JFHB
Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science

Vol. 13 No. 3 September – December 2020

Journal of Food Health and Bioenvironmental Science

http://jfhb.dusit.ac.th/
http://research.dusit.ac.th/new/e-journal/
ISSN 2630-0311 (Online)

Journal of Food Health and Bioenvironmental Science

Vol. 13 No. 3

September – December 2020

ISSN 2630-0311 (Online)

Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science is an international scientific journal which is the official publication of Research and Development Institution, Suan Dusit University, Thailand. The journal publishes papers in the categories of peer reviewed original articles, review article and book review from all areas related to food, health, biological and environmental disciplines in order to exchange knowledge and concepts with the scientific community. The journal is published thrice annually, with the 1st issue covering the months of January to April, the 2nd issue May to August and the 3rd issue September to December. The journal is distributed in online (http://www.researchold.dusit.ac.th/new/e-Journal/jfhb.php) format. Those who are interest can submit the manuscripts via scholar one system (https://mc03.manuscriptcentral.com/jfhb).

All the manuscripts submitted to the journal must be plagiarism free and has never been published or submitted elsewhere unless the manuscript was published in another language and has permission from the journal. Before being published, the manuscripts must be peer reviewed by at least 2 specialized reviewers via double blinded review system.

The following areas are covered in the journal:

• Food science including food chemistry, food biotechnology, food processing, food technology, food microbiology, food safety, food nutrition, product development, sensory analysis and related fields

• Health sciences including epidemiology, nursing, pharmaceutical science, cosmetic, public health and related fields

• Biological science including microbiology, molecular biology & genetics, cell biology, biochemistry, biotechnology, biodiversity, biomonitoring & biomarkers, ecology, plant & animal biology, toxicology, applied biology, agricultural science and related fields

• Environmental science including aquatic environment, atmospheric environment, environmental pollution, environmental chemistry, alternatively fuels, green technology and related fields

All manuscripts accepted for publication are copyrighted by Suan Dusit University; reproduction, in whole or in part, requires written approval from Suan Dusit Unversity. Excluding errors incurred during the printing process, all content contained within articles is the author's responsibility.

Office of Management Research and Development Institute, Suan Dusit University
295 Nakhon Ratchasima Road, Dusit, Bangkok, Thailand 10300
Phone: +662 244 5801-2 Fax: +662 668 7460
e-mail: jfhb@dusit.ac.th

Copyright Research and Development Institute, Suan Dusit University
295 Nakhon Ratchasima Road, Dusit, Bangkok, Thailand 10300
Phone: +662 244 5801-2 Fax: +662 668 7460

Printing Graphicsite
295 Nakhon Ratchasima Road, Dusit, Bangkok, Thailand 10300
Phone: +662 244 5080-2 Fax: +662 243 9113

Journal of Food Health and Bioenvironmental Science

Vol. 13 No. 3 September - December 2020 ISSN 2630-0311 (Online)

Editorial Advisory Board

Sirote Pholpuntin, Ph.D.	Associate Professor President of Suan Dusit University, Thailand
Chanasuek Nichanong, Ph.D.	Associate Professor Vice President in Research and Education Development Suan Dusit University, Thailand

Editorial in Chief

Yutthaya Yuyen, Ph.D.	Assistant Professor, Suan Dusit University, Thailand

Editorial Board

Randall C. Kyes, Ph.D.	Professor, University of Washington, United States
Puangtong Kraiphibul, Ph.D.	Emeritus Professor, Suan Dusit University, Thailand
Thaweesakdi Boonkerd, Ph.D.	Emeritus Professor, Chulalongkorn University, Thailand
Vilai Rungsardthong, Ph.D.	Professor, King Mongkut's University of Technology North Bangkok, Thailand
Chonlaphat Sukasem, Ph.D.	Associate Professor, Mahidol University, Thailand
Khanok Ratanakhanokchai, Ph.D.	Associate Professor, King Mongkut's University of Technology Thonburi, Thailand
Seyed Hossein Hoseinifar, Ph.D.	Associate Professor, Gorgan University of Agricultural Sciences and Natural Resources, Iran
Helmy Yusuf, Ph.D.	Associate Professor, Universitas Airlangga, Indonesia
Tita Foophaow, Ph.D.	Assistant Professor, Suan Dusit University, Thailand
Tri Nhan Nguyen, Ph.D.	Vietnam National University Ho Chi Minh City, Vietnam
Muhammad Ayaz, Ph.D.	University of Malakand, Pakistan
Kamrul Islam	The University of Tokyo, Japan

Managerial Team

Pornpassanan Dechprasittichoke, Ph.D.	Assistant Professor, Suan Dusit University, Thailand
Nuttabodee Viriyawattana, Ph.D.	Assistant Professor, Suan Dusit University, Thailand
Rungnapa Pongkiatchai, Ph.D.	Suan Dusit University, Thailand
Nongnuch Utakud	Suan Dusit University, Thailand
Taweewat Poolsawat	Suan Dusit University, Thailand

Peer Reviewers

Assoc. Prof. Dr. B. Pharm, Chonlaphat Sukasem	Ramathibodi Hospital, Thailand
Assoc. Prof. Dr. B. Pharm, Narisa Kamkaen	Rangsit University, Thailand
Assoc. Prof. Dr. Pongsak Laudee	Prince of Songkla University, Thailand
Assoc. Prof. Dr. Pattana Sripalakit	Naresuan University, Thailand
Assoc. Prof. Dr. Tasanee Panichakul	Suan Dusit University, Thailand
Assoc. Prof. Dr. Mikalai Filonchyk	Lanzhou Jiaotong University, China
Assist. Prof. Dr. Sasikan Kupongsak	Chulalongkorn University, Thailand
Assist. Prof. Dr. Piyaporn Chueamchaitrakun	Mae Fah Luang University, Thailand
Assist. Prof. Dr. Sukhumaporn Saeng-ngam	Srinakharinwirot University, Thailand
Assist. Prof. Dr. Pornpassanan Dechprasittichoke	Suan Dusit University, Thailand
Assist. Prof. Dr. Panyapathk Pinkaew	Suan Dusit University, Thailand
Assist. Prof. Dr. Owais Yousuf	Integral University, India
Assist. Prof. Dr. Musfirah Zulkurnain	Universiti Sains Malaysia, Malaysia
Dr. Thani Isara	Mahasarakham University, Thailand
Dr. Utthapon Issara	Rajamangala University of Technology Thanyaburi, Thailand
Dr. Korawinwich Boonpisuttinant	Rajamangala University of Technology Thanyaburi, Thailand
Dr. Muhammad Ayaz	University of Malakand, Pakistan
Dr. Nor Shariffa Yussof	Universiti Sains Malaysia, Malaysia
Andri Frediansyah	Indonesian Institute of Sciences, Indonesia

Content

Original Articles

The Study of Citrus's Fibers Production for Power Bar and the Effect on Consumer's Acceptance **1**
Nathamon Somrajlichinda

Free Radical Scavenging Activity of Riceberry and Pathum Thani Rice Extracts for Developing Face Powder **9**
Watchara Senajuk, Sasamol Phasuk, Poonyanuch Nilsang & Pannraphat Takolpuckdee

Effects of Components on Qualities of Crispy Bamboo Shoot Snack **16**
Narin Charoenphun & Kanokporn Pakeechai

The Improvement of Hydroponics Growth Media by Using the Corncob Biochar **26**
Pannipha Dokmaingam, Sitang Kongkratoke, Amornrat Anuwatnonthakate & Sarayut Yongprapat

Life Cycle and Larval Feeding Habits of *Macrostemum indistinctum* Banks 1911 (Trichoptera: Hydropsychidae) in the Stream Flows into Krasiow Reservoir, Thailand **32**
Orawan Khamrak & Taeng-On Prommi

Cholinesterase's Enzymes Inhibition and Michaelis-Menten Kinetics Studies on Ethnomedicinally Important Plant *Chenopodium botrys* **40**
Naveed Iqbal, Farhat Ullah, Abdul Sadiq, Muhammad Saeed Jan, Muhammad Shahid & Muhammad Ayaz

Rice Bran Oil Emulsion Organogels as Fat Baking for Brownies **47**
Sakunta Manakla, Pattamaporn Jareonnon & Supathra Lilitchan

Review article

Aphrodisiac Food Ingredients from the Twelve Thai Ancient Formulary Books of Police Captain Bhiam Bunyachot **54**
Vadhana Jayathavaj & Ubol Chuensumran

Book Review

The Handbook of Environmental Chemistry Volume 58 **61**
Author Wagner, M., & Lambert, S.
Penkhae Thamsenanupap

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

The Study of Citrus's Fibers Production for Power Bar and the Effect on Consumer's Acceptance

Nathamon Somrajlichinda*

Chemistry Department Academic Division, Chulachomklao Royal Military Academy (CRMA), Nakornnayok 26001, Thailand

Article info

Article history:
Received : 25 July 2020
Revised : 3 October 2020
Accepted : 12 October 2020

Keywords:
Dietary fiber, Citrus peel, Power bar

Abstract

This research is a study into the production of citrus fiber from 4 citrus plants: pomelo, tangerines, sweet orange, and limes. Produced dietary fibers were analyzed and the amounts of fiber were found to be 58.21 %, 48.45 %, 44.90 %, and 41.70 % in the pomelo, tangerines, sweet orange, and limes, respectively. According to appropriate material supply, fiber amounts, and the production time, fiber from selected tangerines was added to a power bar product. The ingredients of the power bars were investigated and controlled based on the standard of Acceptable Macronutrient Distribution Ranges (AMDR). The nutrient energy of the power bar was analyzed, and the results showed a calorie ratio of carbohydrate 48.02 %, protein 11.40 %, and fats 40.56 %. These power bars were produced with the amount of citrus dietary fiber being added in the ratio of 5 %, 7 %, and 10%. The result showed that the power bar containing 5 % ratio of citrus's fiber had average scores for the highest of all aspects. Appearance had an average score of 8.60, color with an average score of 8.66, taste with an average score of 8.73, texture had an average score of 8.46, and finally, overall acceptance had an average score of 8.73. The amount of orange peel added to the energy bar at 7 % ratio and 10 % ratio are different from 5 % ratio with a 95 % confidence in statistics.

Introduction

In the military, cadets training in the field or on operations require quick deployment, and therefore carry dried food or canned meal. Despite the convenience of these foods, the nutritional balance is a concern. Therefore, there has been the development of Meal, Ready-to-Eat (MRE) that is not only lightweight but also has sufficient nutrition for a daily meal. This is the fundamental energy for supporting the tasks efficiently. Nevertheless, ready meals such as the power bar do not contain enough fiber; therefore, the increment of fiber in the power bar will improve cadets' health during field operations. Moreover, people in modern life are increasingly concerned about their health. The daily meals are a hot topic because the proper amount of nutritional food can balance the necessary energy and keep the body working efficiently. Consequently, the

* Corresponding Author
e-mail: nathamon.so@crma.ac.th

research about the increment of fibers in the power bar is intended to provide another choice for consumers who expect sufficient nutrition, proper energy and resolve the malnutrition problem in dieters. The power bar that increased the level of fiber from the citrus is also environmental-friendly because they utilize fruit peel which was previously a waste product from the food industry. The daily energy requirement in humans relies on all-day energy metabolism. For example, the energy of the muscle movement can be assumed from the body's size, type of movement, and the value of the Basal Metabolic Rate (B.M.R.). According to the statistical data, the average energy which is required daily for human, is categorized in the following age range; children (1-3 years) require 1,000-1,200 kcal/day; children (4-8 years) require 1,200-1,400 kcal/day; adult boy and girl (9-18 years) require 1,600-2,400 kcal/day; men (19-60 years) require 1,800-2,200 kcal/day; women (19-60 years) require 1,500-1,800 kcal/day; and the seniors men and women (older than 60 years) require 1,500-1,800 kcal/day (Bureau of Nutrition, 2020). Energy from the principal nutrients are composed of three elements, carbohydrate, protein, and fats. The ratio of these energetic nutrients is essential for nutritional balance. There is the recommendation ratio among the energetic nutrients in daily food, which is referenced by the various standard value. In this research, we refer the ratio of energetic nutrients with the Acceptable Macronutrient Distribution Ranges (AMDR) that shows the required amounts of each energetic nutrient in Table 1 (Weight Watchers Research Department, 2019).

Table 1 The requirement percentage of nutrient energy

Nutrient	Requirement percentage (%)
Carbohydrate	46 – 65
Protein	10 – 15
Fats	20 – 35

Remark: AMDR-Macronutrient Ranges and Recommendations, Weight Watchers Research Department

Fiber is the composition of the cell wall in plant-based foods. Due to the indigestible element, it cannot absorb and does not give any energy to the human body. There is 2 type of fibers, soluble dietary fiber and insoluble dietary fiber. The fiber amounts in this research are both soluble and insoluble dietary fibers. According to the study about the fiber utilization from wasted fruits and vegetables, the fiber amounts in the orange rind are about 63.7% by dry weight (Tanongkankit, 2014). Thailand's Food and Drug Administration (F.D.A.) makes the table of Thai Recommended Daily Intake (Thai R.D.I.) for Thai people older than six years old. These standard values are the references used for the nutrient calculation in the food label. It shows that the required daily intake of fiber should be more than 25 grams (Watthanataweekul, 2002).

The peel of orange-species fruit and orange can be categorized into four groups; 1) orange group 2) mandarins 3) pomelos or grapefruits and 4) common acid members (Janluay et al., 2009). As the results of fiber-contained in citrus, the fiber amounts inside the orange rind are greater than the fiber amounts in pulp. It also found that the amounts of fiber in fresh rind is high; 93.07% by weight (Saengthongpinit, 2008). Therefore, the objectives of this study are the studying of fiber preparing method and developing the ingredient of fiber added power bar. The developed power bar must meet the nutrient standard of Thai R.D.I. and be satisfying and desirable for the consumer.

Materials and methods

The experiments were composed of 3 steps; (1) Fiber preparation: the dietery fibers of four citrus plants were prepared; pomelo, tangerines, *Citrus sinensis*, and limes, and then a chemical analysis was made to determine the fiber amounts. (2) Power bar production: soybean meal was used as the main component of the power bar and adjusted an ingredient ratio for the appropriated nutrients balanced. (3) The customer's acceptance of the power bar, which added the fiber amounts by weight ratio 5%, 7%, and 10% was evaluated by CLT method. The popular level is a quantitative expression by 9- point hedonic scale.

1. Fiber preparation from orange-species fruit

There are 4 main processes of the fiber extraction from orange rind, (1) wet milling process, (2) washing process, (3) drying process, and (4) dry milling process, respectively (Larrauri, 1997). The experiment proceeds as follows (Pichaiyongvongdee & Rattanapun, 2014). Each of step were shown in the Fig.1

1.1 Wash and cut each type of orange rind into a rectangle shape with a small size (1 x 1 cm).

1.2 Decrease the bitter taste by soaking the rind pieces in sodium chloride solution and adjust the pH value to 7 with the concentration 1:10 (w/v) for 24 hours.

1.3 Water steaming at 80˚C for 10 minutes.

1.4 Heating at 60˚C for 6-8 hours. The orange rind will be in a dehydrated state.

1.5 Finely milling the rind pieces by herb grinder and

sieving the powder using 60 mesh screens.

1.6 Determine the amounts of dietary fiber by analyzing fiber powder with the In-house method (CH-TH-076) base on AOAC (2016) at the Central Lab (Thailand) company.

