbers of plant-based food products (9,233 products), and Thailand had product number equals to 113 products, which "plant-based" was highly claimed in processed meat, dairy and snack category. The survey of processed meat products sold in 5 markets in Bangkok in September 2021 found that there were 94 products.

Study of behavior, attitudes and needs of target consumers using online questionnaire showed that 97.3% of consumers were interested in plant-based nuggets, in a square shape, with plant protein sources from soy protein, corn and potato. In addition, taste, flavor, price, nutrition value and appearance were the extremely important factor affecting their buying-decision.

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Effect of modified starch and storage condition on the cooking properties and texture of non-gluten chicken pasta

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ABSTRACT: Worldwide consumption of pasta is continuously increasing, especially highly nutritious pasta. The objective of this research was to investigate the effect 3 modified starches: acetylated di-starch phosphate (ADP), hydroxypropyl starch (HS) and hydroxypropyl di-starch phosphate (HDP) at either 2.5% or 5% concentrations and two storage temperatures (ambient and 4°C) on the physical characteristic and cooking quality of non-gluten chicken pasta. The results showed that both type and concentration of modified starches significantly ($p \leq 0.05$) affected on their cooking quality. The control (with no additional starch) and samples with either 2.5 or 5% HS showed no significant differences ($p \leq 0.05$) on cooking loss, cooking weight and cooking time except for HS at 5%, which had a significantly shorter cooking time ($p > 0.05$). The addition of either HDP or ADP resulted in significantly ($p > 0.05$) higher cooking loss than the control. The texture of the samples with HS at 5% had the lowest hardness values while sample with ADP at 5% had the highest hardness values. The addition of ADP to samples gave them a higher tensile strength compared to the other samples. Storage at ambient temperature and 4°C affected both hardness and tensile strength of the pasta but were not significantly different ($p > 0.05$) on cooking loss and cooking time. In conclusion, the addition of HS at 5% concentration resulted in better physical properties of non-gluten chicken pasta.

Keywords: Non-gluten chicken pasta, modified starch, cooking quality, hardness

INTRODUCTION

Pasta, a typical main dish food, is popular in all age groups and social classes (Simonato *et al*., 2015) and is a common food in countries throughout the world. There are broad categories of pasta, depending on their shape, size and compositions, (Da *et al*. 2013). Generally, wheat flour is their main ingredient, but wheat allergies are common and can cause symptoms that include: swelling, itching, irritation of the mouth or throat, hives, itchy rash or swelling of the skin, and nasal congestion. Wheat allergy is often conflated with gluten intolerance but it's completely different. Research has shown that, alternative ingredients to wheat must have the capacity to maintain or increase the nutritional value of pasta. Many ingredients for examples; whey protein concentrate, egg albumen, rice bran protein concentrate, and soy protein concentrate have been successfully used to replace wheat in pasta while maintain nutritional properties (Phongthai, 2017). Another aim is to prevent allergenicity and intolerance in particular to wheat. in the production of pasta. Many supplement ingredients have been reported to

meet this requirement while maintaining the desirable characteristics of pasta including sorghum, cassava, corn and rice flour (Ferreira, 2016). Therefore, there is a demand for pasta that can provide economic benefits for local ingredients and that are nutritional and meet consumer's demand who suffer wheat allergy or celiac disease.

Rice flour is a gluten free ingredient that can be used for making pasta and commonly available in Thailand. Rice flour has been used as an alternative to wheat flour products including rice cakes, rice breads, cereals, gluten-free baked, pizza and fermented rice products. For pasta, there are many reports that substitution of rice flour with rice protein improved quality of gluten-free rice spaghetti processed using single screw extrusion (Detchewa, 2022). The addition of protein, fiber or other nutrients will lead to improve the nutritional value in this product. Protein is a promising ingredient for adding to pasta for example, soybean and chick pea. Also chicken is a possible cheap alternative source of protein.

In wheat pasta formulations, starch and protein play an important role in maintaining its structure, textural and sensory characteristics (Larrosa *et al*., 2016; Padalino *et al*., 2016) and using non-gluten ingredients, can change those characteristics. Therefore, many hydrocolloids have been used to improve the quality of pasta products including xanthan gum, guar gum, hydroxypropyl methylcellulose, carboxy-methyl cellulose and various modified starches (Ko, Kim, Baek, and Park, 2015; (Laura, 2020; Larrosa *et al*., 2016).

Native starches have generally been cross-linked with cross-linking agents such as sodium trimetaphosphate, sodium tripoly-phosphate (Hirsch, B. J., and Kokini, L. J., 2002). This modified starch forms cross link between two starch molecules which stabilizing and improving the mechanical properties and water binding stability of starch products. Also, it is more resistant to process conditions, for example, high temperature or shear forces (Somayeh, et al., 2020). Acetylated distarch phosphate (ADP, E 1414), Hydroxypropyl distarch phosphate (HDP, E 1442) Hydroxypropyl starch (HS, E 1440) have been used for various food products. Therefore, the objective of this research was to determine the effect of type and concentration of three modified starches and two storage temperatures on the texture and other characteristics of non-gluten chicken pasta.

MATERIAL AND METHODS

Rice flour, cassava starch and rice bran oil were purchased from a local supermarket. Three modified starches were used: acetylated di-starch phosphate (E1414), hydroxypropyl starch (E1440) and hydroxypropyl di-starch phosphate (E1442). Fresh chicken breast skin was purchased from a supermarket and minced then stored in sealed vacuum bags at -16˚C until use, which was within a month.

Preparation of pasta

The basic formula of gluten free chicken pasta that was used consisted of minced chicken skin (50%), rice flour (31%), cassava starch (13%), oil (5%) and calcium carbonate (1%). Two concentrations, 2.5 and 5%, of the three modified starches was substituted with rice flour. The dried ingredients were completely mixed and sifted to eventually distribute all powder together. The minced chicken skin was gradually added to the dried ingredients into a blender and kneaded until the dough was smooth. The dough was spread out on a plastic sheet and rolled into thin layers and flatten through a pasta machine. It was then passed through the machine again in order to decrease the thickness setting each time. Finally, the pasta was cut into flat sheets with the width and thickness of 0.2 x 0.1 cm. The fresh pasta was steamed for 2 minutes, cooled, packed and sealed in plastic bags. The samples were kept at either ambient (about 25°C) and

chilled (4°C) temperatures for 2 days before analysis.

Cooking quality of pasta

Cooking time, cooking loss and water absorption were measured according to the AACC methods 66-50 (20).

For cooking time, 10 g of sample were weighed and placed in 300 mL of boiling water. Sample was taken every 30 s to check whether they were fully cooked. The optimum cooking time was defined as the minimum time needed to completely gelatinize starch in the pasta until the pasta was soft and chewable.

For cooking weight, 10 g of sample were weighed and fully cooked. The cooked pasta was drained and weighed. The water absorption of pasta was calculated as the weight difference and expressed as a percentage of the original sample weight before cooking. Then the cooking water was measured and recorded. 10 mL of cooking water was dried at 105°C in a hot air oven and the residual was weighed and expressed as a percentage of solid content (%). The cooking loss and cooking weight of pasta were calculations using the following

$$Cooking\ loss(\%) = \frac{weight\ of\ dried\ residue\ in\ cooking\ water}{dry\ weight\ of\ the\ sample}\ x\ 100$$

$$Cooking\ weight(\%) = \frac{weight\ of\ cooked\ sample\ x\ 100}{dry\ weight\ of\ the\ sample}$$

Texture properties of cooked pasta

For evaluation of texture, the pasta was cooked in boiling water for 1.5-2 min, rinsed in cold water for 1 min and left on a stand for 1 min to allow the remaining water to drain. The pasta was place in a covered pack for texture profile analysis (TPA) and spaghetti tensile (A/SPR) using a TA-XTplus texture analyzer (Stable Micro Systems Ltd., Godalming, UK), using a p/35 with 20 mm distance. The probe teats, before, during and after the test were 1, 1 and 5 mm/s. The strain was 75% and trigger force was 5 g for A/SPR with 100 mm distance. The speeds of the probe before, during and after the test were 1, 3 and 10 mm/s trigger force at 5 g. The parameters for hardness and tensile were calculated. The measurements were completed within 15 minutes of cooking.

Statistical analysis

All experimental results were expressed as means and standard deviations and data was subjected to analysis of variance (ANOVA) using IBM SPSS Statistics. Duncan's Multiple Range Test was used for multiple comparison tests at statistical significance of $p \leq 0.05$.

RESULTS AND DISCUSSION

Cooking quality

It has shown to be an important quality of pasta that should have a short cooking time, less solid loss in the cooking water and more water absorption (Chung *et al*., 2012). The effect of type and concentration of modified starch on the cooking qualities are shown in Table 1.

The cooking time refers to the time needed for the pasta to be completely cooked in boiling water. The longer cooking time is unfavourite for consumer. The cooking time of all samples ranged from 1.17 to 2.33 min and was significantly different ($p \leq 0.05$) between samples. During cooking, rice flour and cassava starch is completely gelatinized and chicken proteins are denatured. Gluten-free pasta requires less time to cook due to its looser structure, which allows water to infiltrate the pasta structure more quickly due to the lack of a compact gluten network. (Phongthai, 2017). When adding modified starch, the cross linking acts as a bridge between two starch molecules affecting the gel structure of rice flour and cassava starch. The cross linker such as phosphate or hydroxyl group in cross linked starch help the products quicker absorb water resulting in less

cooking time. In addition, increasing the concentration of each modified starch tended to decrease in cooking time but no differences were observed between types for modified starch. The more amount of crosslinking agents could faster the absorption of water in pasta. The shorter cooking time indicated that the pasta was easier to cook, but chilled pasta required a longer cooking time compared to pasta that had been stored in ambient conditions. When pasta is stored at chilling temperatures, retrogradation occurs, which results in amylose rapidly recrystallizing followed by slow recrystallization of amylopectin. This arranged structure may be the cause of the change in cooking time. Feng *et al.* (2020) also found wet starch noodles that had been stored at 4°C for 24 h had a more porous structure than that of freshly made noodles and attributed these changes to the starch retrogradation during cold storage.

The percentage cooking loss defines an index of resistance to disintegration during cooking and is a critical factor for pasta quality (Larrosa *et al.*, 2016) and a low percentage indicates high quality. A cooking loss of 7.0-8.0% was reported to be acceptable. (Susanna and Prabhasankar., 2013). In this experiment, the cooking loss percentage for the control was 5.82 and 5.50% respectively for ambient and chilled storage while the samples with added modified starch ranged between 5.52 and 8.77% for ambient storage and between 6.41 and 9.38%, for chilled storage. Different modified starches also showed significantly different ($p \leq 0.05$) cooking loss values compared to control. HDP and ADP had significantly higher ($p \leq 0.05$) cooking loss compared to HS. HDP ADP and HS are modified with trimeta-phosphate/propylene oxide and trimeta-phosphate/vinyl acetate and only propylene respectively. The different cross linkers make difference stability in pasta structure resulting in different cooking loss. However, as the concentration of HDP and ADP increased, the cooking loss tended to decrease. These cooking losses were considered high compared, for example, with durum wheat pasta, which has a cooking loss of only 3%. This effect was due to the lack of a gluten network in all non-gluten pasta. Starch polymers are less effectively entrapped in the matrix, resulting in substantial cooking losses (Lucisano et al., 2012; Marti et al., 2013)

The percentage cooking weight is defined as the weight of pasta after cooking in boiling water compared to the weigh prior to cooking. Samples with higher cooking weights have higher amounts of water absorbed by the pasta which makes the pasta softer. All the samples had cooking weights 110% for ambient storage and 131% for chilled storage and the controls were 116% for ambient storage and 123% for chilled storage. From the hydrogen bond between the hydroxyl group in modified starch molecules, it decreased the cooking weight of the spaghetti. The starch's strength increased due to the creation of hydrogen bonds. As a result, the starch's ability to absorb water and expand was reduced (Sriroth and Piyajomkwan, 2007). ADP and HDP showed significantly lower ($p \leq 0.05$) in cooking weight than controls and HS. These results was correspond to cooking loss. The higher cooking loss , the lower cooking weight. However, with increasing starch concentration, there were no significant difference ($p > 0.05$) between treatments. The effects of storage temperature on the controls and samples with HS showed that at chilled conditions there was higher cooking weight while samples with HDP and ADP had lower cooking weights. Therefore, the type and concentration of modified starch affected the quality of non-gluten chicken pasta. Pasta made of 5% HS had lower cooking times and lower cooking losses but higher cooking weights. This effect was due to the water binding and gel forming characteristics of the utilization of the proteins, with the result that the structure of pasta absorbed more water. Protein gelation is a four-phase process consisting of unfolding of the globular protein structure (i), aggregation of the unfolded protein

molecules(ii), string formation of the aggregates (iii), and linkage of the strings to a three-dimensional network (iv) (Lorenzen et al., 2006).

Table 1 Cooking quality of non-gluten chicken pasta with various types and amount of modified starch kept at different temperatures

Sample	Conc. (%)	Stored Temperature	Cooking time (s)	Cooking loss (%)	Cooking weight (%)
Control	0	Ambient	$1.83^{a-c} \pm 0.29$	$5.82^{d} \pm 0.86$	$116^{d-f} \pm 2$
		Chilling	$2.17^{ab} \pm 0.29$	$5.50^{d} \pm 1.81$	$123^{b} \pm 4$
HS (Hydroxy propyl starch)	2.5	Ambient	$2.00^{ab} \pm 0.50$	$5.52^{d} \pm 1.20$	$116^{d-f} \pm 1$
		Chilling	$2.17^{ab} \pm 0.29$	$6.41^{cd} \pm 0.43$	$124^{b} \pm 2$
	5	Ambient	$1.33^{cd} \pm 0.29$	$6.26^{cd} \pm 0.50$	$113^{e-g} \pm 1$
		Chilling	$1.67^{b-d} \pm 0.29$	$6.84^{bcd} \pm 0.29$	$131^{a} \pm 2$
HDP (Hydroxy propyl di-starch phosphate)	2.5	Ambient	$2.17^{ab} \pm 0.29$	$8.13^{abc} \pm 2.20$	$117^{c-e} \pm 4$
		Chilling	$2.17^{ab} \pm 0.29$	$9.38^{a} \pm 1.58$	$110^{g} \pm 1$
	5	Ambient	$1.33^{cd} \pm 0.29$	$6.86^{bcd} \pm 0.35$	$119^{cd} \pm 1$
		Chilling	$1.67^{b-d} \pm 0.29$	$6.08^{d} \pm 0.82$	$112^{fg} \pm 1$
ADP (Acetylated di-starch phosphate)	2.5	Ambient	$2.00^{ab} \pm 0.50$	$8.77^{ab} \pm 0.19$	$120^{c} \pm 1$
		Chilling	$2.33^{a} \pm 0.29$	$8.55^{ab} \pm 0.18$	$110^{g} \pm 0$
	5	Ambient	$2.33^{a} \pm 0.29$	$7.25^{bcd} \pm 1.12$	$115^{ef} \pm 2$
		Chilling	$1.17^{d} \pm 0.29$	$7.32^{bcd} \pm 0.63$	$112^{fg} \pm 1$

Mean value±SD with diferent small letters in the same column are signifcantly diferent ($p \leq 0.05$)

Texture

The addition of starch to the samples had different impacts on the textural properties of rice chicken pasta (Figure 1). González, *et al*. (2000) reported that texture of the cooked pasta is considered as the most critical characteristic in the evaluation of the quality of pasta and its acceptance by the consumer.

Hardness of food products was reported to be the force used to compress them until the sample deforms (Sirilert, 2007). The results showed the hardness of samples with different modified starches had significantly different ($p \leq 0.05$) effects on hardiness. Increasing the concentration of HS and HDP decreased hardiness but increase in ADP increased hardness values. HS and HDP are hydroxylpropyl starches while ADP is acetylated distarch phosphate. The different cross linkers result in different structure of pasta (Miller and Whistler, 2009). Similar results were reported for partially substituting rice starch with cassava starch, which tended to provide softer gel and retard retrogradation (Seetapan *et al*., 2015). The hardness of sample tended to be higher after being stored in a chilled room except sample with ADP. In general, the visual observation found that the hardness for good quality of non-gluten chicken pasta was within the range of 2,500 and 5,000 N. This effect is supported by Kim and Wiesenborn (1996) who reported that enhanced hardness appeared to limit starch granule swelling, which might have contributed to lower cooking loss. This result was corresponded with the cooking quality, the higher hardness in the pasta, the lower cooking weight was observed. If product absorbs less water, the product becomes harder. From the result, the least hardness and highest cooking weight were observed in pasta with 5% HS.

Tensile strength defines as the measure of the maximum force required to break pasta and relates to the toughness of the pasta. The higher the tensile strength a product possesses, the more difficult it is to stretch (Stable Micro Systems, 2022). The samples from the control pasta had higher levels of tensile strength than all other samples with modified starches. Increasing the starch concentration did not have significant effect ($p>0.05$). The tensile strength of samples was generally higher after storage at chilled temperature. Also, in this experiment, it was found that the non-gluten chicken pasta should have the tensile strength in the range between 13.5 and 23.5 g. In contrast, Sereewat *et al.* (2014), found that the addition of the modified starch resulted in an increase in cooking time, cooking weight, and tensile strength of the rice spaghetti after cooking. The tensile strength of 5% HS at chilling was lower than the others. It was correspond to hardness, the product had lowest hardness which was required least force to break down.

Springiness is a measure of the ability of compressed pasta to return to its original height and is expressed as a ratio or percentage of a product's original height (Stable Micro Systems, 2022). The springiness of samples stored at ambient temperature was similar to flexibility. The results indicate that the unchanged structure was related to the water retention capacity of the protein aggregates. However, sample stored at the chilled temperature were insignificantly different ($p>0.05$) to those stored at ambient temperature and samples with 2.5% HDP and 2.5% ADP had higher value of springiness. These results agreed with the previous rheological properties that the increase of intermolecular cross-linking of starch chains strengthened the gel networks and increased water retention. The strength of the gel networks could increase cohesiveness and resilience of steamed dumpling wrappers (Li et al., 2019). The results indicate that the springiness of non-gluten chicken pasta should be about 0.65-0.75. Witek *et al.*, (2020) reported that egg proteins had an impact on the internal structure and strength of pasta. Supplementation of the pasta with egg preparations had no effect on springiness, affected the degree to which distorted pasta strips return to their original condition after being removed from a probe.

In noodle it was reported that chewiness is an indicator of the degree of rupture of the structure during mastication (Singh, *et al.*, 2009). This value is calculated from the multiple of hardness cohesiveness and elasticity. The less chewiness indicates the less energy and easier to eat. In this study, chewiness of pasta decreased when modified starch was added. The cross linked starch may change the gel structure in the product and make the pasta easier to swallow.

Also, chewiness increased in pasta that had been kept in chilled storage. The results indicate that chewiness of non-gluten chicken pasta should be about 1,600-2,000 (g x mm).

It has previously been shown that during the chilling phase, after gelatinization, starch undergoes retrogradation, in which the starch chains tend to reassociate in an ordered structure. While starch retrogradation is a negative phenomenon in baking, it has the potential to improve the quality of extruded products such as pasta. (Karim *et al.*, 2000).

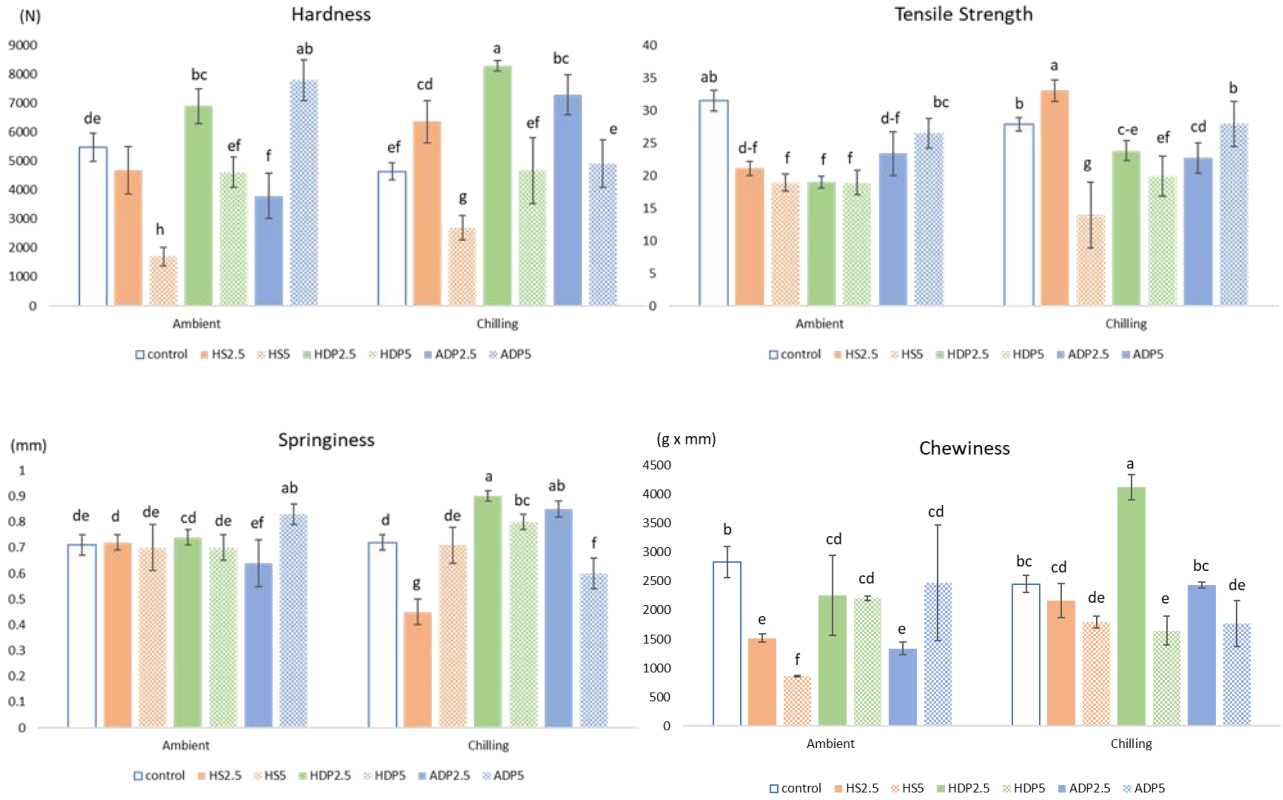

Figure 1 Effect of types and amount of modified starch kept at different temperatures: (A) hardness; (B) Tensile Strength; (C) Springiness; (D) Chewiness.

CONCLUSIONS

The addition of modified starch into non-gluten chicken pasta showed different effects on its cooking quality and texture characteristics. Of the starches that were tested, the addition of 5% hydroxypropyl starch resulted in the best quality pasta in terms of the lowest cooking losses, shortest cooking time and highest cooking weight retention. From the data, it was concluded that the texture of good quality pasta should have hardness, tensile strength, springiness, and chewiness within the range of 2,500-5,000 N, 13.5-23.5 g, 0.65-0.75 mm and 1,600-2,000 g x mm, respectively. It is recommended that further studies should be on sensory evaluation and shelf-life of this optimum treatment.

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Consumer need analysis and development of healthy jelly from fingerroot (*Boesenbergia rotunda* (L.) Mansf.)

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ABSTRACT: The purposes of this research were to survey consumer needs by applying outcome driven innovation (ODI) and in-depth interview and to develop healthy snack product. From consumer need analysis by ODI method, Gen Y participants (n=121) were interviewed. The collected statements demonstrated that the main goals of consumers' need were to live a longer and get healthier life, be absence of illness and have healthy food to consume as medicine. All achieve statements were classified to the same interests and presented 34 desired outcome statements to identify importance and satisfaction. The desired outcomes were plotted on opportunity landscape and the graph showed appropriately served and underserved outcomes. The key messages of underserved need as following; "pleasure and happiness", "hygienic (clean, non-toxic)", "chewability", "immunity-boosting", "low energy/low-calorie", "Thai herbal ingredients" and "gel type food". Targeted Gen Y participants (n=15) were joined in-depth interview for creating desired healthy jelly. The interview results demonstrated that a healthy snack in gel type product which is chewable, contain immune boosting ingredients from Thai herb and contain low calories should be developed. The healthy jelly from fingerroot were produced and evaluated sensorial acceptance. The prototype of honey fingerroot lemon jelly received the preference scores (7.13 $\pm$ 0.90) in overall liking as "like moderately".

Keywords: Fingerroot jelly, Generation Y, Outcome-Driven Innovation, In-depth interview, Value Proposition Canvas

INTRODUCTION

Food consumption was changed during the COVID-19 pandemic. Consumers have had to adopt healthier habits. They have increased focusing on their personal well-being, nutrition, and fitness because many people continue to work from home and use food delivery services. The pandemic has accelerated the need of functional food, beverage, and snack. The recommendation of dietary guidelines, people should consume a variety of food products in proper proportions, more fruit and vegetables, less sugar, fat, and salt [1]. Therefore, people may try to increase eating more healthy food in an attempt to boost their immune system. The concept of new product development should be developed by satisfying a consumer demand.

Customer needs analysis is the process to clearly identify customers' needs and expectation for a product or service by extracting insight from customer feedback [2] and could create the right product for the consumer. Conducting customer research to understand customers' needs can be done by customer interviews, focus groups and surveys, etc. Customers buy products or services to get their *jobs* done [3]. *Job-to-be-done* is generally

defined as the problems that customers want to solve by products or services. Outcome Driven Innovation (ODI) is an evaluation technique represents customer hidden needs which focuses on understanding what are outcomes that customers want to achieve. Outcomes represent as a measure of how well the *job-to-be-done* are achieved [4]. ODI increases the chances to discover hidden product opportunities [5]. Following the ODI method, Hankammer *et al*. [6] interviewed consumers in order to identify their problems and found revealing 30 consumer needs. Then, they evaluated these needs in a survey to quantify their potential. The result analysis (n=183) showed that 11 of the 30 needs are currently unmet by existing business models. Therefore, the presented underserved needs can be considered as first ideas for the development. Num *et al*. [7] studied hotel service opportunities and applied ODI to identify customer hidden needs. The analysis showed that ODI can identify customer needs in hotel service and can be used as a tool for the effective development of a business strategy. The in-depth interview is another qualitative data collection method that is particularly effective in researching a person's behavior, attitudes, needs, beliefs, values, and personality traits. This technique uses open-ended questions to give the interviewees the opportunity to express their opinions which can tell the needs thoroughly [8].

Generation Y, age 25-40 years old, is a working age and has a high purchasing power. This generation pay greater attention to be more health conscious. The survey results showed that 83 % of millennials inform that health is important when choosing a snack [9]. Department of Health, Ministry of public health [10] categorizes Thai snacks into four groups. There is crispy snack, fish snack and nut, jelly and candy. The jelly market was expected to grow significantly, with a market value of 2,000 million baht in 2017 and 3,500 million baht in 2021 [11]. The demand of jelly products is increasing due to various taste, flavors, easy to add functional ingredients and easy to consume. Jellies are made from a variety of fruits, either individually or in combination with other ingredients. The reduction of sugar and the addition of healthy ingredients such as antioxidants, herb extract in jellies brings some improved health characteristics to a product. Also, a new type of jelly which can solve the problems of health and beauty become more interesting. Fingerroot (*Boesenbergia rotunda* (L.) Mansf.), in Thai is called Kra-Chai, is one of local herbs in Southeast Asia and Thailand [12]. This root is medicinal plant and gets much attention currently due to its bioactive compounds, antioxidants, and antimicrobial. Fingerroot has been processed to various food products; for examples, dried fingerroot, pickled fingerroot, fingerroot extract powder, fingerroot tea and fingerroot mixed with fruit juices, etc. Herbs show high potential as functional ingredients in Thai snacks. Therefore, there is opportunity to develop healthy snack.

The objectives of this study were to survey consumer needs by applying outcome driven innovation and in-depth interview and to develop healthy snack product.

MATERIAL AND METHODS

Consumer need analysis by using Outcome-Driven Innovation

The interview study was conducted with 121 consumers who were Gen Y (age between 25-40 years old) have been living in Bangkok and nearby areas, consuming jelly regularly within three months. The ODI process is composed of six steps [5], in this study the steps were adapted to fit the process as follows:

1. Define the customer's "*Jobs-to-be-Done*". What is the goal that interviewees are trying to achieve? without mention any relation to the product. The objective for this step is to discover consumers' actual needs. This process helps generating loads of outcome from interviewees.

Questions in step 1 were divided into 4 parts:

Part 1: Questions about interviewees personality; age, occupation, and weight, etc.

Part 2: Questions on consumer behaviors; personal habits, pattern of lifestyles and consumption behaviors.

Part 3: Questions in relation to health goals; What are the long-term health outcome that consumers wish to achieve?

Part 4: Questions to ask customers in order to know their needs and expectations; for examples, What types of healthy products are you interested in? Did you find the products you were looking for easily? What is your preferred snack?

2. Expose the customer's need, "*Job-to-be-Done needs framework*" is used to categorize, define, capture, and organize all customers' need. Define a need in the form of a desired outcome statement. A set of desired outcomes was obtained.

3. Quantify the degree to which each outcome is over/underserved. To obtain the data needed to discover opportunities, a survey instrument is used to ask customers about what solution (product/service) they are using today to get the *job done*, and then ask them to evaluate the importance and satisfaction of each outcome statements. The survey structure can be seen in **Figure 1**.

	When [job step], how important is it to you that you are able to:					When using [solution] for [job step], how satisfied are you with your ability to:				
	1	2	3	4	5	1	2	3	4	5
Desired outcome 1	○	○	○	○	●	○	●	○	○	○
Desired outcome 2	○	●	○	○	○	○	○	○	●	○

Figure 1 Survey Structure to obtain importance and satisfaction data [5]

4. Discover hidden opportunity. The data set will present which outcomes some people think are important and unsatisfied while others think they are satisfied and unimportant. The results of data analysis will be plotted in the Opportunity Landscape (**Figure 2**). It reveals which outcomes are over/underserved. A need is overserved means that outcome is unimportant to the customer because customers are very satisfied with solutions are using today. Underserved market segment is a segment of customers with many desired outcomes that are very important and poorly satisfied. The landscape provides a visual demonstration of the market opportunities.

The opportunity will be calculated by the equation [5]:

Opportunity = Importance + max (importance – satisfaction, 0)

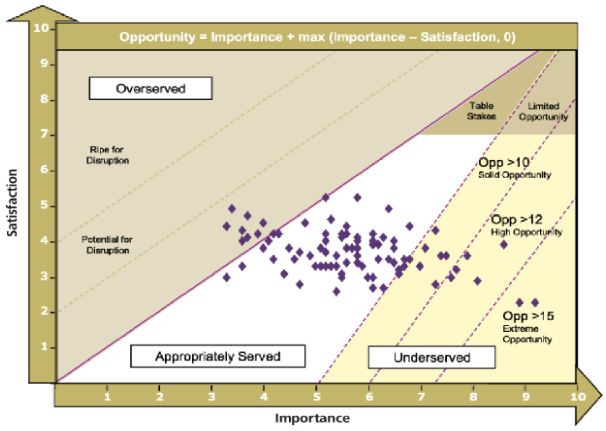

Figure 2 Opportunity Landscape [5]

5. Align existing products with market opportunities. According to the outcome-based segment, the insight can be guided to the solution that will best address underserved outcomes. It leads to right value proposition and a clear understanding of unmet needs and link to optimize the product portfolio for new development.

6. Conceptualize new products to address the underserved outcomes. An effective product portfolio strategy will provide information about (1) how to improve its existing products (2) what are new opportunities in the market (3) what solution should be created. ODI provides the insights that are needed to effectively make these decisions.

Consumer In-depth interview

With in-depth interviewing, fifteen Gen Y, who has healthy eating habits and always choose or consume healthy snacks and consumes jelly regularly within 3 months, were

carefully selected to be a group of participants. The open-end questions were used to let interviewees expressed their opinions [13] by the 5W1H method. 5W1H is an abbreviation in which every letter refers to a question: what, who, where, when, how, and why [14], to define the attitude and behavior of jelly product consumption. The interview took about 30 minutes for each person. The questions were developed and listed to be explored during the interview. Examples of themes and sub-themes questions are provided in **Table 1**.

Table 1 Samples of a set of interview questions

Theme 1: Personal information questions. [name, age, gender, and health concerns]
Theme 2: Questions for problem analysis and demand for health products by using 5W1H. [What are your currently health concerns? Who has health problems? What are the health problems? When do you have health problems? Why do these health problems occur? How to solve your health problems?]
Theme 3: Consumer behaviour interview questions and What are the motives which influence healthy snack purchase decision?
Theme 4: Questions for new product concept development focusing on jelly.
Subtheme 4a: Which types of jelly do you prefer? (For example; soft, sugar-coated, or drinkable jellies)
Subtheme 4b: What are the characteristics of good or healthy jelly?
Subtheme 4c: What kind of packaging do you want?

The interview data was collected and analyzed. The Value proposition canvas (VPC) is a tool that focuses on understanding customers problems and producing products or services that solve them. In presenting results of in-depth interview, data can be displayed as consumer profile section in a value proposition canvas (**Figure 3**). Also, the obtained information was used to create healthy jelly products that related with consumers' need.

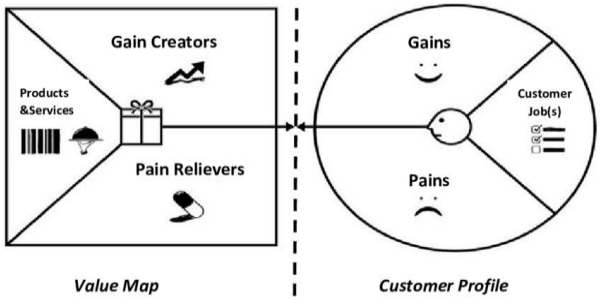

Figure 3 Value Proposition Canvas [15]

Materials

The ingredients used in the formulations included honey (20% w/w), commercial spray dried fingerroot extracted powder (2% w/w), lime powder (2% w/w), mixed hydrocolloids (carrageenan and konjac in a 1:1 ratio) (1, 1.5 or 2% w/w), citric acid (0.2 %w/w), potassium chloride (0.06 %w/w). Water was added to make up to 100% of the total.

Production of samples

Each lot of honey fingerroot lemon jelly (HFL jelly) was produced to obtain 500 g of final product. All ingredients were weighed individually, then mixed hydrocolloid (3 concentration; 1, 1.5 and 2% w/w) was dissolved in a half portion of water and standing it for 5 minutes to adsorb water completely. Then honey, fingerroot extract powder, lime powder, and the rest amount of water were mixed with hydrocolloid solutions. All ingredients were blended together and heated up to 70 ^{o}C, after that citric acid and potassium chloride were added. The samples were heated continuously for 5 minutes. Ten grams of heated samples were hot-filled in heat-resistant aluminum foil bags and sealed completely. Packed samples were pasteurized by heating them in hot water at 80 ^{o}C for 10 minutes, and then samples were cooled immediately and refrigerated at 8$\pm$2 ^{o}C before sensory analysis.

Sensory evaluation

Three formulations of HFL jelly samples were prepared for the consumer

acceptance test using a 9-point hedonic scale (9=like extremely; 5=neither like nor dislike; 1=dislike extremely) with 30 consumers. A commercial fruit juice jelly which produced with similar hydrocolloids type was used to be a control. The panelists were Gen Y, who has healthy eating habits and always choose or consume healthy snacks and consumes jelly regularly within 3 months. For the sensorial test, all samples and a control were served when their temperatures were around 8±2 °C, drinking water and unsalted crackers were served in the sensorial set for mouth washing. The evaluation considered the preference in appearance, odor, texture, elasticity, taste and overall liking.