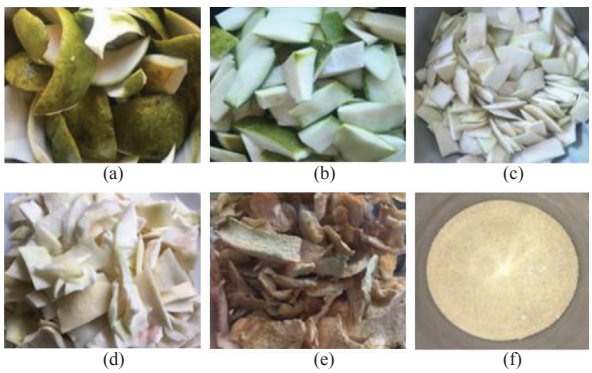


Fig. 1 Physical appearance during the fiber extraction process (pomelo's fiber powder (a) pomelo peel, (b) pomelo peel cut of rind into a rectangle shape, (c) pomelo peel soaked in sodium chloride solution, (d) steamed at 80°C for 10 mins pomelo peel, (e) hydrated pomelo peel, (f) pomelo's fiber powder

2. Production and development of power bar using the prepared fiber as a base ingredient

2.1 The main components of a power bar, butter wheat, and soybean meal were adjusted for the appropriated ingredient recipe. The ingredient of the power bar recipe performed the weight ratio as 100% total compositions. According to the balance of mixing energetic ingredients in which protein ingredient (50% soybean meal content) was controlled to be constant, carbohydrate and fats ingredients were varied in 3 types of ingredients, as shown in Table 2.

Table 2 Various ingredients of wheat and butter contents in power bar production

Ingredients no.	Wheat (%)	Butter (%)	Soybean meal and others. (%)
1	30	20	50
2	35	15	50
3	40	10	50

2.2 The method of chemical property analysis such as a moister content analysis (AOAC., 2019), ash contents analysis (AOAC., 2019), carbohydrate contents analysis (In-house method CH-TH-169 base on method of Analysis for Nutrition Labelling of Sullivan & Carpenter, 1993), protein contents analysis (In-house method CH-TH-042) and fats contents analysis (AOAC., 2019) are supported by the laboratory test at the Central Lab (Thailand). The test results were compared and selected the ingredient recipe, which had a nutrient value, mostly like the requirement of the daily nutrient standard. Consequently, ingredients no. 3 was applied as the base recipe. The basic composition of the power bar which has a nutrient energy ratio based on AMDR standard. Add the fiber powder into the power bar until the fiber amounts are sufficient for the daily requirement amount, 25-20 grams. Fig.2 shows the appearance of the base products approximately 12-13 grams per piece.

Fig. 2 The appearance of base products

3. Customer's acceptance investigation

Made the questionnaire style survey about the customer's satisfaction with the power bar, which added the fiber amounts by weight ratio 5%, 7% and 10%.

3.1 The base ingredient of the power bar was added the dietary fiber by weight ratio of 5%, 7% and 10%. This weight ratio is calculated by comparing the weight of the orange rind and the weight of the products. The products are a circular disc with a diameter of 4 cm. The weight per piece is 12 grams. The products are baked in the oven at 180˚C heat for 10 minutes. Fig. 3 shows the appearance of the final products. These fibers added power bars were surveyed for the degree of consumer satisfaction. The degree of consumer satisfaction will be verified by the 9-point hedonic scale method. The popular scores are averaged, and a statistical analysis was performed.

Fig. 3 The samples of final products with the fiber weight ratio 5%, 7% and 10%, respectively

3.2 The survey, the consumer acceptance for fiber added power bar was verified by the Central Location Test (CLT) method. The popular level is a quantitative expression by 9- point hedonic scale (1 = least popular to 9 = most popular). The details of the questionnaires were the opinion about the product characteristics, appearance, color, smell, taste, and the overview image. The survey was given to 30 subjects with the 3 types of products varied in fiber amounts; 5 %, 7 % and 10 % by weight ratio. The survey results were conducted into a data analysis program SPSS (Kongkaew et al., 2019). The analysis of variance (ANOVA) and multiple comparisons, Duncan's new multiple range test methods, were applied with the condition $p<0.05$.

Results and discussion

1. The process of reducing the bitterness in orange peels

In the production of citrus's fiber 4 citrus plants; pomelo, tangerines, sweet orange, and limes, the bitter taste was reduced by a process of soaking. The citrus peel was soaked in the 10% (w/v) NaCl solution with pH 7 for 24 hours then steamed for 10 minutes. The fiber of pomelo was found to be the most bitter. Because the bitter taste in pomelo peel composed of the derivative of triterpenes called Limonin ($C_{26}H_{30}O_8$), which readily dissolved in acetone or chloroform but were only slightly soluble in aqueous (Maier et al., 1977). The result was consistent with the investigation about the production of fiber powder from pomelo's internal peel by Pichaiyongvongdee & Rattanapun (2014). The verified result of the physical properties of citrus's fiber found that bitterness of pomelo is in Limonin which can be reduced by using 1% NaCl. The result showed that the bitterness can be reduced for 10.3% compared to 7.74% of bitterness reduction using pure water, the results did not have any statistical significance in the difference. Therefore, this research used fiber powder from the mandarin orange peel with high fiber amounts of 48.45%. Moreover, the bitter test in mandarin orange was naringin that could be dissolved in warm water readily. In contrast, the hard-dissolved substance, limonin mainly found in the seed of mandarin orange (Sansawat, 2006).

For the application of a hot air oven in the drying process, the results of the drying time and temperature by Pichaiyongvongdee & Rattanapun (2014) showed that the higher temperature could reduce the drying time, the fiber amounts could be reduced. While drying the process with high temperature, the structure of the cell's wall was damaged, and the absorption performance in both water and oil was increasing (Gould et al., 1989). This effect is also found in Chinnasarn et al. (2015) in the drying process with low temperature, a long time for drying reduced sugar in pectin substance and the performance of water conservation was decreased. According to the drying process for apple peel by Constenla et al. (2002), the structure of fiber did not show any change at drying temperature 60-80˚C. However, at the drying temperature 105˚C, the amounts of pectin are reduced significantly. Consequently, this research controlled the drying temperature at 60˚C and dying time for 6 hours. All types of orange peel were dried entirely and became a dark color, as shown in Fig. 5.

Fig. 5 The appearance of each citrus peel after dried entirely (a) dried pomelo peel, (b) dried mandarin orange peel, (c) dried sweet orange peel, (d) dried lime peel

After the drying process, each of orange peel was finely milled by the herb grinding machine, then make a sieved the powder by 60 mesh screens. Each of fiber powder was shown in Fig. 6

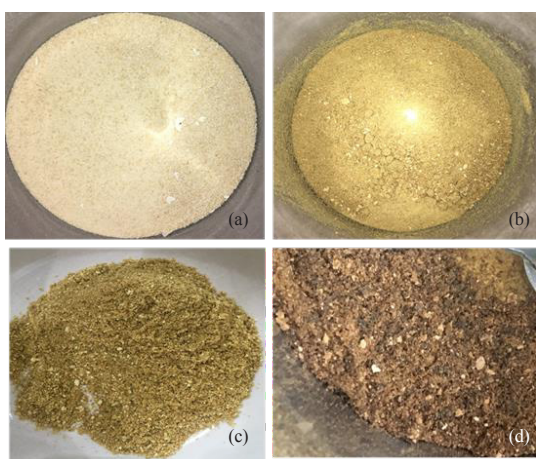


Fig. 6 Fiber powder from the citrus peel (a) pomelo's fiber powder, (b) mandarin orange's fiber powder, (c) sweet orange's fiber powder, (d) limes's fiber powder

The amounts of dietary fiber were measured at the Central Lab (Thailand) company. The results have shown the amounts of fiber from 100 grams of sample. The percentage of weight ratio was summarized in Table 3.

Table 3 Summary the fiber amounts from 4 types of citrus peel

Type of citrus peel	Dietary fiber amounts (%)
Sweet orange	44.99
Mandarin orange	48.45
Pomelo	58.21
Lemon	41.70

The comparison results from 4 types of citrus peel found that the highest-fiber amount was pomelo (58.21%), following by the mandarin orange (48.45%), sweet orange (44.99%), and the lowest fiber amounts were lemon (41.7%). In the investigation of dietary fiber from the pomelo peel, 92% was a water-insoluble fiber. The ratio between soluble and insoluble fiber varied from 1: 5.9 to 1:12.7. It depends on the pomelo species and the production process (Saengthongpinit, 2008). The results corresponding with the research by Pichaiyongvongdee & Rattanapun (2014) that showed the fiber amounts from pomelo was higher than other fruits, peach, pear, sugarcane, the pericarp of cumin, apple, banana, carrot and the other citrus species such as grapefruit, mandarin orange and lemon. However, the fiber amounts from pomelo in this study were lower than Saengthongpinit (2008). In an experimental result, the amount of fiber in pomelo depends on the processing method. Fiber amounts produced by the boiling and heating process tend to lower than the producing by non-heating. The fiber amount from boiled and heated pomelo peel is lower than fresh pomelo peel (Saengthongpinit, 2008).

2. The investigation of power bar base ingredients and chemical properties analysis results

Three base ingredients were verified for the quantity analysis. The weights of moisture, ash, carbohydrate, fat and protein in power bar 100 grams are shown in Table 4.

Table 4 The chemical analysis of 3 base ingredients of the power bar

Chemical analysis	Power bar (g/100 g)		
	No. 1	No. 2	No. 3
Moisture	5.27	7.34	7.05
Ash	1.28	0.98	1.72
Carbohydrate	58.83	56.84	68.85
Protein (%N x 6.25)	11.47	13.50	11.81
Fat	23.15	21.34	10.57

Compared to the AMDR standard, nutrients energy (carbohydrate, protein and fat) in table 5 were calculated for the energy ratios by using the energy rate, 4 kcal/g in carbohydrate and protein, 9 kcal/g in fats. Each of the base ingredients in the power bar showed the ratios of nutrients energy compare to the AMDR standard in Table 5.

Table 5 The results of energetic nutrient ratios

Nutrient	Power bar (%)			The AMDR's standard amounts (%)
	No. 1	No. 2	No. 3	
Carbohydrate	48.06	48.02	65.92	46 – 65
Protein	9.37	11.40	10.11	10 – 15
Fats	42.56	40.56	22.77	20 – 35

The comparison between the ratios of nutrients energy and the daily-requirement energy, the AMDR standard value in Table 5, the base ingredient No. 3 found the most consistent with the AMDR standard. Therefore, this research will apply this base ingredient to the fiber-adding process.

3. The investigation about the consumer acceptance of the power bar added the citrus fiber by weight ratio 5%, 7% and 10%

The consumer's acceptance was evaluated by Central Location Test (CLT) method. The popular level is a quantitative expression by 9-point hedonic scale (1 = least popular to 9 = most popular). The questionnaires will be about the opinion about the product characteristics, appearance, color, smell or flavor, texture and the overview image given to 30 subjects. The results of consumer's acceptance for the power bar, which added the 3 fibers ratios, are summarized in Table 6.

Table 6 The results of consumer satisfaction in fiber addition products

Evaluated Item	The percent amounts of citrus's fiber from an orange peel added to the products		
	5%	7%	10%
Appearance	8.60 ± 0.56^{a}	7.76 ± 0.72^{b}	7.23 ± 1.16^{c}
Color	8.66 ± 0.47^{a}	7.76 ± 0.85^{b}	6.86 ± 1.35^{c}
Flavor	8.73 ± 0.44^{a}	7.63 ± 0.99^{b}	6.50 ± 1.35^{c}
Texture	8.46 ± 0.57^{a}	7.33 ± 0.92^{b}	6.06 ± 1.98^{c}
Overall acceptance	8.73 ± 0.44^{a}	7.80 ± 0.88^{b}	6.96 ± 1.1^{c}

Remark: a-c means the average data in the same rows which have a different character. The statistical significance of the difference was a 95% confidence interval

The average score of flavors from the power bar with amounts of citrus's fiber 5%, 7% and 10% is between medium popular much more popular with the score of 8.73, 7.63 and 6.50, respectively. The popular score of

5% ratio fiber amounts had the highest average score of flavors. The comparison among 3 ingredient ratios showed that the 7% ratio and 10% ratio are different in statistical significance from 5% ratio at 95% confidence interval. According to the removal of naringin in mandarin orange peel did not completely do, the addition fibers in the power bar increased the bitter taste. In the investigations by Attavanich & Anprung (2003) found that, although naringin in mandarin orange peel was soluble in warm water, the aqueous extraction and pH adjustment method could reduce the bitter teste just slightly ($P \leq 0.05$).

The average score of texture from the power bar with amounts of citrus's fiber 5%, 7% and 10 % was found between the range of least popular to more popular in the score 8.46, 7.33 and 6.06, respectively. The popular score of 5% fiber amounts had the highest average score with regards to texture. The comparison among 3 ingredient ratios shown that 7% ratio are 10% ratio are different in statistical significance from 5% ratio at in 95% confidence interval. The results showed that the increase of fiber ratio increased the hardness of texture. The dried fiber could absorb the water contents in products (Siwnguan, 2017). Increasing hardness affected the consumer's acceptance in the point of view of texture was decreased. Consequently, a low fiber addition ratio received higher acceptance scores than the power bar with high fiber addition ratios.

The average score of overall acceptance from the power bar with amounts of 5%, 7% and 10% citrus's fiber was found between the range of least popular to more popular in the score 8.73, 7.80 and 6.96, respectively. The popular score of 5% fiber amounts had the highest average score of the overall acceptance. The comparison among 3 ingredient ratios shown that 7% ratio and 10% ratio are different in statistical significance from 5% ratio at in 95% confidence interval. This research found that the 5% fiber addition of power bars got the highest score in all points of view. The acceptance scores seem to be decreased when the added fiber ratio in the power bar was higher than 7%.

Table 7 The results of the acceptable total score in the additional fiber products

No.	Power bar	Average of the acceptable total score
1.	Fundamental formula with orange fiber 5%	8.73 ± 0.44^{a}
2.	Fundamental formula with orange fiber 7%	7.80 ± 0.88^{b}
3.	Fundamental formula with orange fiber 10%	6.96 ± 1.18^{c}

Remark: a-c means the average data in the same rows which have a different character. The statistical significance of the difference was a 95% confidence interval

Table 6 shown the summarized total acceptance scores of power bar due to the base ingredients with 5%, 7% and 10% orange fiber. They were in the range of little popular to much more popular with the scores 8.73, 7.80 and 6.96, respectively. The power bar that added 5% citrus peel fiber powder has the highest total score and the comparison result of the differences among 3 ratios shows that the 7% and 10% has the statistical significance in the difference with 5% in 95% confidence interval. Consequently, fiber additional 5% of the power bar was accepted due to the highest average scores. It was good in appearance, appropriate color, mild taste and crunchy. This delicious sensation caused the best taste results. Then the sample with the highest score of satisfaction consumers was analyzed for the total energy, ratios of energetic ingredients (carbohydrate: protein: fats) and dietary fiber at the Central Lab (Thailand) company. The results were shown in Table 8.

Table 8 The analysis results of the highest score of the satisfaction consumer product

Chemical analysis	Powerbar with 5% orange fiber (%)	Requirement ratio of AMDR Standard
Carbohydrate	56.84	55-60 %
Protein	13.50	10-15 %
Fats	21.34	< 30 %
Total energy (in 100 grams)	473.42 kcal	-
Fiber	6.22	25-30 g/day

Using these products instead of regular food, serving size for daily required energy will be concerned. Although the daily requirement of total energy depends on the personal activity, 2,000 kcal is the typical average value that was converted to the weight of 1 meal, 150 grams with the equivalent energy 710.13 kcal and including fiber weight 9.33 grams.

Conclusion

The production of fiber analysis from 4 types of citrus fruit, sweet orange, mandarin orange, pomelo, and lemon, which had the drying process for an orange peel 300 grams, thick peel of pomelo and lemon required dehydrating time longer than 6 hours. Whereas the thinner peel, sweet orange, and mandarin orange, in which water absorption is limited, required 6 hours for dehydrating. All dehydrated orange peel was dried entirely, and their color became dark green. Dehydration temperature was stable at 60˚C during the drying process.

The analysis results from Central Lab (Thailand) showed the fiber amounts from 4 types of citrus. Fiber powder from pomelo peel has the highest fiber amounts of 58.21 %, followed by 48.45 % in sweet orange and 44.99 % in mandarin orange while lemon has the lowest fiber 41.70 %.

In conclusion, the results of the investigation about the basic components of a power bar, which has an energetic nutrients ratio based on the AMDR standard, the fundamental formula which composes of 40% wheat, 10% butter and 50% soybean meal and others showed that the ratios of the energetic nutrient is similar to the AMDR standard. In the power bar 100 grams, the study found 65.92% carbohydrate, 10.11% protein and 22.77 % fats.

The results of the investigation about the fiber addition ratio and the verification of consumer satisfaction by a 9-point hedonic scale showed that the power bar, which added 5 % fiber, had the highest in an average score of an appearance, color, smell, flavor, texture and the overview image. The difference results among 3 gradients ratio shown the ingredient ratios 7 % and 10 % have the statistical significance differed from ingredient ratio 5 % in 95 % confidence interval. Consequently, fiber additional 5% of the power bar was acknowledged for the highest average scores and the best of taste evaluation.

Acknowledgments

This work received financial support from the Chulachomklao Royal Military Academy (CRMA) research foundation. I would like to thank the National Research Council of Thailand for the opportunity of presentation in 2020 Thailand Inventors' Day and also the CRMA chemistry department for the experimental devices, the Central Lab (Thailand) company for providing the analysis equipment and test apparatus. Lastly, I would like to thank Dr. Jureemart Deeammart, Instructor of the home economics department, Valaya Alongkorn Rajabhat University for the statistics analytical program supports.

References

AOAC. (2016). *Official Methods of Analysis* (20th ed.). Maryland: Author.

AOAC. (2019). *Official Methods of Analysis* (21st ed.). Maryland: Author.

Attavanich, N., & Anprung, P. (2003). Dietary fiber powder from *Citrus reticulata* Blanco peel and its application. *Food Journal, 33*(1), 45-55.

Bureau of Nutrition. (2020). *Dietary Reference Intake for Thais 2020.* Bangkok: Author.

Chinnasarn, S., Boriboon, S., & Songpra, P. (2015). Preparation of kumquat residue powder and its application in food. *Agricultural Sci. J., 46*(3), 557-560.

Constenla, D., Ponce, A.G., & Lozano, J.E. (2002). Effect of pomace drying on apple pectin. *LWT Food Science and Tecchnology, 35,* 216-221.

Gould, J.M., Jasberg, B.K., & Cote, G.L. (1989). Structure-function relationship of alkaline peroxide treatment lingnocellulose from wheat straw. *Cereal Chemistry, 66,* 213-217.

Janluay, K., Sertwittaya, S., Wongmaneeroj, M., & Sertpakdee, R. (2009). *The variation species of citrus plants.* Retrieved February 7, 2020, from http://www3.rdi.ku.ac.th/exhibition/52/04-plant/kanchana/plant_00.html

Kongkaew, N., Jangsawang, W., Pattanasiri, S., & Malai, D. (2019). Kao-Numpueng pomelo (*Citrus grandis* L. Osbeck) peel powder used as a fat replacer in chocolate milk ice cream. *Agricultural Sci. J., 50* (2), 21-24.

Larrauri, J.A. (1997). *Preparation of new types of dietary fibers from tropical fruit byproducts: Properties and uses in dietetic foods* (Doctoral dissertation). Madrid, Spain: Universidad Politecnica de Madrid.

Maier, V.P., Bennett, R.D., & Hasegwa, S. (1977). Limonin and other limonoids. In S. Nagy, P.E. Shaw, & M.K., Veldius (Eds.). *Citrus Science and Technology* Vol. 1 (pp.355-396). Inc., USA: The AVI Publishing Company.

Pichaiyongvongdee, S., & Rattanapun, B. (2014). *Dietary fiber prepared from Pomelo albedo which to reduce the bitterness and study on physical and functional properties* (Research report). Bangkok: Suan Dusit Rajabhat University Publication.

Saengthongpinit, W. (2008). Production and properties of dietary fiber from *Pomelo albedo* for food products. In *1st NPRU Academic Conference* (pp. 8-14). Nakhon Pathom: Nakhon Pathom Rajabhat University.

Sansawat, T. (2006). *Production of dietary fiber powder from orange (Citrus reticulata Blanco) pomace* (Master's thesis). Chiang Mai: Chiang Mai University.

Siwnguan K. (2017). Using of *Pomelo albedo* powder as dietary fiber in cracker products. *PRSU Journal of Science and Technology, 2*(1), 14-23.

Sullivan, D.M., & Carpenter, D.E. (1993). *Methods of analysis for nutrition labeling.* Arlington, VA: AOAC International.

Tanongkankit, Y. (2014). Utilization of vegetable and fruit residues for production of dietary fiber powder. *Journal of Food Technology, 9*(1), 31-38.

Watthanataweekul, J. (2002). Fibers for wellness. *Journal of Science Service Department, 50*(159), 8-31.

Weight Watchers Research Department. (2019). *AMDR-Macronutrient Ranges and Recommendations*. Retrieved February 7, 2020, from https://www.weightwatchers.com/us/blog/food/acceptable-macronutrient-distribution-range.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Free Radical Scavenging Activity of Riceberry and Pathum Thani Rice Extracts for Developing Face Powder

Watchara Senajuk*, Sasamol Phasuk, Poonyanuch Nilsang & Pannraphat Takolpuckdee

Science Education Department, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani 13180, Thailand

Article info

Article history:
Received : 17 July 2020
Revised : 14 November 2020
Accepted : 18 November 2020

Keywords:
Free radical scavenging activity, Color, Sun protection factor, Face powder

Abstract

Thai rice today is not limited to staple food, but it has been transformed into a variety of medicinal and beauty products, as part of a growing industry with a bright future. We measured total phenolic content, total flavonoid content, free radical scavenging activity, lycopene, β-carotene, color and SPF of raw materials in face powder. Riceberry extract sample contained phenolic and flavonoid with the highest level at 26.46±0.008 mg GAE/g of extract and 101.63±0.034 mg Rutin/g of extract. Free radical scavenging activity was measured by DPPH assay: the Riceberry extract sample contained antioxidants that indicated the EC_{50} at 108.31±0.024 µg/mL. All raw materials showed SPF, which could be used as foundation data for making face powder. However, wood apple powder has the highest SPF for a material, that can be used as a raw material, for further development of face powder.

Introduction

Rice is Thailand's main economic crop; it has been developed to meet the people's basic needs. The development is consistently extended from local wisdom, inherited from the past generations, and with new technology and innovation in producing rice that meets modern consumer needs. Riceberry is a newly registered rice variety from Thailand in 2002, which was derived from a cross-breed between Jao Hom Nin (JHN), the local non-glutinous purple rice, and Khoa Dawk Mali 105 (Jasmine rice), the Thai Hom Mali rice, by the Rice Science Center, Kasetsart University, Thailand. After 4 years of careful selection for nutritional properties, anthocyanin stability, physical and cooking properties, the outcome was a deep purple whole grain rice, that was soft and palatable. Riceberry has come a popular brown rice, known for health promoting properties. Attracting people to consume more brown rice is significant contributor to solving food-related chronic diseases like diabetes, heart disease, high blood chloresterol, obesity and cancers (Suttiarporn et al., 2016; Leardkamolkarn et al., 2011). Pathum Thani rice is a new strain of fragrant rice, developed by a rice institute in Pathum Thani Province, it's kernel and fragrance is so similar to Thai Hom Mali Rice Kernel, that consumers are often unable to tell it from the latter. Pathum Thani rice is mainly grown in the central provinces, which have abundant water. Two or three crops of Pathum Thani rice can be harvested each year, and it is considered as a replacement for Thai Hom Mali rice (Sreethong et al., 2018).

The current consumer trends stimulate strong

* Corresponding Author
e-mail: senajuk.w@gmail.com

interests and preferences in choosing cosmetics that contain natural extracts, because many natural extracts are now comparable to synthetic substances, obtained from laboratories including ability to nourish and protect the skin, antioxidant ability and the aging rejuvenation ability. Consumers are excited by these features and demand them, so cosmetic products, containing natural extracts, are therefore extremely interesting and in high demand by consumers. Free radicals refer to molecules that are unstable and sensitive to chemical reactions and encourage continuous destruction of other molecules in chain reactions. Free radicals are therefore toxic to the body cells. If there are many, it can be dangerous. They will destroy the cell membranes and other structures. In the short term, free radicals cause inflammation and tissue destruction while, in the long term, they affect the degeneration or aging of cells. At present, both domestic and international studies have shown that free radicals are associated with the occurrence of many non-communicable chronic diseases, especially cancer, which is one of the top causes of death for globally (Freddie et al., 2018). Free radicals are created both by the body's metabolic processes and in unhealthy conditions, for example, illnesses or polluted environments. These abnormal condition will cause the body to accumulate more free radicals. Therefore, it is vital for the body to find a way to prevent from being destroyed by free radicals. Antioxidant compounds promote health by protecting the cells of the body from damage caused by free radicals and reactive oxygen species (Pukumpuang & Seansrimon, 2020). They are various substances or enzymes with a low concentration that can delay or prevent the oxidation of substrates, which are sensitive to the reaction. These substrates include almost all substances in the body, for example, proteins, fats, carbohydrates and DNA. However, to some certain extent, the imbalance between free radicals and antioxidants in favor of free radicals results in oxidative stress (Khanthapok & Sukrong, 2019). This affects living cells, in various way, e.g. oxidation of DNA proteins, carbohydrates and destruction of molecules with S-H bonds and cell membranes, that have detrimental effects on cells and cell destruction. This directly leads to aging and some chronic diseases, such as stroke, autoimmune disease, diseases caused by the blood, returning to the organs, that have been constricted, due to short-term stroke, and cancer (Flachenecker, 2012). Antioxidants prevent formation of free radicals by inhibiting free radical chain reactions, stop new free radicals forming, repair damage to the body cells destroyed by free radicals, eliminate and replace the damaged molecules (Lobo et al., 2010). Fig. 1 showed the DPPH assay, an odd electron displays a strong absorption band at a wavelength of 519 nm, which loses absorption once the odd electron is paired off by a hydrogen or electron-donating antioxidant (Ningjian & David, 2014).

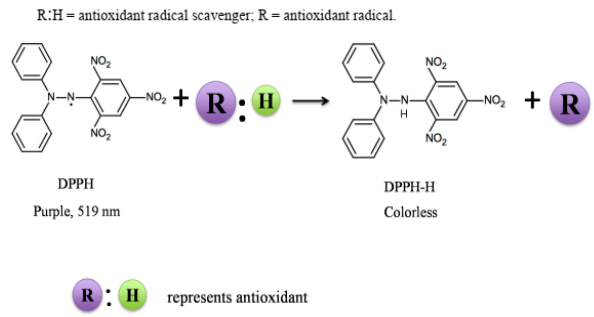


Fig. 1 Reaction mechanism of DPPH with antioxidant (Ningjian & David, 2014)

"Phenolic" refers to molecules containing at least one phenol group and many phenolic substances are found in plants. The total phenolic content varies with type of plant, planting method, degree of ripeness, processing and storage. Heat, used in the processing, reduces the amount of phenolic compounds. Many phenolic compounds have antioxidant properties. The hydroxyl group of the phenol, can be replaced by other cluster functions, *e.g.* flavonoids, lignins, cinnamic acid and coenzyme-Q (Moeiklang & Ruangviriyachai, 2014). Phenolic compounds have chemical formulas ranging from simple structural groups, the phenolic acids, to structural polymers, *e.g.* lignin. The largest group is the flavonoid compounds. Phenolic compounds found in plants often embedded in the sugar molecules in the form of glycosides. The most common type of sugar in the phenolic molecules is glucose and it was found that this perhaps is a recomposition between the phenolic compounds, or phenolic compounds with other compounds such as organic acids including in the molecules of the protein alkaloids and terpenoids (Moeiklang & Ruangviriyachai, 2014). They have been classified into six subgroups in Fig. 2 (Ali & Neda, 2011).

Carotenoid are yellow, orange or red pigments, commonly found in plants, algae, animals and microbes (Costache et al., 2012; Dhir et al., 2013). Carotenoid in plants can be found in different parts, *e.g.* fruits, flowers and roots. Carotenoids are highly effective antioxidants especially β-carotene, lycopene, and lutein which are

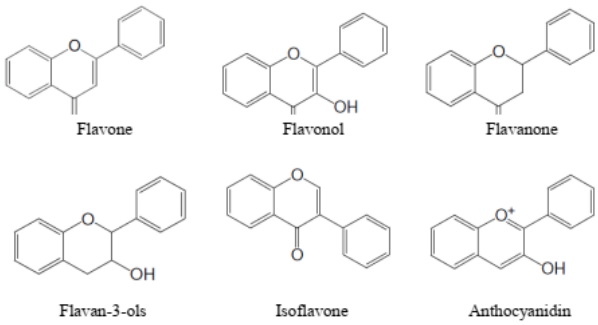


Fig. 2 Structure of some phenolics acids (Ali & Neda, 2011)

used as supplements to help prevent heart disease, cancer, aging and skin disorders caused by sunlight (Sadighara et al., 2016; Stahl & Sies, 2012; Aust et al., 2003; Fazekas et al., 2003). However, carotenoids are also anti-inflammatory and empowering the removal of foreign bodies from the human body. Thus carotenoids are important for the promotion of human health, *e.g.* β-carotene, lutein, cisanthin, lycopene and astaxanthin (Jia et al., 2011).

The sunlight reaching the surface of the earth consists of a broad spectrum, but, the radiation affecting the skin is quite obvious and labeled UV, which is divided into 2 types according to the wavelength: UV-A -in the range 320-400 nm and UV-B-in the range 290-320 nm (Ulrike et al., 2006). UV has positive effects on the skin, e.g. stimulating vitamin D production and skin pigmentation to protect the skin from sunlight (Anitha, 2012). However, UV is also somewhat harmful, in that UV-A can penetrate the skin to the dermis and destroy the collagen tissue and elastic fibers, which causes premature aging for skin cells, while UV-B, if consistent exposure, will cause the skin to swell, become red, swell and peel, causing sunburn, and it may develop skin cancer with long term exposure. The Sun Protection Factor (SPF) measures the effectiveness of sun protection, it tells consumers the length of exposure of the skin to sunlight, that the skin will tolerate without burning. For example, people normally exposed to sunlight for 10 minutes will feel burning pain or skin burn. However, after applying cosmetics to the skin, with SPF 15 sunscreen, exposure can be prolonged by 15 times or 150 minutes, without skin burn. Therefore, if consumers know and understand sunscreen products, they can more accurately choose the right sunscreen items and achieve the highest efficiency in protecting the skin from UV.

Thus, here we measured total phenolic content, total flavonoid content, free radical scavenging activity, lycopene, β-carotene, color, SPF, morphology and elemental composition for use as foundation data to use these raw materials, for developing effective face powder formulae.

Materials and methods

1. Extraction preparation

10 g Riceberry powder (or Pathum Thani rice powder) was soaked in 100 mL 95 % ethanol in a closed container, left it for 24 hr, then filtered with vacuum filter on Whatman filter paper No.1 and evaporated ethanol in a vacuum rotary evaporator at 75˚C.

2. Total phenolic content

Following a Folin-Ciocalteu assay method, modified from Butsat & Siriamornpun (2010), 100 mL Riceberry extract sample (or Pathum Thani rice extract sample) with a concentration of 2 mL per mL and 500 µL of 10% Folin Ciocalteu reagent were transferred to a test tube. It was shaken and left at room temperature for 1 min. After that, 1 mL 20 % w/v Na_2CO_3 was added shaken and left in a dark room for 60 min. Absorbance was measured at a 760 nm, using a UV-Vis spectrophotometer. Three replicates were measured. Total phenolic content was determined by comparing absorbances to the gallic acid calibration graph. Results were reported as mg gallic acid equivalent to 1 g of extracts (mg GAE/g of extract).