Statistical analysis

For HFL jelly experiment, randomized complete block design (RCBD) was used for sensory evaluation. Analysis of variance (ANOVA) with Duncan's new multiple range test (DMRT) was applied. The level of significance used was 95% ($p<0.05$) using the SPSS program version 20.0 (IBM Corp., New York, USA).

RESULTS AND DISCUSSION

Outcome-Driven Innovation (ODI)

The participants in this research were Gen Y (n=121). 62% were female while 38% were male. Most of the participants were 25-30 years old (58%), followed by 31-35 years old (35%) and 36-40 years old (7%). The occupations of respondents were private company employees (48%), business owners/contractors (30%), public company employee (13%) and freelance (9%).

Information obtained in an interview process about "what are outcomes that customers want to achieve" was collected. The data indicated that there were load of uncategorized statements around 573 outcomes. It revealed that the main goals of consumers' need were to live a longer and healthier life, absence of illness and consuming healthy food as medicine. All achieve statements were then classified according to the same interests. There were 34 desired outcome statements as shown in **Table 4**.

Then, participants evaluated the importance (IMP) and their satisfaction (SAT) with various needs. Every panel statement was assigned a score (1 to 5) for importance and satisfaction (5 means critically important or satisfaction and 1 means not important or satisfaction at all). The IMP and SAT scores were calculated using the percentage of participants that rated a panel statement high in terms of IMP and SAT (ranking $\geq$ 4). Additionally, the IMP and SAT scores were plotted into correlation graph as shown in the Opportunity Landscape (**Figure 5**), with the satisfaction on the y-axis and importance on the x-axis. Every point (number 1-34) in the Opportunity Landscape represents a specific outcome. The opportunity (OPP) scores were calculated by using equation of Ulwick [16, 17] as presented in **Figure 4**. The position of the point shows whether existing solutions in the market appropriately served the outcome, underserved or overserved [18]. It can be seen that if the importance score exceeds the satisfaction score, the need can be classified as under-served and is regarded as an innovation potential for the development.

Ulwick [19, 20] stated that the opportunity (OPP) scores can be interpreted in the following: OPP scores between 12 and 15 are "low-hanging fruits" ripe for improvement and OPP scores between 10 and 12 are worthy of consideration especially when discovered in a broad market.

Table 4 The opportunity scores to identify the outcome with importance and satisfaction.

Outcomes	Importance (IMP)	Satisfaction (SAT)	Opportunity (OPP)
1. Healthy body	8.7	5.9	11.5
2. Weight maintenances	7.9	4.0	11.8
3. Beautiful skin	7.6	5.3	9.9
4. Recovery of excretory system	8.4	5.0	11.8
5. Longevity	7.3	5.4	9.2
6. Pleasure and Happiness	8.8	3.5	14.1
7. Full of vitality	8.4	6.1	10.7
8. Alleviate illness	7.9	4.6	11.2
9. Energy boosting	7.7	5.7	9.7
10. Medical expense reduction	7.9	4.1	11.7
11. Hygienic (clean, non-toxic)	9.0	5.7	12.3
12. Easy for ingestion	7.8	4.1	11.5
13. Filling	7.6	5.2	10
14. Cost-worthy	7.9	4.0	11.8
15. Improve cognitive function	8.1	5.6	10.6
16. Clear nutrition labels	8.5	6.5	10.5
17. Attractive packaging	6.9	6.4	7.4
18. Easily digestible	7.9	4.3	11.5
19. Eco friendly / sustainable	7.8	5.8	9.8
20. Vitamin supplement /antioxidant	7.6	4.3	10.9
21. Natural ingredients	8.0	4.2	11.8
22. Innovative technologies	7.2	3.4	11
23. Chewability	7.9	3.1	12.7
24. Immunity-boosting	8.5	3.7	13.3
25. Improve sleep quality	6.9	4.6	9.2
26. Friendly for vegetarians	8.1	4.4	11.8
27. Convenient packaging	7.5	3.9	11.1
28. Low energy / Low-calorie	7.9	3.7	12.1
29. Thai herbal ingredients	7.5	3.0	12
30. Long shelf life	6.9	3.9	9.9
31. Vegan	7.0	3.9	10.1
32. Gel type food	7.8	3.2	12.4
33. Usage of natural sweetener	7.9	4.3	11.5
34. Healthy snacks	7.0	3.7	10.3

Opportunity Score = Importance + max (Importance - Satisfaction, 0).

	Importance						Satisfaction						Opportunity
Outcome	**1**	**2**	**3**	**4**	**5**	**IMP**	**1**	**2**	**3**	**4**	**5**	**SAT**	**OPP**
Gel type food	2	7	13	49	29	7.8	3	11	54	24	8	3.2	12.4
				78						**32**			**7.8+(7.8-3.2)**

Figure 4 Example of calculating opportunity scores

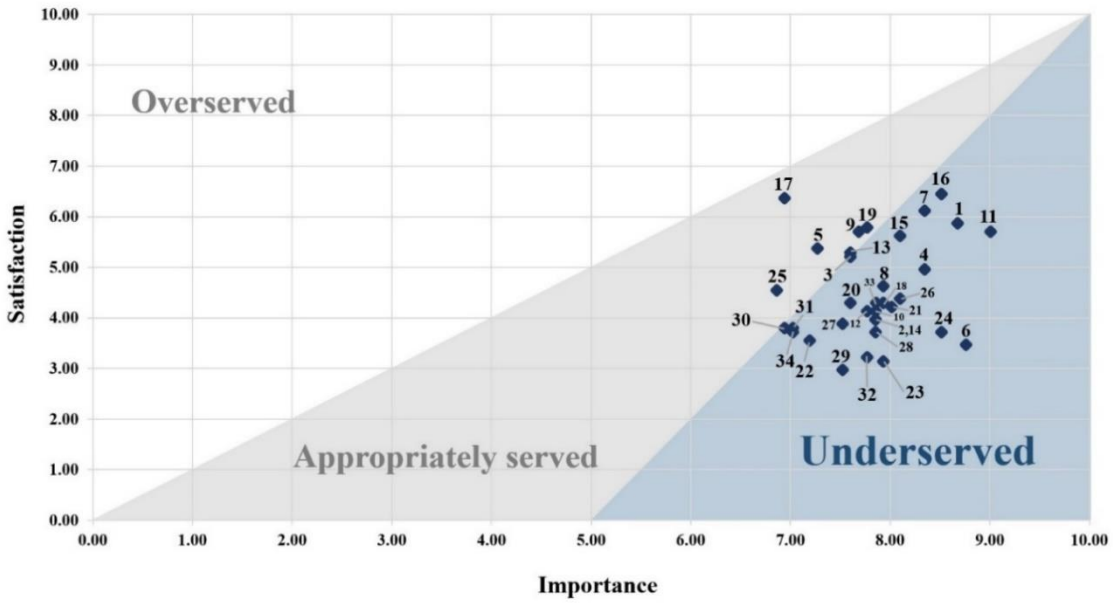

Figure 5 Opportunity Landscape of healthy food products.

In this research, outcome segmentation revealed appropriately served and underserved segments, it discovered that many identified needs were not satisfactorily served. With regard to identified outcomes, the desired outcomes obtained OPP scores $\geq$ 12 that informed key points of underserved need as following; "pleasure and happiness", "hygienic (clean, non-toxic)", "chewability", "immunity-boosting", "low energy/low-calorie", "Thai herbal ingredients" and "gel type food". The results demonstrated that a healthy snack in gel type product which is chewable, contain immune boosting ingredients from Thai herb and contain low calories. According to underserved outcome characteristics mentions, jelly is a snack which identifying and meeting consumer needs and possible to new product development.

In-depth interview

A result of findings from the in-depth interviews about the attitude and behavior of jelly product consumption were defined and used a value proposition canvas (VPC) (**Figure 6**) as a tool for filling key messages of *job to be done* (pain and gain of consumer) and solution development (product or value map). The customer profile identified during interview were translated into a value map.

Identification of customer profiles

Fifteen Gen Y participants mentioned approximately 50 different values. The customer job in this VPC described only the most importance value.

Customer job

According to the collected statements, it revealed that consumers are becoming more concentrate on eating for their health. Most participants focused on increasing longevity, having a healthy body, maintaining beautiful skin, feeling happy life and pleasure. These statements involved task goals, which consumers would like to achieve in the end to get *job done* [21].

Pain point

The participants informed the main problems of jelly consumption including high calories intake and high in sugar content (other than natural sugar from the fruit) within recipe. It causes of health risks such as diabetes, tooth decay, weight gain, and increased triglyceride levels which could be developed to serious illness. Some hidden ingredients: for examples, food preservatives and artificial color might be

used. The texture (hardness and elasticity) of jelly snack unmet consumers' needs properly. Some of jelly products had a firm and chewy texture. Therefore, it was not easy-to-swallow.

Gain point

Form in-depth interview, it proved that consumer is looking for jelly product which contains functional ingredients from natural to promote immunity-boosting, contain vitamins and antioxidants. Also, the product should be easily ingestion and highly digestible.

Identification of value map

Based on the customer profile, a value map was created that describes product and service that are achieved to consumers' need, pain relievers and gain creators.

Product and service

Honey fingerroot lemon jelly (HFL jelly) was the developed snack product in order to solve consumer *job*. The prototype of this snack will be formulated relating to consumers' needs. The key characteristics of healthy jelly snack as follow; containing natural functional extract from Thai herb (fingerroot), sweet and sour taste boosting by addition of honey and lemon, setting gel texture by natural hydrocolloids, packing in an easy and convenient squeeze packaging. The value of this snack associated with the needs of customer and leaded to outcomes that customers want to achieve.

Pain Reliever

These are the aspects of products or services that can improve consumers' pain. From the in-depth interview, it was found that consumers desire to consume jelly which has low calorie, non-fat, no sugar or sucrose added, contain high dietary fiber, made from natural ingredients. The final texture should be soft and chewability. Lasty, consuming healthy food can actually offer some real stress relief.

Gains Creator

This jelly provided immunity-boosting and antioxidants from fingerroot extract. Therefore, it may help immune system to against disease and infection. Also, a snack high in vitamins and antioxidants may reduce the risk of many diseases. The food that easy to ingest and digest will have beneficial effects on consumer health.

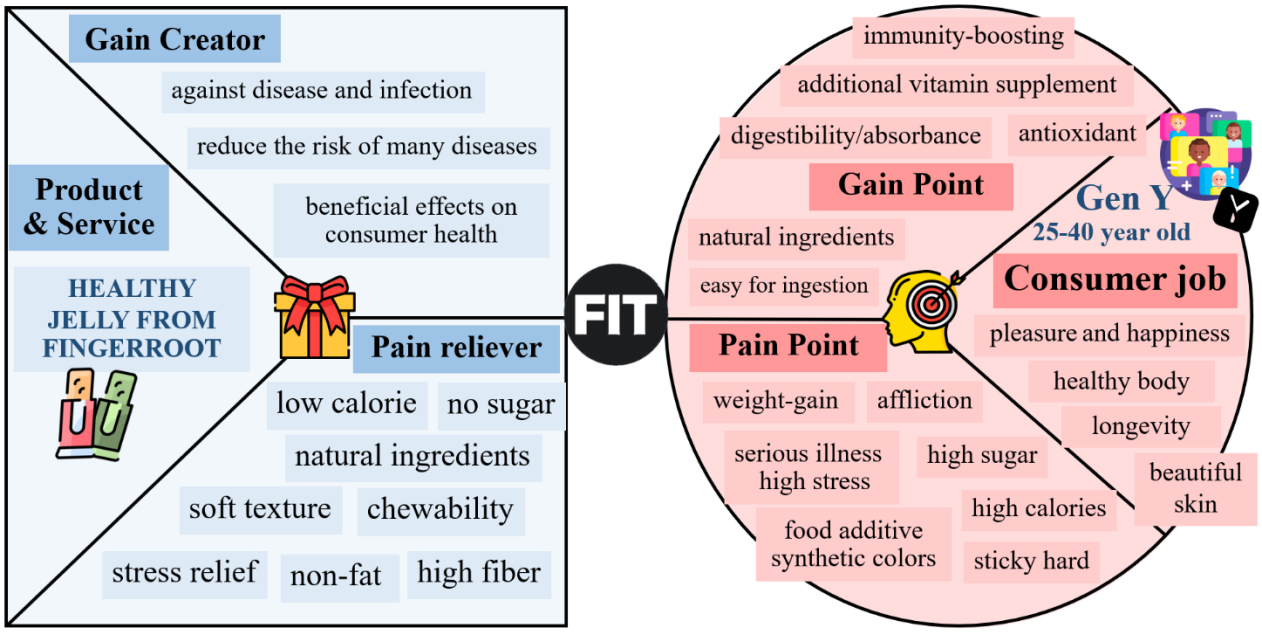

Figure 6 Value Proposition Canvas of healthy jelly product

Treatment	Characteristics					
	Appearance	Odor	Texture	Elasticity	Taste[ns]	Overall liking
Commercial	7.20±0.76[a]	7.33±0.88[a]	7.20±0.85[a]	6.93±0.69[ab]	7.13±0.82	7.13±0.73[a]
H1	7.27±0.78[a]	7.20±0.85[a]	7.13±0.73[a]	7.00±0.74[a]	7.00±0.91	7.13±0.90[a]
H1.5	6.60±0.81[b]	6.60±0.81[b]	6.40±0.97[b]	6.20±0.92[c]	6.73±0.94	6.67±0.96[b]
H2	6.67±0.80[b]	6.67±0.88[b]	6.67±0.96[b]	6.53±0.90[bc]	6.80±0.92	6.87±0.73[ab]

Table 5 Sensorial scores of honey fingerroot lemon jelly.
Note: H1, H1.5 and H2 mean samples added mixed hydrocolloid 1%, 1.5% and 2%, respectively
[ns] Not significantly different ($p > 0.05$)
[a,b,c] Mean±Std.deviation with different superscript letters indicating significant differences ($p \leq 0.05$)

Figure 7 Photographs of honey fingerroot lemon jelly added hydrocolloid 1% (H1), 1.5% (H1.5) and 2% (H2)

Sensory evaluation

The photograph of HFL jelly samples present in **Figure 7**. The levels of consumers' acceptability based on the 9-point hedonic scale of three formulated jelly products and commercial sample are presented in Table 5. The results showed that significant ($p < 0.05$) differences in all attributes (appearance, odor, texture, elasticity, and overall liking) except taste were observed among three formulated jelly samples. The addition of hydrocolloid 1% was appropriate concentration to develop honey fingerroot lemon jelly formulation which received the highest preference scores (7.13 ± 0.90) in overall liking as "like moderately". Comparing with commercial jelly which made from fruit juice and added similar of hydrocolloid types, the preference score in texture, elasticity, and overall liking of H1 was comparable with a commercial product. It was noticed that the increase of hydrocolloids concentration decreased texture and elasticity acceptance scores. The favorable jelly should have a soft texture and elasticity. The liking scores of texture attribute for H1.5 and H2 jelly decreased because high concentration of carrageenan and konjac produced strong and brittle gel which was not accepted in jelly texture.

CONCLUSIONS

According to consumer need analysis by Outcome-Driven Innovation (ODI) and the In-depth interview, it concluded that Generation Y consumers interested in healthy jelly products and would like to purchase when the product characteristics certain qualities following; no sugar added, non-fat, has low calorie, made from natural ingredients, contain functional substances such as immune boosting compounds, antioxidants, has soft texture and chewability. The sensory evaluation of honey fingerroot lemon jelly by using 9-point hedonic scale, demonstrated that healthy jelly product with 1% hydrocolloids addition received the highest acceptance scores in all attributes and its texture and elasticity was comparable with commercial jelly.

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Study of Thai consumer's behavior in purchasing plant-based meat and dairy alternatives

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ABSTRACT: Alternative plant-based meat and dairy products have been raised over years. This research aimed to study extrinsic and intrinsic factors which influence consumers' purchasing behaviors towards plant-based products including barriers and entourage from the norms. Demographic, behavior, and consumption of plant-based milk products were investigated by an online survey. There were 198 responses obtained from online communities. The major consumer was omnivorous although plant-based product consumers are mostly 56.02%. Taste was the most important motivation ($p \leq 0.05$) but not influenced by the emotional or concern of situation issue animal and environmental awareness ($p > 0.05$) besides the attitude but also environmental surroundings. Social expectations involved consumers trying plant-based and dairy alternatives related by open-minded to accept a new thing. Plant-based meat and dairy alternatives are occasionally consumed on the vegetarian festival, rite, or consumers who intended to get weight loss by some diet types: ketogenic diet, clean diet, etc. Consumers' intention on plant-based meat and dairy alternatives purchase is increased by informing how good products taste and word-of-mouth impact by households.

Keywords: Consumer behavior, Plant-based replacements, Purchasing, Motivation

INTRODUCTION

Nowadays, the climate change issue has accumulated and shows its current impact. As the world's population increased, there was a growing demand for food consumption. The food production system has been expanded according to the needs of the increasing world population. Livestock and agriculture were expanded as a consequence leading to more resource use and waste emissions, including carbon dioxide and methane. Most the production plant-based foods have low methane emissions. (Cleveland et al., 2017) Plant-based meat and dairy products alternatives become more popular over years. Since issues of health and health-conscious such as cow's milk protein allergy, digestive problems, and lack of lactose intolerance, environmental issues, animal welfare impact, and sustainability, including the result of an increasing trend of vegetarian and vegan consumption in many countries. In Thailand, plant-based meat has a trade value of 28 billion baht and is likely to grow by an average of 10 percent or increase to 45 billion baht in 2024 (Krungthai COMPASS Research Center., 2020) dairy alternatives also have rising become 40 percent of the milk market share. The major of plant-based milk is soy milk. The data expressed major important opportunities for growth in the plant-based product market. Consumers' behavior toward plant-based products is necessary to study for better understanding of consumer needs. (Phachara, 2018) Thai consumers' perception and purchasing decisions of non-dairy milk products depend on taste and health benefits, social trends, and nutrition options similar to the consumers in Sweden also have health and environmental factors in driving (Mousel et al., 2016) The objective of this research is to study consumer's behaviour in purchasing plant-based meat and dairy alternatives by using

combines the existing Theory of Reasoned (TRA) model to investigate consumer's behavior that influenced by intention person's attitude, subjective norm, and barrier.

MATERIALS AND METHODS

Online Survey

An online survey was developed and launched through the vegetarian online community. The questionnaire construct was created and verified, internal consistency of the questionnaire by factor analysis and reliability analysis by using SPSS statistics software. The survey was fielded by 198 consumers which consisted of consumers who had not consumed plant-based meat and dairy alternatives (n=84) and consumed plant-based meat and dairy products (n=114). The type of questionnaire was separated into three parts. Demographic information was the first part of an online survey consisting of gender, age, education, income, occupation, and diet type. Attitudes, entourage from norms, and barriers were investigated with a Likert-type scale on statements and weighting questions ranging from 1 (strongly disagree) to 7 (strongly agree).

Data variation

After attitudes entourage from norm and barriers were collected by Likert-type scale ranging from 1 (strongly disagree) to 7 (strongly agree) (ref.) Weighting and statement values were combined by multiplying. Each weighting value was maintained from 1 to 7 while the statement value has recorded by SPSS from -3 to 3. Therefore, values were in the range of -21 to 21.

Descriptive Analysis

Demographics' distribution consisting of gender, age, education, income, occupation, and diet type was evaluated the frequency and normal distribution by SPSS descriptive statistics and Kurtosis.

Factor Analysis and Reliability Analysis

Factors barriers and entourage from norm were reduced by using SPSS principal components analysis with direct varimax rotation.

Regression analysis

Exploring the consumer's behaviour intention, which is related to behavior factors including barriers and entourage from norms were using SPSS simple linear regression and multiple linear regression to test hypotheses between each factor and customer's behavior intention of purchasing plant-based meat and dairy alternatives. The following formula relates those factors (Ajzen & Fishbein 1980):

$$B \approx BI = A_{act}\,(\omega 1) + SN\,(\omega 2)$$

Where:
B is a specific behavior
BI is the intention to engage in that specific behavior
A_{act} is the personal attitude towards engaging in that specific behavior
SN is the subjective norm; it tells us whether the entourage of a person wants them to engage in that specific behavior.
ω1, ω2, are the weights reflecting the relative influence each factor has on the behavior intention (BI).

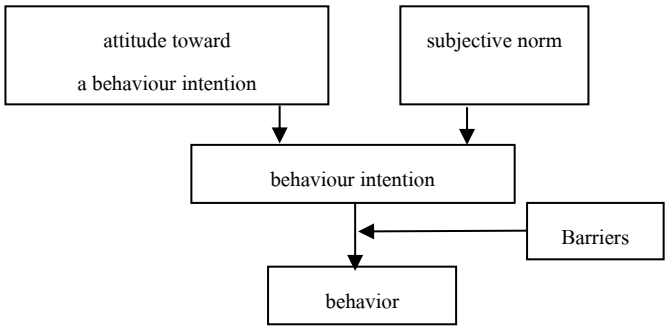

Figure 1 The basic TRA model as defined by Aijzen & Fishbein (1980)

RESULTS AND DISCUSSION

1. Socio-demographic characteristics

A total of 198 consumers (Thai nation) responded to the survey. Major of the respondents were women 88.38 %. Most of the consumers were in the age range of 19-30 years (47.98%) followed by 31-45 years (38.38%), 62.13% of them completed bachelor's degree. Most of the participants are company employees (48.48%) and income were spread between 5,000-50,000 baht. The majority of consumers (94.5%) were omnivorous and did not follow a specific diet (vegans, vegetarians, or ketogenic diet) (as shown in Table 1). Consumption of plant-based meat and dairy alternatives was used as a criteria to classify types of consumers. The results showed that 57.58% of consumers consumed plant-based meat and dairy alternatives. Thirty-five percent of consumers purchased plant-based meat and dairy alternatives more than once a week, 11.6% purchased 3 to 4 times per week, and 10.1% purchased over than 5 times per week. A large number of regular diet in the previous was caused by a low frequency of consuming plant-based meat and dairy alternatives on the other hand frequency of consumed meat and dairy every day was 74.7%. as shown in the Figure1. Purchase frequency and demographics were no significant difference ($P > 0.05$). As consequence consumers in Thailand were on stage to increasing of the number of vegetarians. the result in the year 2017, Thai consumers more vegetarian food, about 7 million people, especially those in Bangkok, are more than around 1.2 million people in the year 2018. Vegetarian food was the alternative of consumption according to the vegetarian festival but nowadays found a lot of consumers eating behavior more vegetarian food choose to buy vegetarian food, consumers will shop for convenient occasions. and not limited in range Vegetarian Festival While most people today are starting to reduce lower meat consumption which is caused by the trend of health care (Pitchayapan, 2020).

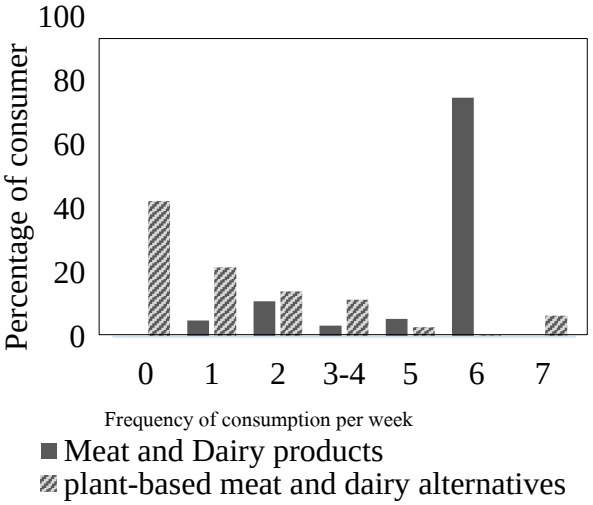

Figure 2 The frequency of consumption by type of product of consumers per week

from the results of studies, plant-based meat and dairy products were low frequency of consumption per week than meat and dairy products may be the result of consumers preferring less processed foods and more whole foods. (Mousel et al., 2016)

2. Reliability Analysis

The reliability test result of each part of the questionnaires which are attitude and barriers showed Cronbach's Alpha of attitude (0.946) entourage from the norm (0.952) and barriers (0.868) (Kaiser 1970, 1974) also factors analysis Kaiser-Meyer-Olkin values (KMO) were attitude (0.825) entourage from the norm (0.865) and barriers (0.822). All factors were highly discriminant validity and significant as the result shown in Table 1, component factor was cut-off by eigenvalues above 1, and factor loading was ranged from 0.713 to 0.929 (Hair et al., 2014)

3. Factor Analysis and Regression analysis

The behavioral intention (BI question: How often do you plan on consuming meat and dairy replacement products in the future) has a strong relationship with behaviour (B . How often do you consume meat and dairy replacements on an average) ($P \leq 0.05$), among consumers with frequent consumption of meat and dairy replacement products this is why they

consumed more often in the future. (Mousel et al., 2016) A study of the theory of planned behavior to predict intention to consume plant-based yogurt alternatives products also found conformable results but has some differences in component subjective norms did not have a positive and significant influence. This finding was inconsistent with several of these studies. (Pandey, 2021). Besides, the attitude was significantly different ($P \leq 0.05$) on taste.

Table 2 Results from the regression analysis

Component	Beta	S.E.
Attitude	0.32*	0.015
Entourage from norm	0.31*	0.070
Price barriers	0.04	0.015
Information barriers	0.08	0.016
Availability barriers	0.21	0.017
Decision barriers	0.30*	0.036
Food phobia barriers	-0.08	0.013
Cultural barriers	0.05	0.015

* Significant difference ($P \leq 0.05$)

Table 1 Socio-demographic characteristics of the study population (n=198)

Socio-demographic Characteristics		Frequency	Percent	Non plant-based Consume (n= 84)^{NS}	Plant-based consume (n=114) ^{NS}
Gender	men	23	11.62	13.10	10.53
	women	175	88.38	86.90	89.47
Age (years)	18	4	2.02	1.19	2.63
	19-30	95	47.98	48.00	47.37
	31-45	76	38.38	40.00	37.72
	46-59	20	10.10	9.52	10.53
	over 60	3	1.52	1.19	1.75
Education	Primary school	1	0.51	-	0.88
	Secondary school	4	2.02	1.19	2.63
	High School	17	8.59	8.33	8.77
	Diploma	8	4.04	3.57	4.39
	Bachelor	125	63.13	66.67	59.65
	Postgraduate	43	21.72	20.24	23.68
Occupation	Student	32	16.16	15.48	16.67
	Self-employed	30	15.15	14.29	15.79
	Company employee	96	48.48	48.81	48.25
	Government officer	40	20.20	21.43	19.30
Income Baht/month	Under 5,000	21	10.60	10.71	10.53
	5,001 - 10,000	15	7.57	7.14	7.89
	10,001-15,000	23	11.61	11.90	11.40
	15,001-30,000	78	39.39	34.52	42.11
	30,001-50,000	33	16.66	21.43	14.04
	50,001 and up	28	14.14	14.29	14.04
Diet	Omnivorous	189	95.45	98.81	92.98
	Pescatarian	3	1.52	1.19	1.75
	Flexitarian	6	3.03	0	5.26

* NS No significant difference ($P > 0.05$)

Table 3 Component Matrix and total variance explained of attitude and entourage from the norm

Component	*eigenvalue*	*Variance explained*	*Factor loading*	*item*
Attitude	4.26	53.23	0.763	*Healthy alternatives*
			0.568	*Tasty*
			0.870	*Animal welfare*
			0.828	*Environment concern*
			0.794	*Good emotional*
Barriers	1.79	16.29	0.808	*Households*
			0.871	*Friends*
			0.883	*Colleague*

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
[a] Rotation converged in 3 iterations.

Similar to several consumer studies, the food consumption behavior survey conducted in 2017 with 27,960 samples of Thai consumers showed the consideration factor for food selection is liking (22.1%) followed by taste (18.5%). This indicated that sensory attributes, especially taste, is an important determinant factor for plant-based diets (Reipurth. et al., 2019) (National Statistical Office, 2018). Surprisingly animal welfare, environmental issues concern, healthy choice, and good emotions were not significantly different ($P>0.05$). Households were the most important positive influence on behavioral intention ($P\leq0.05$) in previous studies, the family is the first social unit to mold life and educate which serves the needs of members of society (Sriwan, et al., 2017). Associated with the barriers was found significantly different ($P\leq0.05$) on decision barriers as shown in Table 2.

CONCLUSIONS

According to this study, attitudes, an entourage by norms, and barriers to Thai consumers' behavior in purchasing plant-based meat and dairy alternatives indicated the attitudes entourage by norms and decision barriers are affecting the behavior of consumers in Thailand. Taste becomes an important attribute that plays an active role in customer attention. The entrepreneur should innovate tasty products, create marketing activities for a free tasting, conduct interesting advertisements making consumers perceive the great health benefits, as well as initiate promotions to gain an increase in the customer trial and create word-of-mouth by the household for inducing new customers.

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Consumer situation on salt reduction product and the effect of flavors and MSG on consumer perception and acceptance

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ABSTRACT: Salt (Sodium Chloride) is a commonly used food additive for enhancing taste and flavor. Sodium-rich diets have been largely demonstrated to promote hypertension that associated with cardiovascular diseases. The aim of this research was to investigate consumer behavior towards salt reduction products and study effect of different flavors (bacon, seafood, and smoked), monosodium glutamate (no MSG and 0.01%MSG), and salt content (0.05 (control), 0.0375, 0.025, 0.0125, and 0%W/V) on saltiness perception (Degree of Different test) and liking (9-point hedonic scale) in cream and clear soup model. According to the consumer study, there were 104 Thai consumers and 151 Taiwanese consumers participated in the survey. Both Thai and Taiwanese consumers cared about fat, vitamins and minerals contained in their diets. Based on findings from Health and Taste Attitude Scale responses, consumer had positive opinion about light product and believed it might improve their health. Focusing on consumer test, it was found that there were effect of MSG, salt content, and flavor on saltiness perception in both clear and cream soup while significantly different in liking score were found in all three factors in clear soup, but different flavor did not affect on consumer acceptance in cream soup. Adding MSG in both types of soup responded to less differentiation in saltiness perception but more acceptable by the consumer when comparing within the same type of soup and same level of salt content that less than control level. Consumer perceived the differences as the salt was reduced in both clear and cream soup. With the presence of MSG and decreasing of salt content, consumer liked all the products significant less at 0.025%W/V (50% reduction) salt content except for the samples with bacon flavor that found lower acceptance score at 0.0125 %W/V (75% reduction) salt content.

Keywords: Consumer, Flavor, Monosodium Glutamate, Salt reduction, Soup

INTRODUCTION

Salt is one of the most important food ingredients. It is multi-functional ingredient used as a food preservative to inhibit microbial growth and to ensure good texture and taste. Due to low cost and several functional properties, salt is the most widely used additive within the food industry. Both food and beverage product contain salt in different amount depends on using purpose. Interestingly, salty taste plays important role in food choice due to the feeling of pleasure created after consuming it. Moreover, salt is an essential mineral that act as electrolytes in the body which help in overall body's fluid balancing, nerve transmission and muscle function. It is estimated that human need about 500 mg of sodium daily for these vital functions. However, with the lifestyle change, people are looking for something fast and convenient. There are more than 5500 instant foods and drinks in the market since 2015, these kinds of industrialized diets are approximately provide 75% of the daily salt intake [1][2], packaged and prepared foods such as lunch meats and canned soups, often have sodium added during manufacturing. The sodium-rich diets (more than 5 gram per day) have been largely demonstrated to promote hypertension, which is a risk factor associated with cardiovascular diseases [2][3]. Unfortunately, people might consume too much salt from their daily food without realizing it since salt was also contained in liquid food like soup and beverages.

World Health Organization (WHO) aware of the issues and members have agreed to reduce the global population's intake of salt by relative 30% by 2025. Reducing salt intake has been identifies as one of the most cost-effective measures countries can take to improve population health outcomes. Indeed, Flegel and Magner (2009) reported that consume less than 1 g of salt per day is able to prevent high blood pressure even when age is increasing [4]. Many food industries are now focusing on producing low salt/sodium foods. Most of the time, salt reduction impact on overall perception and lead a decrease of acceptability by consumers. Therefore, salt reduction without reducing saltiness perception has become a critical challenge for researchers. There were various attempts and researchers were trying to develop lower salt products to provide consumer with the healthier choices. In order to develop a successful product, it is essential to understand both consumer and market nature as well. Therefore, aim of this study were to study effect of flavors and monosodium glutamate with different salt level on saltiness perception using chicken soup and chicken cream soup as food model.

MATERIAL AND METHODS

Consumer Survey

Study consumer's attitude, opinion and behavior on low salt/ low sodium product by using self-administered questionnaire with target 200 consumers (Taiwanese and Thai consumer) answer via Google form. The questionnaire was divided into 2 parts. Part 1 included stages of change model questions and the questions about consumer's understanding, opinion, and behavior about their diet. Part 2 gather demographic information.

Preparation of Chicken Soup

There were two types of soup included in the experiment, chicken clear soup and chicken cream soup. Three-factor factorial design arrangement in RCBD was applied in each type of soup. The factors were mono sodium glutamate (add or not-added), different flavoring agent (not-added, bacon, lobster, and smoked), and different level of salt (0.05%W/V (control), 0.0375%W/V (25% salt reduction), 0.025%W/V (50% salt reduction), 0.0125%W/V (75% salt reduction), and 0%W/V).

Sample Presentation

Consumer were presented with one set of samples per one session. The set of samples was either MSG added or non-added with the

same flavor added containing different level of salt. Consumer were served with one sample at a time. The serving order was random using William's Square design. The samples were served at 65 ± 5° C

Sensory Evaluation

Both male and female consumers were recruited to participate in the product testing with the total number of 50 consumers. Consumers were asked to taste tested samples compare with given reference by focusing on saltiness and rate the different score comparing with control sample using 11-point Degree of Difference (DOD) scale where -5 = extremely less salty, -4 = much less salty, -3 = moderately less salty, -2 = slightly less salty, -1 = very slightly less salty, 0 is no different detected, 1 = very slightly saltier, 2 = slightly saltier, 3 = moderately saltier, 4 = much more salty, and 5 = extremely more salty. Each tested sample was also determined the liking level using 9-point hedonic scale where 1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like nor dislike, 6 = Like slightly, 7 = Like moderately, 8 = Like very much, and 9 = Like extremely

Data Analysis

The data obtained from the survey was processed by using Microsoft Excel (2018) and SPSS Program (IBM SPSS Statistics 19) for descriptive and in inferential statistics.

RESULTS AND DISCUSSION

Consumer survey

There were total of 104 Thai consumers and 151 Taiwanese consumers participated in the survey. There were 39 male and 65 female from Thailand, 45 male and 106 female from Taiwan. Most Thais were at the age between 23-30 years old which has 63 people, and most Taiwanese were at the age between 31-40 years old which has 74 people. For personal status, most Thais (95 people) were single, and most Taiwanese (93 people) were married. Moreover, both Thais and Taiwanese had 4 to 5 family members. The education level of most Thais and Taiwanese were bachelor's degree (76 and 79 people, respectively). The income of most Thais was in a range of 10,001 to 30,000 baht per month, and that of Taiwanese were in a range of 20,0001 to more than 50,000 bath per month. For health condition, the unhealthy Taiwanese (58 people, 38.41%) was higher than the unhealthy Thais (27 people, 25.96%).