3. Total flavonoid content

Following a method, modified from Prabnok et al. (2016), 1 mL Riceberry extract sample (or Pathum Thani rice extract sample) with concentration of 2 mg/mL was pipetted into a test tube and added 4 mL distilled water, shaken and added 300 µL 5% $NaNO_2$. The mixture was shaken again and left for 5 min at room temperature. After that, 300 µL 10% $AlCl_3$ was added and left at room temperature for 6 min. Then, 225 µL of 1 M NaOH and 775 µL distilled water were added. The mixture was shaken and measured the absorbance at wavelength 510 nm using UV-Vis spectrophotometer. The results were reported in mg Rutin/g of extract.

4. DPPH radical scavenging assay

Following a method, modified from Butsat & Siriamornpun (2010), 1 mL of Riceberry extract sample (or Pathum Thani rice extract sample), with concentrations of 31.25, 62.50, 125.00, 250.00 and 500.00 µg/mL was pipetted into a test tube and 3 mL 0.2 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) was added. It was shaken and left in the dark for 30 min. Then, it

was measured the absorbances at 517 nm by a UV-Vis spectrophotometer, using 95 % ethanol as blank and using 0.1 mM DPPH as control. The fraction of DPPH inhibition was calculated by plotting a graph of the relationship between % DPPH inhibition and sample concentration to indicate efficient concentration 50 (EC_{50}), which represents the antioxidant content that reduces DPPH concentration to 50 % by using butyl hydroxytoluene (BHT) as a standard substance. Three replicates were measured. The free radical scavenging activity was calculated as follows:

$$\text{Free radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

where Abs_{sample} is the absorbance of DPPH with sample and $Abs_{control}$ is the absorbance of DPPH without sample. A linear regression was calculated from activity vs concentration to find EC_{50} in µg/mL.

5. Lycopene and beta-carotene content

Following a method, modified from Nagata & Yamashita (1992), 1 g of gac fruit (or wood apple) powder was added 20 mL 4:6 acetone: hexane and stirred for 15 min. Absorbances were measurement at 453, 505, 645 and 663 nm. The following equations were used to find concentration from absorbances:

$$\text{Lycopene (mg/100 mL)} = -0.0458Abs_{663} + 0.204Abs_{645} + 0.372Abs_{505} - 0.0806Abs_{453}$$
$$\beta\text{-carotene (mg/100 mL)} = 0.216Abs_{663} - 1.22Abs_{645} - 0.304Abs_{505} + 0.452Abs_{453}$$

6. Color measurement

Colors were measured in the CIELab L* a* b* space, when L* represents "lightness", ranging from 0 to 100, where 0 means low brightness and 100 means high brightness, a* measures green vs red and b* measures blue vs yellow (Mir et al., 2013). Total color differences, ΔE, where computed as:

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

where ΔL = L* - L, Δa = a* - a , and Δb = b* - b and L*, a*, b* refer to colors of the reference object and L, a, b refer to colors of the sample.

7. Sun protection factor (SPF)

Following a method, modified from Dutra et al. (2004), 100 g sample was weighed and transferred to a 100 mL volumetric flask, diluted to volume with ethanol, shaken for 5 minutes and filtered through a Whatman No.1 filter paper. The filtrate was collected after rejecting the first 10 mL of filtrate. Then a 5.0 mL aliquot was transferred to a 50 mL volumetric flask and diluted to volume with ethanol. Subsequently, a 5.0 mL aliquot was transferred to a 25 mL volumetric flask and the volume completed with ethanol. The filtered solution was put in a quartz cell and the absorbance was measured from 290 to 320 nm at 5 nm steps. The measurement was repeated three times. SPF was calculated from the absorbances (Mansur et al., 1986):

$$SPF = CF \, x \sum_{290}^{320} EE\,(\lambda) x\, I(\lambda) x\, Abs(\lambda)$$

where $EE(\lambda)$ is an erythemal effect spectrum, $I(\lambda)$ mean solar intensity spectrum, $Abs(\lambda)$ means absorbance of sunscreen products and CF means correction factor (=10).

8. Morphology and elemental composition

Morphology and elemental composition of samples were checked by scanning electron microscopy (SEM); model LEO 1455 VP, Germany, coupled with energy dispersive X-ray (EDX Oxford, ISIS 300, England) analyzer.

9. Formula for face powder from rice and natural substance

Samples of two types of broken rice were ground, until they passed a 200 mesh filter by peeling off the rice shells. The gac fruit powder was made from the flesh covering its seeds. The wood apple powder was derived from wood apple tree branches. Samples were prepared using mixtures listed in Table 1, ground, mixed together, and packed in containers.

Table 1 Percentage of the components in the form of face powder formula

Component (%)	Formula 1	Formula 2
Talcum	-	60
Pathum Thani rice	22	-
Riceberry	6	-
Wood apple	3	-
Gac fruit	44	-
Zinc oxide	3	5
Zinc stearate	3	10
Magnesium carbonate	3	10
Kaolin	3	5
Calcium carbonate powder	13	10
Total	**100**	**100**

Results and discussion

Table 2 showed that Riceberry extract sample had total phenolic content at 26.46±0.008 mg of gallic acid/g of extract, while Pathum Thani rice extract sample had 3.67±0.010 mg. The higher phenolic content for Riceberry extract sample was likely due to the darker color of Riceberry seed, showing that it containing more flavonoids. For flavanoid content, the Riceberry extract sample had 101.63±0.034 mg rutin/g and Pathum Thani extract sample had 56.04±0.004 mg of rutin/g.

Table 2 Total phenolic and flavonoid content of rice extract sample

Sample	Total phenolic (mg of gallic acid/g of extract)	Total flavonoid (mg of rutin/g of extract)
Pathum Thani rice	3.67±0.010	56.04±0.004
Riceberry	26.46±0.008	101.63±0.034

Fig. 3 illustrates calculation of the efficient concentration 50 (EC_{50}) of Riceberry extract sample, based on the DPPH assay, using the plot of % free radical scavenging activity *versus* concentration. As expected, free radical scavenging activity increased with sample concentration-dose-dependent curve. Table 3 indicates that the antioxidant activities, EC_{50}, were 108.31±0.024 for Riceberry extract sample and 430.46±0.062 µg/mL for Pathum Thani rice extract sample. This confirms reports from Chakuton et al. (2012), Chen et al. (2012) and Moko et al. (2014), who found that colored rice had higher amounts of phenolic compounds and antioxidant activity than colorless rice. That is, the extracts with a low EC_{50} hold good free radical scavenging activity.

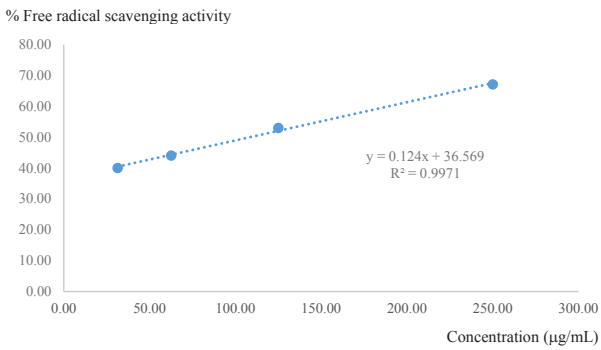


Fig. 3 The relationship between concentration and % free radical scavenging activity of Riceberry extract sample

Table 3 Antioxidant activity of rice extract sample

Sample	EC_{50} (g/mL)	Linear regression	R^2
Butylated hydroxytoluene (BHT)	128.68±0.024	y = 0.124x + 34.034	0.9971
Pathum Thani rice	430.46±0.062	y = 0.108x + 3.337	0.9983
Riceberry	108.31±0.024	y = 0.124x + 36.569	0.9971

Table 4 shows free radicals capturing ability, measured by DPPH assay method. Gac fruit had the highest EC_{50} at 290.39±0.032 µg/mL, lycopene at 1.1099±0.099 mg/100 mL and β-carotene at 0.6292±0.081 mg/100 mL on a dry basic. Gac fruit had the clear pigment at L* = 56.70±0.02, a* is 42.77±0.02, b* = 40.94±0.05. SPFs in Table 5 showed that wood apple offered the highest of SPF at 6.78±0.097.

Table 4 Antioxidant activity, Lycopene and β-carotene of natural extract

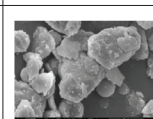
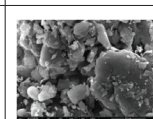
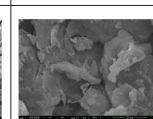
Properties	Gac fruit	Wood apple
Antioxidant activity (g/mL)	290.39±0.032	55.67±0.045
Lycopene (mg/100 mL dry basic)	1.1099±0.099	0.0346±0.000
β-carotene (mg/100 mL dry basic)	0.6292±0.081	0.0400±0.000

Table 5 Sun protection factor of samples

Sample	Riceberry	Phatum Thani	Gac fruit	Wood apple
SPF	1.83±0.083	1.12±0.159	1.58±0.155	6.78±0.097

Table 6 indicates that face powder made from rice and natural extract offers ΔE of color closer to commercial face powder No.3 than talcum face powder. Face powder from rice and natural extracts offered better SPF than talcum powder, which means that rice and natural extracts can protect from UV even though the sun protection value is not very high, compared to those of synthetic sunscreens in the market. However, natural extracts also contained useful antioxidants, which may be used in combination with synthetic sunscreen, to enhance sunscreen efficiency and reduce the use of synthetic sunscreen. Analysis for metals content showed no heavy metals according to TIS 981-2013 standards, which prescribes no more than 20, 5, 1 and 0.05 ppm of lead, arsenic, mercury and barium by weight. The SEM images indicated that the powder formula was similar to that of the commercial formula which had round particles, while the talcum powder formula showed sheet-like particles.

Table 6 Chemical composition and morphology of samples

Item	Commercial face powder No.3	Formula 1	Formula 2
Color			
L*	62.61±0.00	75.73±0.02	92.81±0.02
a*	13.66±0.02	8.09±0.06	-1.48±0.01
b*	23.86±0.02	13.50±0.04	2.06±0.01
ΔE	-	17.62±0.05	40.20±0.01
SPF value	39.39±0.00	1.19±0.06	0.60±0.12
Element by weight (ppm)			
• Lead	nd	nd	nd
• Arsenic	nd	nd	nd
• Mercury	nd	nd	nd
• Barium	nd	nd	nd
Morphology			

Remark: nd = not detected

Conclusion

We showed that face powders based on natural ground rice with added natural ingredients provided a useful level of sun protection, additional antioxidants and free radical scavenging, yet contained no toxic heavy metals, thus were beneficial and safe. Riceberry and Phatum Thani rice both showed significant levels of phenolic content-26.46±0.008 mg GAE/g and 3.67±0.010 mg GAE/g; flavonoids-101.63±0.034 mg Rutin/g and 56.04±0.004 mg Rutin/g-and antioxidants at 108.31±0.024 µg/mL and 430.46±0.062 µg/mL. SPFs were Riceberry -1.83±0.083, Pathum Thani rice-1.12±0.159, gac fruit -1.58±0.155 and wood apple-6.78±0.097.

Thus natural extracts are highly appropriate for use as an ingredient in cosmetic products; further, they show unique local identity and pride for the community, that, could earn more income and promote career choices for people in it. Antioxidants in these natural extracts can additionally offer rejuvenation of the cells and reduce the use of synthetic sunscreens. Therefore, they could be developed further for future research projects.

References

Ali, G., & Neda, G. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research, 5*(31), 6697-6703.

Anitha, T. (2012). Medicinal plants used in skin protection. *Asian Journal of Pharmaceutical and Clinical Research, 5*(3), 35-38.

Aust, O., Wilhelm, S., Helmut, S., Hagen, T., & Dan, U.H. (2003). Supplementation with tomato based products increase lycopene, phytofluene, and phytoene levels in human serum and protects against UV-light-induced erythema. *International Journal for Vitamin and Nutrition Research, 75*(1), 54-60.

Butsat, S., & Siriamornpun, S. (2010). Antioxidant capacities and phenolic compound of the husk, bran and endosperm of Thai rice. *Food Chemistry, 119,* 606-613.

Chakuton, K., Puangpronpitag, D., & Nakornriab, M. (2012). Phytochemical content and antioxidant activity of colored and non-colored Thai rice cultivars. *Asian Journal of Plant Sciences, 11*(6), 283-293.

Chen, X.Q., Nagao, N., Itani, T., & Irifune, K. (2012). Anti-oxidative analysis, and identification and quantification of anthocyanin pigments in different coloured rice. *Food Chemistry, 135,* 2783-2788.

Costache, M.A., Campeanu, G., & Neata, G. (2012). Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. *Romanian Biotechnological Letters, 17*(5), 7702-7708.

Dhir, R., Harkess, R.L., & Bi, G. (2013). Physiological responses of ivy geranium beach and butterfly to heat stress. *Journal of the American Society for Horticultural Science, 138*(5), 344-349.

Dutra, E.A., Oliveira, D.A.G.C., Kedor-Hackmann, E.R.M., & Santoro, M.I.R.M. (2004). Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. *Brazilian Journal of Pharmaceutical Sciences, 40*(3), 381-385.

Fazekas, Z., Gao, D., Saladi, R.N., Lu, Y., Lebwohl, M., & Wei, H. (2003). Protective effects of lycopene against ultraviolet B-induced photodamage. *Nutrition and Cancer, 47*, 181-187.

Flachenecker, P. (2012). Autoimmune diseases and rehabilitation. *Autoimmunity Reviews, 11,* 219-225.

Freddie, B., Jacques, F., Isabelle, S., Rebecca, L.S., Lindsey, A.T., & Ahmedin, J. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer Journal Clin, 68*(6), 394-424.

Jia, D., Fan, L., Liu, G., Shen, J., Liu, C., & Yuan, Y. (2011). Effects of genotypes and bagging practice on content of ß-carotene in apple fruits. *Journal of Agricultural Science, 3*(4), 196-202.

Khanthapok, P., & Sukrong, S. (2019). Anti-aging and health benefits from Thai food: Protective effects of bioactive compounds on the free radical theory of aging. *Journal of Food Health and Bioenvironmental Science, 12*(1), 54-67.

Leardkamolkarn, V., Thongthep, W., Suttiarporn, P., Kongkachuichai, R., Wongpornchai, S., & Wanavijitr, A. (2011). Chemopreventive properties of the bran extracted from a newly-developed Thai rice: The riceberry. *Food Chemistry, 125*(3), 978-985.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Review, 4*(8), 118-126.

Mansur, J.S., Breder, M.N.R., Mansur, M.C.A., & Azulay, R.D. (1986). Determinacao do factor de protecao solar por espectrofotometria. *Anais Brasileiros de Dermatologia, 61,* 121-124.

Mir, S.A., Bosco, S.J.D., & Sunooj, K.V. (2013). Evaluation of physical properties of rice cultivars grown in the temperate region of India. *International Food Research Journal, 20*(4), 1521-1527.

Moko, E.M., Purnomo, H., Kusnadi, J., & Ijong, F.G. (2014). Phytochemical content and antioxidant properties of colored and non colored varieties of rice bran from Minahasa, North Sulawesi, Indonesia. *International Food Research Journal, 21*(3), 1053-1059.

Moeiklang, N., & Ruangviriyachai, C. (2014). Determination of phenolic compounds and antioxidant potential in fruit beverages. *KKU Research Journal, 14*(4), 69-79.

Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Journal of the Japanese Society for Food Science Technology, 39,* 925-928.