According to Transtheoretical model, before individual change their behavior, they would go through 5 steps, which were precontemplation (individuals have no intension of changing behavior into eating healthy), contemplation (individuals plan to change their diet to be healthier), preparation (individuals plan to change to healthier diet within one month), action (individuals already changed their diet with in the last three months) and maintenance (individuals changed for at least six months). Table 1 showed that Thai consumer perceived as consumer they were in contemplation stage (25%), they already eating healthy diet (24%), and that already change to healthier diet for about 3 months (18%). consumer who was in contemplation stage (25%). Taiwanese consumer perceived as they already made changes to adopt healthy diet more than 6 months (30%) and within the past 3 months for 26%. From this data can be observed that more people now are considering their health, and they are willing to eat healthier.

Table 1 Numbers and percentage of Thai and Taiwanese consumer belong in different stage of change.

Stages of change	Thai		Taiwanese	
	Number of consumer	%	Number of consumer	%
Precontemplation	16	15.39%	9	5.96%
Contemplation	26	25.00%	31	20.53%
Preparation	18	17.31%	25	16.56%
Action	19	18.27%	40	26.49%
Maintain	25	24.04%	46	30.46%

Focusing on the consumer's attitude towards health and taste aspects of food product, it was found that Thai consumers had higher score

in health aspect than taste aspect while Taiwanese consumers had highest score in general health interest and lowest in consuming food as reward (Table 2).

Table 2 Average of Likert score of Thai and Taiwanese towards health and taste subscales

Health and Taste Attitude Scale	Thai	Taiwanese
Health aspects		
General health interest	4.03± 1.32	4.85± 1.18
Light product interest	4.32± 1.37	3.57± 0.70
Natural product interest	4.03± 1.70	3.13± 0.69
Taste aspects		
Craving for sweet foods	3.72± 1.76	3.28± 0.98
Consuming food as reward	3.63± 1.62	2.86± 0.90
Pleasure	3.90± 1.63	3.57± 0.75

Table 3 Effect of each factor and interaction on degree of difference (DOD) and overall liking toward chicken clear soup

Source of variation	DOD		Overall liking	
	F Value	Pr > F	F Value	Pr > F
MSG	41.08	<.0001	17.02	<.0001
flavor	9.06	<.0001	19.43	<.0001
msg*flavor	5.91	0.0005	0.34	0.7954
salt	1760.60	<.0001	219.10	<.0001
msg*salt	3.77	0.0047	1.01	0.4026
flavor*salt	3.96	<.0001	5.10	<.0001
msg*flavor*salt	2.79	0.0009	1.71	0.0598

Study Effects of Factors and Interaction on Saltiness Perception and Overall Liking: Chicken Clear Soup

As shown in Table 3, all of main factors (msg, flavor, and salt) and combination of factors (msg*flavor, msg*salt, flavor*salt, and msg*flavor*salt) had significant effect on saltiness perception. It can be assumed that consumers were able to detect the difference in saltiness comparing between samples. Changing in amount of salt, MSG or different flavors can significantly affect saltiness perception. Otherwise, combination of factors (msg*flavor, msg*salt and msg*flavor*salt) had no significant effect on overall liking. It can be assumed that changing in amount of salt or different flavors or addition of msg can alter the consumers' preference, and others combination (msg*flavor, msg*salt and msg*flavor*salt), some factors could cover another factor's characteristic or the interaction between factors can blend in the characteristic with each other which leads to the non-significant effect on liking.

From Table 4, there was a significant difference in saltiness perception comparing between sample and control in every flavor. It can be assumed that consumers can perceive the difference in saltiness between control and sample, the more salt is reduced, the more difference can be detected. And the situation happened no matter chicken clear soup with or without MSG.

Table 4 DOD score of chicken clear soup with/ without MSG

% salt (w/v)	With MSG			
	None	Bacon	Lobster	Smoke
0.05	Control	Control	Control	Control
0.0375	-1.3 *	-1.5 *	-1.1 *	-0.6 *
0.025	-2.6 *	-2.9 *	-2.1 *	-2.0 *
0.0125	-3.5 *	-3.9 *	-3.5 *	-3.2 *
0	-4.4 *	-5.0 *	-4.4 *	-4.3 *
% salt (w/v)	Without MSG			
0.05	Control	Control	Control	Control
0.0375	-1.4 *	-1.3 *	-1.2 *	-1.4 *
0.025	-2.7 *	-2.2 *	-2.5 *	-2.1 *
0.0125	-3.1 *	-3.5 *	-4.0 *	-3.5 *
0	-4.1 *	-4.2 *	-4.5 *	-4.3 *

Note: * indicates significant difference at alpha = 0.05

According to Table 5, no matter chicken clear soup contained MSG or not, the overall liking had no significant different between control and reduced 25% salt soup in every flavor. The

significant difference in overall liking began to appear when salt was reduced to 50% in original flavor, lobster and smoke flavor whereas there was no significant difference in overall liking in soup with 50% salt with bacon flavor. Soup with less than 25% salt showed the significant difference in overall liking. It can be assumed that salt can be reduced by 25% in every flavor without effect on saltiness perception.

However, soup without MSG has larger gap in DOD and overall liking. Thus, it can be assumed that MSG had effect on salty taste and liking score due to the Na+ in its structure and glutamate that promote umami flavor. Moreover, as shown in previous result, consumers tended to prefer salty soup. Thus, the overall liking score of soup with higher %salt or with MSG was slightly higher.

Table 5 Average of overall liking score of clear soup with/ without MSG

% salt (w/v)	With MSG			
	None	Bacon	Lobster	Smoke
0.05	6.0 ^{A}	6.0 ^{A}	6.3 ^{A}	6.5 ^{A}
0.0375	6.1 ^{A}	5.8 ^{A}	6.4 ^{A}	6.6 ^{A}
0.025	5.2 ^{B}	5.8 ^{A}	5.2 ^{B}	5.7 ^{B}
0.0125	3.9 ^{C}	5.7 ^{A}	4.4 ^{C}	4.6 ^{C}
0	3.1 ^{D}	4.1 ^{B}	3.3 ^{D}	3.8 ^{D}
% salt (w/v)	Without MSG			
0.05	5.8 ^{A}	5.6 ^{AB}	6.3 ^{A}	6.5 ^{A}
0.0375	5.5 ^{A}	6.1 ^{A}	6.1 ^{A}	6.3 ^{A}
0.025	4.3 ^{B}	5.3 ^{BC}	5.1 ^{B}	5.2 ^{B}
0.0125	4.3 ^{B}	4.8 ^{C}	3.7 ^{B}	3.9 ^{C}
0	2.6 ^{C}	4.0 ^{D}	3.4 ^{C}	3.7 ^{C}

Note: Different subscript letter indicates significant difference at alpha = 0.05

Study Effects of Factors and Interaction on Saltiness Perception and Overall Liking: Chicken Cream Soup

As shown in Table 6 all individual factors (msg, flavor, and salt) and some combination of factors (flavor*salt) had significant effect on saltiness perception. It can be assumed that consumers were able to detect the difference in saltiness comparing between samples. Changing in amount of salt or different flavors as well as MSG adding can significantly affect saltiness perception. While some interaction between factors (msg*flavor, msg*salt and msg*flavor*salt) had no significant effect on saltiness perception. It can be assumed that milky taste or mouthfeel and fat molecule in milk could mask the effect of these interactions. Therefore, it was more difficult to perceive the difference.

Additionally, there were some individual factors and combination of factors (msg, salt, flavor*salt and msg*flavor*salt) that had significant effect on overall liking. It can be assumed that changing in amount of salt or different flavors or msg adding can alter the consumers' preference as well as different flavor was an individual preference. Otherwise, other factors and combination of factors (flavor and msg*flavor) had no significant effect on overall liking. It can be assumed that, in factor combination, some factors could conceal other's factor characteristic or the interaction between factors blended the characteristic of each other which leaded to the non-significant effect on liking.

Table 6 Effect of each factor and interaction on degree of difference (DOD) and overall liking towards chicken cream soup

Source of variation	DOD		Overall liking	
	F Value	Pr > F	F Value	Pr > F
MSG	12.13	0.0005*	9.64	0.0019*
flavor	5.26	0.0013*	0.92	0.4327
msg*flavor	2.57	0.0530	1.94	0.1217
salt	2362.85	<.0001*	150.15	<.0001*
msg*salt	0.96	0.4263	3.52	0.0071*
flavor*salt	3.20	0.0001*	4.49	<.0001*
msg*flavor*salt	0.87	0.5769	1.89	0.0313*

According to Table 7, there was a significant difference in saltiness perception

comparing between sample and control in every flavor, no matter chicken cream soup with or without MSG. It can be assumed that consumers can perceive the difference in saltiness between control and samples. The more salt was reduced, the more difference could be detected.

Table 7 DOD score of chicken cream soup with/ without MSG

% salt (w/v)	With MSG			
	None	Bacon	Lobster	Smoke
0.05	Control	Control	Control	Control
0.0375	-0.9 *	-0.7 *	-0.8 *	-1.3 *
0.025	-2.0 *	-2.3 *	-2.4 *	-2.6 *
0.0125	-3.2 *	-3.5 *	-3.7 *	-4.0 *
0	-4.1 *	-4.2 *	-4.3 *	-4.4 *
% salt (w/v)	Without MSG			
0.05	Control	Control	Control	Control
0.0375	-0.8 *	-0.9 *	-1.0 *	-1.4 *
0.025	-2.0 *	-2.3 *	-2.5 *	-2.5 *
0.0125	-3.3 *	-3.4 *	-3.8 *	-3.6 *
0	-4.1 *	-4.1 *	-4.5 *	-4.5 *

Note: * indicates significant difference at alpha = 0.05

According to Table 8, there was no significant difference in overall liking when salt was reduced by 25% in original, bacon and smoke flavor, as well as when lobster flavor with MSG added in soup. The significant difference in overall liking began to appear when salt was 50% reduced in every flavor. It can be assumed that salt can be reduced by 25% in every flavor without effect on preference either with or without flavor added. Moreover, consumers still accepted soup with 50% salt when soup contained MSG based on 5 score in 9-point hedonic scale, and as shown in previous result, consumers tended to prefer salty soup. Thus, the overall liking score of soup with higher %salt or with MSG was slightly higher.

Table 8 Average overall liking score of cream soup with/without MSG

% salt (w/v)	With MSG			
	None	Bacon	Lobster	Smoke
0.05	6.4 ^{A}	6.2 ^{A}	6.8 ^{A}	6.8 ^{A}
0.0375	6.3 ^{A}	6.0 ^{A}	6.0 ^{B}	6.5 ^{A}
0.025	5.5 ^{B}	5.3 ^{B}	5.5 ^{C}	5.0 ^{B}
0.0125	4.6 ^{C}	5.1 ^{B}	4.4 ^{D}	4.0 ^{C}
0	3.8 ^{D}	3.5 ^{C}	3.6 ^{E}	3.9 ^{C}
% salt (w/v)	Without MSG			
0.05	6.7 ^{A}	6.2 ^{A}	6.8 ^{A}	6.7 ^{A}
0.0375	6.8 ^{A}	6.1 ^{A}	6.5 ^{A}	6.4 ^{A}
0.025	5.8 ^{B}	5.5 ^{B}	5.6 ^{B}	5.6 ^{B}
0.0125	4.8 ^{C}	4.7 ^{C}	4.8 ^{C}	4.6 ^{C}
0	4.7 ^{C}	4.3 ^{C}	4.3 ^{D}	4.3 ^{C}

Note: Different subscript letter indicates significant difference at alpha = 0.05

CONCLUSIONS

In conclusion, it was found that with- and without flavor added making a significant difference in saltiness perception and overall liking. It was clearly shown that with flavor replacement salt can be reduced by 25% and can be reduced up to 50% from original formula in chicken clear soup with bacon flavor. Furthermore, MSG had a significant effect on both saltiness perception and overall liking. Thus, addition of MSG could lead to reducing of salt addition and improve overall liking toward the soup.

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Effect of flour types on qualities and freezing points of frozen plant-based shrimp

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ABSTRACT: The objectives of this study were to investigate the effect of flour types, namely tapioca flour (S1), chickpea flour (S2), soy flour (S3) and wheat flour (S4) on the qualities of plant-based shrimp and to compare their qualities and freezing point with commercial plant-based shrimp product. The water activity and moisture content of plant-based shrimp products were in the range of 0.973-0.980 and 71.10-73.62 % wet basis, respectively. Plant-based shrimp made from soy flour had the highest freezing point (-4.1°C), followed by chickpea flour (-4.7°C), tapioca flour (-5.6°C), and wheat flour (-6.2°C). Plant-based shrimp made from wheat flour had the highest drip loss value after freezing of 1.04 $\pm$ 0.05% whereas the percentage of drip loss of other plant-based shrimp samples was 0.28-0.41%. The hardness, cohesiveness, gumminess, and chewiness of plant-based shrimp made from chickpea flour and soy flour were comparable to commercial plant-based shrimp. The formulation from this study indicated that there is a possibility to develop and use chickpea or soy flour as ingredients to produce good quality of frozen plant-based shrimp.

Keywords: Chickpea flour, Freezing point, Frozen food, Plant-based shrimp

INTRODUCTION

Plant-based food market has been increasing due to high demand of animal-based food consumption by the growing of global population, lead to the rising of environmental pollution and global warming [1]. Other reasons, unhealthy lifestyle, as well as the intensity of animal welfare, are turning people to plant-based food consumption in order to develop their health improvement and protect animal rights [2]. The changes in customer's behaviour such as becoming flexitarians or reducing meat intake cause rapidly growing demand of plant-based food. Therefore, the creation of satisfying meatless products to fulfil the demand of replacing meat in diets has been developing [3]. Ground or minced meat analogue products mostly appear in the market, but plant-based seafood is rarely found in the section [4]. Consequently, this is an opportunity to develop plant-based seafood for multiplying customer choices.

From another point of view, the consumption of seafood also has negative effect on biodiversity, environment and human health risks because of toxic contamination from

water pollution [1]. The plant-based seafood is alternative meal to avoid mercury poisoning, microplastic, heavy metal, and contaminated toxins that can be found in traditional seafood. Thus, vegan seafood becomes increasingly popular since customers are more concerned about health care consumption [4]. Plant-based seafood can play a crucial role in expanding the global supply of vegan seafood to meet healthy and sustainable food production [5].

Textural attributes are important factors for customer overall acceptability, relate to the rheology properties of the product [1]. Binders are significant ingredients to improve texture of plant based-food product by setting gels, providing emulsion stability and reducing oil and water leakage [6]. Starch is commonly used as a binder in food products to improve their physical, chemical properties and sensory attributes. The characteristics of quality end-products depend on the proportion of molecules in different types of flour [7]. For plant-based food production, the ingredients such as bean flour, chickpea flour, wheat flour and soy flour has been recommended [6]. Chickpea flour can be used in various plant-based food and beverage products to act as a binder and provide high protein content [8]. Soy is regularly used in imitated meat due to its protein quality like meat and its gels emulsifying property to stabilize the emulsion [9]. Wheat flour is starch containing gluten which provide the structure for finish products by forming gluten structure [10]. Tapioca flour is made from cassava plant roots and is fortified in various vegan food formulation. It is a thickener used in food products due to its high viscosity, and adhesive properties [11].

Freezing is a common method of food preservation. Food with high moisture content has less stable than dried food because water is a cause of biochemical deterioration of foods. Freezing can reduce liquid water form by establishing ice crystal [12]. The temperature at -18^{o}C and lower is recommended to store frozen food and it will retain the quality of foods during storage [13]. The key factor in freezing process to confirm that food products reach low storage temperature is a freezing point [14]. The freezing point is a temperature that first ice crystal appears. The freezing point of pure water is 0 ^{o}C but in food system the freezing point is below 0 ^{o}C due to the presence of free and bound water [15].

The objectives of the research were to study effect of different types of flour (tapioca flour, soy flour, chickpea flour, and wheat flour) on qualities of frozen plant-based shrimp and to compare their qualities and freezing points with commercial plant-based shrimp product.

MATERIALS AND METHODS

Materials

Tapioca flour, chickpea flour, soy flour, wheat flour, sugar, and seasoning powder were purchased from a supermarket nearby Thammasat university, Thailand. Konjac flour was supplied from Chemipan corporation Co., Ltd. Shrimp flavour and gac fruit powder was obtained from food center Co., Ltd.

Plant-based shrimp production

The formulations of plant-based shrimp are given in Table 1. Konjac flour and water were blended using a mixer at high-speed level for 3 minutes. After that all ingredients (flour, sugar, seasoning) were added and mixed until a homogeneous mixture was visible. The mixture was shaped as shrimp by using a shrimp silicone mold making. In order to create imitated shrimp appearance, strips of orange colour were painted and dyed with natural orange colour from gac fruit solution (the mixture of gac fruit powder to water at 5:2). The dyed samples were steamed for 15 minutes. Cooked plant-based shrimps were packed into vacuum plastic bags and frozen at -18 ^{o}C for 24 hours.

Four types of flour (tapioca flour, chickpea flour, soy flour and wheat flour) were used as ingredients to produce plant-based shrimp. The commercial frozen plant-based shrimp, NBT brand, (the ingredients of commercial plant-based shrimp formula are

following; 80% water, 13.8% tapioca flour, 3% konjac flour, 2% soybean oil, 1% salt, and 0.2% seasoning) was used as a control for quality and freezing point measurement.

Table 1 Formulation of plant-based shrimp with various types of flour.

Materials	Percentage of ingredient in S1-S4 recipes			
	S1	S2	S3	S4
Tapioca flour	4	-	-	-
Chickpea flour	-	4	-	-
Soy flour	-	-	4	-
Wheat flour	-	-	-	4
Konjac flour	16	16	16	16
Sugar	4	4	4	4
Seasoning powder	5.5	5.5	5.5	5.5
Gac fruit solution	0.5	0.5	0.5	0.5
water	70	70	70	70
Total	100	100	100	100

Note: Plant-based shrimp made from tapioca flour (S1), chickpea flour (S2), soy flour (S3), and wheat flour (S4)

Quality determination

Appearance of steamed and thawed products

Plant-based shrimp made from tapioca flour (S1), chickpea flour (S2), soy flour (S3), and wheat flour (S4) were steamed for 15 minutes and then were taken photos. After cooked samples were frozen at -18 °C for 12 h and thawed at 4 °C for 12 h, thawed products were taken photos for comparing with commercial vegan shrimp.

Water activity

Water activity (a_w) of cooked plant-based shrimp products were measured by using a water activity meter (Aqualab PRE, TDR, USA)

Moisture content

Moisture contents of cooked plant-based shrimp were measured by hot air oven method. Five grams of cooked samples were filled in dried crucibles and placed in an oven at 105 ± 2 °C for 24 h. The moisture contents were calculated following formula (1)

$$\text{Moisture content} = \frac{(w_2 - w_1) - (w_3 - w_1)}{(w_2 - w_1)} x100 \quad (1)$$

Where w_1 = weight of the dried empty crucible, w_2 = weight of the crucible and fresh sample before drying, and w_3 = weight of the crucible and sample after drying.

Texture profile analysis

The texture profiles of steamed plant-based shrimp samples and commercial product (control) after thawing and steaming were determined by using a texture analyzer (TA-XT Plus, stable Micro System, UK). All samples were cut into cubes shape with 10 mm side and then were compressed twice by a cylinder probe (p/50) under the following condition: Pre-test speed: 1 mm/s, test speed: 5 mm/s and post-speed: 5 mm/s. The results of averaged 10 times measurements were presented as hardness, springiness, cohesiveness, gumminess, and chewiness.

Drip loss

Each recipe of frozen plant-based shrimp samples was weighed and then thawed in a refrigerator at 4 °C for 12 h. Completely thawed samples were weighed once again after thawing done. The percentages of drip loss were calculated following equation (2)

$$\% \text{ drip loss} = \frac{w_1 - w_2}{w_1} x100 \quad (2)$$

Where: w_1 = Weight of sample before thawing
w_2 = Weight of sample after thawing

Freezing point

The cooked plant-based shrimp was inserted by a thermocouple into the centre position of sample and then frozen to -18°C in

a freezer (FENICE S13, Italy). The temperature of sample was recorded during freezing every 1 minute. The freezing curve was plotted as a graph of temperature change against time.

Statistical analysis

The data was analysed using SPSS software version 26. One-way analysis of variance (ANOVA) was used to determine significant difference between samples ($p < 0.05$) The mean difference was analysed using Duncan's new multiple range test to determine the difference of means value.

RESULTS AND DISCUSSIONS

The appearance of samples after steaming and thawing

The photos of frozen commercial product and four formulas of plant-based shrimp samples after steaming and thawing were taken and shown as in figure 1 and 2. It can be seen that, after freezing and thawing all plant-based shrimp samples retained their original shape and did not differ from products before freezing (after steaming). However, the colour tone of all samples after thawing was opaquer than that of samples before freezing. This could be explained by the retrogradation of amylose will accelerate aggregation and aging process, which in turn decrease transparency of starch paste [16].

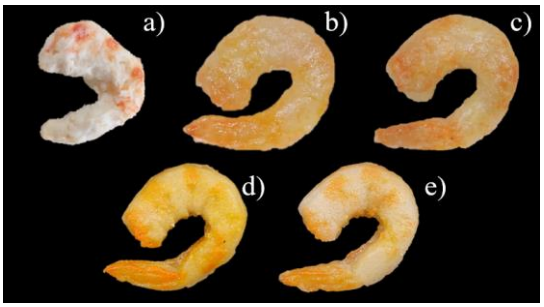

Figure 1 Photographs of frozen commercial vegan shrimp (a), plant-based shrimp made from tapioca flour (S1), chickpea flour (S2), soy flour (S3) and wheat flour (S4) after steaming.

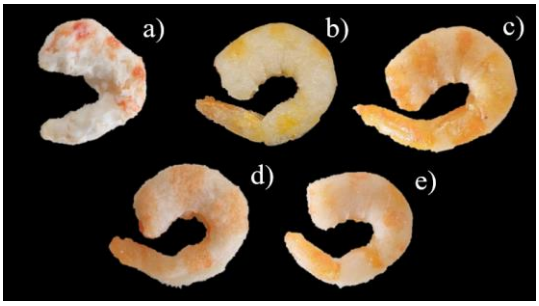

Figure 2 Photographs of frozen commercial vegan shrimp (a), frozen plant-based shrimp made from tapioca flour (S1), chickpea flour (S2), soy flour (S3) and wheat flour (S4) after thawing

Water activity and moisture content

Water activity and moisture content of commercial vegan shrimp (control) and four formulas of plant-based shrimp are shown in Table 2. The values of water activity and moisture content of plant-based shrimp made from different types of flour were 0.973-0.980 and 71.10-73.62 %wb, respectively. The lowest water content was presented in plant-based shrimp made from wheat flour (S4). This might relate to amylose and amylopectin content since the ratio of amylose and amylopectin and their structures have an effect on the quality of final product [16]. Starch with high amylose content has more tendency to retrograde after cool down than starch with low amylose content [17]. The content of amylose in wheat flour was 28%, while tapioca flour, chickpea flour, and soy flour contain amylose content, 19, 11 and 1%, correspondingly [18-20]. The retrogradation of amylose results in the separation of water [21] and might affect water content in final product.

Table 2 Water activity and moisture content of plant-based shrimp samples

Samples	Water activity	Moisture content (%wb)
Commercial	0.995 ± 0.002^{a}	80.71 ± 0.22^{a}
Tapioca (S1)	0.980 ± 0.003^{b}	73.62 ± 0.24^{b}
Chickpea (S2)	0.973 ± 0.001^{c}	72.00 ± 0.07^{c}
Soy (S3)	0.978 ± 0.002^{ab}	71.68 ± 0.18^{c}
Wheat (S4)	0.977 ± 0.005^{ab}	71.10 ± 0.19^{d}

$^{a,b,\cdots}$Different letters in the same column indicate that values are significantly different ($p<0.05$). ($n=3$)

Temperature change of plant-based shrimp during the freezing

Four types of cooked plant-based shrimp samples after steaming and thawed commercial vegan shrimp were frozen at -18°C. The temperature of overall samples was measured and recorded during freezing by thermocouples every minute for 20 h. The freezing curves of control and plant-based shrimp samples against time were plotted as figure 2. The highest freezing point was found in plant-based shrimp made from soy flour (-4.1°C), followed by chickpea flour (-4.7 °C), tapioca flour (-5.6°C), and wheat flour (-6.2°C). The freezing points of plant-based shrimp products were different because the appearance of free and bound water in the system [15]. Gluten in wheat flour has great water holding capacity through the interaction between gluten protein and water in food [22]. According to Sim *et al*. [23], the appearance of unfrozen phase in wheat dough result on low freezing point. However, the reduction of temperature during freezing process affects water holding capacity of gluten [22], cause the present of

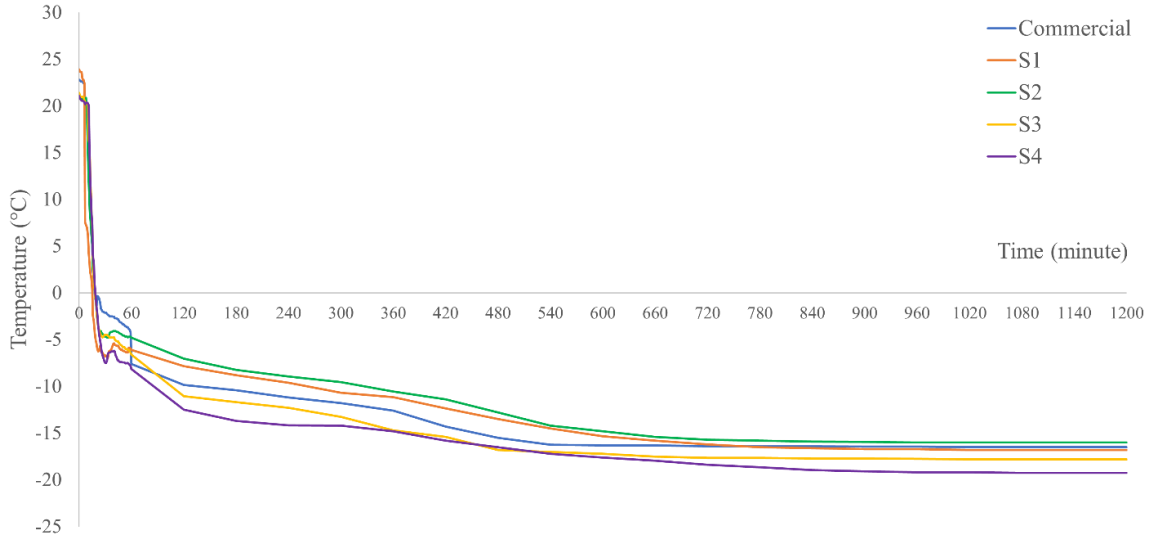

Figure 3 Freezing curves of all four plant-based shrimp formulas compared to commercial vegetarian shrimp

high drip loss after thawing, the result presents in Table 3. Plant-based shrimp made from soy flour and chickpea flour showed higher freezing points, which were comparable to the freezing point of commercial plant-based shrimp.

Drip loss

The percentage of drip loss of plant-based shrimp products made from various flour are presented in table 3. The occurrence of drip loss in frozen products after thawing were explained by the formation of large ice crystal which is able to damage cell's structure and lead to an increase of water leak out from the product after thawing [24]. High percentage of drip loss indicated that the product loss their quality in term of appearance, texture and nutrition value [25]. Plant-based shrimp made from wheat flour had the highest drip loss value, 1.04 $\pm$ 0.05%. The explanation is that water in structure might be migrated during freezing, causing the formation of large ice crystal and disruption of gluten network in wheat flour, lead to a decrease of water retention capacity [22]. The water loss of plant-based shrimp made from tapioca flour, chickpea flour, and soy flour were in the range of 0.28-0.41% and there were no significant

differences among these three formulas. Gelatinization and retrogradation of the added starch affect the formation of ice crystal [26]. When starch gels were frozen after setting, no disintegration was found in the starch particles and derived from ice crystals was absorbed by starch particles. However, under some freezing conditions the addition of starch could increase drip loss and water excreted from syneresis of starch gel product also affect the drip loss value [31].

Table 3 Drip loss of plant-based shrimp

Samples	%Drip loss
Tapioca (S1)	0.41 ± 0.05^{b}
Chickpea (S2)	0.36 ± 0.23^{b}
Soy (S3)	0.28 ± 0.02^{b}
Wheat (S4)	1.04 ± 0.05^{a}

[a,b] Different letters in the same column indicate that values are significantly different ($p<0.05$). (n=3)

Table 4 Texture properties of plant-based shrimp samples

Shrimp samples	Textural properties				
	Hardness ($g._{force}$)	Springiness	Cohesiveness	Gumminess	Chewiness
Commercial	1643.56 ± 390.85^{a}	0.927 ± 0.07^{a}	0.714 ± 0.05^{c}	1208.70 ± 324.06^{a}	$1055.57 \pm 3\ 11.78^{a}$
Tapioca (S1)	691.57 ± 104.26^{c}	0.870 ± 0.04^{b}	0.748 ± 0.03^{b}	517.33 ± 80.58^{c}	449.56 ± 69.78^{c}
Chickpea (S2)	1169.15 ± 204.19^{b}	0.878 ± 0.04^{b}	0.783 ± 0.03^{a}	924.96 ± 176.44^{b}	830.53 ± 182.27^{b}
Soy (S3)	1055.25 ± 161.61^{b}	0.850 ± 0.04^{b}	0.783 ± 0.02^{a}	826.33 ± 127.71^{b}	732.25 ± 124.16^{b}
Wheat (S4)	693.84 ± 167.01^{c}	0.879 ± 0.05^{b}	0.759 ± 0.04^{b}	517.65 ± 120.95^{c}	437.60 ± 92.39^{c}

[a,b,…] Different letters in the same column indicate that values are significantly different ($p<0.05$). (n=10)

Texture profile analysis

Table 4 shows texture profile analysis of cooked plant-based shrimp. The results indicated that hardness, cohesiveness, gumminess, and chewiness of chickpea shrimp (S2) and soy shrimp (S3) were significantly higher than tapioca shrimp and wheat shrimp. This might be because soy and tapioca flour contain high protein content, which are 20 and 40% [27, 28]. Moreover, gel formation and properties of konjac flour could dispute by the presence of protein [29]. Ran *et al.* [30] found that konjac glucomannan could be formed entangled network with protein, which enhances hardness, chewiness, and gel strength of plant-based fish ball. In this study, plant-based shrimp made from chickpea flour and soy flour showed the most comparable of textural properties with commercial vegan shrimp that produced from tapioca flour. This indicated that the use of only 4% of high protein flour could replace the amount of 13.8% tapioca flour addition and also improve nutritional especially protein of plant-based shrimp products.

CONCLUSIONS

Different types of flour directly affected the quality of frozen plant-based shrimp. Plant-based shrimp made from wheat flour had the lowest freezing point, while drip loss was highest. Plant-based shrimp made from chickpea flour and soy flour had higher freezing point and lower drip loss than plant-based shrimp made from tapioca flour and wheat flour. The use of 4% chickpea flour or 4% soy flour in formulations could produce products which were comparable in textural properties with commercial vegan shrimp made from 13.8% tapioca flour. This indicates that chickpea flour and soy flour can be used as ingredients for producing good quality and

enhancing nutritional values of plant-based food product.

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DIVISION D
Food Microbiology, Food Biotechnology, Fermentation

ORAL PRESENTATION

Growth profiles of probiotic yeast (*Saccharomyces boulardii*) in different culture media; fruit, vegetable juices, and standard media for development of alternative culture substrate in probiotic cell production

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ABSTRACT: *Saccharomyces cerevisiae var. boulardii* (*S. boulardii*), is a probiotic yeast that exhibits numerous benefits on the human gastrointestinal tract. Generally, *S. boulardii* is cultured in standard medium and fermented in fruit juice. Viability and therapeutic property of *S. boulardii* is greatly affected by composition of culture medium, especially, glucose content. The objective of this study was to evaluate the growth property of *S. boulardii* in a different culture media - vegetable (Gotu kola) juice and fruit (red grape) juice and compared against standard medium (YMB). The results demonstrated that the viable cell count in Gotu kola juice was lower than red grape juice but higher than the standard medium. Physicochemical profiles of two cultured juices were relatively similar. A decreased in total soluble solids and pH, and increase in the acidity of citric and lactic acid were observed. Cells in the two juices showed an oval shape morphology with budding reproduction while the cells in standard media showed a round shape morphology, indicating fission reproduction, after 48 hours of fermentation. The bioactive compounds were also changed during fermentation; antioxidant activity of post-fermented red grape juice was higher than pre-fermented juice. In contrast, activity in post-fermented Gotu kola juice dropped from pre-fermented juice. Therefore, this study demonstrated that among three media, red grape juice was the most effective while Gotu kola juice could be a potential alternative. Nevertheless, further research is required for accurate viable cell property, particularly, therapeutic property to verify the alternative substrates.

Keywords: Saccharomyces boulardii, Probiotics yeast, Fermented juice, Alternative medium, Culture media

INTRODUCTION

Probiotics are living micro-organisms, which when administered in adequate amounts confer health benefits on the recipient [9, 13]. Among the traditional probiotics, Lactic acid bacteria are the main group, Non-Lactic acid bacteria as a second group and yeast probiotics, *Saccharomyces cerevisiae var. boulardii* as only commercial yeast probiotics strain [12].

In 1923, *S. boulardii* was first found in Lychee and mangosteen by Henri Boulard, French scientist [21, 22]. This probiotics yeast cell wall contains 2 layers that outer layer composes mainly of Mannose (Phosphopeptidomannan, PPM and Phospholipomannan, PLM) and internal composes Chitin (β(1,3)- and β(1,6)-glucan) [9]. This cell structure allows this yeast to resist acidity and high temperature and other stress

conditions. *S. boulardii* has been used for intestinal surface adhesion, the balance of enteric microbiota, immune enhancement, prevention and treatment of human gastrointestinal diseases such as Traveleriarrhea, acute diarrhea, inflammatory bowel disease and disease caused by *Clostridium difficile* [2, 8, 9, 26]. *S. boulardii* has been used as commercial products in powder and tablet forms (Biocodex, Inc., United States.) and lyophilized form (Perenterol forte, Germany) [4, 10, 20, 21].

Generally, yeast is cultured in a standard medium such as Yeast Extract Peptone Dextrose (YPD) (g/L: 10 g peptone, 20 g glucose and 10 g yeast extract), Potato dextrose (PD) (g/L: 200 g potato, 20 g Dextrose) [13, 14] Yeast malt (YM) (g/L: 3 g yeast extract, 3 g Malt extract, 5 g peptone and 10 g glucose) and YPG (g/L: 20 g peptone, 20 g glucose and 10 g yeast extract) [3], which sugars, particularly glucose are the main composition. Hossain and the research group [13] studied the effect of carbon, organic nitrogen and inorganic nitrogen sources on viability of *S. boulardii*. This study demonstrated that glucose, yeast extract and sodium nitrate, were the keys for the probiotic yeast growth rate, respectively.

Since glucose is the key carbon source for yeast growth, the substrates rich in glucose, fruit juices have been widely used for cultivation of *S. boulardii* such as mixed berry juice [9] and grape juice [18]. Specifically, Grape juice and its properties in supporting yeast to grow have been systematically characterized and are well known. Its components including protein 0.26% fat 0.29% carbohydrate 15.6% (consist of 7.42% Fructose, 6.61% Glucose and a few of sucrose, lactose, maltose and galactose), minerals (mainly Phosphorus, 50 mg) and Vitamins (including Vitamin B) have been reported as key substances for increase growth. Vegetable juices for yeast cultivation have been received less attention relative to fruit juices. However, there are some interesting aspects of vegetable juice associated with yeast growth and cell properties. Potato juice for instance, showed new advantage for the production of probiotic yeast in terms of biomass with therapeutic properties [3]. Yang and the research group [30] reported that probiotic yeast could grow in Chinese yam juice and produced volatile compounds (esters, alkanes, alcohols and ketone) which improved the taste, odor and flavor of the juice a long with high cell biomass.