Ningjian, L., & David, D.K. (2014). Antioxidant property of coffee components: Assessment of methods that define mechanisms of action. *Molecules, 19,* 19180-19208.

Prabnok, J., Phasuk, S., & Takolpuckdee, P. (2016). Chemical compounds and antioxidation efficiency of *Livistona speciosa* Kurz. Seed crude extract. *Thai Journal of Pharmaceutical Sciences, 40*(Supplement Issue), 112-115.

Pukumpuang, W., & Seansrimon, J. (2020). Antioxidant activity, total phenolic content and anti-tyrosinase activity of Thai colored rice cultivar extracts. *Journal of Food Health and Bioenvironmental Science, 13*(1), 34-42.

Sadighara, P., Saghafi, M., Erfanmanesh, A., & Mahdaviyekta, M. (2016). Antioxidant activity and properties of outer shell pistachios in different temperature of cooking. *Der Pharmacia Lettre, 8*(12), 263-266.

Sreethong, T., Prom-u-thai, Ch., Rerkasem, B., Dell, B., & Jamjod, S. (2018). Variation of milling and grain physical quality of dry season Pathum Thani 1 in Thailand. *Chiang Mai University Journal of Natural Sciences, 17*(3), 191-202.

Stahl, W., & Sies, H. (2012). Photoprotection by dietary carotenoids: Concept, mechanisms, evidence and future development. *Molecular Nutrition & Food Research, 56,* 287-295.

Suttiarporn, P., Sookwong, P., & Mahatheeranont, S. (2016). Fractionation and identification of antioxidant compounds from bran of Thai black rice cv riceberry. *International Journal of Chemical Engineering and Applications, 7*(2), 109-114.

Ulrike, P.K., Dan, L., Marisa, O., Karl, S., & Thomas, M.R. (2006). Short and long wave UV light (UVB and UVA) induce similar mutations in human skin cells. *Journal of Investigative Dermatology, 126*(3), 667-675.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Effects of Components on Qualities of Crispy Bamboo Shoot Snack

Narin Charoenphun[a]* & Kanokporn Pakeechai[b]

[a] *Faculty of Agricultural Technology, Burapha University Sakaeo Campus, Sakaeo 27160, Thailand*
[b] *Faculty of Business Administration and Information Technology, Rajamangala University of Technology Suvarnabhumi, Phranakhon Si Ayutthaya 13000, Thailand*

Article info

Article history:
Received : 5 October 2020
Revised : 9 November 2020
Accepted : 18 November 2020

Keywords:
Crispy snack, Bamboo shoot, Tapioca flour

Abstract

The objective of this research was to study the effect of ingredients on the quality of crispy bamboo shoot snack. The effects of different ratios of tapioca flour and bamboo shoots on the quality of crispy bamboo shoot snack were studied. It was found that the optimum ratio of tapioca flour and bamboo shoot was 50:50. It had good appearance and swelling rate after frying. Moreover, the aroma of bamboo shoots was clear. The effects of different flour types on the quality of crispy bamboo shoot snack were investigated. It was found that tapioca flour was suitable flour for crispy bamboo shoot snack production. It had good appearance and swelling rate after frying that it was higher than the formula using glutinous rice flour, rice flour and wheat flour. In addition, the texture of the crispy bamboo shoot snack was crispy at an appropriate level. The effects of flavoring with various flavors on the sensory acceptance of consumers were investigated. The original, seaweed mixed with black sesame and spicy flavors had a higher score in appearance, flavor, taste, texture and overall liking score than other flavors. The analysis of the chemical composition of crispy bamboo shoot snack was investigated. The moisture, protein, fat, total carbohydrate, ash, and energy were 2.73-3.50 %, 1.04-2.39 %, 26.91-28.53 %, 66.36-66.67 %, 1.89-1.98 % and 513.08-523.81 kcal, respectively. It can be used as a guideline for crispy bamboo shoot snack production for commercial distribution.

Introduction

Bamboo shoots are the young shoots of bamboo trees. It is a raw material for food products that is delicious and rich in nutritional value. It is considered one of the five most popular health foods in the world. Bamboo shoots are used as raw materials for cooking in many countries such as China, India, Japan and Southeast Asian countries. Currently, bamboo shoots are processed into food products at the industrial level, such as dried bamboo shoots, pickled bamboo shoots, bamboo shoots soaked in brine, and canned bamboo shoots, etc. In terms of nutritional value, the chemical composition of fresh bamboo shoots is high in protein content between 1.49 to 4.04 (average 2.65 grams) per 100 grams of fresh bamboo shoots and contains 17 amino acids. There are 8 types of essential amino acids, especially tyrosine, which is approximately 57-67 % of all amino acids and

* Corresponding Author
e-mail: narinch@buu.ac.th

it has a low-fat content of approximately 0.26-0.94 % (Chongtham et al., 2011).

Bamboo shoots have a high fiber content of about 6-8 grams per 100 grams of fresh weight. Furthermore, it has high phytosterols, which is a useful chemical substance. It helps reduce cholesterol and has anti-cancer activity (Brufau et al., 2008). In addition, bamboo shoots are a good source of vitamin B, niacin, vitamin A, vitamin B6 and vitamin E. There are many important minerals in bamboo shoots such as potassium, calcium, manganese, zinc, chromium, copper, and iron (Nirmala et al., 2007). The benefits mentioned above, thus bamboo shoots are a potential raw material in the food industry. Nowadays, planting bamboo shoots in Thailand can be found in every region throughout the country. Bamboo shoots that are planted for sale tend to continuously grow. If properly maintained, it can produce throughout the year. The bamboo shoots have very low price in the rainy season, which is a problem encountered by agriculturists. If there is no proper management process after cutting the bamboo shoots, the quality of fresh bamboo shoots will decrease rapidly. Therefore, the food products from bamboo shoots processing is an interesting way that helps to extend the shelf life of bamboo shoots and value adding to agricultural products.

Crispy snack is a product obtained from mixing flour with seasoning. It may contain meat, vegetables, or fruits such as fish, shrimp, pumpkin, taro, black sesame, and white sesame, etc. It is mixed and molded into the desired shape. Next, the mixture is steamed until cooked and cut into thin strips or shaped as desired. It is dried using heat from sunlight or other energy sources. It may be fried before packing or not fried (Thai industrial standards institute, 2006). Generally, there are two types of crispy snack products, including raw crispy snack and ready-to-eat crispy snack. Currently, there are many crispy snacks producing research from a variety of raw materials, such as fish (Tantasuttikul & Samuankid, 2014) Champedak (Wongsudaluk, 2016) mushroom (Khaepimyi & Nepthangdee, 2018) Burma bean (Suriya et al., 2011) etc. The fresh bamboo shoots are used as raw materials for crispy snack production that help increase the nutritional value of products and as an alternative food to consumers. Normally, there are many factors affecting on the quality of the crispy snack including the proportion of flour and other ingredients, the type of flour, the process of partially making the dough, kneading, steaming, drying, and frying (Tangkanakul, 2003).

The objective of this research is to study the effect of ingredients on the quality of crispy bamboo shoot snack. The apparent, physiochemical, and sensory acceptance were investigated to select the suitable formula for production as a prototype of crispy bamboo shoot snack. The knowledge gained from this research can be used as a guideline for the crispy bamboo shoot snack production. Moreover, it is way for value adding to agricultural products as well as further expanding into production for commercial distribution.

Materials and methods

1. Raw materials

The ingredients used in crispy bamboo shoot snack were composed of sugar (Mitrphol, Suphan Buri, Thailand), salt (Prungthip, Nakorn Ratchasima, Thailand), glutinous rice flour and rice flour (Erawan brand, Nakhon Pathom, Thailand), tapioca flour (Fish brand, Nonthaburi, Thailand), wheat flour (Kite, Samut Prakan, Thailand), vegetarian mushroom seasoning powder (Fah Thai, Nakhon Pathom, Thailand), Barbecue seasoning powder (OK, Samut Sakhon, Thailand), black sesame and ground black pepper (Raithip, Nonthaburi, Thailand), dried seaweed and garlic (Tontawan, Samut Prakan, Thailand). The bamboo shoots (*Dendrocalamus asper* Backer) were obtained from the agriculture group in Sa Kaeo province. The fresh bamboo shoots were cut. The bamboo shoots with similar growth stages of equal size and length approximately 30 cm were selected. The fresh chilli (*Capsicum annum* L.) young galangal (*Alpinia galanga* (L.) Willd.) and Yanang leaves (*Tiliacora triandra* (Colebr.) Diels) were bought from a local market in Sa Kaeo province.

2. Study on the effect of different ratios of tapioca flour and bamboo shoots on the quality of crispy snack

The bamboo shoots preparation, the shell was removed. It was washed with clean water, and was cut into small pieces before boiling in hot water for 30 min. It was removed from the hot water and was blended until fine. The ratio of tapioca flour to bamboo shoots was, 100:0, 75:25, 50:50 and 25:75, respectively. The blended bamboo shoots were heated with steaming for 1 min before mixing with tapioca flour that It was kneaded easily. The 60 % hot water was added in 100 % tapioca flour formula. The 25 % hot water was added in 75 % tapioca flour formula, while 50 % and 25 % tapioca flour formula without water added. The crispy snack production process was modified from Tangkanakul

(2003). First of all, tapioca flour and bamboo shoots according to the recipe were mixed and kneaded together. Secondly, the mixture was molded into a rectangle 40 mm long and 20 mm wide before steaming for 1 hr. Then, it was put in room temperature and cooled at 4˚C for 12 hr. It was cut into sheets with a thickness of about 2 mm and dried at 60˚C for 6 hr. The raw crispy snack products were kept in sealed container. The ready-to-eat crispy snack preparation, the raw crispy snacks were fried at 180˚C for 30 sec or until or until cooked throughout the sheet by observing from the blooming of the crispy snack and were kept in sealed container.

The appearance of crispy snack was observed, consisting of forming ability after mixing, swelling after steaming, gel stability after steaming, cutting ability after chilling, swelling rate after frying, hardness of raw crispy snack, hardness of ready-to-eat crispy snack, bamboo shoots flavor intensity of ready-to-eat crispy snack and yellow color intensity of ready-to-eat crispy snack. The analysis of the quality of the crispy snack in various areas as follows:

2.1 The weight loss value of ingredients after steaming, cooling, drying, and frying were analyzed. The crispy snacks before and after processing were weighted by balance (Zepper EPS-3001, China). Weight loss was defined as follows: weight loss (%) = [(Weight of crispy snacks before processing - Weight of crispy snacks after processing)/ Weight of crispy snacks before processing] x 100 (Kotoki & Deka, 2010).

2.2 The moisture content in raw crispy snacks and ready-to-eat crispy snacks were measured (Moisture Meter, GM640, China).

2.3 The swelling rate of crispy snacks was analyzed by replacing sesame seeds (Tiwthao, 2014). The swelling rate was defined as follows: swelling rate = (Volume of raw crispy snack (ml) / Weight of raw crispy snacks (g)) / (Volume of ready-to-eat crispy snacks (mg) / ready-to-eat crispy snacks (g))

2.4 The hardness of raw and ready-to-eat crispy snacks were measured using a hardness instrument (Daiichi FG 520K, Japan). The cylindrical probe was used, and the unit of force was newton (N).

2.5 Color of raw and ready-to-eat crispy snacks were measured by color meter (Colorimeter, WR10QC, China). The CIE system was defined by L * or brightness (0 = black, 100 = white), a * (+ a = red, -a = green) and b * (+ b = yellow, -b = Blue).

The optimum ratio of bamboo shoots and tapioca flour was selected to produce crispy bamboo shoot snacks in the next step.

3. Study on the effect of different flour types on the quality of crispy bamboo shoot snacks

The qualities of crispy bamboo shoot snack products using tapioca flour, glutinous rice flour, rice flour and wheat flour were compared. The ratio of tapioca flour to bamboo shoots selected in the previous step was used in the crispy bamboo shoot snack production. The crispy bamboo shoot snacks production, according to the steps mentioned above was carried out. The appearance of crispy bamboo shoot snack during the production process was observed, consisting of forming ability after mixing, swelling after steaming, gel stability after steaming, cutting ability after chilling, swelling rate after frying, hardness of raw crispy snack, hardness of ready-to-eat crispy snack, bamboo shoots flavor intensity of ready-to-eat crispy snack and yellow color intensity of ready-to-eat crispy snack. The weight loss, moisture content, swelling rate, hardness, and color value were investigated as follows above method. The suitable flour that had good quality of crispy bamboo shoot snacks was selected for production in the next step.

4. Study on the effects of flavoring of crispy bamboo shoot snacks with various flavors on the sensory acceptance

Raw material preparation: (1) The fresh garlic was peeled and washed before blending until fine. (2) Young galangal was washed and cut into small pieces before blending until fine. (3) Yanang leaves were washed and cut into small pieces. Yanang leaf to water ratio was 1: 1 by weight before blending until fine. It was filtered with a thin white cloth. Yanang water was boiled with bamboo shoots for 30 min. (4) Dried seaweed was blended into fine powder. (5) The fresh chilli was washed before blending until fine.

The crispy bamboo shoot snacks production using various flavors, consisting of original, garlic and pepper, young galangal and Yanang leaves, seaweed and black sesame, spicy and barbecue were investigated. The 1% sugar and 0.3% vegetarian mushroom seasoning powder were added in original formula and other formulas of crispy bamboo shoot snacks. The 0.5% garlic and 0.1 % pepper were added in garlic and pepper formula. The 0.5% young galangal was added in galangal and Yanang leaf formula. The 2% black sesame and 1% seaweed were added in seaweed and black sesame formula. The 0.5% fresh chilli was added to spicy formula. The 1% barbecue flavor was added to barbecue formula. The production of crispy bamboo shoot snacks

according to the steps mentioned above was carried out. The sensory evaluation by 30 untrained panelists was investigated. The importance of liking of appearance, flavor, taste, texture, and overall liking were expressed by 9-point hedonic scale. The suitable formula was selected for crispy bamboo shoot snacks prototype products.

5. Chemical composition analysis

Chemical composition of the crispy bamboo shoot snacks including moisture content, protein, fat, carbohydrate, ash and total energy were investigated (Association of Official Analytical Chemists, 2012).

6. Statistical analysis

The completely randomized design (CRD) was used. The statistical technique one-way ANOVA was used for calculating. Duncan's new multiple-range Test (DMRT) was used to compare the difference in the average values at the 95% confidence level.

Results and discussion

1. Effect of different ratios of tapioca flour and bamboo shoots on the quality of crispy snack

Effects of different ratios of tapioca flour and bamboo shoots on the observable characteristics of crispy snacks are shown in Table 1. It was found that the ratio of tapioca flour to a bamboo shoot at 25:75 was not suitable for crispy snack production. Obviously, it was formulated with a low tapioca flour and high bamboo shoots content. It was difficult to mold due to there being too little tapioca flour resulting in the low agglomeration of the mixture. The texture of crispy snacks during forming was very soft. There was a low swelling rate after steaming. The gelling structure after steaming was weak and unstable. It was quite difficult to cut into pieces after chilling. The crispy snack had the lowest swelling rate after frying. The texture of raw crispy snack and ready-to-eat crispy snack were quite fragile. Apparently, the ratio of tapioca flour and bamboo shoots at 100:0, 75:25 and 50:50 was suitable for crispy snack production. It was molded easy due to the appropriate ratio of tapioca flour and bamboo shoots, resulting in better agglomeration of raw materials. The texture of crispy snacks during forming molds easily, not too sticky or too messy. There was a high swelling rate after steaming. The gel structure was quite strong and stable. It was quite easy to cut into pieces after chilling. The crispy snack had the highest swelling rate after frying. The suitable texture of raw crispy snack and ready-to-eat crispy snack were suitably crispy were not easily broken, not sticky or hardened.

The ratio of tapioca flour and bamboo shoots were an important factor in the quality of the crispy snack. If the bamboo shoots add too little, there will be no fragrance of bamboo shoots such as the ratio of tapioca flour to bamboo shoots at 100:0 and 75:25. Conversely, the bamboo shoots were added in high volume such as the ratio of tapioca flour to bamboo shoots at 25:75, resulting in the low swelling rate. In terms of color, 100 % tapioca flour was used in crispy snack which was white in color. Bamboo shoots were added at 25 %, 50 % and 75 % that was yellow color of crispy snack. The intensity of the yellow color will increase, according to the amount of bamboo shoots in the formula. Basically, the production of crispy snack relies on the basic principle of gelatinization of the flour granule is occurring, which must be completely produced to get a good product (Tangkanakul, 2003). In general, characteristics of crispy snack according to product standards are specified. The raw crispy snack must be dry, non-sticky, and in separate pieces. The ready-to-eat crispy snack must be crispy, not sticky, or more hardened, good, and consistent swelling (Thai industrial standards institute, 2006). The flour solution is gelatinization when heated during steaming. It causes changes in the internal molecules because the hydrogen bonds within the starch molecules was destroyed by the heating process. The amylose and amylopectin polymers that are tightened in the granule will relax and combine with the surrounding water, resulting in changes in appearance, puffed of grain, and the viscosity of the flour solution continuously increasing (Damondaran & Parkin, 2017).

Table 1 Effect of different ratios of tapioca flour and bamboo shoots on the observable appearance of crispy bamboo shoot snack

Observable appearance	The ratio of tapioca flour to bamboo			
	100 : 0	75 : 25	50 : 50	25 : 75
Forming ability after mixing	+++++	+++++	+++++	++
Swelling after steaming	+++++	++++	++++	+
Gel stability after steaming	+++++	+++++	+++++	+
Cutting ability after chilling	+++++	+++++	+++++	++
Swelling rate after frying	+++++	+++++	+++++	+
Hardness of raw crispy snack	+++++	++++	++++	++
Hardness of ready-to-eat crispy snack	++++	+++	+++	+
Bamboo shoot flavor intensity of ready-to-eat crispy snack	-	++	++++	+++++
Yellow color intensity of ready-to-eat crispy snack	-	++	++++	+++++

Remark: + = very low, ++ = low, +++ = moderate, ++++ = high, +++++ = very high

The effects of different ratios of tapioca flour and bamboo shoots on weight loss, moisture content, swelling rate and hardness of crispy bamboo shoot snack are shown in Table 2. It was found that the weight loss after steaming varied directly as the amount of bamboo shoots. The weight loss after freezing, drying and low frying were inversely proportional to the amount of bamboo shoots. Specifically, the amount of tapioca flour had the lowest content that it caused the gel weakness after gelatinization. Moreover, the high initial moisture content of boiled bamboo shoots was 91.90-93.50 % (Mogkhuntod et al., 2017). It caused an unbalanced composition with a higher amount of liquid than solid. The free water had a higher evaporation during steaming, resulting in greater water loss than other formulas. In contrast, the formulas using the ratio of tapioca flour to bamboo shoots at 100:0 75:25 and 50:50 were observed. It was formulated with the right amount of tapioca flour and bamboo shoots. The gelatinization during steaming had a strong structure that the low weight loss.

The weight loss after chilling of all formulas have no significant difference to the 95% ($P > 0.05$). The weight loss after chilling may result from starch retrogradation. It occurs when a gelatinized starch paste was cooled. The glucose molecules in amylose and amylopectin chains are reconnected with hydrogen bonds and the water is eliminated. It is called syneresis that new crystals were created (Damondaran & Parkin, 2017). The weight loss after drying varied directly as the amount of tapioca flour. The water is eliminated during drying when air or hot air blows over the surface causing weight loss. The heat is transferred to the surface of the crispy snack and the water is eliminated by the latent heat of vapor formed. The water vapor is spread through the air film and is blown away by moving hot air. It causes the vapor pressure at the surface of the crispy snack that it is lower than the pressure on the inside of the crispy snack, resulting in differences in vapor pressure. The inner layer of crispy snack will have high vapor pressure and gradually decrease when the crispy snack layer near the dry air. This difference of vapor causes pressure to expel water from the crispy snack (Fellows, 2017).

The weight loss after frying varied directly as the amount of tapioca flour. In general, the method used for crispy snack frying is deep-fat frying. The heat transfer in the oil, frying is the convection of the hot oil and the heat conduction inside the products. The product surface will receive similar heat, resulting in consistent color and external appearance (Mogkhuntod et al., 2017). Most water is eliminated during the drying process. Therefore, there will be only a small amount of free water in raw crispy snack, resulting in a slight weight loss during the frying process. There are oil particles replacing the amount of evaporated water and the space inside the product, causing the oil content to increase and moisture to be reduced (Rattanathammawat et al., 2003).

The moisture content of all raw crispy snacks had an average of 9% by weight and ready-to-eat crispy snack has an average of 3% by weight. It was in the value range as according to the standard crispy snack products. The moisture content of raw crispy snacks must not exceed 12% by weight and the moisture content of ready-to-eat crispy snack must not exceed 4% by weight (Thai industrial standards institute, 2006). Drying was an important step affecting on puffing and pore consistency which was related to the moisture content in the crispy snacks. Generally, a crispy snack is heated from oil, which causes the water in the flour to vaporize. The vapor pressure in the crispy snack expands into a pore. The optimum moisture of raw crispy snack is approximately 12% by weight. If the raw crispy snack has moisture below 7-8%, that is baked too long, the raw crispy snack will crack. Conversely, if it is baked, or dried in the sun is a short time, will get a raw crispy snack with high humidity. There are some large, small, irregular porous holes when deep-frying that is characteristic of low-quality crispy snack (Tangkanakul, 2003). The hardness and swelling rates from raw crispy snack to ready-to-eat crispy snack after frying were observed. It is found that all formulas of crispy snack, a swelling rate and hardness have significance differences of the 95% ($P \leq 0.05$). The swelling rate and hardness of crispy snack varied directly as the amount of tapioca flour. Basically, the swelling of crispy snack is caused by many factors, such as the tapioca flour processing, kneading, steaming, chilling, drying and heating under high temperature by frying until causing the puffiness of the crispy snack (Fellows, 2017). The swelling rate of the crispy snack using the ratio of tapioca flour to bamboo shoot 25:75 had the lowest value because it is a low amount of tapioca flour. The swelling rate is related to the hardness of the crispy snack. If the amount of tapioca flour is high, it will cause the high gelatinization. It affects the viscosity of the crispy snack and making the strong structure of crispy snack. The hardness of raw and ready-to-eat crispy snacks were compared. It was found that ready-to-eat crispy snack was lower hardness than a raw crispy snack. Because the frying causes the expansion

of the structure that the structural strength to decrease (Kengkhetkit et al., 2019).

Table 2 Effect of different ratios of tapioca flour and bamboo shoots on the quality value of crispy bamboo shoot snack

Quality value of crispy snack	The ratio of tapioca flour to bamboo			
	100 : 0	75 : 25	50 : 50	25 : 75
Weight loss after steaming (%)	-1.67±0.29^{b}	-1.07±1.51^{b}	-0.33±0.47^{b}	9.33±1.70^{a}
Weight loss after chilling ns (%)	2.46±0.50	2.12±1.37	1.99±0.80	1.85±1.07
Weight loss after drying (%)	32.98±1.39^{a}	29.62±1.36^{ab}	26.09±3.37^{bc}	23.98±0.34^{c}
Weight loss after frying (%)	-0.95±0.78^{a}	-1.53±0.54^{a}	-1.85±0.69^{a}	-3.96±0.79^{b}
Moisture content of raw crispy snack (% by weight)	9.20±0.00^{a}	9.19±0.01^{a}	9.18±0.00^{ab}	9.17±0.02^{b}
Moisture content of ready-to-eat crispy snack^{ns} (% by weight)	3.14±0.04	3.11±0.02	3.10±0.00	3.09±0.01
Swelling rate after frying	1.02±0.01^{a}	0.90±0.01^{b}	0.85±0.02^{b}	0.56±0.02^{c}
Hardness of raw crispy snack (N)	17.63±0.14^{a}	14.30±0.13^{b}	12.32±0.04^{c}	7.23±0.02^{d}
Hardness of ready-to-eat crispy snack (N)	11.23±0.09^{a}	9.20±0.06^{b}	7.27±0.04^{c}	3.29±0.10^{d}

Remark: mean±SD, $^{a-d}$ means within each row indicate significant differences ($P \leq 0.05$) and ^{ns} means not statistically significant ($P > 0.05$) using Duncan's multiple range test

The effects of different ratios of tapioca flour and bamboo shoots on the color values of raw and ready-to-eat crispy snacks are shown in Table 3. It was found that the brightness (L *) varied directly as the amount of tapioca flour. The brightness of ready-to-eat crispy snack is greater than raw crispy snacks. The a * shows the red to green color values. If a * is negative, is in the green range and a * is positive, is in the red range. The a * value of ready-to-eat crispy snacks varied directly as the amount of bamboo shoots. The b * value indicates the yellow to blue range. If the b * value is negative in the blue range and the b * positive value is in the yellow range. The b * value of crispy snacks varied directly as the amount of bamboo shoots. Specifically, the crispy snacks without adding bamboo shoots had white color from tapioca flour. The yellow color of bamboo shoots is caused by the chemical composition which affects the color change such as carotenoids and chlorophyll (Bal et al., 2011). Carotenoids are yellow, orange, red, and red orange pigments. It is found in plants and organisms that can be synthesized by light. It is worked with chlorophyll, which is a green pigment. The chlorophyll absorbs energy from sunlight, photosynthesis and helps plant growth and protection from light hazards. Generally, chlorophyll is unstable to heat. If it exposed to heat, it changes to phytotin causing the green color to turn greenish brown (Damondaran & Parkin 2017). The crispy snack is heated by steaming, drying, and frying. It is resulting in chlorophyll being destroyed and changing color. The observable appearance and various quality values were considered. The suitable ratio was selected for development into crispy bamboo shoot snack in the next step. The tapioca flour and bamboo shoot equal to 50:50 was suitable formula. It is easy molding, good swelling, and strong structure after steaming, cut into pieces easily, good swelling after frying, and a clear aroma of bamboo shoots.

2. Effect of different flour types on the quality of crispy bamboo shoot snack products

The effect of different types of flour at the ratio of flour and bamboo shoots equal to 50:50 on the observable characteristics of crispy snacks shown in Table 4. It was found that tapioca flour has better the observable appearance than the other flours, such as forming ability after mixing, swelling after steaming, gel stability after steaming, cutting ability after chilling, swelling rate after frying, hardness of raw crispy snack, and hardness of ready-to-eat crispy snack. The aroma of bamboo shoots in all formulas has no significance differences of the 95% ($P > 0.05$). The tapioca flour and wheat flour formulas had a darker yellow color than glutinous rice flour and rice flour formulas. The kneading and molding process using glutinous rice flour, rice flour and wheat flour were more difficult than tapioca flour and sticky of texture, the puffiness after steaming of all formulas was compared. It was found that tapioca flour had the highest swelling rate, followed by glutinous rice flour, wheat flour and rice flour, respectively. The rice

Table 3 Effect of different ratios of tapioca flour and bamboo shoots on the color value of crispy bamboo shoot snack

The ratio of tapioca flour to bamboo	Raw crispy snack			Ready-to-eat crispy snack		
	L*	a*	b*	L*	a*	b*
100 : 0	70.74±0.21^{a}	-0.82±0.03^{d}	-0.25±0.02^{d}	77.19±0.17^{a}	-0.13±0.01^{d}	0.81±0.04^{d}
75 : 25	54.17±0.19^{b}	-0.32±0.02^{c}	2.83±0.03^{c}	65.58±0.10^{b}	0.45±0.06^{c}	9.31±0.03^{c}
50 : 50	52.59±0.05^{c}	-0.27±0.10^{b}	3.72±0.06^{b}	60.29±0.04^{c}	4.92±0.01^{b}	12.24±0.09^{b}
25 : 75	45.50±0.07^{d}	1.70±0.07^{a}	9.73±0.06^{a}	48.84±0.07^{d}	8.43±0.04^{a}	16.28±0.09^{a}

Remark: mean±SD, $^{a-d}$ means within each column indicate significant differences ($P \leq 0.05$) using Duncan's multiple range test

flour formula had the lowest swelling rate and high gel stability after steaming. The rice flour gel had a rather more the dense texture than other flour. It had low flexibility that different from the tapioca flour gel. It was soft and flexible. The glutinous rice flour and wheat flour gel had low stability. The cutting ability after chilling was investigated. It was found that the tapioca flour and rice flour formulas were cut easily. The glutinous rice flour and wheat flour formulas were difficult for cutting due to being sticky. In terms of swelling after frying, the tapioca flour had the highest swelling rate, followed by glutinous rice flour, wheat flour and rice flour, respectively. The texture of raw crispy snacks was stronger than ready-to-eat crispy snacks. The ready-to-eat crispy snacks using tapioca flour had a suitable hardness. The rice flour formula had the least hardness, brittle and easily broken.

The type of flour is an important factor that affects the quality the crispy snack. In various flour had different quality, such as the characteristics of cooked flour, temperature for making the dough cooked, the texture of the toughness, swelling, and crispy. Because each type of flour contains amylose and amylopectin in different proportions. The amount of amylose to amylopectin in tapioca flour, rice flour, wheat flour, and glutinous rice flour were, 17:83, 17:83, 28:72, and 0:100, respectively. The texture of cooked flour that contains high amylose had high hardness while the high amylopectin flour had highly sticky and flexible. The ratio of amylose to amylopectin was related to swelling rate. The flour that high amylopectin was good swelling, lightweight, and brittle products 1 (Tangkanakul, 2003).

Table 4 Effect of different flours on the observable appearance of crispy bamboo shoot snack

Observable appearance	Flours			
	Tapioca	Glutinous rice	Rice	Wheat
Forming ability after mixing	++++	++	++	++
Swelling after steaming	++++	+++	+	++
Gel stability after steaming	+++++	++	+++++	+++
Cutting ability after chilling	++++	++	+++++	++
Swelling after frying	++++	+++	+	++
Hardness of raw crispy snack	++++	+++	+	++
Hardness of ready-to-eat crispy snack	+++	++	+	+
Bamboo shoot flavor intensity of ready-to-eat crispy snack	++++	++++	++++	++++
Yellow color intensity of ready-to-eat crispy snack	++++	+++	+++	++++

Remark: + =very low, ++ = low, +++ = moderate, ++++ = high, +++++ = very high

The effects of different flour types on weight loss, moisture content, swelling rate and hardness of crispy snack are shown in Table 5. It was found that the weight loss after steaming, after cooling, and after drying of all formulas have no significant differences of the 95% ($P > 0.05$). The weight loss of tapioca flour and rice flour formulas were higher than rice flour and wheat flour lightly. The moisture content of raw and ready-to-eat crispy snacks after frying were 8.94-9.21 % by weight and 2.98-3.13 % by weight. It was in the standard range of general crispy snack products (Thai industrial standards institute, 2006). The swelling rate and hardness of crispy snacks after frying using tapioca flour had the highest, followed by glutinous rice flour, wheat flour and rice flour, respectively. Totally, the hardness of raw crispy snacks was higher than ready-to-eat crispy snacks.