Centella asiatica L. (Gotu kola), a local vegetable in the every part of Thailand, is commonly used as food, juice and traditional medicine [5, 6]. This vegetable contains several key bioactive compounds particularly phenolic compounds, antioxidants (Triterpenoid). These phenolic compounds have antioxidants and are also used as indicators of Gotu kola quality. Nutritional properties of this vegetable mainly include 4% protein, 0.2% fat and 6.7% carbohydrate, minerals (mainly Calcium, Magnesium and phosphorus) and vitamins (vitamin B, C, E, K,) [6, 24]. Thus, based on composition, this juice with sugar added could be an alternative medium for probiotic yeast biomass production with therapeutic properties. Therefore, the objective of this study was to evaluate the feasibility of using Gotu kola juice as a substrate for *S. boulardii* cultivation. The growth profile and kinetics of *S. boulardii* in Gotu kola juice were investigated in comparison with fruit juice and standard medium by monitoring yeast cell population and dynamic changes in physicochemical properties and bioactivity of the cultured media.

MATERIAL AND METHODS

Yeast strains and culture maintenance

Saccharomyces cerevisiae var. boulardii CNCM I-745 Bioflor® (*S. boulardii*) was isolated from one sachet of dried yeast (commercial product of Biocodex, Inc.). The colony isolated was maintained in glycerol stored at -20°C used throughout the whole experimental regime. It was reactivated in YMB (Yeast Malt Broth; culture medium; brand Merck co.), at 150 rpm, 30°C for 48 hours. Cells were pelleted by centrifugation (4°C) and washed with sterile physiological

solution (NaCl 8.5g/L) and resuspended in the same solution for use as fresh culture.

Cultivation medium preparation and yeast cultivation

Cultivation medium included red grape juice and Gotu kola juice were prepared. Red grape juice was prepared from Tipco (Ayutthaya, Thailand) diluted to 12°Brix with distilled water, adjusted pH 4.0 with Sodium bicarbonate and pasteurized at 63°C for 30 minutes. For Gotu kola juice (Baijai, Pathumthanni, Thailand) was added with sucrose syrup to 12°Brix, adjusted pH 4.0 with Lactic acid and pasteurized at 63°C for 30 minutes.

Yeast was cultured in 100 mL prepared medium by adding 10% fresh culture (initial cell population 6 log CFU/mL), at 150 rpm, 30°C for 48 hours. Yeast was also cultivated in YMB following the same condition as grape and Gotu kola juice. Dynamic changes of viable population and juice properties (TSS, pH and TTA) during cultivation were determined 0, 8, 24, 32 and 48 hours. The bioactivity in terms of antioxidant and total phenolic compound were determined in 48 hours fermentation of red grape and Gotu kola juices.

Analytical methods

Viability of yeast during cultivation were determined by cultural plating methods. The cultured medium was serially diluted and spreaded onto YM Agar, incubated at 30°C for 48 hours. Total soluble solid (TSS) and pH of cultured medium were determined using refractometer (handhold, Fisherbrand, Thailand) and pH meter (LAQUAtwin–pH-22, Horiba, Japan). Total Titratable acidity (TTA) was determined and reported as percentage of lactic and citric acid [16, 21]. Cell morphologies of 48 hours cultured yeast were inspected using crystal violet staining under optical microscope (BX51, Olympus, Thailand).

Antioxidant activity using the DPPH inhibition method and the probiotic juices were measured based on the inhibition of the DPPH from [9, 18, 25] 1 mL of juices were centrifuged (Tuttlingen, Germany) at 4000 x g, 10 minutes. 0.25 mL suspension of probiotic juices was transferred into a new tube mixed with 4.75 mL of DPPH (Sigma Aldrich, USA) solution (1 mM in Methanol) and incubated at room temperature for 30 minutes in a dark place. Then, absorbance of juice samples was measuring at 517 nm by spectrophotometer (Biospectrometer basic, Eppendorf) using methanol as a blank. The percent DPPH inhibition was calculated by the following equation:

$$\text{DPPH inhibition (\%)} = \frac{(\text{A initial} - \text{A sample})}{(\text{A initial})} \times 100$$

Where, A initial was the absorbance of 0.25 mL of methanol mixed with 4.75 mL of DPPH solution and A sample was the absorbance of juices.

The total phenolic content (TPC) in juices was determined based on [9, 18] using the Folin-Ciocalteu method. 0.5 mL of juices was mixed with 10 mL of distilled water and added to 0.5 mL of Folin-Ciocalteu reagent. Solutions were left to react for 5 minutes at room temperature in the dark; to which 2 mL of Sodium bicarbonate (10% w/v) was added. After 10 minutes, the absorbance was measured at 760 nm using a spectrophotometer and applied the linear regression equation ($y = 0.1225x + 0.2713$). The results were showed in equivalents of Gallic acid (mg GAL /L)

Growth kinetic and Statistical analysis

Growth kinetic of yeast in each cultivation medium were presented in term of specific growth rate was (μ/h) = $dX/(X \cdot dt)$; X: cell (log CFU/mL); t: fermentation time (h). Data were presented as mean ± standard deviation conducted in triplicates. Analysis of variance (ANOVA) and multiple comparisons by Duncan's test were performed using IBM-SPSS statistics package version 22 (SPSS Inc., Chicago, IL, USA). Statistically significant

difference was calculated at significant level of $p<0.05$.

RESULTS AND DISCUSSION

Growth profile of *S boulardii* in different medium

To find the key condition for the cultivation of *Saccharomyces cerevisiae var. boulardii* CNCM I-745 Bioflor®, the optimum pH and sugar level were preliminarily evaluated. Red grape juice adjusted pH in ranges 3.0-5.0 and TSS at 5, 8, 12°Brix were used to cultivate the yeasts. From this screening, pH 4.0 and TSS at 12°Brix were observed as optimum levels for the CNCM I-745 Bioflor® to grow (data not shown). Thus, cultivation medium from red grape and Gotu kola juices were prepared by adjusting pH and sugars as mentioned in the above materials and methods. The growth of yeasts as shown in Figure 1 (a) displayed that among three different medium, in red grape juice, the viable population of yeast significantly increased from 7 log to 11 log CFU/mL 32 hours and remained through 48 hours. In a standard medium, the growth profile of this yeast was relatively similar to the red grape juice but the total viable populations were 1 log-1.5 log CFU/mL lower throughout the cultivation days and the total population reached just 9 log CFU/mL at 48 h. In Gotu kola juice, a relatively different growth pattern was observed. During the first 10 hours, the populations were decreased for around 1 log CFU/mL. Then significantly increased and higher than YMB after cultivation for 24 hours. In this juice the final population at around > 10 log was observed at 48 h cultivation time and trended to continuously increase. The growth profiles of yeast in these media were corresponded to specific growth rates as shown in Figure 1 (b). It was found that the highest specific growth rates of the yeast in red grape juice and YMB were observed during 0-10 hours of cultivation while in Gotu kola juice, the highest specific growth was observed during 10-24 hours. From the results in figure 1 (b) showed the specific growth rate (μ) at 8 hours, viable cells increased in red grape and YMB, and decreased in Gotu kola juice at 0.018, 0.005 and -0.021 log CFU/mL hour. After that, viable cells in Gotu kola juice slightly increased and the specific growth rate reached to the highest point at 24 hours. However, viability of *S. boulardii* was highly 10.5 log CFU/mL in both media at 48 hours. [4, 22, 29] Yang and the research group [30] demonstrated that *S. boulardii* was the glucophillic yeast, firstly utilizing glucose as a carbon source in the substrate system [4]. Thus, in grape juice that contained glucose up to 6.61% also YMB, the yeast could therefore directly utilize this glucose molecule for growth since the early stage of cultivation as observed. In Gotu kola juice, only carbon source in this system was sucrose that was added. *S. boulardii* had to produce enzyme to hydrolyze sucrose during the initial cultivation stage [17], prior to growth with significantly higher specific growth rate during the middle phase relative to the other cultivation media. This observation reflected the performance of the Gotu kola juice in support yeast growth. Other than glucose, minerals and vitamin in Gotu kola juice could also support yeast growth such as Phosphorus, Magnesium, Calcium, Sodium and Thiamine [14].

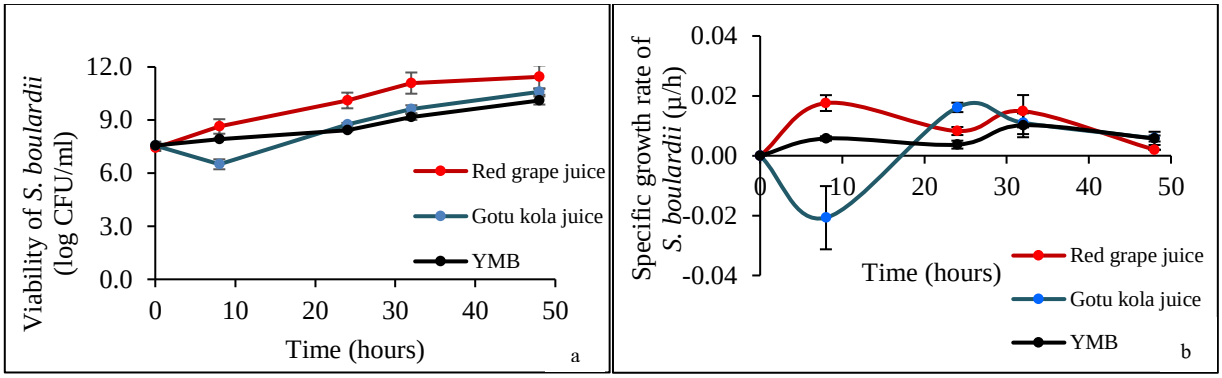

Figure 1 a) The viability of *S. boulardii* (log CFU/mL) and b) the specific growth rate (log CFU/mL hour) in different medium during fermentation time (Results shown as mean ± standard deviation)

For TSS profiles, pH and total acidity of juices during fermentation 48 hours, the initial yeast count was 7.42 log CFU/ml in red grape juice and 7.57 log CFU/ml in Gotu kola juice. After fermentation 48 hours, yeast count was 11.44 and 10.59 log CFU/ml, respectively. As the results in Figure 2, in red grape juice, the TSS value slightly dropped from 12 to 4°Brix, the pH value also dropped from 4.00 to 3.58, and the titratable acidity of citric acid and lactic acid slightly increased from 0.42±0.05 to 0.50±0.05% and 0.20±0.03 to 0.23±0.03%, respectively. While in Gotu kola juice, the TSS value decreased from 12 to 6°Brix, the pH value dropped from 4.00 to 3.33. The titratable acidity of citric acid and lactic acid increased from 0.33±0.03 to 0.83±0.03 % and 0.15±0.01 to 0.39±0.01 %, respectively.

In agreement with Udeagha et. al [29] demonstrated that amount of yeast and TSS value had effect on pH and titratable acidity of juices; as increased TSS levels lead to increased acidity. Although the results of pH and titratable acidity in red grape juice were in contrast with above research, this could be because it is the commercial product with added additive with acid stability that was resistant to increased acidity of citric and lactic acid in red grape juice. Whereas Gotu kola juice was similarly pattern due to it is an organic fresh product with no additive.

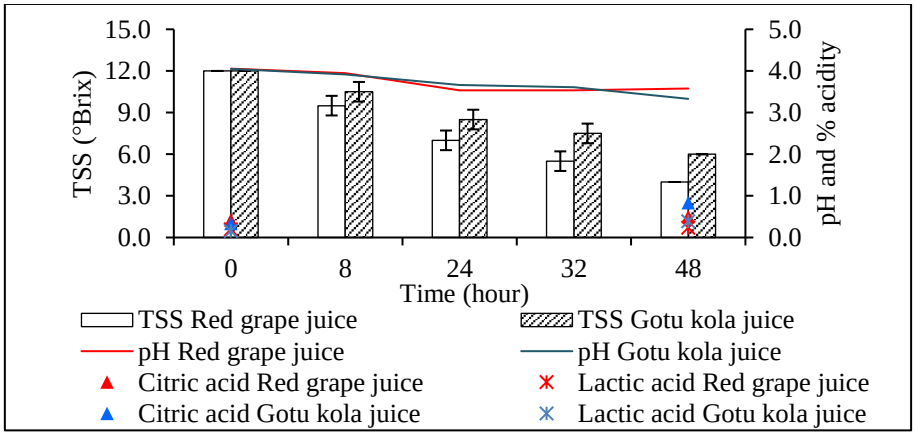

Figure 2 Effect on physical properties in fruit and vegetable juice; Total soluble solids, pH, percent of citric and lactic acidity during fermentation time.
(Results shown as mean ± standard deviation)

As mentioned above, the growth rate is related to the morphology of cells. This is generally reflected by the mode of reproduction. Commonly, *S. boulardii* reproduces asexually by budding which causes asymmetry division in oval and apiculate shape that differ in cell size with mother cells and the site of budding on cells can usually have more than one pole. As shown in figure 3 (b), (c) showed the characteristic of *S. boulardii* in oval and apiculate shape in red grape juice and Gotu kola juice. The arrows in the figures indicated the cells reproduction by budding.

However, *S. boulardii* can adapt into sexual reproduction by fission, which is the duplication of vegetable cells and divides symmetry in a round and/or spherical shape under stressful conditions such as low substrate and/or high acidity during fermentation. As shown in figure 3 (a) presented round and oval shape of *S. boulardii* in YMB and the arrows indicated the cells reproduction by fission [15, 23, 27, 28, 32]. It is interesting to note that *S. boulardii* in fruit and vegetable juice using budding reproduction produced higher cell count (high viability), as compared to standard media. As budding on cells usually has more than one pole per cell.

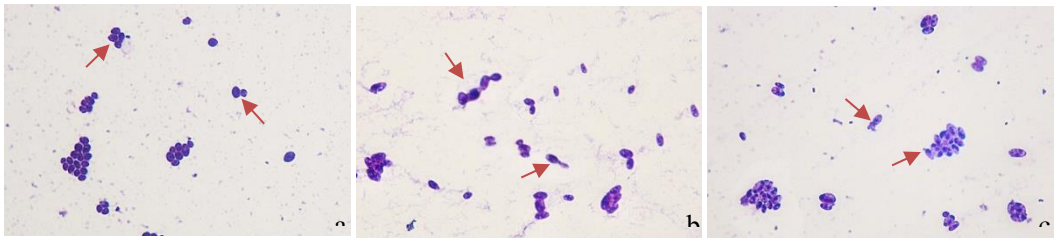

Figure 3 Observation after Crystal violet staining of *S. boulardii* under optical microscope (100X) in different medium A) oval and/or spherical shape of *S. boulardii* in YMB B) *S. boulardii* in red grape juice C) *S. boulardii* in Gotu kola juice

Bioactive compounds

Results mentioned above indicated the viability of cells had effect on physical properties such as low pH, high acidity and/or enzyme from yeast. These could be influenced by bioactive compounds and juice properties. Fruits and vegetables are rich sources of antioxidants, which are the phenolic compounds that prevent oxidation, scavenging and chain-breaking of free radicals. Antioxidant activity depends on structure, the number and position of hydroxyl groups such as phenolic compound, flavonoids, glycoside, organic acid and triterpenoid.

Flavonoids (e.g., flavonols, flavones, flavanones and isoflavones) are classified into phenolic compounds. Flavonoids are mainly in red grape juice which is contained in the skin, seed and pulp whereas in Gotu kola juice is contained in leaf and root.

Quercetin is the main flavonols which present in red grape juice, while in Gotu kola juice mainly contains naringin as flavanones. Quercetin and naringin differ in the number and location of the redox-active hydroxyl group and the structure. Thus, quercetin is more stable and active than naringin. For this reason that can be affected to reach antioxidant and TPC in probiotic red grape juice during fermentation. In contrast, antioxidant and TPC in probiotic Gotu kola juice reduced due to unsaturated bond, hence, unstable structure [2, 7, 19]

The result in Figure. 4 showed percent inhibition of antioxidant in probiotic red grape juice slightly increased to 62.12±0.33% compared with control red grape juice 59.83±0.57% ($p \leq 0.05$). TPC expressed using Folin-Ciocauteu method showed a slightly increased from 9.43±0.27 to 9.52±0.12 mg GAL/L in probiotic red grape juice. On the other hand, the antioxidant in probiotic Gotu kola juice significantly dropped from 39.92±0.45 to 26.82±0.81% ($p \leq 0.05$). TPC in

probiotic Gotu kola juice also decreased from 9.12±0.13 to 4.57±0.08 mg GAL/L.

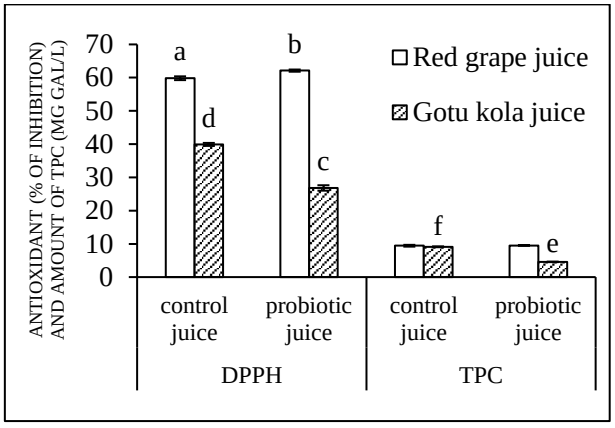

Figure 4 Effect of antioxidant using DPPH method (% of inhibition) and effect of total phenolic content using Folin-Ciocauteu method (mg GAL/L) on red grape and Gotu kola juice. (Results shown as mean ± standard deviation and paired sample test was used to determine the significant difference, $p \leq 0.05$ between the control and probiotic juices)

CONCLUSIONS

To summarize, different compositions and medium had a varying effect on the viability of cells. The growth of *S. boulardii* in Gotu kola juice was higher than standard media but lower than red grape juice. Yeast cell morphology in two juices was oval shape (budding reproduction) whereas, in standard media, it was round shape due to cells being under stressful environment and adapted into sexual reproduction (fission). Thus, it appeared that the viability of cells in juices was higher than standard media. On the other hand, fermentation had effect on bioactivity in red grape and Gotu kola juice. Due to the production of enzyme and by-product by yeast cells, changes in the antioxidant and TPC of juices was observed. Based on the findings of this study, fruit and/or vegetable juice are relatively more suitable for cultivations of *S. boulardii* species. Further research is required to develop and optimize alternative substrates, for value-addition and profitable production of fruit and vegetable juice substrate.

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Altering surface area of fermenters and volume of pineapple juice for enhancing bacterial cellulose production and property with selected strains

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ABSTRACT: Bacterial cellulose (BC) has many benefits as dietary fiber in the food industry. Pineapple is a tropical fruit commonly found in Thailand, and research has shown that it can be used to culture cellulose-producing bacteria that require proper nutrients and acidity. Unqualified pineapple for processing in the canned food industry can be utilized to add value as a culture media for BC production. This study aimed to assess the condition for cultivation of cellulose-producing bacterial strains in unqualified pineapple juice. Four strains included *Gluconacetobacter xylinus* TISTR 1064 (ATCC 23767), *Komagataeibacter xylinus* TISTR 086, *K. xylinus* TISTR 428, and *K. xylinus* TISTR 1061, *K. xylinus* TISTR 428, were evaluated for their BC generation properties in pineapple juice under room temperature for 14 days. Results demonstrated that *K. xylinus* TISTR 428 significantly yielded the highest BC production (105.10±0.08 g/L) ($p<0.05$). Thus, the strain TISTR was selected to further investigate effects of shape and surface area of fermenters, and volume of pineapple juice on cellulose production. The results indicated that cuboid shape fermenter significantly yielded more BC than cylinder ($p<0.05$). The surface area had a positive correlation to BC production ($p<0.05$). On the contrary, increase medium volume caused a significant decrease in BC production ($p<0.05$). There were significant effects of surface area and volume on the texture profile (TPA) of BC ($p<0.05$). Reduction in surface area and volume, also decreased moisture content of BC, directly affecting the springiness which affects the elasticity of BC. With the increase in the surface area and volume, springiness was increased while hardness was reduced. Consequently, this study contributed to revealing key information in BC production for the value-addition of unqualified pineapple.

Keywords: *Bacterial cellulose production, Komagataeibacter xylinus, Pineapple juice*

INTRODUCTION

Pineapple is one of the famous tropical fruits cultivated in Thailand, due to its attractive aroma and nutrition values. The different varieties of pineapples cultivated in Thailand include Cayenne, Queen, and Spanish. Smooth Cayenne has mainly been grown for industrial purposes such are canned pineapple [1]. Due to their varying shapes and sizes, some of them fail to reach the standard requirement and hence are wasted. However, since their nutrition value remains the same, they can be used by value-addition for bacterial cellulose production.

Bacterial cellulose (BC) is an extracellular polysaccharide with β-1,4-glycosidic bonds produced by various types of bacteria which form protective envelopes around the cell. Compared to plant cellulose, BC has no hemicellulose and lignin. Furthermore, the nanomolecular structure of BC cause-outstanding physicochemical properties such as high Young's modulus value, high water holding capacity, and high tensile strength [2]. BC has been produced from a lot of different bacteria species, counting gram-negative bacteria species such as *Acetobacter*, *Azotobacter*, *Rhizobium*, *Agrobacterium*, *Pseudomonas*, *Salmonella*, *Alcaligenes*, plus gram-positive bacterial species such as *Sarcina ventriculi*. Among these, *Acetobacter xylinum*, also called *Komagataeibacter xylinus* or *Gluconacetobacter xylinus*, has been popularly used as the inoculum in the food industry because of its high cellulose productivity.

In addition, BC is a dietary fiber, approved as "generally recognized as safe" (GRAS) food by the US Food and Drug Administration (FDA). Such Dietary fibers are famous in terms of health benefits. Some studies support using BC as a dietary fiber to inhibit obesity-induced in mice and to reduce cholesterol in hamsters [3, 4].

In the food industry, BC has been used as raw material which is sliced BC soaking in syrup named Nata. The well-known commercial one is nata de coco. Bacteria produce this from coconut water medium creating white, gelatinous, and chewy material that has been used for desserts [5]. Nevertheless, using other juices than coconut water for bacterial cellulose production needs to be adjusted to support proper conditions and nutrient balance. Carbon sources and acidity conditions played the main role in BC yield because BC biosynthesis requires glucose and fructose as carbon sources producing UDP-glucose which is a substrate for cellulose synthase further low pH value enhances high BC production rate along with bacterial growth [6, 7].

According to the above mentioned, pineapple is another interesting option since it has high sucrose content including glucose and fructose with a low pH value. There have been many reports on pineapple as an alternative substance for BC production. Vatakit et al. [8] studied immobilizing probiotics in BC and used pineapple juice as a substrate to produce BC by *A. xylinum* TISTR 893. Their pineapple juice was adjusted total soluble solid to 10°Brix using sucrose and was added inducer using 0.05% (w/v) magnesium sulfate. The pineapple was pasteurized at 90°C for 15 minutes and added 2% (v/v) ethyl alcohol before adjusting pH to 4.5 using acetic acid [8]. Waste liquid pineapple peel could be a cultivation media for BC production from *A. xylinum* as reported by Juwita et al. [9]. Result turned out that 40% (v/v) starter in this media incubated at 29°C for 21 days obtained the highest yield [9]. Neera et al. [10] supported that two-time diluted pineapple juice with no additive with *Gluconacetobacter spp.* could better yield BC than corn starch media [10].

The activity of the key enzyme in bacterial cellulose synthesis, cellulose synthase is induced by dinucleotide c-di-GMP containing phosphate [11], thus phosphate is generally required and/or added as inducer in culture medium for BC production. Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) has been widely used as supplement substrates (as an inducer) for BC production in industries. Also nutrients, surface area, and volume of media

were considered one of the factors of BC production. AL-Kalifawi and Hassan [12] found that, in kombucha fermentation, BC production of *A. xylinum* increased with the increase of surface area and depth of tea broth. [12] The increase in surface area/volume ratio of culture medium was expected to increase BC production of *G. xylinus*, an aerobic microorganism [13].

This study aimed to select bacterial cellulose-producing strains for production of BC from juice of unqualified pineapple and to investigate the effect of shape, surface area and volume on yield and texture profile of BC obtained.

MATERIAL AND METHODS

Starter culture preparation

Gluconacetobacter xylinus TISTR 1064 (ATCC 23767), *Komagataeibacter xylinus* TISTR 086, *Komagataeibacter xylinus* TISTR 428, and *Komagataeibacter xylinus* TISTR 1061 used in this research were obtained from the Thailand Institute of Scientific and Technological Research (TISTR). Bacteria were cultured in 10 ml glucose yeast extract broth (GYEB) under agitated conditions (200 rpm) at 30°C for 48 hours [14].

Pineapple juice from unqualified pineapple from the canned industry was used throughout this study. The pineapple juice was pasteurized at 95°C for 10 minutes. The GYEB culture was inoculated in 100 ml of pasteurized pineapple juice with 0.6% (w/v) ammonium dihydrogen phosphate ($NH_4H_2PO_4$), adjusted pH 4.0-4.5 with 0.1% NaOH. Cultivation was conducted under agitation (200 rpm), at 30-32°C for 48 hours and used as a starter culture.

Strains selection for BC production

Culture medium was prepared from 45 ml of pasteurized pineapple juice with 0.6% (w/v) ammonium dihydrogen phosphate ($NH_4H_2PO_4$), adjusted pH 4.0-4.5 with 0.1% NaOH. Starter culture of each strain (10% (v/v)) was inoculated to prepare pineapple juice, incubated under static condition at 30°C for 14 days. BC productions were inspected every 48 hours by measuring the thickness of BC using vernier caliper. The fermented juice was collected every 48 hours to evaluate pH, total titratable acidity (TTA) as per the AOAC reference method [15], and total soluble solids (TSS) by refractometer. After incubation, BC was obtained to determine the wet weight (g/L). One strains with best performance in BC production was selected for further investigation.

Effect of surface area and volume of media on BC production

Fermenters with different shapes (cylinder and cuboid) and surface areas (15.6, 24.6, 26.0, 42.0, 55.0, 64.3, and 128.0 cm^2) were sanitized by UV light for 30 minutes, prior to media addition. Pineapple juice medium, prepared as previously described, at different volumes (25, 50, 100, and 150 cm^3) was added to fermenters as shown in Table 2. Starter culture of selected strains (10% v/v) was inoculated to prepare pineapple juice and incubated under the static condition at 30°C for 14 days. The bacterial celluloses were collected to measure thickness by vernier caliper and determined texture profile analysis (TPA). TPA was done on 2x2 cm dimension samples of BC using a texture analyzer (TA-XT2i; Stable MicroSystems, England) with a 50 mm diameter cylinder aluminum (P/50) adaptor at pretest, test, and posttest speeds set at 1, 0.5, and 0.5 mm/s, respectively. The trigger force was 5 g and each sample was compressed to 40% strain of the original height twice [16].

Statistical analysis

The results obtained in this research were presented as mean ± standard deviation for three replications. Completely randomized design (CRD) was used in this experiment. Analysis of variance (ANOVA) and multiple comparisons were determined by Duncan's multiple range test at significant level of $p<0.05$ using SPSS statistic version 22. All experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Strains selection

Since each strain has different phenotype, differently expresses and/or responds to each environmental condition. Therefore, strains with efficient BC production in pineapple juice were selected prior to use as specific stains for investigation of external factors associated with BC production. Results demonstrated that reference bacterial cellulose-producing strains, including *G. xylinus* TISTR 1064, *K. xylinus* TISTR 086, *K. xylinus* TISTR 428, and *K. xylinus* TISTR 1061 displayed different properties in BC production under pineapple condition. The BC thickness as fermentation progressed, increased with incubation time. The thicknesses of BC were different among each strain while fermented juice properties were no different as shown in figure 1 and table 1.

Figure 1 shows that TSS of *K. xylinus* TISTR 428 was the lowest, while TTA was the highest. This demonstrated the ability of this strain to consume carbon sources in pineapple medium and convert into acetic acid. In addition, BC's wet weight of the *K. xylinus* TISTR 428 was also the highest (105.10 ± 0.08 g/L) as shown in Table 1. This demonstrated that this strain could utilize acetic acid as a carbon source (Acetic acid-derived ATP) for producing bacteria via acetic acid oxidation [17]. Based on the performance in BC production under pineapple condition, *K. xylinus* TISTR 428 was selected as specific strain for optimization of surface area and volume of fermenter and media used for BC production.

Table 1 Wet weight of BC (g/L) after fermentation

Strains	Wet weight of BC (g/L)
(A) *G. xylinus* TISTR 1064	38.20 ± 0.08^{c}
(B) *K. xylinus* TISTR 086	57.00 ± 0.07^{b}
(C) *K. xylinus* TISTR 428	105.10 ± 0.08^{a}
(D) *K. xylinus* TISTR 1061	33.40 ± 0.05^{c}

$^{a\text{-}c}$ Values with the different superscript letters in the same column show significant differences ($p < 0.05$).

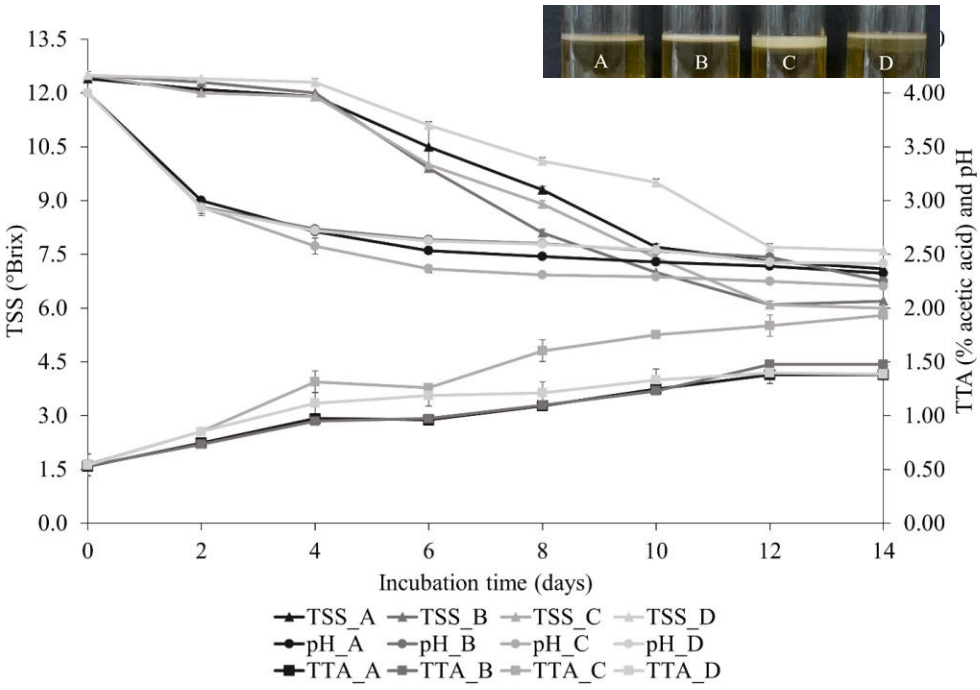

Figure 1 Bacterial cellulose production by commercial strains (A) *G. xylinus* TISTR 1064, (B) *K. xylinus* TISTR 086, (C) *K. xylinus* TISTR 428, and (D) *K. xylinus* TISTR 1061 and fermented PP properties

Table 2 Effect of surface area and volume on % BC yield/volume of PP and texture profile of BC in medium

Container's shape	Volume (cm^3)	Surface area (cm^2)	% BC yield/ volume	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness
cylinder	50	24.6	27.80±0.14^{de}	2,120.91±17.35^{b}	0.19±0.01^{c}	0.20±0.01^{c}	398.10±36.69^{a}	72.58±7.73^{bc}
cuboid	50	55.0	65.18±5.06^{bc}	1,896.32±7.65^{c}	0.24±0.05^{bc}	0.27±0.09^{bc}	518.14±171.75^{a}	133.57±69.19^{ab}
cuboid	25	15.6	31.20±0.00^{de}	2,577.86±105.72^{a}	0.28±0.00^{bc}	0.22±0.03^{c}	556.30±52.55^{a}	154.85±15.64^{ab}
cuboid	25	26.0	54.70±5.29^{c}	1,978.96±63.39^{bc}	0.33±0.03^{bc}	0.31±0.02^{bc}	598.63±7.28^{a}	192.39±12.96^{a}
cuboid	25	42.0	71.65±3.21^{b}	985.95±14.41^{e}	0.37±0.02^{b}	0.39±0.04^{bc}	384.00±48.35^{a}	141.59±25.03^{ab}
cuboid	25	128.0	120.32±10.86^{a}	1,627.84±125.93^{d}	0.39±0.02^{b}	0.24±0.03^{c}	391.13±75.21^{a}	153.54±35.68^{ab}
cuboid	50	64.3	55.30±1.27^{c}	2,181.00±17.40^{b}	0.25±0.00^{bc}	0.18±0.02^{c}	384.57±27.52^{a}	95.56±8.08^{abc}
cuboid	100	64.3	39.22±5.46^{d}	1,864.15±77.02^{c}	0.24±0.00^{bc}	0.20±0.02^{c}	381.38±54.09^{a}	88.55±11.69^{bc}
cuboid	150	64.3	27.03±0.15^{e}	62.13±3.17^{f}	0.57±0.14^{a}	0.92±0.07^{a}	56.63±1.25^{b}	32.55±8.56^{c}

$^{a-f}$ Values with the different superscript letters in the same column show significant differences ($p < 0.05$)

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Effect of surface area and volume of media on BC production

The selected strain, *K. xylinus* TISTR 428, was used to determine the effect of surface area and volume by varying the fermenter's shape, volume of medium, and surface area at room temperature between 30-32°C for 14 days. There are several reports demonstrating the effect of container shapes on BC production since they showed that the shape of the container affected BC spheroid formation [18]. Table 2 shows that 2 shapes (cylinder and cuboid) and surface area were varied to investigate their impact and found that cuboid fermenter significantly yielded more BC than cylinder ($p<0.05$). In the same fermenter's shape, %BC yield expressed direct variation with surface area, in contrast with volume. As the BC formation mechanism in the liquid phase starts with consuming dissolved oxygen in medium to obtain cell biomass and to produce cellulose, only bacteria on the surface could maintain their BC production activity, further affecting the depth [19]. Previously, the effect of surface area and volume on BC production was studied by Krystynowicz et al. (2002) by altering media volume in plastic trays and incubating with *A. xylinum* E25 for 7 days at 30°C. Results indicated that the surface area to volume ratio increased along with BC yield [20]. Cakar et al. varied surface area and medium volume incubating with *G. xylinus* for 6 days at 30°C and provided results in the same way [21].

Table 2 shows TPA parameters for BC produced on 9 different surface areas and volumes. Results significantly revealed the effect of surface area and volume on the TPA ($p<0.05$). With the decrease in the surface area and volume, hardness increased, and springiness decreased. On the other hand, increasing volume with the same surface area initiated reducing hardness, gumminess, and chewiness but build springiness and cohesiveness. Study by Department of Gastronomy and Culinary Arts, Turkey, explained that springiness has direct correlation to moisture content and hence, the elasticity of BC. With the reduction in surface area and volume, moisture content also decreases, herein decreasing the elasticity of BC [22]. While the fermenter's shapes cylinder and cuboid had significantly no effect on TPA parameters ($p<0.05$).