There are many factors affecting on the swelling of flour, such as non-carbohydrate composition in starch granules, types of starch, strength, and appearance of the network structure within the grain, etc. Non-carbohydrate composition in starch granules, gluten in wheat flour which is a glycoprotein found in the endosperm of wheat. It is caused by the combination of glutenin and gliadin proteins in equal proportions. It is creating a disulfide bond, insoluble in water, making the gluten tough and flexible (Damondaran & Parkin, 2017). The glutinous rice flour, rice flour and wheat flour, which is flour from cereals, have a 2-puffing pattern. The bonding forces in flour are crystal and the amorphous bond. The cereal flour has the highest number of bonds but has the lowest swelling and dissolving. It has a high amount of amylose which amylose will strengthen the network structure and low swelling (BeMiller & Whistler, 2009). Glutinous rice flour is without amylose but is high amylopectin. It had good swelling, light weight, but easily brittle (Tangkanakul, 2003). Tapioca flour made from the root section or the middle of the stem. There is only one layer of swelling. Puffing power is higher than flour from cereals since there are fewer bonds. In addition, the flour from the root section causes gelatinization at lower temperatures than cereal flour, which is related to the hardness of the crispy snack. It may be caused by the number and type of bonds within the starch molecules. The factors affecting on the number of bonds including, size, shape, composition, distribution of the network structure within the starch granule, the ratio of amylose and amylopectin, molecular weight, molecular distribution, number of branches, the arrangement of molecules and the length of the branch in the amylopectin structure, etc

(BeMiller & Whistler, 2009). These factors resulted in different hardness values of crispy snack produced from different types of flour. The rice flour formula had the lowest hardness, brittle and easily broken that consistent with the observable appearance.

Table 5 Effect of different flours on the quality value of crispy bamboo shoot snack

Quality value of crispy snack	Flours			
	Tapioca	Glutinous rice	Rice	Wheat
Weight loss after steaming ^{ns} (%)	-0.33±0.47	-1.00±1.63	0.33±1.70	-2.00±1.63
Weight loss after chilling ^{ns} (%)	1.99±0.80	2.63±1.22	3.66±0.88	1.94±1.35
Weight loss after drying ^{ns} (%)	26.09±3.37	25.76±0.20	28.12±1.26	26.32±2.45
Weight loss after frying (%)	-1.85±0.69^{a}	-2.75±1.15^{a}	-5.81±1.25^{b}	-5.45±1.15^{b}
Moisture content of raw crispy snack (% by weight)	9.18±0.00^{b}	9.10±0.01^{ab}	8.94±0.00c	9.21±0.02^{a}
Moisture content of ready-to-eat crispy snack (% by weight)	3.10±0.00^{b}	3.13±0.00^{a}	2.98±0.01^{d}	3.00±0.02^{c}
Swelling rate after frying	0.85±0.02^{a}	0.72±0.01^{b}	0.06±0.02^{d}	0.54±0.01^{c}
Hardness of raw crispy snack (N)	12.32±0.04^{a}	10.40±0.03^{b}	5.19±0.07^{d}	8.20±0.03^{c}
Hardness of ready-to-eat crispy snack (N)	7.27±0.04^{a}	5.43±0.01^{b}	2.13±0.0^{2d}	4.00±0.02^{c}

Remark: mean±SD, $^{a-d}$ means within each row indicate significant differences ($P \leq 0.05$) and ^{ns} means not statistically significant ($P > 0.05$) using Duncan's multiple range test

The effects of different types of flour on the color value of raw and ready-to-eat crispy snack are shown in Table 6. The results indicated that raw crispy snack using rice flour for the production had the highest brightness, followed by glutinous rice flour, tapioca flour and wheat flour, respectively. In terms of ready-to-eat crispy snack, glutinous rice flour had the highest brightness, followed by tapioca flour, rice flour and wheat flour, respectively. The a * value of raw crispy snack produced from tapioca flour had a negative value that was in the green range. There was a positive value for other formulas in the red range. The a * value of ready-to-eat crispy snack was positive in the red range. The b * values of raw and ready-to-eat crispy snack in all formulas were positive in the yellow range. The yellow color of the crispy snack was the result of the pigment in bamboo shoots that mentioned above. In addition, using different flours results in the brightness of ready-to-eat crispy snack was different. The tapioca flour formula and glutinous rice flour had a high swelling rate, resulting in the structure of the cracker to expand. The brightness of ready-to-eat crispy snack was higher than a raw crispy snack. The rice flour formula and wheat flour to produce raw crispy snack had a higher brightness value than ready-to-eat crispy snack that due to having a low swelling rate. The fried crispy snack had darker color, result in the Maillard reaction. It is a non-enzymatic browning reaction. It occurs between reducing sugar and amino acids, protein or other nitrogen compounds under the heat catalyzed. The various compounds that give brown color and various flavorings were the products of Maillard reaction (Damondaran & Parkin, 2017).

The observed appearance and quality of the crispy snack were considered. It was found that tapioca flour has better the observable appearance than the other flours, such as forming ability after mixing, swelling after steaming, gel stability after steaming, cutting ability after chilling, swelling rate after frying, hardness of raw crispy snack, and hardness of ready-to-eat crispy snack. Therefore, the tapioca flour was suitable flour for crispy snack production in the next step.

3. Effects of flavoring of crispy bamboo shoot snacks with various flavors on the sensory acceptance

The results of flavoring of crispy snacks with various flavors on the sensory acceptance are shown in Table 7. It was found that all crispy snacks flavored had a high average score in appearance, flavor, taste, texture, and overall liking in the medium to like very much. The original, seaweed and black sesame, and spicy flavor had a higher average score in appearance, flavor, taste, texture, and overall liking than other flavors. All crispy snacks flavored had unique characteristics. The original flavor had the sensory characteristics like the crispy snacks that was commercially available in the market. The garlic and pepper flavor were outstanding in the aroma of garlic-pepper mixture. The young galangal flavor mixed with Yanang leaves was the aroma of young galangal like herb

Table 6 Effect of different flours on the color value of crispy bamboo shoot snack

Flours	Raw crispy snack			Ready-to-eat crispy snack		
	L*	a*	b*	L*	a*	b*
Tapioca	52.59±0.05^{c}	-0.27±0.10^{d}	3.72±0.06^{d}	60.29±0.04^{b}	4.92±0.01^{d}	12.24±0.09^{b}
Glutinous rice	53.29±0.08^{b}	2.26±0.08^{a}	4.87±0.06^{c}	70.41±0.07^{a}	5.35±0.02^{c}	13.57±0.07^{a}
Rice	70.47±0.07^{a}	0.45±0.03^{c}	14.33±0.06^{a}	56.74±0.04^{c}	7.37±0.02^{b}	10.40±0.11^{c}
Wheat	50.06±0.04^{d}	1.38±0.06^{b}	9.29±0.08^{b}	45.35±0.03^{d}	8.39±0.04^{a}	7.38±0.07^{d}

Remark: mean±SD, $^{a-d}$ means within each column indicate significant differences ($P \leq 0.05$) using Duncan's multiple range test

flavor. There are black sesame and seaweed distributed in pieces of crispy snacks with the fragrant aroma of black sesame seeds. The spicy flavor had fresh chilies and slightly spicy flavor. It was suitable for consumers who like spicy taste. The aroma of barbecue had the taste like the crispy snacks on the market that is flavored with barbecue flavor. All crispy snacks flavored had an average score in the medium to like very much. It can be used as a guideline to develop crispy bamboo shoot snacks for further commercial distribution.

Table 7 Effect of different flavors on sensory evaluation of crispy bamboo shoot snack

Crispy snack flavors	Attribute				
	Appearance	Flavor	Taste	Texture	Overall liking
Original	7.83±0.83^{ab}	7.53±0.82^{ab}	7.77±0.68^{ab}	7.73±0.87^{ab}	7.87±0.73^{ab}
Garlic and pepper	7.76±1.21^{ab}	7.40±0.81^{b}	7.73±0.98^{ab}	7.67±0.92^{ab}	7.53±0.97^{b}
Galangal and Yanang leaves	7.53±1.10^{b}	7.47±0.88^{ab}	7.50±1.10^{b}	7.57±0.93^{b}	7.50±1.06^{b}
Seaweed and black sesame	8.13±0.90^{a}	7.93±0.87^{a}	8.10±0.71^{a}	8.10±0.84^{a}	8.13±0.94^{a}
Spicy	8.03±0.93^{ab}	7.87±0.82^{ab}	8.00±0.91^{ab}	7.87±0.73^{ab}	8.07±0.91^{a}
Barbecue	7.53±1.11^{b}	7.57±1.01^{ab}	7.80±0.92^{ab}	7.67±0.99^{ab}	7.53±0.97^{b}

Remark: mean±SD, $^{a-b}$ means within each column indicate significant differences ($P \leq 0.05$) using Duncan's multiple range test

4. Chemical composition of crispy bamboo shoots snack products

The original, seaweed and black sesame, and spicy flavor of crispy bamboo shoots snack products (Fig.1) had the highest score of sensory acceptability in appearance, flavor, taste, texture and overall liking. It was selected for chemical composition analysis (Table 8). The crispy bamboo shoot snack produced is rich in nutrition. It is an interesting alternative product for consumers who enjoys eating snacks.

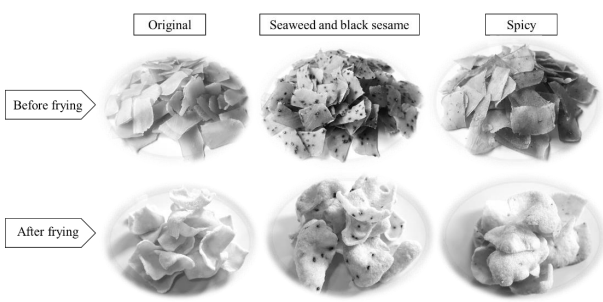


Fig. 1 Appearance of of crispy bamboo shoots snack products

Table 8 Chemical composition of crispy bamboo shoots snack products

Chemical composition	Crispy snack flavors		
	Original	Seaweed and black sesame	Spicy
Moisture content (%)	3.50 ± 0.25	2.73 ± 0.22	3.46 ± 0.19
Protein (%)	1.06 ± 0.39	2.40 ± 0.41	1.04 ± 0.35
Fat (%)	26.91 ± 0.45	28.53 ± 0.51	26.93 ± 0.55
Total carbohydrate (%)	66.64 ± 0.15	64.36 ± 0.12	66.67 ± 0.15
Ash (%)	1.89 ± 0.50	1.98 ± 0.66	1.90 ± 0.86
Energy (kcal)	513.08 ± 0.49	523.81 ± 0.60	513.12 ± 0.52

Conclusion

The optimum ratio of tapioca flour and bamboo shoots in the production of crispy snack was 50:50, which has easy molding, good swelling rate and strong structure of the gel after steaming, easy cutting, and good swelling rate after frying. Tapioca flour was a suitable raw material for crispy snack producing because of obtaining good quality products. The original, seaweed and black sesame, and spicy flavor had a higher score in appearance, flavor, taste, texture, and overall liking than other recipes. The prototype crispy bamboo shoot snack can be used as a guideline for further development of commercial production as alternative food products for consumers. It also creates added value for agricultural products. There may be additional studies on the packaging. That helps extend the shelf life of the product including modern packaging designs that attract consumer attention.

Acknowledgment

This work was financially supported by the Ministry of Higher Education, Science, Research and Innovation of Thailand through the research and innovation project for transferring technology to foundation communities of Burapha University (Grant no. 1/2020).

References

Association of Official Analytical Chemists. (2012). *Official methods of analysis* (19th ed.). Washington, D.C.: Author.

Bal, L.M., Kar, A., Satya, S., & Naik, S.N. (2011). Kinetics of colour change of bamboo shoot slices during microwave drying. *Journal of Food Science and Technology, 46,* 827–833.

BeMiller, J.N., &Whistler, R.L. (2009). *Starch : Chemistry and technology.* London: Academic Press.

Brufau, G., Canela, M.A., & Rafecas, M. (2008). Phytosterols: Physiologic and metabolic aspects related to cholesterol-lowering properties. *Nutrition Research, 28,* 217-225.

Chongtham, N., Bisht, M.S., & Haorongbam, S. (2011). Nutritional properties of bamboo shoots: Potential and prospects for utilization as a health food. *Comprehensive Reviews in Food Science and Food Safety, 10,* 153-169.

Damondaran, S., & Parkin, K.L. (2017). *Fennema's Food Chemistry.* Florida: CRC Press, Taylor &. Francis Group.

Fellows, P.J. (2017). *Food Processing Technology: Principle and Practice.* Uxbridge: Woodhead Publishing.

Kengkhetkit, N., Mopoung, R., Tassanaudom, U., & Yodphet, A. (2019). Effect of wheat flour content on characteristics of spicy fermented fish paste crackers. *NSRU Science and Technology Journal, 11,* 1-14.

Khaepimyi, N., & Nepthangdee, P. (2018). *Consumer satisfaction per craker mushroom products.* Retrieved November 16, 2020, from https://dspace.bru.ac.th

Kotoki, D., & Deka, S.C. (2010). Baking loss of bread with special emphasis on increasing water holding capacity. *Journal of Food Science and Technology, 47,* 128-131.

Mogkhuntod, S., Denrungruang, P., & Chitchak, C. (2017). *Nutrients and toxins in the bamboo shoots.* Retrieved November 16, 2020, from http://forprod.forest.go.th

Nirmala, C., David, E., & Sharma, M.L. (2007). Changes in nutrient components during ageing of emerging juvenile bamboo shoots. *International Journal of Food Sciences and Nutrition, 58,* 345–352.

Rattanathammawat, R., Suwonsichon, T., Chompreda, P., Sriroth, K., & Haruthaithanasan, V. (2003). *Effect of moisture content of half-snacks and frying time on physicochemical properties of taro flour snack.* Retrieved November 16, 2020, from http://www.lib.ku.ac.th/KUCONF/KC4106006.pdf

Suriya, P., Thamaragsa, N., Kaewtein, S., Pocarat, R., & Arkanit, K. (2011). Product development of Burma bean chip. In *The 2nd MJU-Phrae National Research Conference* (pp. 276-283). Phrae: Maejo University Phrae Campus.

Tangkanakul, P. (2003). Making cracker as a quality product. *Food, 33,* 90-93.

Tantasuttikul, A., & Samuankid, N. (2014). Development of seasoning fish cracker. *Rajabhat Agriculture Journal, 13,* 71-79.

Thai industrial standards institute. (2006). *Community product standards: Crispy snack, khaogriab (107/2554).* Retrieved November 16, 2020, from http://tcps.tisi.go.th/pub/tcps107_54.pdf

Tiwthao, O. (2014). *Study of salmon skin puffing by microwave oven and frying* (Master's Thesis). Retrieved November 16, 2020, from http://kb.psu.ac.th/psukb/bitstream/2010/10010/1/388040.pdf

Wongsudaluk, W. (2016). *Application of champedak* (Artocarpus integer) *seed flour in cracker product.* Retrieved November 16, 2020, from http://ird.skru.ac.th/RMS/file/21388.pdf

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

The Improvement of Hydroponics Growth Media by Using the Corncob Biochar

Pannipha Dokmaingam[a,b]*, Sitang Kongkratoke[a,b], Amornrat Anuwatnonthakate[a] & Sarayut Yongprapat[c]

[a] *School of Health Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[b] *Research Group Circular Economy for Waste-free Thailand, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[c] *Venus supply Co., Ltd., Bangkok 10900, Thailand*

Article info

Article history:
Received : 10 September 2020
Revised : 10 December 2020
Accepted : 14 December 2020

Keywords:
Corncob, Biochar, Hydroponic growth media, Crop production

Abstract

Corncob is one of the main agricultural wastes in the northern part of Thailand. It is utilized in many proposes such as wood pellet, activated carbon or biochar. Biochar has several excellent properties that could provide benefits for agriculture applications such as water absorption, ionic exchange, water holding capacity, and conductivity. These properties could also offer an advantage for the hydroponics system. This study selected corncob biochar for improving the growth of hydroponic plants. Firstly, the commercial biochar will be characterized it's chemical and physical properties and then hydroponic plants, namely Red Oak lettuces, Green Oak lettuces, and Cos lettuces, were grown in the growth medium with and without biochar in order to compare the growth of plants. It was found that corncob biochar has high aliphatic carbon content with 99.27 % fixed carbon. As the morphology properties, it's surface area, total pore volume and mean pore diameter are 40.4695 m^2/g, 0.0385 cm^3/g, 3.85875 nm., respectively. These advantage for promote nutrients utilization in the hydroponics system. These are also affected to different number of leaves of Cos lettuce and dry weight of Green Oak lettuce from growth media with and without biochar which is reached a significant difference level ($p<0.05$). It was suggested that adding a small amount of corncob biochar could endorse nutrients used in a hydroponics system. This would advantage for put forth shoots of hydroponic plants. However, the biochar would benefit for microbial activity that is important to water and nutrient uptake. This would more advantage if biochar is applied to the soil.