CONCLUSIONS

Unqualified pasteurized pineapple juice was the potential to be culturing medium for producing BC. *K. xylinus* TISTR 428 producing the highest BC yield was selected to observe the effect of surface area and volume on BC production. It appeared that cuboid container obtained higher % BC yield than cylinder. BC production was in line with the surface area but inversed with medium volume. Surface area and volume had a significant effect on the texture profile of BC. However, there are still many factors affecting BC production that require further study to develop production.

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POSTER PRESENTATION

Screening of lignocellulolytic fungi from decayed mango strum

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ABSTRACT: The aim of this study was to isolate and screen for lignocellulolytic fungi from decay strum of mango tree (*Mangifera indica*) found in the Khon Kaen University, Nong Khai Campus. Total 10 isolates were found but only 4 isolates exhibited lignocellulolytic abilities with similar capacity as assessed by a qualitative method. Based on the microscopic characterization, those isolates likely belong to the genus of *Aspergillus.* These fungal isolates could be the source of alternative cellulolytic enzymes, applying in food industries, agricultural and environmental management.

Keywords: Fungal isolate, Cellulolytic activity, Xylanolytic activity, Lignolytic activity

INTRODUCTION

Wood decay is caused by a wide variety of fungi that could decompose the wood tissue by secreting lignocellulolytic enzymes, lignified plant cell walls. Lignocellulose is the major structural component of woody plant cell walls and consists of lignin, hemicellulose and cellulose. Degradation of lignocellulose can occur in trunk, branches, and roots, particularly in the dead tree [1]. However, decomposition performance is dependent on fungal species and ability to secret lignocellulolytic enzymes.

Cellulose hydrolysis is achieved by endoglucanases and cellobiohydrolases, collectively termed cellulases. Hydrolysis of hemicellulose, a mixed polymer, occurs via the action of xylanases, mannanases, and other hydrolytic enzymes [2]. However, hydrolytic ability of these components by the action of different fungi might be varied due to several factors such as optimum temperature and substrate specificity. Therefore, screening and characterization of lignocellulolytic enzymes suitable for decomposition of tropical wood may result in useful information regarding production of an alternative source of valuable enzymes. Lignocellulolytic enzymes have been applied in numerous industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture [3].

Mango (Mangifera indica) is believed to have originated from India and widely distributed in Thailand including the mixed deciduous forest in the area of Khon Kaen University, Nong Khai Campus. Those tree species decay by the action of microorganisms, particularly fungi. Isolation and characterization of those fungi responsible for hardwood decomposition would gain some information regarding the alternative source of lignocellulolytic enzymes. Application of fungal cellulase for extracting antioxidants

from mango peels has been reported [4]. Therefore, this study aimed to isolate and characterize the morphology of fungi from decayed strum from mango trees. In addition, lignocellulolytic ability from fungal isolates was also evaluated.

MATERIAL AND METHODS

Sample collection

The three sources of decay strum of mango tree *(Mangifera indica)* were collected from Nong Khai province in Northeast of Thailand. Gloves and sterile zip lock bags were used. All samples in zip lock bags were taken to the laboratory and stored at 4° C until used.

Fungal isolation

Each sample was crushed. Fungi counting and isolation were performed by taking 25 grams of crushed sample to 225 ml of normal saline and tenfold serially diluted up to 10^{-6}. Each dilution was spreaded on PDA agar by spread technique, triplicate plate/dilution. All plates were incubated at 25° C for 7 days. Each plate was counted of colonies and calculated for colony forming unit/gram (CFU/g). The differences between colonies in color and diameter were collected and code for isolated names.

Ligninolytic capacity

The lignin basal medium (LBM) agar was prepared according to the method described by Pointing [6]. Briefly; g/L of KH_2PO_4 1 g, $C_2H_{12}N_2O_6$ 0.5 g, $MgSO_4.7H_2O$ 0.5 g, $CaCl_2.2H_2O$ 0.01 g, Yeast extract 0.01 g, $CuSO_4.5H_2O$ 0.001 g, $Fe_2(SO_4)_3$ 0.001 g, $MnSO_4.H_2O$ 0.001 g. and agar 1.6 g. for LBM media. The sterilized LBM was supplemented with 1 ml/100 ml of each separately sterilized 20% w/v glucose solution and 1% of sterilized tannic acid solution in media before solidifying. Then inoculated each isolated fungi on the center of each LBM agar plate and incubated at 25° C for 10 days. The positive of lignin degradation is the appearance of a brown oxidation zone around colonies. Each isolate was performed for triplicate. The ligninolytic capacity was calculated with the ratio of diameter of the brown zone and colony. Only positive ligninolytic isolates were selected for hemicellulolytic, cellulolytic capacity and identification based on mitosporic identification [5].

Hemicellulolytic capacity

This capacity was analyzed by dye staining of xylan agar [6]. The xylanolytic basal medium (XBM) was prepared. Briefly; g/L of $C_4H_{12}N_2O_6$ 5 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, Yeast extract 0.1 g $CaCl_2.2H_2O$ 0.001 g and agar 1.6 g. Before sterile, the 4% w/v xylan powder was added. Each isolate of fungi was inoculated on the center of the xylan agar plate and incubated at 25° C in dark for 3-5 days. Flood plates with iodine stain (0.25% w/v aqueous I_2 and KI) for 5 min. Then pour off the stain and wash with distilled water. The xylanolytic isolate showed the hydrolysis zone as a yellow-opaque area against a blue / reddish purple color. The capacity of xylanolytic was estimated by the diameter ratio of hydrolysis zone and colony. Each isolate was performed in triplicate.

Cellulolytic capacity

This capacity was analysed in the same manner with that of hemicellulolytic, according to Pointing [6]. The CMC agar was prepared the same with XMB but supplemented with 2% w/v of CMC powder instant xylan powder. Inoculation of each isolate was on the center of CMC agar plate and incubated at 25° C in the dark for 3-5 days. Then flood plate with 2% w/v aqueous congo red. After 15 minutes, pour off stains and wash with distilled water and flood again with 1 M NaCl for 15 minutes and pour. Similarly, the cellulolytic isolate showed the hydrolysis zone around the colony. The yellow-opaque zone against a red color of undegraded CMC. The cellulolytic capacity was estimated by the diameter ratio of hydrolysis zone and colony. Each isolate was performed in triplicate.

Morphology characteristic of selected fungi

The isolated fungi were preliminary identified based on mitosporic characteristics of fungi [5]. Each fungus was prepared by slide culture technique and observed under a bright field microscope.

RESULTS AND DISCUSSION

Isolation of fungi

Normally, the decay of hardwood stems is caused by the action of microorganisms, particularly tropical fungi. The degradation of hardwood found in the area of Khon Kaen University was selected to isolate important fungi taking action on wood decay. The decay of tropical woods was used for isolation of fungi by spread plate technique on PDA agar. The total isolates of 10 were found and coded as listed in Table 1. In addition, the concentration of total count from was 4 x 10^3 CFU/ml. It can be seen that each colony could grow on PDA agar with a different extent as indicated by the different diameter. These suggested that the ability to degrade lignin xylan and cellulose in the hardwood tissue would be different among those isolates.

Ligninolytic ability of isolates

Ligninolytic ability indicates the performance of microorganisms to degrade hardwood since the main component in hardwood tissue is lignin. The ability could be primarily screened by the Bavendamm test on lignin agar. Fungi isolated from all hardwood showed lignolytic ability although the amount of positive isolate from each wood was different. The number of positive tests were 4 isolates as shown in Figure 1.

The lignin degradation capacity of each isolate was estimated as the diameter ratio of the brown zone and colony. All isolates showed the value between 1.153-1.311 without significant difference (Figure 2). The results suggest that all isolated fungi have the ability to decompose lignin within the plant cell wall and might be the main spice to decompose the dead hardwood [7]. However, application of ligninolytic enzymes very useful in biomass management [8] but, in food might be rather limited than that of cellulase or pectinase. Therefore, screening for the cellulase and hemicellulase activity is necessary for wider application.

Table 1 Fungi isolated from decayed strum from mango tree found in the Faculty Interdisciplinary Studies, Khon Kaen University, Nong Khai province.

Colony count (CFU/ml)	Codes	Colony Ø (cm)
4 x 10^3	NKM 1-1	4.08
	NKM 1-2	5.74
	NKM 1-3	0.10
	NKM 1-4	1.80
	NKM 2-1	4.74
	NKM 2-2	0.38
	NKM 2-3	9.00
	NKM 3-1	4.30
	NKM 3-2	0.10
	NKM 3-3	9.00

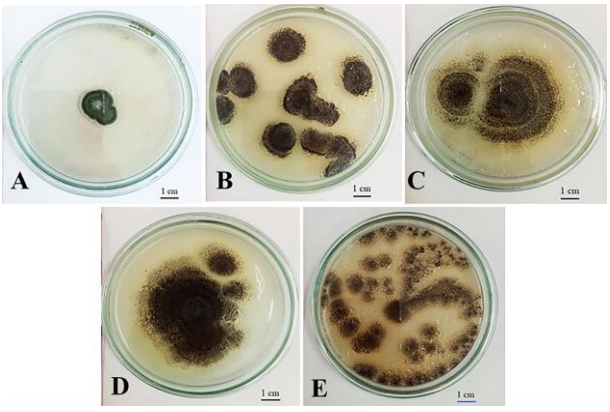

Figure 1 Brown zone of tannin degradation around fungi colonies on tannic acid agar. A = negative control, B = NKM 1-1, C = NKM 1-2, D = NKM 2-1 and E = NKM 3-1

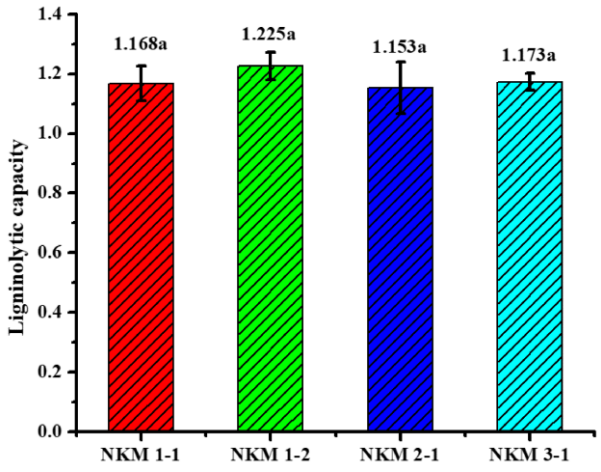

Figure 2 Tannin degradation capacity fungi isolated from *Mangifera indica.*

Cellulose and hemicellulose degradation

Then cellulolytic and hemicellulolytic abilities were studied since these were the indicator for degradation of cellulose of softwood. The yellow-opaque zone around the colony on xylan agar and CMC agars indicated the positive test for both activities (Figures 3 and 4). These results suggested that fungi isolated from this hardwood secretes either xylanase or cellulase to degrade cellulose and hemicellulose. The results suggested that all isolates have a potential as a good source for producing alternative enzymes, particularly xylanase and cellulase for further application in food industries. Isolation of lignocellulolytic enzymes from dairy manure soil has been successfully reported [9]. The hydrolytic capacity of cellulase and xylanase were found in between 1.13-1.3 and 1.18-1.3, respectively (Table 2).

Morphology

In order to identify the species of each isolate, morphological characteristics of each colony were examined. It can be seen in Figure 5 that morphology of all isolates was likely to be genus *Aspergillus* sp. since the short white hypha and brown to black conidia on PDA agar. In addition, the septate hyphae, spherical vesicle on conidiophore, bottle like phialide (conidiogenous) and circular conidia arenment in chain were observed under a microscopic picture (Figure 5) [10]. In previous study, Andlar [3] reported that *Aspergillus oryzae* can produce catechol oxidase as lignin degradation, *Aspergillus niger* has hemicellulolytic enzyme; Exo - β - 1,4 - manosidase, Feruloyl esterase, Endo - α - 1,5 - arabinanase and α-L-arabinofuranosidase and *Aspergillus fumigatus* can produce Xylan α 1,2 - glucuronidase enzyme. In this study, results showed that the 4 isolates have potential in producing all lignocellulolytic enzymes; ligninolytic, hemicellulolytic and cellulolytic enzymes. Therefore, determination of enzyme activity and specific enzymes could be useful for evaluation the potential to apply either fungal isolate or secreted enzymes commercially.

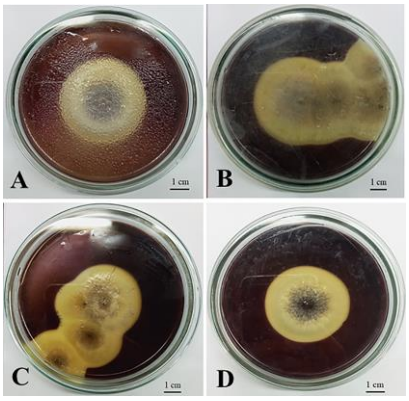

Figure 3 Yellow-opaque zone of xylanolytic ability of fungi isolated from *Mangifera indica.* A = NKM 1-1, B = NKM 1-2, C = NKM 2-1 and D = NKM 3-1

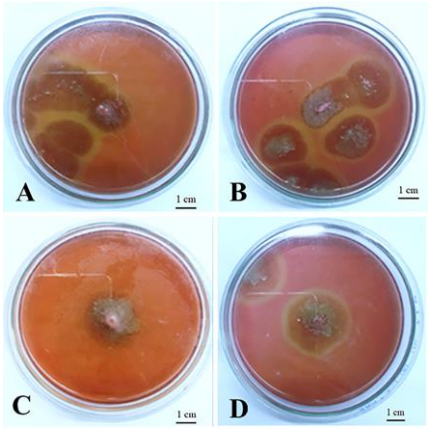

Figure 4 Yellow-opaque zone against a red color of cellulolytic ability of fungi isolated from *Mangifera indica.*. A = NKM 1-1, B = NKM 1-2, C = NKM 2-1 and D = NKM 3-1

Table 2 Degradation capacity of fungi isolated from *Mangifera indica.*

Isolate	Degradation capacity	
	Xylanolytic	**Cellulolytic**
NKM 1-1	1.176 ± 0.06a	1.248 ± 0.09a
NKM 1-2	1.132 ± 0.13a	1.194 ± 0.01a
NKM 2-1	1.289 ± 0.15a	1.147 ± 0.12a
NKM 3-1	1.189 ± 0.05a	1.298 ± 0.18a

** The different letter within column indicates statistically different ($p < 0.05$)*

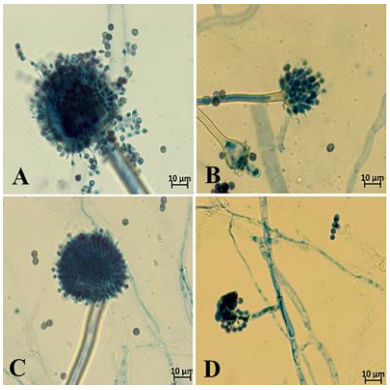

Figure 5 Morphology of fungi isolated from *Mangifera indica* under bright field microscopy. A = NKM 1-1, B = NKM 1-2, C = NKM 2-1 and D = NKM 3-1

CONCLUSIONS

The decay strum from mango trees could be the source of tropical fungi and tentatively belonged to *Aspergillus* sp. The fungal isolates (40%) exhibited lignolytic and cellulolytic activities with similar degradation capacity and could be the source of commercial cellulolytic enzymes.

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Selection of commercial molds and yeasts for sato production

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ABSTRACT: Sato is an alcoholic beverage produced by dough balls containing molds as starch hydrolyzers, yeasts as alcohol formers, and bacteria as acid producers. Products are thus relied on the composition of starter cultures. However, there is often a variation in types and numbers of microorganisms in dough balls from the same origin, leading to the uncontrollable fermentation and uncertain products. This research is, therefore, aimed to select the commercial molds and yeasts for Sato fermentation from San-pah-tawng 1 rice that is commonly cultivated in northern Thailand. When two mold species, namely *Aspergillus oryzae* TISTR 3019 and *Rhizopus oryzae* TISTR 3052, were used to hydrolyze starch, the former demonstrated higher amylase activity and reducing sugars on day 3 of fermentation. After that, seven strains of *Saccharomyces cerevisiae*, namely 71B, BM4x4, EC-1118, ICV/D-47, K1-1116, QA23 and RC-212, were compared for alcohol formation. Strain QA23 yielded the product with high total soluble solids but low acid and glycerol contents. As a result, appearance, odor, flavor, and overall liking of Sato made by this yeast strain were most acceptable. Finally, the same two mold species were used as starch hydrolyzers before alcohol fermentation was continued by QA23. Results suggested that the chemical composition of Sato was indifferent but the product from *Rhizopus oryzae* revealed higher sensory scores. Therefore, *Rhizopus oryzae* TISTR 3052 and *Saccharomyces cerevisiae* QA23 are recommended for the Sato production from San-pah-tawng 1 rice in order to control the fermentation and the product consistency.

Keywords: Sato, Mold, Yeast, San-pah-tawng 1 rice

INTRODUCTION

Thai locals have a long history of rice wine production which is involved in two stages – the aerobic and anaerobic fermentation. Starter cultures are commonly added as dough balls containing several types of microorganisms such as *Amylomyces*, *Rhizopus*, *Aspergillus*, *Mucor*, *Saccharomyces*, *Saccharomycopsis*, *Endomycopsis*, *Hansenula*, *Candida*, *Torulopsis*, *Pichia*, *Issatchenkia*, *Torulaspora*, *Rhodotorula*, *Trichosporon*, *Pediococcus*, *Lactobacillus*, *Acetobacter*, *Gluconobacter*, and *Bacillus* [1]. In general, molds would hydrolyze starch for yeasts to ferment sugars into alcohol [2]. Dough balls are thus considered as a ready-to-use microbial source. However, a variety of bacteria present in dough balls not only proceeds to the product identity but also the fermentation failure if their levels are increased during preparation or storage.

This research is, therefore, aimed to select the commercial molds and yeasts for Sato fermentation from San-pah-tawng 1 rice that is commonly cultivated in northern Thailand. Moreover, the fermentation time would be determined to ensure the consumer acceptance. Application of commercial pure cultures could be a practical and consistent way to control the Sato production at an industry level.

MATERIAL AND METHODS

Raw materials and chemicals

San-pah-tawng 1 rice was obtained from a local farmer in Lampang, Thailand. *Aspergillus oryzae* TISTR 3019 and *Rhizopus oryzae* TISTR 3052 were purchased from the culture collection of Thailand Institute of Scientific and Technological Research (TISTR). Seven strains of *Saccharomyces cerevisiae*, namely 71B, BM4×4, EC-1118, ICV/D-47, K1-1116, QA23, and RC212, were purchased from Lallemand (Montreal, Canada). Sodium periodate, ethylene glycol, sodium hydroxide, sulfuric acid, bromothymol blue, citric acid, sodium citrate, and glucose were purchased from Kemaus (Cherrybrook, Australia) while Dinitrosalicylic acid (DNS) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Comparison of starch hydrolysis by molds

100 g of rice was washed in a tap water before soaked in 500 ml of reverse osmosis (RO) water for 17 h. Rice was again washed in a tap water before transferred to high density polyethylene (HDPE) bag. RO water was added to a total weight of 200 g and rice was autoclaved at 121°C for 30 min. After cooled down, cooked rice was mixed with 2 g of *Rhizopus oryzae* or *Aspergillus oryzae* spores at 10^8 spores/g before incubated at 30°C for 5 days. 1 g of samples was daily removed and mixed with 9 ml of distilled water before centrifuged at 3000×g for 10 min. Supernatant was collected to analyze for reducing sugars and amylase activity. Experiment was conducted in triplicate for each mold species.

Comparison of alcohol formation by yeasts

1,750 g of rice was washed in a tap water before soaked in 8,750 ml of RO water for 17 h. Rice was again washed in a tap water before transferred to HDPE bag. RO water was added to a total weight of 3,500 g and rice was autoclaved at 121°C for 30 min. After cooled down, cooked rice was mixed with 35 g of mold that yielded the highest reducing sugar content and amylase activity at 10^8 spores/g before incubated at 30°C for 3 days. 500 g of fermented rice was transferred to seven polyethylene terephthalate (PET) bottles. 500 ml of RO water and 0.2 g of yeast powder at 10^{10} cells/g was added before incubated at 25°C. 50 ml of samples was removed every other days and centrifuged at 3000×g for 10 min. Supernatant was collected to analyze for alcohol content. When 7% alcohol was reached, products were centrifuged at 3000×g for 10 min. Supernatant was stored in PET bottles at -4°C for analysis of total soluble solids, reducing sugars, pH, acetic acid, glycerol, and sensory attributes. Experiment was conducted in triplicate for each yeast strain.

Comparison of sato production by molds

250 g of rice was washed in a tap water before soaked in 1,250 ml of RO water for 17 h. Rice was again washed in a tap water before transferred to HDPE bag. RO water was added to a total weight of 500 g and rice was autoclaved at 121°C for 30 min. After cooled down, cooked rice was mixed with 5 g of *Rhizopus oryzae* or *Aspergillus oryzae* spores at 10^8 spores/g before incubated at 30°C for 3 days. Fermented rice was transferred to a PET bottle. 500 ml of RO water and 0.2 g of yeast powder at 10^{10} cells/g that yielded the highest sensory scores was added before incubated at 25°C. 50 ml of samples was removed every other days and centrifuged at 3000×g for 10 min. Supernatant was collected to analyze for alcohol content. When 7% alcohol was reached, products were centrifuged at 3000×g for 10 min. Supernatant was stored in a PET bottle at -4°C for analysis of total soluble solids, reducing sugars, pH, acetic acid, glycerol, and sensory attributes. Experiment was conducted in triplicate for each mold species.

Chemical analysis

Reducing sugars

1 ml of sample was mixed with 1 ml of 10% DNS before heated at 100°C for 10 min

and cooled down. Absorbance was measured at 570 nm using distilled water as a blank [3]. Glucose was used as a standard at concentrations of 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 µg/ml. Reducing sugar content was obtained by fitting the absorbance of sample on a standard curve of glucose. Each sample was analyzed in triplicate.

Amylase activity

1 ml of sample was mixed with 1 ml of 0.1 M citrate buffer and 1 ml of 2% starch solution before incubated at 40°C for 10 min. The mixture was heated at 100°C for 10 min and cooled down before glucose content was analyzed [3]. Each sample was analyzed in triplicate. Amylase activity was calculated as below.

$$\text{Amylase activity (U/ml)} = \frac{R1-R0}{10}$$

R0 = Glucose content after incubation (µg/ml(
R1 = Glucose content in sample (µg/ml(
When 1 U is defined as a glucose content)µg) derived from the amylase activity for 1 min.

Alcohol

Boiling point of samples was compared to one of RO water by Ebulliometer (Dujardin-Salleron, Paris, France) to determine % alcohol by volume.

Total soluble solids

Sample was dropped on Refractometer (Atago, Tokyo, Japan) to determine % total soluble solids (% Brix).

pH

pH was measured by pH meter (Mettler-Toledo, Switzerland).

Acetic acid

5 ml of sample was mixed with 95 ml of distilled water before titrated with 0.1 M NaOH until an end point at pH 8.7 [4]. % Acetic acid was calculated as below.

% Acetic acid = Volume of 0.1 M NaOH × 0.12

Glycerol

1 ml of sample was mixed with 50 ml of distilled water and 0.1 ml of 0.1% bromothymol blue. 0.1 M H_2SO_4 was added until the mixture turned yellow and then 0.05 M NaOH was added until the mixture turned blue. After 50 ml of 6% $NaIO_4$ was added, the mixture was kept in a dark room for 30 min. After 10 ml of 50% $C_2H_6O_2$ was added, the mixture was kept in a dark room for 20 min. Distilled water was added to a total volume of 300 ml before 0.1 ml of 0.1% bromothymol blue was added. The mixture was titrated with 0.125 M NaOH until turned blue [5]. % glycerol was calculated as below.

$$\text{\% Glycerol} = \frac{9.209 \times N \times (T1-T2)}{W}$$

N = A concentration of NaOH (0.125 M)
T1 = Volume of NaOH used for a sample
T2 = Volume of NaOH used for distilled water
W = Volume of sample (1 ml)

Sensory analysis

Appearance, odor, flavor, and overall liking of products were tested for consumer acceptance by 105 untrained panelists using 9 – point hedonic scale.

Statistical analysis

Data were reported as mean ± standard error (SE) and tested for statistical difference at 95% confidence by Analysis of Variance in General Linear Model (GLM) followed by independent t-test or Duncan's Multiple Range Test (SPSS Statistics 17.0).

RESULTS AND DISCUSSION

Starch hydrolysis by molds

Two mold species, namely *Aspergillus oryzae* and *Rhizopus oryzae*, were compared for the ability to hydrolyze starch in San-pah-tawng 1 rice. These molds could be found in dough balls used for Sato fermentation as the suppliers of amylase, which were classified into alpha-amylase, beta-amylase, and glucoamylase, leading to the hydrolysis of starch into sugars [6]. Results demonstrated

that *Aspergillus oryzae* expressed higher levels of reducing sugars and amylase activity during 4 days of fermentation as illustrated in Figure 1. The rationale would be that *Aspergillus oryzae* was able to grown well in cooked rice [7]. However, other study reported that Mucoraceae, such as *Rhizopus*, *Mucor*, *Amylomyces*, released higher levels of amylase but the activity was incomplete, evolving in lower levels of reducing sugars when compared to Moniaceae such as *Aspergillus* [8].

Moreover, the highest level of reducing sugars was found on day 2 of fermentation (Figure 1A) while the amylase demonstrated the highest activity on day 3 of fermentation (Figure 1B). Therefore, further studies would be conducted using *Aspergillus oryzae* to hydrolyze starch for 3 days.

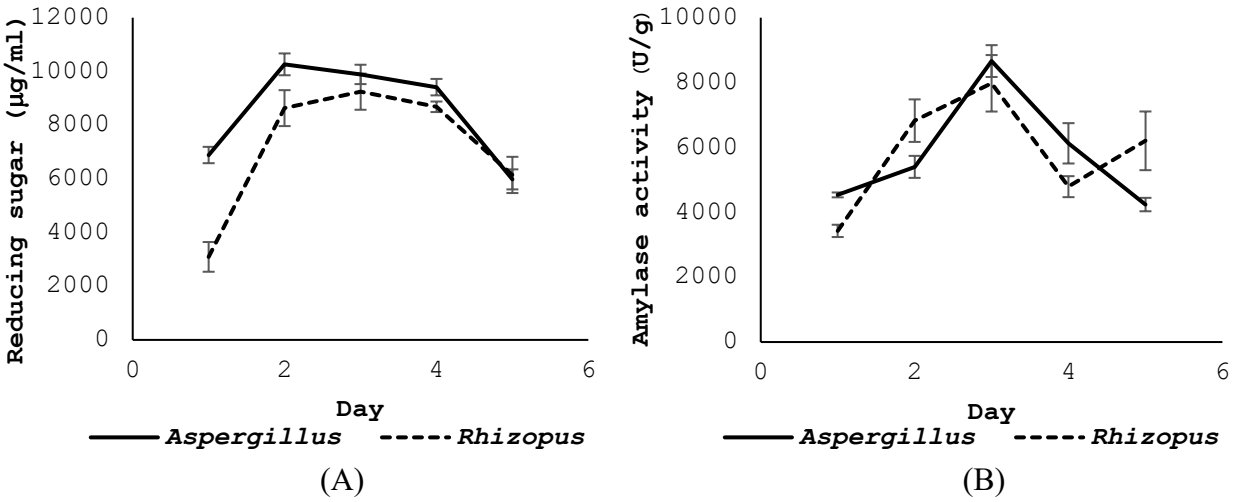

Figure 1 Reducing sugars (A) and amylase activity (B) in rice fermented by *Aspergillus oryzae* and *Rhizopus oryzae* for 5 days.

Alcohol formation by yeasts

Sato production is succeeded by the fermentation of sugars into alcohol [1]. However, current consumers prefer the low alcoholic beverage. This study is thus aimed to develop Sato with 7% alcohol, which is similar to a content found in a wine cooler. It was revealed that strains 71B and RC212 spent the shortest and longest time to yield 7% alcohol, respectively (Table 1). Fermentation time is probably influenced by some biochemical attributes of yeast, for example, the alcohol tolerance of cell membrane, resistance to heat released during fermentation, or acid tolerance during growth [9].

Total soluble solids generally indicate a sugar content in rice wine [10]. Results illustrated that strains BM4×4 and RC212 produced Sato with the lowest and highest levels of total soluble solids, respectively (Table 1). Changes in total soluble solids during Sato production are involved in the release of sugars from starch by molds such as *Rhizopus*, *Mucor*, *Amylomyces*, *Aspergillus* [2, 11, 12]. After that, sugars are utilized by yeast into alcohol and other metabolites, resulting in the decrease of total soluble solids until the end of fermentation.

However, Sato in this study contained a lower level of total soluble solids when compared to previous research that reported 7.8 – 15.6% total soluble solids in rice wine [13]. It is likely that molds in this study was unable to grow well and incompletely hydrolyze starch into sugars under the anaerobic condition in HDPE bag. Moreover, Sato fermented by strain RC212 expressed the highest level of total soluble solids but

contained a low level of reducing sugars and a high level of acetic acid (Table 1). This suggests that total soluble solids also refer to an acid content in rice wine. Total soluble solids, nonetheless, did not highly affect the consumer acceptance since Sato fermented by strain RC212 demonstrated the lowest sensory score (Table 2).

Reducing sugars are products of beta-amylase and glucoamylase. The highest level was found in Sato fermented by strain EC-1118 (Table 1). However, the product fermented by this strain revealed a low sensory score (Table 2), inferring that reducing sugars are not a key factor that affects consumer acceptance.

In addition to alcohol, molds and yeasts are able to form organic acids, such as lactic acid and acetic acid, resulting in the pH reduction to 3.38 – 3.65 (Table 1). Sato produced by seven yeast strains were not statistically different in terms of pH values, which were similar to a previous study that reported the pH values of 3.17 – 4.00 in rice wine [14]. At low pH values, the yeast growth is promoted while other microorganisms are inhibited, leading to the succession of Sato fermentation [15].

Acetic acid is a by-product of wine fermentation via the alcohol oxidation [16]. Yeasts generally form acetic acid at a low level but oxygen exposure could lead to the over-oxidation of alcohol [17]. As the result, Sato flavor is sour and unacceptable as found in products fermented by strains EC-1118 and RC212 that contain the highest levels of acetic acid (Table 1). In the other hand, Sato yielded by strains 71B, K1-V1116, and QA23, which consisted of acetic acid at low levels, received the high sensory scores (Table 2).

Glycerol is a non-volatile compound, thus not affecting the aroma but highly influencing the sweetness and fullness, which are ones of major sensory attributes in wine [18]. This compound is a by-product of alcohol fermentation, being the third metabolite after alcohol and carbon dioxide. Yeasts reduce dihydroxyacetone phosphate into glycerol-3-phosphate by glycerol-3-phosphate dehydrogenase and removes a phosphate group by phosphatase [18]. Therefore, the yeast strain has a significant effect on the glycerol formation. According to the culture provider, strain RC212 is a low glycerol producer, while EC-1118 and 71B are considered the high glycerol formers [19]. Current study also suggested the comparable result that strain 71B yielded the highest glycerol content. Moreover, Sato fermented by seven yeast strains contained the higher level of glycerol when compared to fruit wines [19], implying the effect of raw materials. However, a glycerol content was not contributed to the consumer acceptance as Sato fermented by strain QA23, which consisted of low glycerol amount, received the highest sensory score.

Table 1 Chemical composition of Sato fermented by seven yeast strains.

Yeast	Time (Day)	% Alcohol	% Brix	Sugar (mg/ml)	pH	% Acid	% Glycerol
71B	7.00 ± 0.58 ^{b}	7.63 ± 0.43 ^{a}	6.00 ± 0.50 ^{bc}	2.06 ± 0.14 ^{b}	3.41 ± 0.10 ^{a}	0.50 ± 0.06 ^{bc}	3.17 ± 0.15 ^{a}
BM4x4	9.67 ± 1.20 ^{ab}	7.60 ± 0.57 ^{a}	5.80 ± 0.40 ^{c}	2.26 ± 0.10 ^{b}	3.39 ± 0.15 ^{a}	0.59 ± 0.09 ^{ab}	1.67 ± 0.18 ^{g}
EC-1118	9.00 ± 1.53 ^{ab}	7.70 ± 0.50 ^{a}	6.33 ± 0.68 ^{ab}	2.82 ± 0.30 ^{a}	3.40 ± 0.06 ^{a}	0.67 ± 0.13 ^{a}	2.76 ± 0.05 ^{c}
ICV/D-47	7.33 ± 0.33 ^{b}	7.50 ± 0.50 ^{a}	6.20 ± 0.60 ^{ab}	2.30 ± 0.10 ^{b}	3.62 ± 0.12 ^{a}	0.52 ± 0.12 ^{bc}	2.24 ± 0.14 ^{d}
K1-V1116	7.33 ± 0.33 ^{b}	7.53 ± 0.47 ^{a}	6.20 ± 0.60 ^{ab}	2.17 ± 0.03 ^{b}	3.38 ± 0.32 ^{a}	0.45 ± 0.05 ^{c}	2.05 ± 0.07 ^{e}
QA23	8.00 ± 0.58 ^{ab}	7.53 ± 0.43 ^{a}	6.27 ± 0.64 ^{ab}	2.17 ± 0.18 ^{b}	3.65 ± 0.12 ^{a}	0.47 ± 0.08 ^{bc}	1.92 ± 0.19 ^{f}
RC212	11.67 ± 3.18 ^{a}	7.47 ± 0.49 ^{a}	6.47 ± 0.64 ^{a}	1.89 ± 0.08 ^{b}	3.39 ± 0.03 ^{a}	0.67 ± 0.09 ^{a}	2.92 ± 0.12 ^{b}

Note: Different letters in the same column indicate significant differences ($p \leq 0.05$).

Sensory scores of Sato fermented by seven yeast strains were presented in Table 2. More specifically, strain QA23 expressed the highest scores in terms of odor and overall liking, while strain 71B received the highest flavor score. However, strain RC212 demonstrated the lowest scores of odor and overall liking while strain EC-1118 exhibited the lowest flavor score. In summary, consumers preferred Sato fermented by strain QA23, probably due to the ability to produce beta-glucosidase that could release terpenes from raw materials, making the product more aromatic [20]. Strain QA23 also requires low levels of nutrients and oxygen as well as yields a high alcohol content at low temperature, making this strain suitable for the production of Chardonnay, Sauvignon Blanc, Semillon, Chardonel, and Gewurztraminer [20].

Table 2 Sensory scores of Sato fermented by seven yeast strains.

Yeast	Appearance	Odor	Flavor	Overall	Total
71B	6.23 ± 0.17 ^{a}	6.09 ± 0.16 ^{ab}	6.32 ± 0.18 ^{a}	6.42 ± 0.16 ^{a}	25.06 ± 0.57 ^{ab}
BM4x4	6.10 ± 0.17 ^{a}	5.81 ± 0.17 ^{ab}	5.88 ± 0.18 ^{abc}	5.87 ± 0.17 ^{b}	23.66 ± 0.57 ^{bc}
EC-1118	6.06 ± 0.17 ^{a}	5.84 ± 0.16 ^{ab}	5.61 ± 0.20 ^{c}	5.86 ± 0.20 ^{b}	23.36 ± 0.58 ^{c}
ICV/D-47	6.02 ± 0.18 ^{a}	5.91 ± 0.15 ^{ab}	5.90 ± 0.16 ^{abc}	6.12 ± 0.16 ^{ab}	23.96 ± 0.50 ^{abc}
K1-V1116	6.18 ± 0.16 ^{a}	6.00 ± 0.17 ^{ab}	5.76 ± 0.17 ^{bc}	6.17 ± 0.14 ^{ab}	24.11 ± 0.52 ^{abc}
QA23	6.30 ± 0.17 ^{a}	6.26 ± 0.15 ^{a}	6.26 ± 0.17 ^{ab}	6.50 ± 0.16 ^{a}	25.30 ± 0.50 ^{a}
RC212	5.92 ± 0.18 ^{a}	5.70 ± 0.16 ^{b}	5.64 ± 0.17 ^{c}	5.79 ± 0.15 ^{b}	23.05 ± 0.48 ^{c}

Note: Different letters in the same column indicate significant differences ($p \leq 0.05$).

Sato production by molds

When *Aspergillus oryzae* and *Rhizopus oryzae* were used to hydrolyze starch into sugars before fermented by *Saccharomyces cerevisiae* QA23, it was revealed that *Rhizopus oryzae*, spent non-significantly less time of fermentation to reach 7% alcohol. However, alcohol, total soluble solids, reducing sugars, pH, acetic acid, and glycerol in Sato were not statistically different between two mold species as depicted in Table 3.

Table 3 Chemical analysis of Sato produced by *Aspergillus oryzae* and *Rhizopus oryzae*.

Mold	Time (Day)	% Alcohol	% Brix	Sugar (mg/ml)	pH	% Acid	% Glycerol
Aspergillus	17.00 ± 4.00 ^{a}	7.90 ± 0.00 ^{a}	12.90 ± 0.30 ^{a}	1.79 ± 0.44 ^{a}	4.00 ± 0.01 ^{a}	0.53 ± 0.02 ^{a}	2.62 ± 0.06 ^{a}
Rhizopus	9.50 ± 1.50 ^{a}	8.30 ± 0.00 ^{a}	12.60 ± 0.60 ^{a}	1.59 ± 0.35 ^{a}	4.30 ± 0.10 ^{a}	0.50 ± 0.04 ^{a}	2.82 ± 0.18 ^{a}

Note: Different letters in the same column indicate significant differences ($p \leq 0.05$).

Sato produced by *Aspergillus oryzae* and *Rhizopus oryzae* demonstrated non-significant sensory scores as shown in Table 4. However, *Aspergillus oryzae* received a non-statistically higher odor scores. It was likely that this species spent more fermentation time, the formation of esters, such as ethyl acetate, ethyl lactate, isoamyl acetate [21], was thus extended, leading to the more aromatic product.

Table 4 Sensory scores of Sato produced by *Aspergillus oryzae* and *Rhizopus oryzae*.

Mold	Appearance	Odor	Flavor	Overall	Total
Aspergillus	6.11 ± 0.17 [a]	6.01 ± 0.21 [a]	5.69 ± 0.22 [a]	6.06 ± 0.20 [a]	23.87 ± 0.70 [a]
Rhizopus	6.21 ± 0.18 [a]	5.73 ± 0.24 [a]	6.61 ± 0.22 [a]	6.49 ± 0.22 [a]	25.04 ± 0.72 [a]

Note: Different letters in the same column indicate significant differences ($p \leq 0.05$).

CONCLUSIONS

Rhizopus oryzae TISTR 3052 and *Saccharomyces cerevisiae* QA23 are commercial starter cultures suitable for the Sato production from San-pah-tawng 1 rice.

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DIVISION E
Related Topics (Food Packaging, Food Safety & Quality, Food Laws & Regulations, Food Policy, etc.)

ORAL PRESENTATION

Effect of UV-C curing on properties of ferulic acid-added soy protein film

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ABSTRACT: Due to environmental threat and needs for effective packaging waste management, scientists have been actively conducting research on developing environmentally friendly biodegradable and edible packaging to replace the petroleum-based counterpart. Soy protein has been extensively investigated because of its outstanding film-forming capability. However, soy protein film exhibits inferior mechanical strength and moisture barrier property to those made from synthetic polymers. The objective of this study was to explore the effect of UV-C curing on the properties of ferulic acid-added soy protein film. The films were fabricated from soy protein isolate added with 1.5% ferulic acid by casting method. UV-C radiation was applied at different doses (0.32, 1.56, 4.00, 12.00 J/cm^2) to either preformed film or film-forming solution. It was found that UV-C treatment, particularly at higher dose (12.00 J/cm^2) to both preformed film and film-forming solution resulted in a film with significantly higher tensile strength and elongation at break as compared to the control. Water vapor permeability and water solubility were minimally affected by the irradiation. UV-C curing decreased film transparency and increased yellowness. Surface hydrophobicity of the irradiated preformed film was found to increase while curing of film-forming solution posed a minimal effect on surface hydrophobicity of the resulted film. The findings of this study indicate that UV curing have a potential to improve mechanical properties and modify optical properties of ferulic acid-added soy protein film.

Keywords: Soy protein film, Ferulic acid, UV curing, UV-C, Protein cross-linking

INTRODUCTION

Packaging is designed to contain and extend shelf life while maintaining the quality of the product inside [1]. Globally, over 280 million tonnes of petroleum-based plastics are manufactured each year, with an average annual growth rate of around 5% [2]. Most of the plastic materials produced are non-recyclable and non-biodegradable, contributing to wastage and environmental problems. At present, packaging and food industries are working together to develop environmentally friendly alternatives to petroleum-based plastics.

Proteins are one of the natural biopolymers with the potential to manufacture biodegradable packaging. Soy protein has been extensively investigated because of its outstanding film-forming capability. However, soy protein film possesses inferior mechanical strength and moisture barrier properties for practical applications, particularly under high humidity conditions [3].

Protein cross-linking via chemical and physical means has been reported to improve protein film properties. Ferulic acid, a phenolic compound with low toxicity, has been shown to induce cross-linking in various proteins. Side group of certain amino acid residues of polypeptide chains, such as tyrosine, lysine, and cysteine, can react with ferulic acid and its oxidative product, quinoid ferulic acid, to form covalent C-N and C-S bonds [4-5]. The ferulic acid fortification effectively increased tensile strength, elongation at break, and surface hydrophobicity while decreasing water vapor

permeability and water solubility of soy protein film, particularly at high concentration of its oxidized form [6].

UV radiation can modify the properties of proteins either by inducing covalent cross-links or molecular degradation [7]. Through the absorption of UV radiation by a side group of aromatic amino acids like tyrosine and phenylalanine, the amino acid free- radicals are produced and, in turn, induce a covalent cross-link in proteins [8]. Exposure of soy protein film to UV radiation was reported to result in an increase in tensile strength and yellowness with a decrease in elongation, but the radiation treatment posed no effect on the water vapor permeability of the film [7].

The objective of this study was to investigate the effect of UV treatment, either to preformed film or film-forming solution, on the properties of ferulic acid-added soy protein film.

MATERIALS AND METHODS

Materials

Soy protein isolate (90% protein, wet basis) and glycerol were purchased from Krungthepchemi (Bangkok, Thailand). Ferulic acid was obtained from Chanjao Longevity (Bangkok, Thailand).

Film preparation

Soy protein film was prepared according to the method described by [6] with some modifications. To prepare the film-forming solution, 5 g of soy protein isolate were dissolved in 70 g of phosphate buffer (pH7.4), which contained 2.75 g of glycerol (55% by weight of soy protein isolate) as a plasticizer. After being homogenized for 2 min at 22000 rpm, the protein solution was then heated in a water bath at 70°C for 30 min to partially denature the protein and then cooled to ambient temperature. Separately, 0.075 g of ferulic acid (1.5% by weight of soy protein isolate) was dissolved in 30 g of phosphate buffer (pH7.4). The ferulic solution was then added to the protein solution and homogenized for 2 min at 22000 rpm. The ultrasonic degassing technique was used to remove air bubbles from the solution. Film casting was done by transferring 45 mL of film-forming solution onto a 150 mm × 150 mm acrylic mold.

Non-irradiated soy protein film without added ferulic acid was used as a control. Non-irradiated 1.5% ferulic-added soy protein film (FE) and soy protein film without ferulic acid addition and UV-treated at 0.32 J/cm^2 (UV0.32) were used as references. UV-C radiation at a wavelength of 253.7 nm was applied to either preformed films or film-forming solutions at four different doses (0.32, 1.56, 4.00, and 12.00 J/cm^2). Irradiation treatment was carried out in a UV-C cabinet (model PIS-88C, P Inter Supply, Bangkok, Thailand) with a UV-C intensity of 2500 µW/cm^2. For the UV-C treatment on preformed films, UV-C radiation was applied to the film after being dried at 40°C for 24 h and cooled to room temperature. All film samples were equilibrated at 50% RH and 25°C for 48 h before further analyses.

Thickness and mechanical properties

Film thickness was measured using a thickness gauge (model 7301, Mitutoyo, Tokyo, Japan). Tensile strength and elongation at break were analyzed according to the ASTM D882 standard method (ASTM, 2009) using a Texture Analyzer (model TA. XT plus, Stable Micro System, Godalming, UK) equipped with tensile grips (A/TG) probes. A 30 mm × 100 mm film strip was mounted on both grips, and the film sample was pulled at a constant speed of 8.33 mm/s until failure.

Colour

A chromameter (model CR400, Konica Minolta Sensing, Osaka, Japan) was used to determine the film color in the CIELAB system with a 10° observer and D65 illuminant. Hue angle and chroma were also calculated from the CIE *L**, *a**, *b**.

Water vapor barrier property

Water vapor permeability (WVP) of the film samples was determined following the ASTM E96 method [9]. A film sample without leaks and scratches was cut into a 60 mm × 60 mm piece. Twenty g of dried silica gel was placed

into a glass permeation cup. Silicone grease was applied to the rim of the cup. After being mounted on the cup, the film piece was tightened with a rubber ring and parafilm. The cup was then weighed and placed in a chamber containing distilled water at 25°C. The weight of the permeation cup was taken every 24 h for 7 days and calculated for the WVP using equation (1):

$$WVP = W\ d/A\ t\ (P_2-P_1) \quad \ldots\ldots (1)$$

where W is weight gain of the permeation cup (g); d is the film thickness (m); A is exposed area of the film available for water permeation; t is the time to reach equilibrium (h); and (P_2-P_1) is the difference in partial pressure of water vapor across both sides of the film (Pa).

Water solubility

The water solubility of the film samples, expressed in terms of total soluble matter, was determined according to the method described by [6].

Surface hydrophobicity

Film surface hydrophobicity was determined using a contact angle measuring instrument (Data Physics Instruments, Filderstadt, Germany). A droplet (4 µL) of distilled water was deposited on the film surface. The contact angle of the water droplet and the film surface (θ) was measured, with θ<90° and θ>90° characterizing hydrophilic and hydrophobic surfaces, respectively.

Film density

Film density was calculated from dry mass and dimensions of a film piece using equation (2):

$$\text{Film density} = m/(A\times\delta) \quad \ldots (2)$$

where m is the dry mass of the film piece (g); A is the film area (cm^2); and δ is the film thickness (cm) [10].

Transparency

Transparency of the film samples was expressed as %transmittance. A film sample was cut into a precise dimension (10 mm × 40 mm) and mounted onto the inside of a quartz cuvette. %Transmittance was measured at 500 nm using a UV-Vis spectrophotometer (model GENESYS20, Thermo Scientific, Waltham, MA, USA).

Experimental design and statistical analysis

Experiments were done in three replicates. A completely randomized design was used. Data were analysed using Analysis of Variance. A Duncan's new multiple range tests (Cochran and Cox, 1957) were used to determine the difference among sample means at p=0.05 using SPSS Statistics 22.0 (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

Thickness and mechanical properties

The thickness and mechanical properties of the film samples are shown in Table 1. The thickness of the films was in the range of 0.165-0.193 mm. The film thickness was affected ($p\leq0.05$) by ferulic acid addition and UV-C treatment. This may be due to the induced protein aggregation that may influence the structure of the film [11].

Mechanical properties of the film samples are reported in terms of tensile strength and elongation at break (Table 1). It was found that ferulic acid addition and UV-C curing tended to induce an increase in tensile strength and elongation at break of the soy protein film. However, the difference was statistically insignificant (p>0.05), except at higher doses of UV-C (12.00 J/cm^2 for tensile strength; 4.00 and 12.00 J/cm^2 for elongation at break). The ferulic acid-added films treated with UV-C at 12.00 J/cm^2 exhibited about a 1.3-fold increase in tensile strength and a 1.7-fold increase in elongation at break compared to the control. The increase in both tensile strength and elongation at break upon exposure to high doses of UV-C could be due to the induced protein cross-linking, rendering the film matrix stronger and able to stretch without readily tearing apart. UV radiation could be absorbed by the side group of aromatic amino acids, such as tyrosine and phenylalanine, with a

production of amino acid free radicals. Recombination of these free radicals could lead to the formation of inter-and intra-molecular covalent cross-links in protein [12]. Similar finding was also previously reported by Gennadios et al. [7] for UV-treated soy protein film.

Density and transparency

The density and transparency of the film samples are summarized in Table 2. The density of the films ranged between 0.983-1.073 g/cm^3. In general, film density seemed to be unaffected by ferulic acid addition and UV-C curing (p>0.05). On the other hand, UV-C treatment of ferulic acid-added soy protein films caused a decrease in film transparency (i.e., decrease in %transmittance), particularly at higher UV-C doses (4.00 and 12.00 J/cm^2). The reduction in transparency is most probably due to protein cross-linking and aggregation induced by both ferulic acid and UV-C [13]. Higher doses of UV radiation probably alter the aromatic acids and produce photo products from photochemical reactions, which may reduce the transparency of protein film [2].

Table 1 Thickness and mechanical properties of ferulic acid (FE)-added soy protein films treated with UV-C at 0.32, 1.56, 4.00 and 12.00 J/cm^2

Film samples	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
Control	0.165±0.133^{c}	2.48±0.10^{b}	100.25±12.62^{c}
FE	0.185±0.179^{ab}	2.61±0.14^{b}	117.19±18.90^{bc}
UV0.32	0.188±0.127^{ab}	2.49±0.10^{b}	122.50±17.05^{bc}
UV-treatment on preformed films			
FE+UV0.32	0.186±0.152^{ab}	2.56±0.16^{b}	107.75±16.02^{c}
FE+UV1.56	0.181±0.132^{ab}	2.72±0.05^{b}	124.11±17.02^{bc}
FE+UV4.00	0.193±0.107^{a}	2.74±0.08^{b}	140.64±26.95^{ab}
FE+UV12.00	0.177±0.143^{ab}	3.12±0.16^{a}	166.67±27.65^{a}
UV-treatment on film-forming solutions			
FE+UV0.32	0.184±0.141^{ab}	2.61±0.07^{b}	110.81±30.25^{bc}
FE+UV1.56	0.177±0.130^{ab}	2.64±0.07^{b}	118.98±22.71^{bc}
FE+UV4.00	0.189±0.102^{ab}	2.74±0.39^{b}	150.95±26.92^{ab}
FE+UV12.00	0.178±0.079^{ab}	3.29±0.13^{a}	165.45±21.15^{a}

Mean±SD of three replicates.
Sample means within a column that do not share a common superscript letter differ significantly at p=0.05

Water vapor permeability, water solubility and surface hydrophobicity

Table 3 illustrates the water vapor permeability, water solubility (WVP), and surface hydrophobicity (expressed as contact angle) of the film samples. WVP of the ferulic acid-added soy protein films was not significantly affected by UV-C treatment. Overpowering of the hydrophilic nature of protein film to the increase in cross-linked protein network could be the most probable reason for this phenomenon [14].

In terms of water solubility (Table 3), UV-C treatment on preformed films brought about a gradual decrease in water solubility. Contrastingly, UV-C treatment of film-forming solutions caused a slight increase in water solubility. However, the water solubility of all film samples was not statistically different (p>0.05). This insignificant difference in water solubility may also be due to the highly hydrophilic nature of soy protein film.

Table 2 Density and transparency (expressed as %transmittance) of ferulic acid (FE)-added soy protein films treated with UV-C at 0.32, 1.56, 4.00 and 12.00 J/cm^2

Film samples	Density (g/cm^3)	%Transmittance
Control	0.983±0.042^{bcd}	62.50±2.64^{c}
FE	0.923±0.033^{d}	56.90±1.72^{d}
UV0.32	1.020±0.040^{abc}	70.21±1.53^{a}
UV-treatment on preformed films		
FE+UV0.32	0.969±0.051^{cd}	61.38±0.62^{c}
FE+UV1.56	1.000±0.034^{bc}	56.83±2.47^{d}
FE+UV4.00	1.019±0.015^{abc}	54.91±0.69^{de}
FE+UV12.00	1.039±0.013^{ab}	51.73±0.76^{f}
UV-treatment on film-forming solutions		
FE+UV0.32	1.073±0.020^{a}	67.40±0.32^{b}
FE+UV1.56	1.017±0.026^{abc}	62.73±1.60^{c}
FE+UV4.00	0.966±0.064^{cd}	52.41±1.33^{ef}
FE+UV12.00	0.955±0.042^{cd}	52.36±0.85^{ef}

Mean±SD of three replicates.
Sample means within a column that do not share a common superscript letter differs significantly at p=0.05.

Surface hydrophobicity is reflected from the contact angle of a water droplet to the film surface (Table 3). UV-C treatment of ferulic acid-added preformed films significantly increased the contact angle (i.e., surface hydrophobicity). The preformed film irradiated with 12.00 J/cm^2 UV-C demonstrated the greatest contact angle (60.40°). Fathi et al., [10] proposed that the increase in surface hydrophobicity is probably due to the development of cross-linking and the decrease in free hydrophilic groups in the polypeptide chains. Moreover, the reduction in hydrophilicity after UV treatment on protein film may be due to the exposure of hydrophobic regions of proteins [15].

On the other hand, UV-C curing of film-forming solutions at lower doses (0.32-4.00 J/cm^2) caused a decrease in contact angle. Meanwhile, at high UV-C dose (12.00 J/cm^2), the film obtained possessed a similar contact angle to the control. The higher effectiveness of UV-C treatment on preformed films than the treatment on film-forming solutions could be due to the higher protein concentration of preformed (i.e., dried) films compared to the film-forming solution resulting in the close proximity of the polypeptide chains, thus facilitating the cross-linking of proteins.

Colour

The CIE color parameters of the film samples are presented in Table 4. UV-C treatment was shown to pose an effect on the color of the films, especially the b^* value. The hue angle of all film samples was approximately 95°, close to the angle of yellow hue (90°). Chroma tended to increase with increasing UV-C dose, which corresponds well with the increase in $+b^*$. The addition of phenolics was reported to cause an increase in yellowness of soy protein due to the color product of a reaction between phenolic compounds and protein [4], and the yellowness became more intense upon oxidation of the phenolics [6]. In this study, we have postulated that UV-C may accelerate the oxidation of ferulic acid, leading to the greater chroma of the film samples subjected to higher UV-C doses. Moreover, UV-C was previously reported to induce yellowness in soy protein film without phenolic addition [7]. Schmid et al., [2] suggested that the formation of the conjugated double bond upon UV exposure is probably the cause of the yellowness of protein film.

Table 3 Water vapor permeability (WVP), water solubility, and surface hydrophobicity (expressed as contact angle) of ferulic acid-added soy protein films treated with UV-C at 0.32, 1.56, 4.00, and 12.00 J/cm^2

Film samples	WVP (g m/m^2 h P)	Water solubility (%)	Contact angle (°)
Control	0.55±0.11^{abc}	41.86±1.88^{ab}	47.77±0.99^{de}
FE	0.65±0.03^{a}	40.47±1.11^{ab}	47.17±1.63^{e}
UV0.32	0.54±0.05^{abc}	41.36±2.39^{ab}	49.77±0.95^{d}
UV-treatment on preformed films			
FE+UV0.32	0.62±0.06^{ab}	40.53±3.44^{ab}	46.57±1.07^{e}
FE+UV1.56	0.49±0.05^{c}	38.91±2.70^{b}	53.17±1.55^{c}
FE+UV4.00	0.64±0.05^{a}	38.52±2.79^{b}	56.47±1.20^{b}
FE+UV12.00	0.62±0.05^{abc}	37.49±0.75^{b}	60.40±2.05^{a}
UV-treatment on film-forming solutions			
FE+UV0.32	0.59±0.02^{abc}	45.94±5.32^{a}	38.90±1.30^{g}
FE+UV1.56	0.50±0.06^{bc}	45.57±4.99^{a}	39.90±1.47^{g}
FE+UV4.00	0.62±0.04^{ab}	43.45±3.24^{ab}	42.53±1.07^{f}
FE+UV12.00	0.55±0.01^{abc}	40.23±2.66^{ab}	47.43±1.33^{de}

Mean±SD of three replicates.
Sample means within a column that do not share a common superscript letter differ significantly at p=0.05.

Table 4 Colour parameters of ferulic acid-added soy protein films treated with UV-C at 0.32, 1.56, 4.00 and 12.00 J/cm^2

Film samples	*L**	*a** ^{ns}	*b**	Hue angle (°)	Chroma
Control	90.44±0.87^{ab}	-1.87±0.13	17.91±2.01^{d}	96.40±0.75^{ab}	18.77±2.28^{cd}
FE	90.01±0.77^{bc}	-2.01±0.16	18.65±2.29^{cd}	96.40±0.75^{ab}	18.77±2.28^{cd}
UV0.32	90.47±0.92^{ab}	-1.88±0.13	17.94±1.78^{d}	95.99±0.32^{b}	18.04±1.18^{d}
UV-treatment on preformed films					
FE+UV0.32	90.28±0.66^{ab}	-2.11±0.16	18.88±1.89^{cd}	96.39±0.19^{ab}	18.99±1.89^{cd}
FE+UV1.56	90.79±0.69^{ab}	-2.18±0.24	19.53±1.95^{cd}	96.38±0.54^{ab}	19.66±1.96^{cd}
FE+UV4.00	91.13±0.76^{a}	-1.87±0.13	19.53±2.29^{cd}	95.50±0.34^{c}	19.62±2.38^{cd}
FE+UV12.00	90.57±1.06^{ab}	-1.66±0.15	21.35±2.70^{bc}	94.48±0.39^{d}	21.42±2.71^{cd}
UV-treatment on film-forming solutions					
FE+UV0.32	90.32±0.81^{ab}	-2.16±0.20	18.76±2.40^{cd}	96.61±0.34^{a}	18.88±2.41^{bc}
FE+UV1.56	90.49±1.11^{ab}	-2.09±0.24	19.44±306^{cd}	96.18±0.40^{ab}	19.56±3.06^{cd}
FE+UV4.00	90.12±0.74^{bc}	-2.13±0.27	22.45±3.07^{ab}	95.42±0.16^{c}	22.54±3.09^{ab}
FE+UV12.00	89.36±1.25^{c}	-1.96±0.20	24.89±4.80^{a}	94.58±0.49^{d}	24.97±4.80^{a}

Mean±SD of three replicates.
Sample means within a column that do not share a common superscript letter differ significantly at p=0.05.
^{ns} Sample means within the column do not differ significantly at p=0.05.

CONCLUSIONS

UV-C curing was shown to be effective in terms of improving tensile strength and elongation at break of ferulic acid-added soy protein film, particularly at high radiation dose (12.00 J/cm^2). The radiation could be applied to either preformed film or film-forming solution. The ferulic acid-added films treated with UV-C at 12.00 J/cm^2 exhibited about a 1.3-fold increase in tensile strength and a 1.7-fold increase in elongation at break from the control. Regarding optical properties, UV-treated ferulic acid-added soy protein films demonstrated a decrease in transparency

and an increase in yellowness. UV-C treatment of ferulic acid-added preformed films significantly increased surface hydrophobicity. Meanwhile, UV-C irradiation at lower doses (0.32-4.00 J/cm^2) to film-forming solution caused a decrease in surface hydrophobicity but for that treated at 12.00 J/cm^2 had similar surface hydrophobicity to the control. In general, UV-C curing posed a minimal effect on density, water vapor permeability, and water solubility of the ferulic acid-added soy protein films.

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Antimicrobial effect of essential oils with Nisin and Natamycin in combination against *Escherichia coli* O157:H7

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ABSTRACT: This research aimed to determine the antimicrobial activities of (S)-(-)-Limonene and Geraniol in combination with Nisin and Natamycin against *Escherichia coli* O157:H7. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidial Concentration (MBC) were determined using Macrobroth dilution method. The MIC of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin were 8,500, 350, 4 and 8,700 mg/L, respectively. The MBC were 11,500, 400, 110 and >70,000 mg/L respectively. In addition, the results from the determination of Fractional Inhibitory Concentration Index (FIC_{index}) of the binary, ternary and quaternary combination system using Checkerboard assay against *Escherichia coli* O157:H7 indicated synergistic effect of the combination of essential oils with Nisin or Natamycin with the FIC_{index} as 0.5. However, the combination of Nisin with Natamycin showed an antagonistic effect with the FIC_{index} as 1.25. Moreover, the combination of (S)-(-)-Limonene, Geraniol and Nisin, (S)-(-)-Limonene, Geraniol and Natamycin and (S)-(-)-Limonene, Nisin and Natamycin presented synergistic effect with the FIC_{index} as 0.75 and combination of Geraniol, Nisin and Natamycin showed additive effect with the FIC_{index} as 1.0. In addition, the quaternary combination of those demonstrated synergistic effect with the FIC_{index} as 0.5 and The MICs of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin in the quaternary system were 1,062.5, 43.75, 0.5 and 1,087.5 mg/L, respectively. Therefore, the combination of essential oils with Nisin and Natamycin lead to reduce the use of amounts of essential oil or food preservative and enhances their antimicrobial activities.

Keywords: *Essential oils, Nisin, Natamycin, Multi antimicrobial system*

INTRODUCTION

Escherichia coli O157:H7 is an important foodborne pathogen that cause diarrhea, hemorrhagic colitis and hemolytic uremic syndrome. It resulted in illness and mortality worldwide [1]. *E. coli* O157:H7 can be transmitted via the fecal-oral route after consumption of contaminated food or water with feces. *E. coli* O157:H7 is commonly found in fresh vegetables, fruits, raw or undercooked meat and raw milk [2]. These contamination lead to unsafe food, food loss and shelf-life reduction [3]. Recently, consumer demand for more healthy foods. therefore, natural preservatives are used in foods for improve the safety of food product. Essential oils are complex natural mixtures of secondary metabolites extracted from plant against a broad range of bacterial, fewer side effect and reduce bacteria resistant to antibiotics. Therefore, they are used in food and beverages industry [4]. (S)-(-)-Limonene is a monoterpene. It is major component of the citrus. (S)-(-)-Limonene has been considered as generally recognized as safe (GRAS) for use as a food preservative that can be against gram- negative bacteria [5]. Geraniol is

an acyclic monoterpene alcohol with a rose-like fragrance. It is major component of Citronella oil, Rose oil and Palmarosa oil [6]. Geraniol has been also considered as GRAS [7] that can be against gram- negative bacteria. These essential oils caused membrane damage and membrane disruption, resulting in a loss of ions and cell contents, leading to death [8]. However, the using only essential oils were limited about strong unfavorable odor characteristic of essential oil and might be use at the high concentration. Thus, Essential oils were used in combination with natural preservatives to improve their antimicrobial activity. Nisin is a natural antimicrobial peptide. It has been considered as GRAS. Nisin can be against gram-positive bacteria by binding to lipid II and forming pores in the membrane but it has limited activity against gram-negative bacteria due to outer membrane of gram-negative bacteria prevents nisin from reaching lipid II. Previous research reported that the combination of Thyme essential oil at 0.6% and Nisin at 500 or 1000 IU/g showed a synergistic activity which decreased the population of *Listeria monocytogenes* in minced meat during storage at 4°C. below the official limit of the European Union [9]. Natamycin is a polyene natural antimycotic that can be against yeasts and molds. It has been considered as GRAS. The combination of Natamycin and Nisin was successful on the reduction of Lactobacilli and Lactococci populations in Galotyri cheese between days 14 and 28 of storage [10]. Therefore, the researchers speculated that binary, ternary and quaternary combination system probably an alternative preservative treatment against microorganism. This research aimed to determine the antimicrobial activities of (S)-(-)-Limonene and Geraniol combination with Nisin and Natamycin against *Escherichia coli* O157:H7.

MATERIAL AND METHODS

Material

(S)-(-)-Limonene ((S)-4-Isopropenyl-1-methyl cyclohexene) was purchased Madrid, Spain Geraniol was purchased from Sigma-Aldrich Corp., St. Louis, Mo., U.S.A. Nisin and Natamycin were purchased from Shandong Freda Biotechnology Co., Ltd., Linyi, Shandong, China. Ethylenediamine tetra acetic acid (EDTA) was purchased from Ajax Finechem Pty Ltd, Taren Point, New South Wales, Australia. Hydrochloric acid 37% Grade AR, Acetic Acid Glacial Grade AR and Sodium hydroxide pellets Grade AR were purchased from QReC®, New Zealand. Nisin stock solution was prepared by dissolving Nisin powder in 0.05 % Acetic acid in a volumetric flask and subsequently the solution was filtered by 0.45 µm syringe filter. Natamycin stock solution was prepared by dissolving Natamycin powder in 0.04N HCl then the solution was heated and subsequently cool down the heated solution. EDTA stock solution was prepared in deionized water in a volumetric flask. The pH was adjusted to pH 8 by Sodium hydroxide pellets in which EDTA was completely dissolved.

Tested microorganisms

Escherichia coli O157:H7 DMST12743 were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. Microorganism was activated in 50 mL of Tryptic soy broth (TSB; Difco, USA) and incubated at 37°C for 18 h before using.

Determination of the Minimum inhibitory concentration (MIC)

The MIC of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin against the test microorganism were determined using the method described in the DVG guidelines [11]. Different concentrations of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin were differently prepared by serial dilutions in the Trypticase Soy Broth (TSB). Essential oils dilution was prepared in TSB supplemented with 10% DMSO. Nisin was prepared in TSB supplemented with 500 µL of 40mM EDTA. Natamycin was prepared in TSB supplemented with 40mM EDTA (maximum volume 500 µL). Each tube was inoculated with 100 µL of bacterial suspension (1.0×10^6 CFU/mL). Two blank TSB tubes, with and without bacterial inoculation, were used as positive control and negative control. The bacteria-containing tubes were incubated at 37ºC for 24 h. After the incubation, the tubes were detected the

MICs by observation the visible of growth. MIC value was the first tube that showing no visible growth of bacteria.

Determination of the Minimum Bactericidal Concentration (MBC)

The MBC of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin against the test organism were determined using a series of steps, undertaken after a MIC test has been completed. One loop full of each tube was transferred on a Trypticase Soy Agar plate. The bacteria-containing plates were incubated at 37ºC for 24 h. After the incubation, MBC value was the first tube that showing no growth on Trypticase Soy Agar plate.

The Fractional Inhibitory Concentration Index (FIC_{index}) in binary and ternary combination system

The Fractional Inhibitory Concentration Index were determined using checkerboard assay according to [12] with some modification. The concentrations of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin were diluted to 0MIC, 1/4MIC, 1/2 MIC, MIC, 2MIC and 4MIC.

The FIC_{index} in binary combination system using the following Formula:

$$FIC_{index} = FIC_A + FIC_B \quad (1)$$

Where:

$$FIC_A = (MIC_{(A \text{ in the presence of } B)})/(MIC_{(A)})$$
$$FIC_B = (MIC_{(B \text{ in the presence of } A)})/(MIC_{(B)})$$

The FIC_{index} in ternary combination system using the following Formula [13]:

$$FIC_{index} = FIC_A + FIC_B + FIC_C \quad (2)$$

Where:

$$FIC_A = (MIC_{(A \text{ in the presence of B and C})})/(MIC_{(A)})$$
$$FIC_B = (MIC_{(B \text{ in the presence of A and C})})/(MIC_{(B)})$$
$$FIC_C = (MIC_{(C \text{ in the presence of A and B})})/(MIC_{(C)})$$

Among of the FIC_{index} calculated for all isoeffective combinations, the FIC_{index} of < 1 indicates synergism effects, = 1 indicates additive effects, > 1 indicates antagonism effect. [14]

The Fractional Inhibitory Concentration Index (FIC_{index}) in quaternary combination system

The Fractional Inhibitory Concentration Index were determined using checkerboard assay according to [12] with some modification. The concentrations of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin were diluted to 0MIC, 1/8MIC, 1/4 MIC, 1/2MIC, MIC and 2MIC.

The FIC_{index} in quaternary combination system using the following Formula:

$$FIC_{index} = FIC_A + FIC_B + FIC_C + FIC_D \quad (3)$$

Where:

$$FIC_A = (MIC_{(A \text{ in the presence of B, C and D})})/(MIC_{(A)})$$
$$FIC_B = (MIC_{(B \text{ in the presence of A, C and D})})/(MIC_{(B)})$$
$$FIC_C = (MIC_{(C \text{ in the presence of A, B and D})})/(MIC_{(C)})$$
$$FIC_D = (MIC_{(D \text{ in the presence of A, B and C})})/(MIC_{(D)})$$

Among of the FIC_{index} calculated for all isoeffective combinations, the FIC_{index} of < 1 indicates synergism effects, = 1 indicates additive effects, > 1 indicates antagonism effect.

RESULTS AND DISCUSSION

The MIC and MBC values of the essential oils, Nisin and Natamycin are presented in Table 1. The results demonstrated that the MIC of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin against *E. coli* O157:H7 was 8,500, 350, 4 and 8,700 mg/L, respectively. The MBC was 11,500, 400 and 110 mg/L, respectively. However, the MBC of Natamycin was higher than 70,000 mg/L due to natamycin insoluble at the high concentration.

Table 1 The Minimum Inhibitory Concentration and The Minimum Bactericidal Concentration of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin against *E. coli* O157:H7.

Substances	MIC (mg/L)	MBC (mg/L)
S-(-)-Limonene	8,500	11,500
Geraniol	350	400
Nisin	4	110
Natamycin	8,700	>70,000

The interaction of binary mixtures against *E. coli* O157:H7 are presented by isobolograms in Figure 1A-1F. On the isobolograms, it was found that the combination of S-(-)-Limonene/Geraniol, essential oils/Nisin, essential oils/Natamycin had a synergistic effect against *E. coli* O157:H7. It could be observed from the mixed MICs fall below the dashed line. While the combination of Nisin/Natamycin had an antagonistic effect. It can be observed from the mixed MICs above the dashed line.

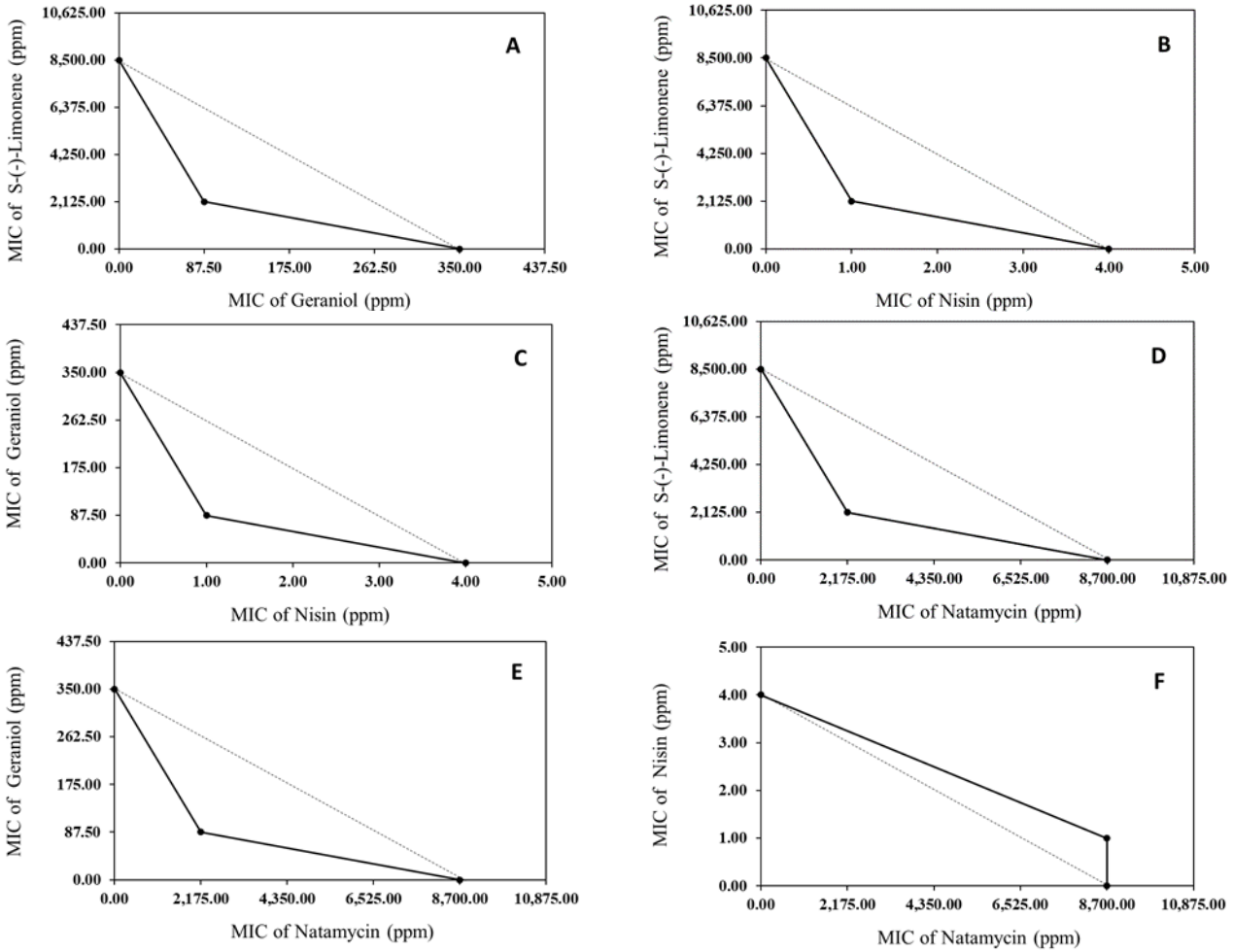

Figure 1 Isobolograms of combination system (S-(-)-Limonene/Geraniol; A, S-(-)-Limonene/Nisin; B, Geraniol/Nisin; C, S-(-)-Limonene/Natamycin; D, Geraniol/Natamycin; E, Nisin/Natamycin; F) against *E. coli* O157:H7.

Binary combination system was showed in Table 2. The Fractional Inhibitory Concentration Index (FIC_{index}) is commonly used to explain the effect of antimicrobial combinations which were correlated with the MIC isobolograms (Figure 1). All the combinations of essential oils with Nisin or Natamycin showed synergistic effect against *E. coli* O157:H7 with the FIC_{index} of 0.5, whereas the combination of Nisin with Natamycin showed an antagonistic effect against *E. coli* O157:H7 with the FIC_{index} of 1.25. In the present study, it was showed that essential oils with Nisin or Natamycin in the combinations system showed antimicrobial potential better than the use of essential oils or Nisin or Natamycin alone. The MICs of essential oils, Nisin and Natamycin were reduce when use essential oils with Nisin or Natamycin combination. In addition, the results showed that Natamycin could inhibit bacteria,

presumed to induce cell injury resulting in a lag phase extension [9].

Table 2 The Fractional Inhibitory Concentration Index (FIC_{index}) in binary combination system

System	FIC				FIC index
	L	G	Ni	Na	
L-G	0.25	0.25	-	-	0.5
L-Ni	0.25	-	0.25	-	0.5
G-Ni	-	0.25	0.25	-	0.5
L-Na	0.25	-	-	0.25	0.5
G-Na	-	0.25	-	0.25	0.5
Ni-Na	-	-	0.25	1	1.25

L= S-(-)-Limonene, G = Geraniol, N = Nisin, Na = Natamycin

Ternary combination system was showed in Table 3. The combination of essential oils with Nisin or Natamycin showed synergistic effect against *E. coli* O157:H7 with the FIC_{index} as 0.75. The combination of S-(-)-Limonene with Nisin and Natamycin also showed synergistic effect against *E. coli* O157:H7 with the FIC_{index} as 0.75, whereas the combination of Geraniol with Nisin and Natamycin showed an additive effect against *E. coli* O157:H7 with the FIC_{index} as 1.0.

Table 3 The Fractional Inhibitory Concentration Index (FIC_{index}) of ternary combination system

System	FIC				FIC index
	L	G	Ni	Na	
L-G-Ni	0.25	0.25	0.25	-	0.75
L-G-Na	0.25	0.25	-	0.25	0.75
L-Ni-Na	0.25	-	0.25	0.25	0.75
G-Ni-Na	-	0.25	0.25	0.5	1

L= S-(-)-Limonene, G = Geraniol, N = Nisin, Na = Natamycin

Quaternary combination system was showed in Table 4. The combination of essential oils with Nisin and Natamycin showed synergistic effect against *E. coli* O157:H7 with the FIC_{index} as 0.5. The MICs of (S)-(-)-Limonene, Geraniol, Nisin and Natamycin in the combinations system were 1,062.5, 43.75, 0.5 and 1,087.5 mg/L, respectively. The results were demonstrated that the combination of essential oils and food preservatives may lead to reduce the use of amounts of essential oil or food preservative compared with The MIC values of essential oils and food preservatives alone due to the mechanisms of these substances are achieved consist in multi target actions [15]. The advantage was reduced unfavourable odour characteristic of essential oil [4]. In a prospective study, the researchers have applied the combination of essential oils with Nisin and Natamycin in orange juice due to essential oils used in study that smell like orange juice. Furthermore, Nisin and Natamycin were widely used in fruit juices. the researchers speculated that the combination of essential oils with Nisin and Natamycin were highly inhibited *E. coli* O157:H7 in orange juice and increase the shelf life of orange juice lead to good quality orange juice.

Table 4 The Fractional Inhibitory Concentration Index (FIC_{index}) in quaternary combination system

System	FIC				FIC index
	L	G	Ni	Na	
L-G-Ni -Na	0.125	0.125	0.125	0.125	0.5

L= S-(-)-Limonene, G = Geraniol, N = Nisin, Na = Natamycin

CONCLUSIONS

This work has shown that (S)-(-)-Limonene, Geraniol, Nisin and Natamycin had antimicrobial activity against *E. coli* O157:H7. All the combinations of essential oils with Nisin or Natamycin in binary combination system showed synergistic effect, whereas the combination of Nisin with Natamycin showed an antagonistic effect against *E. coli* O157:H7. In addition, ternary combination system showed synergistic or additive effects and quaternary combination system showed synergistic effects Therefore, the combination of essential oils and food preservatives have been shown to reduce the use of amounts of essential oil or food preservative and enhances their antimicrobial activities can lead to safe food and it were able to preserve flavor and color providing a good quality food. Because the use of small amount of these substances.

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POSTER PRESENTATION

Development of plant-based minced meat from firm tofu and banana blossom

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ABSTRACT: Nowadays, plant-based meat is an alternative option for health-conscious consumers. However, the big challenges in plant-based meat production are the improvement of texture, color, flavor and nutritional value. Mostly, soy protein isolate and wheat gluten are used as the main ingredients of plant-based meat which, however, are low in fiber content. Tofu and banana blossom have been reported as good sources of nutrients including fiber. This study was aimed to develop a high fiber plant-based minced meat by using firm tofu (FTF) and boiled banana blossoms (BBB). The chemical compositions of FTF and BBB were determined prior to their combinations being employed to produce plant-based minced meat. Physicochemical properties and sensory quality of the products were evaluated. The results showed that the protein, carbohydrate, ash, fat and fiber content of FTF were 55.93, 33.75, 6.02 %, 4.30 and 1.14 % dwb, respectively, and the moisture content was 67.44%. BBB contained less protein but high fiber content compared to FTF. The protein, carbohydrate, ash, fat and fiber content of BBB were 10.97, 79.03, 8.94, 1.06 and 9.73 % dwb, respectively, and the moisture content was 88.70 %. The plant-based minced meat was formulated based on the different ratios of FTF to BBB at 22:38, 26:34, 30:30, 34:26, and 38:22. The results showed that a ratio of 26:34 gave the most acceptable product to consumers having an overall acceptance score of 6.15 (from 9.0). Moreover, it showed comparable texture and flavor scores compared to plant-based minced meat commercial product. The total-, soluble- and insoluble dietary fiber, protein content, and total phenolic content in the best formula were 21.84, 1.33, 20.51, 52.57 (%dwb), and 1.59 mg GAE/ g dwb, respectively which were higher than that of the commercial products being 5.69, 0.37, 5.32, 40.92% dwb, and 0.33 mg GAE/ g dwb, respectively. Therefore, this study demonstrates the potential of using a composite of FTF and BBB for the development of high fiber plant-based minced meat.

Keywords: *Plant-based meat, Firm tofu, Banana blossom, Dietary fiber*

INTRODUCTION

The plant-based food market is progressively growing to reach 8.72% per year recently [1] while the BIS research [2] estimated that the world plant-based food market is worth about $4.2 billion. The expansion of market is foreseen to 14% per year in 2024 whilst that in Thailand is to be about 10%.

One of the factors that influence the growth of the plant-based market is consumer awareness of more healthy food, especially under the Covid-19 pandemic. Consumers tend to choose safe food and reduce their intake of meat. Moreover, the global issue of climate change also has an impact on consumer behavior. It is known that plant-based meat production produces 30-90% less greenhouse gas emissions than meat processing [3]. Therefore, the consumption of plant-based meat is the solution to sustainable living.

The challenge of plant based-meat production is the consumer's perception including texture, taste, nutritional value, and price of the product. Plant-based meat ingredients are predominantly protein including soy protein isolate and wheat gluten which are

low in dietary fiber content. A study reported that dietary fiber supplementation in plant-based meat products increased product acceptance because it provides a range of benefits [4].

Dietary fiber is complex carbohydrates (polysaccharides) in plant cell walls. The total dietary fiber (TDF) can be divided into 2 types: soluble (SDF) and insoluble dietary fiber (IDF). SDF forms a gel in the intestines, resulting in slower intestinal absorption and digestion and also reducing the rate of sugar absorption. IDF does not have gel-forming properties but it helps stimulate the work of the intestines to have good compression [5] because it cannot be digested in the small intestine. For these reasons, it gives a positive effect on the reduction of colon cancer [6]. It is not only good for the digestive system but consumption of food products that are rich in dietary fiber can protect against diabetes and obesity [7].

Tofu is a result of the coagulation of soy milk. There are various types of tofu including soft tofu, tube tofu, firm tofu, and dried tofu [8]. It is an alternative protein source for commercial meat [9]. Moreover, tofu contains calcium, which helps nourish bones and prevent osteoporosis. It can reduce the risk of coronary heart disease and LDL-cholesterol. It is also low in calories but is rich in antioxidants that can prevent cancers. Therefore, tofu is a cheap healthy food that is easy to buy.

Banana blossom (*Musa acuminata*) is an inflorescence at the ends of bananas. They are covered with red leaf sheaths, stacked like lotus buds. In Asian countries, people consume banana blossoms as a vegetable. Banana blossoms can be eaten both cooked and raw [10]. Moreover, it is a good source of dietary fiber and biologically active compounds such as vitamin C, tannin, myoinositol phosphates, and alpha-tocopherol [11]. According to Acharya and colleagues [12], 100g of banana blossoms contain 5.4 g of dietary fiber and antioxidants such as phenolic and flavonoids.

Therefore, this study aimed to improve fiber content of plant-based minced meat by using tofu and banana blossoms. Consumers' acceptance and chemical compositions, as well as antioxidant activities were investigated.

MATERIAL AND METHODS

Soybean and raw wild banana (*Musa acuminate colla*) blossom were bought from the local market in Chiang Rai, Thailand. Coconut oil, yeast extract (Unilever Bestfoods, Thailand co., ltd.), beetroot powder (Global Vision House, Thailand co., ltd.), magnesium sulfate, citric acid, carrageenan, carboxymethyl cellulose, modified starch, and inulin were purchased from Krungthepchemi (Thailand co., ltd.). Boric acid, bromocresol green, ethanol, hydrochloric acid, methyl red, sodium hydroxide, sulfuric acid, copper sulfate, potassium sulfate, tris (hydroxymethyl) aminomethane (THAM), petroleum ether, sodium hydroxide, sulfuric acid, n-octanol, acetone, celite, gallic acid, sodium carbonate, and folin-ciocalteu reagent were purchased from Sigma Aldrich Corporation, USA. α-Amylase, protease, amyloglucosidase were bought from Megazyme (Ireland).

Firm tofu preparation

FTF preparation was conducted using a slightly modified method described by Molamma and colleagues [13]. Soybean was cleaned and soaked for 16 h and then drained prior to blending with water at a ratio of soybean to water at 1: 4 using a blender (Model HR211, Philips, 600W, Thailand) for 3 min. The puree was filtered with cheesecloth and squeezed to obtain the soy milk. Soy milk was boiled at 80-90 ℃ for 20 min with continued stirring. The 1% of magnesium sulfate solution was added to the hot soy milk and stirred for 5 min before cooling at room temperature for 20 min. The soy milk was precipitated and poured into the FTF mold and pressed for 1.30h.

Banana blossom preparation

Banana blossom preparation was conducted using a method described previously [14] with slight modification. Raw banana blossoms from the wild variety were cleaned and peeled until they obtained the white layer of banana blossoms. They were chopped into 3 mm thickness and soaked in a 1 L of 0.2 % citric acid solution for 30 min. The banana blossoms were drained from citric acid solution, blanched in hot water for 2 min and cooled at room temperature. The blanched banana blossom (BBB) was blended using an electric meat chopper (Model HR211, Philips, 600W, Thailand).

Fat analog preparation

Fat analog was prepared by using a method described previously [15]. The fat was prepared in 200 g by homogenizing the coconut oil, inulin, and water at the ratio of 38:19:43 at 24,000 rpm/min for 10 min using a homogenizer (IKA T25 digital ULTRA TURRAX, Bangkok, Thailand). The obtained fat was added with lecithin (2% of the total weight of fat analog). The fat analog was packed in a polyethylene bag and kept at 4 ℃ until used.

Plant-based minced meat preparation

The plant-based minced meat was prepared according to Table 1 with five different ratios of FTF and BBB. The plant-based minced meat was prepared according to Table 1 with five different ratios of FTF and BBB, 60% in total. The rest of the composition in the five samples contained 24 % cooled water, 12 % wheat gluten, 1 % modified starch, 0.6 % carboxymethylcellulose, 0.6% carrageenan, 0.4 % yeast extract, 0.4 % pepper, 0.3 % garlic powder, 0.2 % salt, 0.2 % fat analogue, and 0.2 % beetroot powder. All ingredients were mixed in a meat blender (Model HR211, Philips, 600W, Thailand) for 10 min or until all raw materials were homogeneous. The obtained mixture was molded in an aluminum tray and covered with aluminum foil. They were transferred to an electric oven (Model EO-19K, SHARP, Bangkok, Thailand), baked at 135 ℃ for 1 h, flipped, and continued baking for another 1 h. Then, the obtained mixture was cooled and transferred into a meat grinder machine (the die size is 7 mm). The obtained plant-based minced meat was packed in a polyethylene bag and kept at -18 ℃ until analysis.

Table 1 Formulation of plant-based minced meat with different ratios of FTF and BBB

Treatment	F1	F2	F3	F4	F5
FTF (%)	22	26	30	34	38
BBB (%)	38	34	30	26	22

Determination of physicochemical properties of FTF and BBB

Color determination was performed employing the method described by Dreher and co-workers [16] using a CIE L*a*b* system by colorimeter (Hunter Associates Laboratory, USA). L* indicated blackness (0) and whiteness (100), b* indicated red (positive) and green (negative), b* indicated yellow (positive) and blue (negative).

The chemical properties including moisture, protein, lipids, ash, crude fiber, and total carbohydrate content were carried out according to the American Association of Cereal Chemists approved methods of analysis (AACC International, 2012) [17]. Moisture content was conducted by adding 2 g samples in a moisture can and weighed accurately. The sample was then incubated in a hot air oven at 105 ℃ overnight (Method 44-15.0). Ash content was conducted by adding exact 2 g samples in a crucible. To the muffle furnace, the crucible was added and heated at 525 ℃ for 4 h (Method 08-01.01). Crude protein content analysis was conducted by placing a 2 g sample in a weighing paper and determining the crude protein content (% N x 6.5) using a Kjeldahl digestion and distillation system (Method 46-13.01). Lipids content was performed by using petroleum ether as a solvent in a Soxhlet extractor (Method 30-25.01). Crude fiber content was determined by weighing accurately 1 g samples in the crucible and placed in a crude fiber analyzer. After that, crucibles were dried in a hot air oven at 105 ℃ for 2 h and put in a

muffle furnace at 525 ℃ for 4 h (Method 32-10.01). Total carbohydrate content was carried out by subtracting 100 with moisture, ash, protein, and lipids content.

Determination of dietary fiber content

The determination of dietary fiber content was performed according to the method of Megazyme, AOAC Method 991.43 and 32-07.01 [18]. The 1.0005 g sample (duplicate; m1 and m2) was added to a 250 mL beaker. MES-TRIS blend buffer solution was included (40 mL) and stirred until the samples were completely dispersed in the solution. To the mixture, 50 µL heat-stable α-amylase was added and the beaker was covered with aluminum foil prior to incubation in a shaking water bath at 98-100 ℃ for 30 min. After that, the water bath temperature was decreased to 60 ℃ to cool down. The sidewall of the beaker was rinsed with distilled water and the solution was incubated with protease by adding 100 µL protease solution. The beaker was covered with aluminum foil and incubated in a shaking water bath at 60 ℃ for 30 min. To the beaker, 5 mL of 0.561 N HCl solution was added and adjusted pH to 4.1-4.8 by 5% NaOH or 5% HCl solution. Thereafter, 200 µL amyloglucosidase was added and covered with aluminum foil. The incubated solution was placed in the shaking water bath at 60 ℃ for 30 min.

Determination of IDF was performed by filtering the prepared samples solution through a crucible that contained 0.001 g celite into the receiving flask. The residue was washed twice with 10 mL pre-heated distilled water (70 ℃). The residue was washed in a crucible twice with 10 mL of 95% EtOH and acetone, respectively. The residue was dried in the crucible at 105 ℃ overnight and the weight was recorded. One residue was analyzed for protein and ash. The filtrate was poured into a 600 mL beaker, washed with water and used for further SDF determination.

Determination of SDF was performed by filtering the filtrate from described above through the duplicate crucibles containing 0.001 g celite into the receiving flask. The filtered solution was rinsed with 78 % EtOH and transferred all remaining particles into a crucible. The residue was washed with 78 % EtOH, 95 % EtOH, and acetone, respectively. Then it was dried and the protein and ash content were determined.

The result was calculated by using equation (1)

$$\text{Dietary fiber (\%)} = \frac{\frac{R_1+R_2}{2} - p - A - B}{\frac{m_{1+m_2}}{2}} \times 100 \qquad (1)$$

Where R1= residue weight 1 from m1; R2= residue weight 2 from m2; m1= sample weight 1; m2= sample weight 2; A = ash weight from R1; p = protein from R2; B = blank

Determination of total phenolic content (TPC)

The total phenolic content (TPC) determination was carried out by using a slightly modified method [19]. The sample was weighed 2 g in a centrifuge tube. Then, the sample was extracted by 20 mL of 80% v/v ethanol and centrifuged (Beckman Coulter) at 12,000 rpm/min for 20 min. The supernatant was collected, then 2.5 mL of Folin reagent and 2 mL of 7.5% sodium carbonate (Na_2CO_3) were added. The clear layer was incubated in a water bath at 30℃ for 1 h. The absorbance was measured at 760 nm using a spectrophotometer and compared with the. gallic acid (0-0.1 mg/mL) standard curve. The results were expressed as mg GAE/ g dwt.

Sensory evaluation

Sensory evaluation was divided into 2 parts. Sensory evaluation using a 9-point hedonic scale by 30 untrained panelists in terms of appearance, texture, color, odor, flavor, and overall acceptability was carried out to choose the best ratio of FTF and BBB. The 5 formulas of samples were placed in a plastic cup with a randomized 3-digit number to avoid bias. Panelists drank the water that was provided before testing the other formulas. Moreover, a consumer survey was conducted. A total of 100 random consumers were asked

to evaluate the sensory quality of chosen plant-based minced meat from the experiment and commercial plant-based minced meat available in the market.

Statistical analysis

All analyses were carried out in triplicate and evaluated by one-factor analysis of variance (ANOVA). The mean difference was compared between samples of $p < 0.05$ confidence significantly different using the STAR program (statistical tool for agricultural research software version STAR 2.0.1.)

RESULTS AND DISCUSSION

Physicochemical properties of FTF and BBB

Physicochemical properties of fresh FTF and BBB are presented in Table 2. It can be seen that the color L* of BBB was lower than FTF indicating the dark color in BBB compared to FTF as expected. This can be the result of the pigment in BBB as well as the browning reaction that occurred from exposure to oxygen during preparation even though the citric acid solution was used to prevent such reaction. The higher a* and b* values of BBB than FTF also designated the darker color. In addition, BBB contained high moisture content, TDF, and carbohydrate whereas the protein content of FTF was higher than BBB ($p < 0.05$). Waqas, et al [20] reported that FTF contained high protein content of 36.87±0.02% whereas Wickramarachchi, et al [21] reported a higher protein content of fresh banana blossoms compared to the current work. However, the discrepancy might be the result of a different variety of bananas employed.

Physicochemical properties of plant-based minced meat

The physicochemical properties of the plant-based minced meat are presented in Table 3. It was found that the L* value of F1 was lower than the other samples. This is because the highest content of BBB in this sample, the ratio of FTF to BBB being 22:38. Table 2 shows that lightness of BBB was lower than FTF. Accordingly, F3, F4, and F5 had a higher luminosity value than F1. Similar results were reported by Tasnim et al. [22] who found that an increased amount of banana blossom in a plain cake recipe resulted in a decrease in L* value from 43.68 to 42.04. The chemical compositions were reported on a dry weight basis (g/100 g). The commercial products contained the highest moisture content (70.50 %), while the moisture contents of all five formulations were not significantly different ($p < 0.05$). In terms of protein content, there was an increasing trend with an increasing amount of FTF and a decreasing amount of BBB. Among samples, F4 and F5 had the highest protein content of 55.18 and 56.87%, respectively. Commercial products had the lowest protein content (40.92%), lower than the protein contents of F1, F2, and F3. Sobhy et al. reported that increments of SPI level (5-20 %) in cookie formula significantly increased protein content. In addition, the total carbohydrate content was significantly decreased when the SPI level increased (5-20 %) [23]. The fat content of F1 and F5 were the lowest and highest, respectively. The results showed that an increase in fat content was attributed to the ratio of FTF being higher than BBB. The similar results were found by Abdullah et al. [24] that adding a higher amount of textured vegetable protein (TVP) to chickpea flour in plant-based nuggets increased fat content in the product.

Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) content

TDF content of all five formulations demonstrated a decreasing trend with decreasing amount of BBB and an increasing amount of FTF. As expected, the IDF was found to be higher compared to SDF for all plant-based minced meat formulations and commercial products (Table 3). The results are in line with the findings of Yesmin and Sankar [25] who reported the IDF and SDF content of the inner bracts of culinary banana blossom flowers being 61.86 and 4.36%, respectively.

Likewise, Bhaskar et al. [26] reported that the IDF and SDF content of banana inflorescence was 58.3% and 7.3%, respectively. The F1 and F2 formulations contained the highest TDF of 23.27 and 21.84%, respectively, and commercial products had the lowest TDF (5.69%). TDF, SDF, and IDF content. The TDF of F3 and F4 formulations were not significantly different ($p < 0.05$). The differences in TDF between samples could be attributed to the initial TDF of BBB and FTF used, where the BBB had an initial TDF of 66.22% which was greater than that of the FTF (0.3%).

Total phenolic content (TPC)

The analysis of TPC of plant-based minced meat products and commercial product are shown in Table 3. The TPC of samples demonstrated an increasing trend with an increased amount of BBB and decreased amount of FTF. Therefore, F1 and F2 had the highest total phenolic content being 1.64 and 1.59 (mg GAE/ g dwt), respectively. The commercial product had the lowest total phenolic content (0.33 mg GAE/ g dry weight). A previous study by Marikkar et al. [27], reported that regarding the antioxidant activity of banana blossom (Musa species) the total phenolic content of banana flower was 80.13 mg of GAE / g of extract. In addition, the FTF had a phenolic content lower than banana blossom. Total phenolic content in FTF was 60.02 mg GAE/ g dried weight. [28]

Table 2 Physical and chemical properties of FTF and BBB

Composites	FTF (%)	Blanched banana blossom (%)
L*	75.34±12.96	65.95±9.47
a*	1.53±0.23	2.58±1.12
b*	15.06±2.70	16.27±5.99
Moisture (%dwb)	67.44±0.12	88.70±0.66
Crude protein (%dwb)	55.93±0.51	10.97±0.01
Crude fat (%dwb)	4.30±0.14	1.06±0.39
Crude fiber (%dwb)	1.14±0.05	9.73±0.27
Ash (%dwb)	6.02±0.43	8.94±0.05
Total carbohydrate (%dwb)	33.75±0.93	79.03±0.29

All values are the mean standard deviation of the triplicate sample.

Table 3 Physical and chemical properties of plant-based minced meat from different ratios of FTF and BBB and commercial product

Component	F1	F2	F3	F4	F5	Commercial product
L*	46.01±2.04^{d}	51.10±0.97^{c}	56.83±3.05^{b}	57.48±0.43^{ab}	61.81±0.80^{a}	60.13±0.40^{ab}
a*	6.36±0.33^{b}	5.46±0.14^{c}	4.77±0.05^{c}	4.02±0.14^{d}	3.86±0.11^{d}	11.59±0.52^{a}
b*	9.24±0.07^{c}	11.28±0.59^{bc}	13.57±1.82^{b}	12.32±0.13^{b}	12.83±0.28^{b}	15.90±0.67^{a}
Moisture (%)	62.94±0.46^{b}	61.91±0.57^{bc}	61.31±0.46^{bc}	61.11±073^{bc}	59.06±0.82^{bc}	70.50±1.09^{a}
Protein (%dwb)	51.38±0.58^{c}	52.57±1.69^{c}	53.40±0.28^{bc}	55.18±0.70^{ab}	56.87±0.73^{a}	40.92±0.55^{d}
Fat (%dwb)	0.53±0.02^{d}	0.76±0.05^{c}	0.78±0.05^{c}	0.95±0.08^{bc}	1.34±0.05^{b}	23.19±0.22^{a}
Ash (%dwb)	3.89±0.01^{bc}	4.23±0.03^{b}	4.39±0.03^{b}	4.79±0.09^{ab}	4.88±0.38^{ab}	5.59±0.02^{a}
Carbohydrate (%dwb)	44.39±0.21^{a}	42.44±0.10^{ab}	41.43±0.20^{ab}	39.08±0.32^{b}	36.91±0.47^{c}	30.31±0.25^{e}
IDF (%dwb)	21.94±0.15^{a}	20.51±0.74^{a}	18.01±0.25^{b}	16.86±0.32^{bc}	15.29±0.78^{c}	5.32±0.29^{d}
SDF (%dwb)	1.60±0.12^{a}	1.33±0.04^{ab}	1.06±0.04^{bc}	1.04±0.08^{bc}	0.95±0.11^{c}	0.37±0.03^{d}
TDF (%dwb)	23.27±0.19^{a}	21.84±0.78^{ab}	19.07±0.29^{c}	17.40±0.46^{cd}	15.74±1.45^{d}	5.69±0.32^{e}
TPC (mg GAE/ g dwt)	1.64±0.04^{a}	1.59±0.02^{a}	1.44±0.04^{b}	1.32±0.10^{bc}	1.16±0.01^{c}	0.33±0.00^{d}

All values are the mean standard deviation of the triplicate sample.
Means within a row with different letters are significantly different ($p < 0.05$).

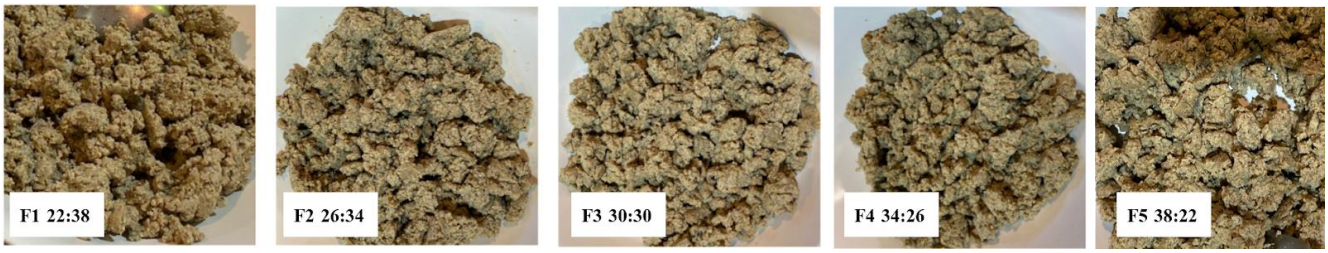

Figure 1 Plant-based minced meat 5 samples (**F1**, FTF 22% and banana blossom 38%; **F2**, FTF 26% and banana blossom 34%; **F3** FTF 30% and banana blossom 30%; **F4**, FTF 34% and banana blossom 26%; **F5**, FTF 38% and banana blossom 22%)

Table 4 Sensory evaluation of plant-based minced meat samples and consumer test of the chosen sample compared to commercial product

Attributes	F1	F2	F3	F4	F5	Consumer test	
						Commercial product	F2
Appearance	5.63±1.10^{b}	6.57±1.61^{a}	6.43±1.50^{a}	6.57±1.57^{a}	6.93±1.53^{a}	7.83±1.66	6.01±1.71***
Texture	6.37±1.47^{ab}	6.87±1.41^{a}	5.07±1.55^{c}	5.27±1.82^{c}	5.80±1.61^{bc}	6.78±1.53	6.54±1.55
Color	4.90±1.41^{cd}	6.70±1.75^{a}	5.87±1.56^{bc}	6.30±1.56^{b}	4.76±1.61^{cd}	6.67±1.87	5.63±1.95**
Odor	6.53±1.61^{a}	6.83±1.42^{a}	6.07±1.41^{ab}	5.67±1.61^{b}	6.20±1.71^{ab}	6.45±2.09	5.94±2.01**
Flavor	6.77±1.43^{a}	6.97±1.43^{a}	5.90±1.56^{b}	5.73±1.72^{b}	6.20±1.79^{ab}	6.51±2.15	6.29±2.05
Overall	7.10±1.32^{a}	7.07±1.46^{a}	5.63±1.52^{b}	5.93±1.66^{b}	6.27±1.50^{b}	7.01±1.63	6.15±1.53***
Acceptance (%)						100	73

All values are mean ± standard deviation.
Means within a row with different letters are significantly different ($p < 0.05$).
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Sensory evaluation

Sensory evaluation of the plant-based minced meat produced from FTF and BBB was carried out. The results showed that the ratio of FTF to BBB at 26:34 was the most acceptable treatment by panelists in terms of appearance, color, smell, taste, texture, and overall preference as shown in Table 4. This might be partially due to the firm texture and low soybean smell as described by panelists (data not shown). Increasing the amount of FTF resulted in lower scores on various sensory attributes. The same results were reported by Akter et al. [29]. It was demonstrated that increasing amounts of soy flour in the cake resulted in the reduction of the taste score from 8.2 to 5.4.

The consumer acceptability on plant-based minced meat from the final product was determined and compared with a commercial product (Table 4). The sensory scores of the texture and flavor were not significantly different between the commercial product compared to the F2 sample. However, the score of appearance, color, taste, and overall acceptability of commercial product were moderately higher than that of the samples.

CONCLUSIONS

The results of this study showed that FTF and BBB had the potential to be used as ingredients for the development of plant-based minced meat. The 26% FTF and 34% BBB was the most preferred formulation. More importantly, all samples in the experiment had lower fat content whereas the protein, TDF, SDF, IDF and TPC were higher compared to the commercial product. Therefore, the cooperation of FTF and BBB is a promising option for the production of even healthier plant-based minced meat compared to the product currently available in the market.

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Influence of phenolic from lime peel on xyloglucan-chitosan film properties

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ABSTRACT: This research aimed to study the quantity of total phenolic compounds from dried lime peel using water solvent extraction method. The amount of the total phenolic compounds was 117.38 milligrams per gram dried lime peel. The ratio of phenolic content and some properties of xyloglucan-chitosan film with phenolic content at 0.25%, 0.50%, 0.75%, and 1.00% concentration to mixing were studied. The hydroxyl group indicating the functional group of phenol and the complex between aldehyde group of xyloglucan and amino group of chitosan were investigated using Fourier Transform Infrared Spectroscopy (FTIR), where peak time in the period of wavenumbers 3362.45 to 3368.63 cm^{-1} and 1637.96 to 1640.02 cm^{-1} were found, respectively. This complex compound of xyloglucan-chitosan was not affected by the amount of phenolic content. Furthermore, xyloglucan-chitosan film with 0.25% phenolic content has the best tensile strength and elongation at break. On the other hand, the increasing amount of phenolic content was inverse with the tensile strength, elongation at break, and water activity. However, the ratio of phenolic content did not affect the water vapor permeability rate. The antimicrobial activity by disc diffusion method found that the ratio of phenolic content at 1.00% concentration was resistant to *Escherichia coli* and *Staphylococcus aureus.*

Keywords: Xyloglucan-chitosan film, Lime peel, Phenolic content, Water solvent extraction

INTRODUCTION

Xyloglucan is composed of three monosaccharides, which are glucose, xylose, and galactose, in a ratio of 3:2.5:1, with (1→4) β-D-glucan and (1→6)-α-D-xylose branch and partially substituted (1→2)-β-D-galactose [7, 17]. Xyloglucan has high water holding capacity and good water solubility. It also has good resistance to heat and pH and shear strength. Therefore, xyloglucan has been widely used in various industries. In the food industry, xyloglucan has been used as a thickener, stabilizer, gelling agent [5] due to its compatibility, bioavailability and film forming ability. According to a study by Adair and Narkrugsa [1], xyloglucan from tamarind kernel powder was mixed with chitosan to

form Chitam, a complex compound formed by covalent bonds between aldehyde group of xyloglucan and amino group of chitosan. The gel was found to be strong and flexible with good antimicrobial ability and can be used as a food ingredient [17]. In this study, phenolic compounds from lime peel were mixed in xyloglucan-chitosan to enhance their antimicrobial properties.

Phenolic compounds are secondary compounds that can be found naturally in a wide variety of plants such as lime, orange, grape, turmeric, pepper, tea and cocoa. The structure of phenolic compounds has a hydroxyl group attached to a benzene ring [11, 18]. The lime peel contains pectin, polyphenols, vitamins, essential oils, dietary fiber, etc. Substances in lime peel include tannins, flavonoids, limonene, sabinene and myrcene [12, 15], etc. These phenolic compounds have antioxidant properties [3], with a mechanism that can inhibit the free radical reaction chain [4]. They also have antimicrobial activity that inhibits the growth of bacteria by destructing the bacteria cell wall, cytoplasm and membrane proteins [9]. In addition, they can also be used as a food additives to prevent rancidity, fatty acid oxidation and preserve food, etc. A study by Riaz et al. (2018), which investigated the incorporation of apple peel polyphenols (APP) with chitosan to improve the antioxidant and antimicrobial properties of packaging films, found that CS-APP films were resistant to microorganisms such as *E. coli, B. cereus, S. aureus* and *S. typhimurium*, and can also control microbial growth, color change, maintain storage quality and extend shelf life of food. It can be seen that mixed phenolic compounds with xyloglucan-chitosan film can enhance the film properties. Therefore, this study studied the effects of phenolic compounds on the antimicrobial properties of xyloglucan-chitosan film using water as solvent in the extraction process and the optimum content of phenolic compounds for xyloglucan-chitosan film was also determined.

MATERIAL AND METHODS

Materials

Lime (Citrus aurantifolia Swingle) was obtained from local farmers around Prachinburi province. Chitosan (degree of deacetylation > 90%, molecular weight of $4.0x10^5$) was purchased from Bio21, Thailand. Jellose was purchased from Pinpetch, Thailand.

Chemicals and Reagents

Glycerol was purchased from Leonid Chemicals, India. Acetic acid was purchased from Qrec, Newzealand. Sodium carbonate, Folin-Ciocalteu reagent and Sodium chloride were purchased from CARLO ERBA Reagents, France. Nutrient Agar (NA) and Nutrient Broth (NB) were purchased from HiMedia Laboratories, India.

Preparation and Extraction of Lime Peel

Limes were washed and peeled. The washed lime peels were dried at 65°C for 6-8 h in a tray dryer (Progress, Thailand) and then ground in a food processor. The dried lime peel powder was collected in sealed bags.

10 g sample of dried lime peel powder was extracted with 100 ml of water. The extraction was performed using water as a solvent at 60 °C for 30 min. The mixture was then filtered by vacuum pump using Whatman No. 4 filter paper. The resulting solution is evaporated using a rotary evaporator (Rotavapor® R-300, BUCHI, Thailand) to remove the excess solvent and increase the solution's concentration.

Total Phenolic Content

Total phenolic content was analyzed by the Folin-Ciocalteu method. 0.2 ml of dried lime peel extract into 0.5 ml of 10% Folin-Ciocalteu solution and 2 ml of 7.5% of sodium carbonate (Na_2CO_3) solution were then added to the test tube and vigorously vortexed. The solution was incubated in darkness for 30 min. The absorbance of the solution was recorded

at 750 nm using a Visible Spectrophotometer (SP-880, Metertech, Taiwan). The amount of total phenolic compound was calculated using the standard curve of gallic acid.

Film Preparation

In the preparation of xyloglucan-chitosan film, the solution was divided into two parts. A solution of xyloglucan was prepared by dissolving 1 g of xyloglucan in 10 ml of hot distilled water at 85 °C with 15 min stirring using a glass rod until xyloglucan is completely dissolved. 25 ml of 4% acetic acid (v/v) was added and stirred at room temperature for 30 min using a Digital Dual-Range Mixer at 220 rpm. For a solution of chitosan, 0.25 g of chitosan was dissolved in 25 ml of 4% acetic acid (v/v) and then stirred using Bamix Hand Blender for 2 min. Both solutions were combined and stirred using a Digital Dual-Range Mixer at a speed of 220 rpm for 15 min. 35% (w/w) glycerol (weight of total solids in solution) was added and the solution was stirred using a Digital Dual-Range Mixer. Phenolic compounds were added at the ratio of 0.25%, 0.50%, 0.75% and 1.00%, and the mixture was stirred for 15 min using a Digital Dual-Range Mixer at 220 rpm. The mixture was poured onto 10x12 cm acrylic sheet for forming a film.

Properties of xyloglucan-chitosan-phenolic mixed film

Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups of xyloglucan-chitosan and xyloglucan-chitosan-phenolic mixed were characterized by Fourier Transform Infrared Spectroscopy (FTIR) (Alpha II, Bruker, Germany). The spectra were recorded in 4000-400 cm^{-1} wavenumber range.

Tensile Strength and Elongation at Break

Tensile strength and elongation at break of the films were analyzed in accordance with ASTM D882, a standard for tensile testing of films and thin plastics 1 mm thick. The film sample was cut to a size of 2x4 cm and analyzed by texture analyzer (TA.XT Plus, Satable Micro systems, UK) with A/TG probe.

Water Activity (Aw)

Water activity in xyloglucan-chitosan-phenolic mixed film was analyzed by Water Activity Meter (4TE, Aqua lab, USA) calibrated with distilled water. The sample film was cut and placed in the test cup for measurement.

Water Vapor Transmission Rate (WVTR)

In this study, water vapor transmission rate analysis has adapted a method reported by Riaz et al. (2018). The film was cut to 12 cm diameter and placed in the test cup. One side of the specimen was exposed to high humidity, while the other side was exposed to low humidity (containing silica gel). Weight changes were recorded at 1 h interval during the first 4 h and after 24 h. The slope of weight loss and time was generated and the WVTR was calculated as follows the equation:

$$WVTR = slope/film\ area \quad (1)$$

Where slope is the slope of the graph of the relationship between weight loss and time, film area is an area of the film.

Antimicrobial Activity

Disc diffusion method (adapted from CLSI, 2009) was used to analyze the antimicrobial activity of xyloglucan-chitosan-phenolic mixed against *Escherichia coli* AIKL 1070 and *Staphylococcus aureus* AIKL 1058. One colony from the petri dish was inoculated in Nutrient Broth (NB) and incubated at 37 ºC for 4-5 h. The turbidity of the culture was adjusted with 0.85% sodium chloride to match the 0.5 McFarland standard. A sterile cotton ball was dipped into the culture and then smeared over the Nutrient Agar (NA) surface. A paper disc containing of xyloglucan-chitosan-phenolic mixed was placed on NA surface using sterile forceps and incubated at 37 °C for 24 h. The results were

observed and recorded using a vernier caliper to measure the diameter of inhibition zone.

RESULTS AND DISCUSSION

Total phenolic content was analyzed by the Folin-Ciocalteu method. For the analysis principle of phenolic compounds, total phenolic compounds react with Folin-Ciocalteu reagent, containing phosphomolybdic-phosphotungstic acid. This compound is reduced by phenolic hydroxyl groups of total phenolic compounds to form tungsten and molybdenum blue, which give a blue color and show absorption spectrum at a wavelength of 750 nm and compared with the standard curve of gallic acid. In this study, 10 g of dried lime peel powder in 100 ml of distilled water at 60°C for 30 min showed the amount of total phenolic content of 117.38 mg/g dry basis.

Properties of xyloglucan-chitosan-phenolic mixed film

Fourier Transform Infrared Spectroscopy (FTIR)

According to FTIR analysis, xyloglucan-chitosan-phenolic mixed film showed peaks in the period of wavenumber 3362.45 to 3368.63 cm^{-1} indicating hydroxyl group (R-OH) of phenol. This indicates that phenolic compounds were produced in xyloglucan-chitosan film. In addition, peaks in the period of wavenumber 1637.96 to 1640.02 cm^{-1} corresponded to aliphatic secondary amides (R-CO-NH-R'), suggesting that with 4% acetic acid, glycosidic bonds of xyloglucan were hydrolyzed by acid (acid hydrolysis). This resulted in opening of xyloglucan ring at C-1 hydroxyl group (-OH) and aldehyde group (-CHO) was formed. The complex of xyloglucan-chitosan formed by covalent bonds between aldehyde group of xyloglucan and amino group of chitosan [17]. After addition of phenolic into xyloglucan-chitosan film, no significant wavelength shifts were observed, indicating that no covalent bonds between phenolic and xyloglucan-chitosan were detected [13].

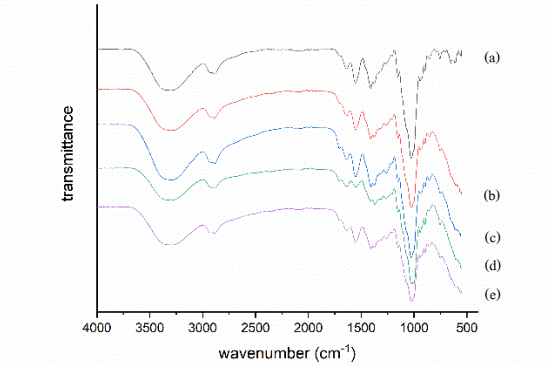

Figure 1 FTIR spectra of (a) xyloglucan-chitosan film (b) xyloglucan-chitosan-phenolic 0.25% mixed film (c) xyloglucan-chitosan-phenolic 0.50% mixed film (d) xyloglucan-chitosan-phenolic 0.75% mixed film (e) xyloglucan-chitosan-phenolic 1.00% mixed film

Tensile Strength and Elongation at Break

Tensile test of xyloglucan-chitosan-phenolic mixed films at different phenolic content using Texture Analyzer showed that the increase in phenolic content in xyloglucan-chitosan film had a statistically significant effect on elongation at break of xyloglucan-chitosan-phenolic mixed film ($p \leq 0.05$). The films with phenolic content of 0.50%, 0.75% and 1.00% showed no statistically significant difference in tensile strength ($p > 0.05$). Similarly, xyloglucan-chitosan-phenolic mixed film with phenolic content of 0.25% showed no statistically significant difference in elongation at break ($p > 0.05$). However, increasing phenolic content in xyloglucan-chitosan film resulted in a statistically significant increase in elongation at break of the film ($p \leq 0.05$). The films with phenolic content of 0.50%, 0.75% and 1.00% showed no statistically significant difference in elongation at break ($p > 0.05$), while the films with phenolic content of 0.25% and the control showed no statistically significant difference in elongation at break compared to other films ($p \leq 0.05$).

Table 1 Tensile Strength and Elongation at Break of xyloglucan-chitosan-phenolic mixed films

Ratio of phenolic	Tensile strength (g)	Elongation at break (mm)
0%	3155.60±1252.92[b]	25.59±0.00[a]
0.25%	2922.50±690.58[b]	25.61±0.04[b]
0.50%	1610.90±826.54[a]	30.80±0.00[c]
0.75%	1312.70±549.79[a]	30.80±0.00[c]
1.00%	989.46±1169.60[a]	30.80±0.00[c]

Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

This is because some phenolic compounds are fatty acids that can penetrate into the film structures [6,8], causing the film to lose its mechanical properties, resulting in reduced tensile strength and elongation at break. The effects of addition of phenolic extract from lime peel on the xyloglucan-chitosan film showed the same trend.

Water Activity (Aw)

Water activity analysis of xyloglucan-chitosan-phenolic mixed films revealed that the increase in phenolic content had no significant effects on water activity of xyloglucan-chitosan-phenolic mixed films ($p>0.05$). This is because water was used as a solvent in extraction of phenolic compounds. The extracted phenolic compounds were then evaporated using a rotary evaporator and water-free phenolic compounds were obtained. Therefore, the increase in phenolic content in xyloglucan-chitosan film did not affect the water activity, which is an important factor determining shelf life and food safety. From Table 2, it can be observed that the water activity of the xyloglucan-chitosan-phenolic mixed films were lower than 0.6 [10], which is unsuitable for microbial growth.

Table 2 Water activity of xyloglucan-chitosan-phenolic mixed films

Ratio of phenolic	Water activity (Aw)
0%	0.4117±0.01[a]
0.25%	0.4109±0.41[a]
0.50%	0.4105±0.00[a]
0.75%	0.4101±0.01[a]
1.00%	0.4115±0.01[a]

Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

Water Vapor Transmission Rate (WVTR)

Preventing food from exposure to water vapor is one of the main functions of food packaging films to extend shelf life and slow down food spoilage [2]. From the results of the water vapor transmission analysis of the xyloglucan-chitosan-phenolic mixed film, it was found that increasing the phenolic content did not result in a statistically significant difference in the water activity of the film produced ($p>0.05$). The increase in water vapor transmission may be due to an increase in the thickness and density of xyloglucan-chitosan [14, 19]. In the film production process, film mold of the same width and height were used, so it did not affect the water vapor transmission rate of the xyloglucan-chitosan-phenolic mixed film with different phenolic contents.

Table 3 Water Vapor Transmission Rate of xyloglucan-chitosan-phenolic mixed films

Ratio of phenolic	WVTR (x 10^{-11} gm^{-1} s^{-1} Pa^{-1})
0%	14.3313±0.64[a]
0.25%	13.7474±0.37[a]
0.50%	13.4820±0.11[a]
0.75%	13.2516±0.35[a]
1.00%	13.7208±0.86[a]

Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

Antimicrobial Activity

The antimicrobial ability against *Escherichia coli* and *Staphylococcus aureus* of xyloglucan-chitosan-phenolic mixed film with the phenolic content of 0.25%, 0.50%, 0.75% and 1.00% were studied. It was found that xyloglucan-chitosan film with 1.00% phenolic content had the highest antimicrobial ability. The diameters of clear zone of *Escherichia coli* and *Staphylococcus aureus* were 5.08 mm and 4.10 mm, respectively. The comparison of antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* revealed that xyloglucan-chitosan-phenolic mixed film had higher antimicrobial activity against *Escherichia coli* than *Staphylococcus aureus*. This indicates that the films had good antimicrobial ability due to the formation of complexes between the aldehyde group of xyloglucan and the amino group of chitosan. Phenolic compounds have a mechanism for destroying the bacteria cell wall membrane, cytoplasmic membrane and membrane protein [9]. Therefore, the film can be used as an antimicrobial packaging film to improve and extend the shelf life of food.

Table 4 Inhibition zone of xyloglucan-chitosan-phenolic mixed

Ratio of phenolic	Inhibition zone diameter (mm)	
	E. coli	*S. aureus*
0%	1.18	1.80
0.25%	3.31	2.90
0.50%	3.41	3.67
0.75%	4.62	4.05
1.00%	5.08	4.10

Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

CONCLUSIONS

Lime peels are waste from lime processing. Lime peel contains phenolic compounds and has good antimicrobial activity. In this study, the influence of phenolic compounds on the properties of xyloglucan-chitosan film was studied. The FTIR analysis revealed peaks in the period of wavenumber 3362.45 to 3368.63 cm^{-1}, indicating hydroxyl group of phenol in xyloglucan-chitosan. Increased phenolic content in the film reduced its tensile strength and elongation at break, but did not statistically affect the water activity and water vapor transmission rate of the xyloglucan-chitosan-phenolic mixed film. It also showed good antimicrobial activity when the phenolic compounds were added at a ratio of 1.00%, indicating that this xyloglucan-chitosan-phenolic mixed film can be further developed into a packaging film for extending the shelf life of food.

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Quality changes of fingerroot (*Boesenbergia rotunda* (L.) Mansf.) juice mixed with honey and lime juice as affected by high pressure processing

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ABSTRACT: The effect of high pressure processing (HPP) on quality changes and antioxidant activity of fingerroot juice mixed with honey and lime juice was studied during storage at 5±1°C. The mixture 100 g of fingerroot juice and 200 mL of water (1:2) was prepared and mixed with honey and lime juice. Sample was treated under different pressure including 200, 400 and 600 MPa for 5 min. The results were compared to conventional heat treatment (CHT) at 90◦C for 5 min. The results showed that higher amount of total phenolic content (TPC), DPPH and FRAP were observed in samples treated under HPP at 600 MPa for 5 min compared to CHT and the value were 34.74±0.02 mg GAE/100 mL, 80.01±0.12 µmol Trolox /100 mL and 381.02±0.05 µmol of $FeSO_4$/100 mL, respectively. The pH value and total soluble solids (TSS) of sample treated with this HPP condition was about 3.58±0.02 and 15.00±0.05◦Brix, respectively. Moreover, aerobic plate count (APC) and yeast and mold (YM) were not detected in this sample. Thus, this condition was selected for further study. Quality changes of HPP treated sample during 12 days storage at 5±1°C were compared to CHT and untreated sample (fresh juice). Sampling was done every 2 days. The results showed that HPP significantly maintained the TPC and antioxidant activity of both DPPH (78.81±0.09 to 80.01±0.05 µmol Trolox/100 mL) and FRAP (349.00±0.05 to 381.02±0.21 µmol of $FeSO_4$/100 mL), during storage time when compared to CHT. In addition, APC and YM were not detected in HPP treated and CHT sample while untreated sample (fresh juice) had 4.88±0.06 log CFU/mL of APC and 2.97±0.10 log CFU/mL of YM at the end of storage time. From this experiment, HPP treatment at 600 MPa for 5 min was recommended for preserving freshness and antioxidant activity as well as safety of fingerroot juice mixed with lime and honey during cold storage.

Keywords: *Antioxidant activity*, *Fingerroot*, *High hydrostatic pressure*

INTRODUCTION

Nowadays, healthy food and organic medicinal products have become worldwide increasingly popular. Medicinal plants can be defined as the plants that possess therapeutic properties or provide beneficial pharmacological effect on the human or animal body. Several bioactive agents can act as antioxidants that are linked to prevent several diseases as well as provide anti-oxidative and anti-inflammation activity [1]. They are used worldwide as an alternative and/or a complementary medicine.

Fingerroot (*Boesenbergia rotunda* (L.) Mansf.) is a native plant of the tropical areas, particularly in South and Southeast Asia as well as China. A common English name is

fingerroot since the rhizome is finger look-like appearance. It is also called ao chun jiang in Chinese; temu kunci in Indonesian; gajutu in Japanese; khchiey in Khmer; neng kieng in Lao; temu kunci in Malay; cu ngai in Vietnamese and krachai in Thai. It is one of local edible and culinary herbs that are reported to provide several medicinal properties and be used as natural ingredient in Thai food. Anti-allergic, antibacterial, anticancer, anti-inflammatory, antioxidant, and antiulcer activities as well as wound healing are reported as a medicinal property of fingerroot [2]. Recently, fingerroot juice as an herbal drink made by mixing the fingerroot extract with honey and lime juice becomes popular due to its health benefit. However, freshly prepare is recommended for fingerroot juice since the conventional thermal process including pasteurization, UHT and sterilization destroy bioactive compounds in the juice. Alternative technology such as high pressure processing should be applied for convenience, shelf life extension and preserving functional properties of fingerroot juice.

High pressure processing (HPP) or high hydrostatic pressure is a novel non-thermal technology capable of extending shelf life, inactivating harmful microorganisms and enzymes while maintaining freshness, sensorial and nutritional quality of food products. A pressure between 100 to 1,200 MPa [3] is commonly transmitted to food products packed in a flexible container by the water as a liquid medium contained inside the HPP chamber. The effect of HPP on quality of food products primary focused on microbial food safety, physical changes in food components and food nutrition as well as functionality. Apart from the safety purpose, HPP was reported to use in various commercial fruit and vegetable products to maintain and/or improve nutritional qualities. The pressure at 600 MPa for 4 min could slow down the degradation rate of ascorbic acid in orange juice [4] and at the same pressure was applied to 'Nanglae' pineapple juice for 5 min [5] could obtain the same trend. In addition, low intensity of HPP at 100 MPa for 10 min was applied to persimmons resulted in increasing total phenolic content [6] and different HPP conditions (pressure: 100, 350 and 600 MPa; temperature: 17-34◦C; 5 min) can differently affect bioactive compounds of prickly pears [7]. Therefore, this study aimed to clarify the effect of HPP on quality changes and antioxidant activity in fingerroot juice mixed with lime and honey during storage at 5±1°C.

MATERIAL AND METHODS

Chemicals and reagents*:* All chemicals and reagents used were analytical grade or above. Folin-Ciocalteu reagents, gallic acid, Trolox (6-hydroxy2,5,7,8-tetramethyl-chroman-2-carboxylic acid) and methanol (HPLC-grade) were purchased from Fluka (Buchs, Switzerland). DPPH (2,2-diphenyl-1-picryhydrazyl) were purchased from Aldrich (Steinheim, Germany). TPTZ (2,4,6-tris (2-pyridyl)-s-triazine) and ferric chloride ($FeCl_3$) were purchased from Ajax Finechem (Seven Hills, Australia). Plate count agar (PCA), potato dextrose agar (PDA) and Peptone were purchased from Merck (Darmstadt, Germany). Water used in this experiment was purified with a Milli-Q water purification system (Millipore, Bedford, USA).

Sample preparation*:* Fingerroot (*Boesenbergia rotunda* (L.) Mansf.) was bought from local market in Chiang Rai, Thailand, immediately transported to the laboratory and stored at 4 ˚C until use. Fingerroot was washed and blended with water in the ratio of 1:2 (fingerroot : water). Mixed sample was then filtered using cheesecloth and the extracted juice was further mixed with honey (30 mL) and lime juice (20 mL). The sample was packed in polyethylene terephthalate (PET) bottle prior to HPP treatment.

HPP treatment: The suitable condition of HPP was first clarified. Sample was treated under different pressure at 200, 400 and 600 MPa for 5 min by a high pressure apparatus (Bao Tou KeFa High Pressure Technology Co., Ltd., China) with a capacity of 3.0 L at room temperature (25°C). Reverse osmosis (RO) water was used as the pressure transmitting fluid. The treatment time in this study did not include the time of increasing and releasing pressure. Conventional heat treatment (CHT) at 90 ºC for 5 min was also applied to the sample. Fresh mixed juice was used as a control since it is a representative of commercial product. Quality changes after treatment were compared among HPP treated, CHT and control. The suitable HPP condition was then selected to study the quality changes of fingerroot juice mixed with honey and lime juice during storage at 5±1 °C. Sampling was done every 2 days until 12 days of storage.

Determination of color parameter, total soluble solid and pH: Color (L^*, a^* and b^*) was measured using CIE colorimeter (Hunter Associates Laboratories, Inc., Reston, VA, USA). Total soluble solid (TSS) was measured using digital hand-held refractometer (ATAGO, Japan) and reported as ˚Brix. The pH was measured using pH meter (Binder, Scientific Promotion Co., Ltd.). All samples were performed in triplicate.

Determination of total phenolic content: Total phenolic content (TPC) was determined according to [8] with some modifications. Sample 250 µL was mixed with 1,250 µL of 10% v/v Folin-Cioculteu phenol's reagent and 1,000 µL of 7.5% w/v of sodium carbonate. The mixture was incubated at room temperature (25°C) for 60 min. Then, the absorbance was measured using a UV-VIS Spectrophotometer (V-630, Jasco, Tokyo, Japan) at 765 nm, and water was used as a blank. The TPC was reported as the mg Gallic acid equivalent (GAE) per 100 mL of sample.

Determination of DPPH radical scavenging activity: DPPH radical scavenging activity assay was determined according to [9] with some modifications. Sample 50 µl was mixed with 1,950 µL of 60 µM DPPH solution prepared by dissolving DPPH in 95% methanol. The mixture was incubated in the dark condition for 30 min and the absorbance was measured at 517 nm using methanol as a blank. The radical scavenging activity was expressed as the µmol Trolox equivalent (TE) per 100 mL of sample.

Determination of ferric reducing antioxidant power activity: Ferric reducing antioxidant power assay (FRAP) was determined according to [10]. Sample 400 µL was added with 2.6 mL of FRAP solution prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) in 40 mM Hydrochloric acid (HCl) and 20 mM Iron (III) chloride ($FeCl_3$) in ratio of 10:1:1 (v/v/v). The mixture was incubated at 37°C for 30 min and measured the absorbance at 595 nm by using water as a blank. FRAP was expressed as µmol $FeSO_4$ equivalent per 100 mL of sample.

Determination of aerobic plate count and yeast and mold count: Aerobic plate count (APC) was analyzed according to [11]. One ml of sample was serially diluted with 0.1% sterile peptone water and 1.0 mL of each dilution was pipetted to plate count agar using pour plate method. Inoculated plates were incubated at 35±1°C for 48±3 h. Yeast and mold count (YM) were analyzed according to [12]. After making serial dilution as APC, 0.1 mL of each dilution was pipetted to potato dextrose agar and spread plating was done. The plates were then incubated at 25°C for 5 days. APC and YM were expressed in log CFU/mL.

Statistical analysis: Data were expressed as means±standard deviations of triplicate determination. Analysis of variance (ANOVA) is performed, followed by the Duncan's test using SPSS 16.0 for Windows to determine significant differences with a level of significance $p < 0.05$.

RESULTS AND DISCUSSION

HPP treatment did not significantly affect to color of fingerroot juice. The L* value of HPP treated sample ranged from 57.39±0.04 to 57.73±0.02 while the fresh juice had the value of 57.85±0.02 and the CHT sample had less lightness as 55.38±0.01. The better color retention was reported in strawberry and blackberry purées submitted to HPP than in those that were thermally pasteurized [13]. HPP is known to have a limited effect on pigments, causing minimal alteration in the natural color of food and beverages. In addition, since the heat evaporates some moisture in product, TSS of CHT sample was significantly higher than other treatments (Data not shown). The pH values were similar in all samples and be in range of 3.16–3.59.

Table 1 TPC and antioxidant activity measured as DPPH and FRAP in fingerroot juice mixed with honey and lime juice after treated with different HPP condition for 5 min

Sample	TPC*	DPPH**	FRAP***
CHT	29.63±0.01^{c}	56.38±0.01^{c}	309.33±0.01^{d}
200 MPa	34.29±0.01^{a}	73.34±0.03^{b}	360.32±0.01^{b}
400 MPa	34.50±0.02^{a}	74.48±0.03^{b}	375.09±0.02^{b}
600 MPa	34.74+0.02^{a}	80.01±0.12^{a}	381.02±0.02^{a}
Fresh	34.12±0.02^{b}	72.03±0.03^{b}	357.66±0.08^{c}

Three replicates were performed for each measurement reported as means and standard deviation. Different uppercase letters in the same column indicate a significant difference ($p<0.05$). *Total phenolic content (TPC) reported as mg of Gallic acid equivalent/100 mL of sample. **DPPH radical scavenging activity (DPPH) reported as µmol Trolox equivalent/100 mL of sample. ***Ferric reducing antioxidant power activity (FRAP) reported as µmol FeSO4 equivalent/100 mL of sample.

The effect of HPP on TPC, antioxidant activity in terms of DPPH and FRAP were presented in Table 1. The TPC in fingerroot juice treated with HPP was similar to that of the fresh sample and significantly higher than that in CHT. Enzymatic oxidation (polyphenol oxidase and peroxidase) plays a major role in the degradation of phenolic compounds in HPP samples. Moreover, Patras et al. (2009) [14] reported that phenolic contents in tomato and carrot purées were not affected by HPP treatment. These results suggested that HPP treatment may increase, have no effect, or decrease in phenolic compounds, depending on the commodity, treatment pressure and time. When considering antioxidant activity, pressurized fingerroot juices had similar DPPH radical-scavenging activities to the fresh juice while the lowest value was observed in CHT sample as shown in Table 1. In addition, the FRAP assay showed the similar trend to DPPH radical scavenging activities, the highest value was observed in 600 MPa treatment while the lowest was found in CHT sample. HPP trends to provide a better retention of the antioxidant activity when compared with CHT which similar to the report in orange juice [4]. HPP could lower the rate of degradation of bioactive compounds which have a positive correlation to antioxidative capacity.

Table 2 Aerobic plate count (APC) and yeast and mold count (YM) in fingerroot juice mixed with honey and lime juice after treated with different HPP condition for 5 min

Sample	APC (Log CFU/mL)	YM (Log CFU/mL)
CHT	< 1.40	< 1.00
200 MPa	4.68±0.11	< 1.00
400 MPa	4.50±0.13	< 1.00
600 MPa	< 1.40	< 1.00
Fresh juice	4.70±0.05	< 1.00

Three replicates were performed for each measurement reported as means and standard deviation.

Aerobic plate count (APC) and yeast and mold count (YM) in fingerroot juice mixed with honey and lime juice as affected by HPP were shown in Table 2. It was found that HPP at 600 MPa for 5 min and CHT were equally effective in reducing microbial counts

for both APC and YM. Several authors have illustrated protein denaturation and cell injury as the two main reasons for microbial inactivation at high pressures. The permeability characteristics of cell membranes are adversely affected as they undergo microporation, on subjecting to high pressure. Also, high pressure impairs protein synthesis causing the inactivation of intracellular enzymes leading to metabolic inactivation of microbes [15].

From the results, HPP at 600 MPa for 5 min was then selected to study the quality changes of fingerroot juice mixed with honey and lime juice during 12 days storage at 5±1°C compare to fresh and CHT samples.

The lightness (L^*) of HPP, fresh and CHT sample slightly decreased from the beginning (day 0) as 57.39±0.03, 57.85±0.02 and 55.38±0.01, respectively to 56.26±0.03, 57.61±0.04 and 55.32±0.03, respectively after 12 days storage but not significantly different along the storage time. TSS and pH of HPP treated sample was quite stable along the storage time (14.90-15.15 and 3.57-3.65, respectively) and the value was also similar to the fresh juice (15.00-15.05 and 3.54-3.59). The comparison appearance of all samples was in Figure 1. CHT seemed to give darker in color among all samples. No change in color during storage up to 40 days at 4 °C was reported in guava puree treated with 600 MPa for 15 min [16]. It might be concluded that HPP had less effect on color change and could provide fresh-like product.

Figure 2 shown the changes in TPC and antioxidant activity (DPPH and FRAP) as affected by HPP treatment. After HPP treatment (Day 0), TPC in juice treated with 600 MPa for 5 min was similar to that of the untreated sample (fresh juice) and higher than that in the CHT.

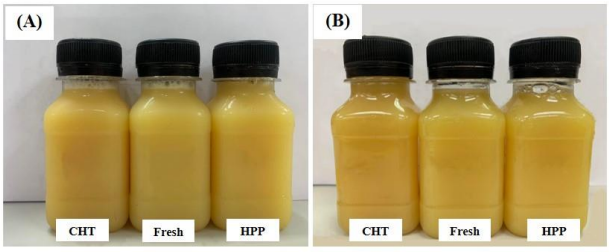

Figure 1 Comparison between CHT, fresh juice and HPP 600 MPA for 5 min treated fingerroot juice mixed with honey and lime juice (A) After treatment (Day 0) and (B) after storage at 5±1°C for 12 days.

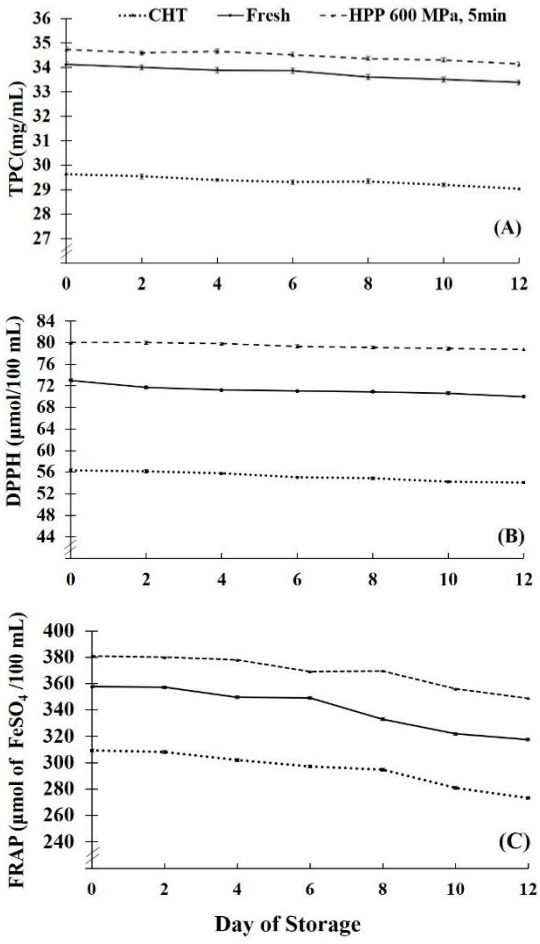

Figure 2 Changes in (A) TPC, antioxidant activity in terms of (B) DPPH and (C) FRAP in fingerroot juice mixed with honey and lime juice as affected by HPP treatment compared to CHT and fresh juice during storage at 5±1°C.

The value of TPC was almost stable along the storage time and was in a range of 33.94 to 34.74 mg of GAE/100 mL of sample. Pressure treatment at 400 and 500 MPa was reported to produce a slight but non-significant increase on TPC. The increasing in TPC may be related to an increased extractability of some of the antioxidant components following high pressure processing [13].

In addition, DPPH of HPP treated sample was higher than that in fresh and CHT juice. The value was quite constant with slightly decreased at the end of storage time and be in a range of 78.81±0.09 to 80.01±0.05 µmol TE/100 mL of sample. For FRAP, the results were found similar to DPPH. Significant increasing of FRAP value was observed in HPP treated juice (381.02±0.21 µmol of $FeSO_4$/100 mL) compared to fresh (357.66±0.05 µmol of $FeSO_4$/100 mL) and CHT (309.33±0.11µmol of $FeSO_4$/100 mL) and the values slightly decreased until the end of storage time. Antioxidant activity in tomato and carrot puree after exposure to HPP (400-600 MPa at 20°C for 15 min) was reported higher than in untreated and thermally processed samples [14]. HPP either increases or maintains antioxidant activity depending on the different pressure level and holding time [17]. In this study, antioxidant activity may relate to the higher present of TPC in sample under HPP treatment.

The effect of HPP on APC and YM in fingerroot juice mixed with honey and lime juice during 12 days storage 5±1 °C were presented in Table 3. The growth of aerobic bacteria and yeast and mold were not observed on agar and the count were reported as less than 1.4 log CFU/mL (APC) and less than 1 log CFU/mL (YM) for both HPP and CHT samples.

However, APC and YM gradually increased in sample without any treatment (fresh juice) and the count were reported as 4.88±0.06 log CFU/mL for APC and 2.97±0.10 log CFU/mL for YM at the end of storage time. Many researchers reported on microbial inactivation by different level of pressure. The main mechanism accounting for microbial inactivation by HPP is probably due to the mechanical disruption of cells caused by spatial pressure, velocity gradients, turbulence and cavitation [18].

Table 4 Changes in aerobic plate count (APC) and yeast and mold count (YM) in fingerroot juice mixed with honey and lime juice after 600 MPa, 5 min HPP treatment during storage at 5±1°C

Sample	APC (Log CFU/mL)	YM (Log CFU/mL)
CHT		
Day 0	< 1.40	< 1.00
Day 2	< 1.40	< 1.00
Day 4	< 1.40	< 1.00
Day 6	< 1.40	< 1.00
Day 8	< 1.40	< 1.00
Day 10	< 1.40	< 1.00
Day 12	< 1.40	< 1.00
HPP Day 0	< 1.40	< 1.00
Day 2	< 1.40	< 1.00
Day 4	< 1.40	< 1.00
Day 6	< 1.40	< 1.00
Day 8	< 1.40	< 1.00
Day 10	< 1.40	< 1.00
Day 12	< 1.40	< 1.00
Fresh juice		
Day 0	4.70±0.05	2.45±0.11
Day 2	4.85±0.11	2.51±0.05
Day 4	4.70±0.11	2.50±0.05
Day 6	4.65±0.05	2.45±0.06
Day 8	4.67±0.07	2.84±0.05
Day 10	4.79±0.03	2.80±0.09
Day 12	4.88±0.06	2.97±0.10

Three replicates were performed for each measurement reported as means and standard deviation.

CONCLUSIONS

High pressure processing at 600 MPa for 5 min was considered to be the best treatment for preserving freshness and maintaining and/or improving antioxidant activity in fingerroot juice mixed with honey and lime juice. It can be stored at 5±1°C for about 12 days. This condition provided the same microbiological safety level as conventional heat treatment. The effect of HPP on other properties such as anti-inflammatory should be carried on in further study. As alternative technology, HPP could be applied in fruit and vegetable industry for creating innovative health products for consumers.

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