Introduction

During 2012-2014, around 4,000,000 tons/year of maize was produced in Thailand (Centre for Agricultural Information, 2017) which are mostly used as raw material for industrial animal feed. One of the main residuals of this industrial is corncob. This is around 15% of the maize yield and is more than 1,000,000 tons/year. (Tangtaweewipat et al., 2012). Burning becomes the most common method to dispose of this large amount of agriculture residual because it is easy and fast. However, this also causes air pollution (especially pm 2.5) in Thailand. This problem

* Corresponding Author
e-mail: pannipha.dok@mfu.ac.th

has been extremely concerning for the last decade. This pollution affects health, economics and environment. The Kasikorn research center reported that the impact of air pollution on the economy of the Bangkok Metropolitan Region could be around 3,200–6,000 million baht for 1 month (Kasikorn research center, 2020). Additionally, the quality of soil is also reduced from the burning. This might be because of the annihilation of the microorganism and porosity destruction in soil by burning. As the environmental problem from agriculture waste burring, many alternative solutions were developed to use the corncob, instead of burning, such as wood pellet (Tangtaweewipat et al., 2012), activated carbon (Khoomsab et al., 2015) or biochar (Angmanee et al., 2017; Tansathit et al., 2016). Among them, biochar is preferred for agricultural proposes. Generally, biochar is produced via the pyrolysis process. Several previous studies applied biochar to improve solid properties (Angmanee et al., 2017; Novak et al., 2009; Zhu et al., 2017). The conditions for pyrolyzed different feedstock types are also studied (Ronsse et al., 2013; Zhu et al., 2017). As the agricultural benefit, biochar is interesting because it could be used as soil amendments. This is the result of the physical properties of biochar which are high porosity and surface area. These properties could improve water absorption, ionic exchange and reduce the density of soil structure, water holding capacity, conductivity, porosity, and adjusting pH (Angmanee et al., 2017; Awad et al., 2017; Fryda & Visser, 2015; Tansathit et al., 2016). Moreover, biochar could limit the heavy metal and over secondary macronutrients into the plant nutrients absorption process (Rizwan et al., 2016). The pyrolysis biochar could improve the water-holding capacity and reduce water and nutrient leaching (Jahromi et al., 2018). As these advantages, biochar is suggested as a growth media for hydroponics (Awad et al., 2017; Blok et al., 2017; Vaughn et al., 2015). Moreover, the biochar molecules are stable and highly resilient to microbial degradation (Blagodatskaya et al., 2014; Singh et al., 2017). Hence, the introduction of biochar may not only maintain suitable nutrients for plant growth but may also restrict the unwanted algal growth around the plant root, preventing the adverse effect of these microbial on crop yield. However, further research is required to proof the practicality and benefits of using corncob biochar as a growth medium in hydroponic systems.

Now a day, biochar could be used for hydroponics growing medium. These could be produced from rice husk biochar (Awad et al., 2017; Blok et al., 2017), rice straw biochar (Vaughn et al., 2015) or agriculture residual biochar (Blok et al., 2017). It might be assumed that the properties of growth medium for Hydroponics could be improved by adding some of the biochar. This study has produced significant evidence backing this claim. The aim of this work is to study the benefits of biochar from corncob as the growth medium for hydroponics plants. Three common hydroponics plants are selected in this study which are Red Oak lettuces, Green Oak lettuces, and Cos lettuces. We hope that by increasing the applicability of the biochar and increasing the productivity of hydroponic plants, the inappropriate burning of the agriculture residual should be lowered.

Materials and methods

In this study, the commercial biochar was sent from Mae Chaem, Chiang Mai, Thailand which are the major source of the corncob. The biochar was pyrolyzed from corn crop at an operating temperature around 650˚C. This biochar was characterized by both chemical and physical properties. The functional group of this biochar is analyzed by using Fourier-transform infrared spectroscopy (FTIR). The surface area and the surface morphology of the activated carbon were determined by Brunauer-Emmett-Teller (BET) and a scanning electron microscope (SEM), respectively. Nuclear magnetic resonance spectroscopy (NMR) is used for analyzing the core-structural characteristics of biochar. It was evaluated via Fourier Transform Nuclear Magnetic Resonance Spectrometer 400 MHz. (Solid) – NMR 400 MHz. (Bruker, Germany). It was operated at 13C frequency of 75.5 MHz. Their chemical composition was studied via Ultimate analysis process. The pH of the biochar was evaluated by mixing biochar with De-ionized water with a ratio of 1 % (w/v) and shaking for 24 h. pH was measured by using multimeter (Multi 350i/SET, WTW, Germany) (Novak et al., 2009).

After that, the benefit of addition biochar in to support culture for hydroponic plants was investigated in the experimental field. The size of hydroponics systems is 160 cm. x 600 cm. x 80 cm., combined with a nutrient solution container for growing 160 plants. All testing samples were cultivated in the hydroponic system with commercial liquid fertilizer (N=268.5 ppm, P=56.724 ppm, K=326.19 ppm). Three types of hydroponic plants were selected in this testing which are Red Oak lettuces (*Lactuca sativa* var.*crispa* L.), Green Oak lettuces

(*Lactuca sativa* L.), and Cos lettuces (*Lactuca sativa* L.). Note that these seed were obtained from Maejo 68 Seed company, Chiang Mai, Thailand.Ten of these plants will be implanted in the support culture with and without biochar. The pots of all testing seed were distributed uniformly in the hydroponic growth systems were placed without a greenhouse. For the support culture with biochar, 1g. of biochar added to the support culture before seeding. Meanwhile, the control sample is directly seeding in the support culture. These were harvested after 45 days of seeding. After that, their growth was measured. The growth of hydroponic plants from these two-difference support cultures was identified by measured width and length of leaf, the number of leaf and dry weight of plants. The differences in each treatment were adjudged by Tukey test ($P \leq 0.05$).

Results and discussion

1. Fourier-transform infrared spectroscopy (FTIR)

From FTIR spectrum from range between 500-4000 cm^{-1}, as presented in Fig. 1, shows the absorption band of aliphatic carbon (2869 cm^{-1}: C-H stretching and 1540: C=C stretching). The band at 2358 cm^{-1} is designated as the O=C=O stretching from the adsorbed CO_2 which absorbed on the alkaline biochar. The wideband from 1700-1600 cm^{-1} are assigned to C=O stretching of ketone, aldehyde or carboxyl groups. As a result, the carbonyl carbon has a large partial positive charge and the oxygen has a large partial negative charge as denoted (Smith, 2016). These functional groups benefit for ionic exchange of nutrient utilization at the roots of plants.

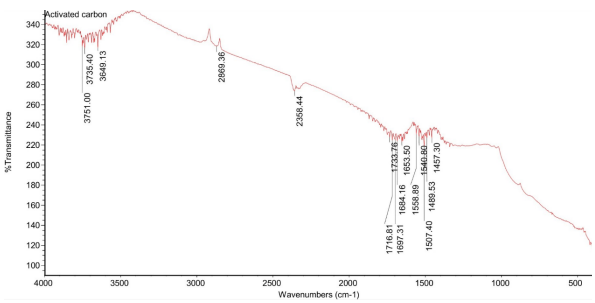


Fig. 1 The FTIR spectrum of the biochar from corn crop

2. Nuclear magnetic resonance spectroscopy (NMR)

As seen in Fig. 2, the ^{13}C NMR of biochar was dominated by the aliphatic C=C and aromatic-C centered at 124 ppm. Two other peaks centered at 206 and 47 ppm indicates the presence of carboxyl and aliphatic C-C. Biochar with high aromatic-C contents is probably appropriate for long-term C sequestration. Moreover, biochar with high aromatic-C are resistant to microbial mineralization (Novak et al., 2009).

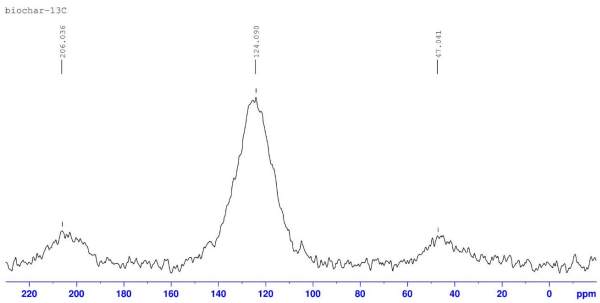


Fig. 2 ^{13}C NMR spectral scan of the biochar from corn crop

3. Biochar porosity

In this study, SEM images of biochar from corncob shown in Fig. 3 with different magnifications. The surface morphology of the biochar was structure with different diameters of pores. In Fig.3 (b), the pore size in range of 1 to 7 µm. were observed, which is classified as storage pores (0.5 to 50 µm.) (Batista et al., 2018). The BET results are shown in Table 1. This is influence to capable of holding water and the retention of nutrients in the growth media.

Table 1 Porosity properties of corncob biochar

Parameter	Porosity Property
Surface area	40.4695 $m.^2/g$
Total pore volume	0.0385 $cm.^3/g$
Mean pore diameter	3.85875 nm.

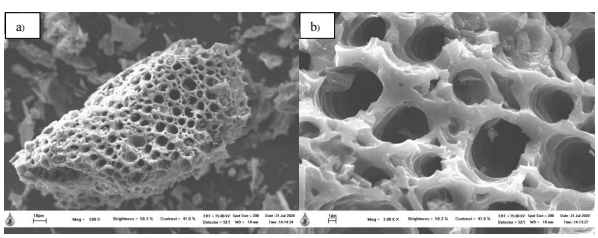


Fig. 3 SEM of the biochar from corncob at magnification of (a) 500 x and (b) 3000 x.

4. Chemical properties of biochar

The solution from biochar is in the alkaline pH region (Table 2). However, Nurhidayat & Mariati (2014) recommends that pH in range of 5.0 - 7.5 is the suitable range for nutrient accessibility for the plant. Outside this range, the nutrient loading for the plant will be imbalanced. This might advantage to change the acidic

growth media to improve nutrient utilization for plants.

The proximate results indicate that the fixed carbon was a dominant content at more than 90% in the biochar which is consistent with the ^{13}C NMR result.

Table 2 Chemical properties of corncob biochar

Sample	pH	Volatile (%)	Ash (%)	Fixed carbon (%)
1	8.33	0.58	0.11	99.32
2	8.32	0.70	0.06	99.24
3	8.30	0.67	0.09	99.25
Average	8.32	0.65	0.09	99.27

5. Effect of biochar on plants growth

The effect of biochar on hydroponic plants growth were shown as followed. The plants were seeded and grown in hydroponics growth media with and without biochar, as seen in Fig. 4 and 5. All of them were put in the control condition and nutrient.

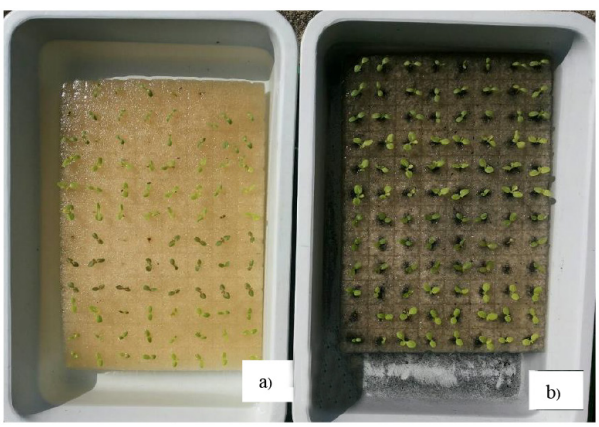


Fig. 4 Hydroponic plants seeding in the support culture with biochar (a) and without biochar (b)

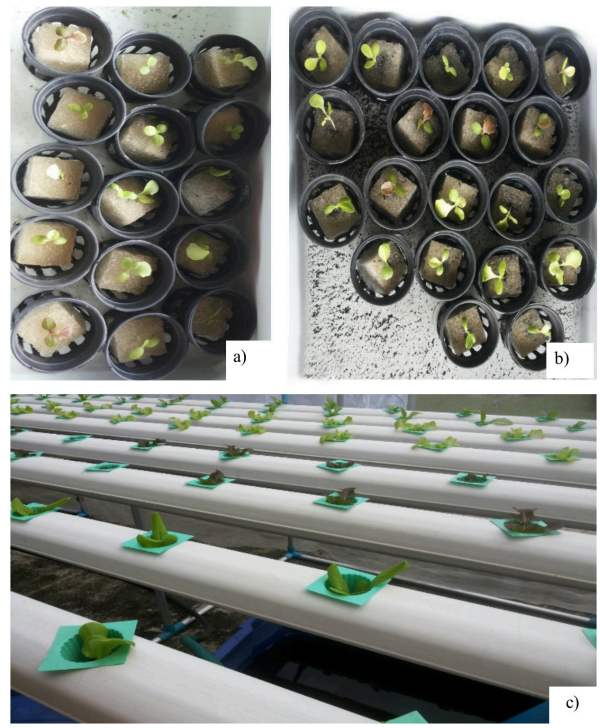


Fig. 5 Hydroponic plants growth from the support culture (a) with biochar and (b) without biochar in the (c) hydroponic system

All results compare the growth in the term of number length and width of leaves. It could be seen in Tables 3-6, the different number of leaves of Cos lettuce from growth media with and without biochar is significant ($p<0.05$). The number of leave greatly increased for all three plants. Meanwhile, the length and width of Cos lettuce, Red Oak lettuce, Green Oak lettuce from growth media with and without biochar are not different.

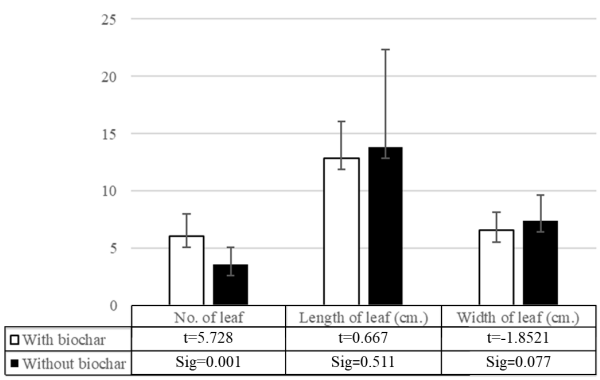


Fig. 6 Comparison of Cos lettuce growth from hydroponic growth media with and without biochar (n=10)

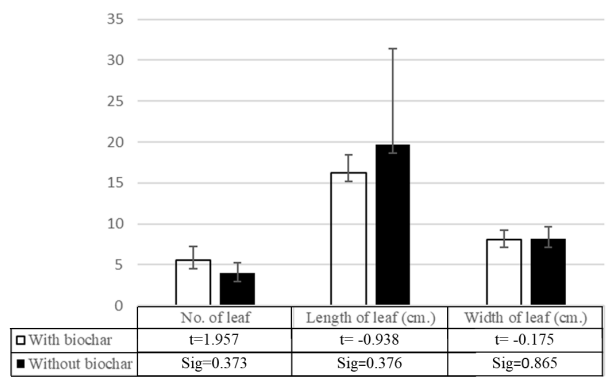
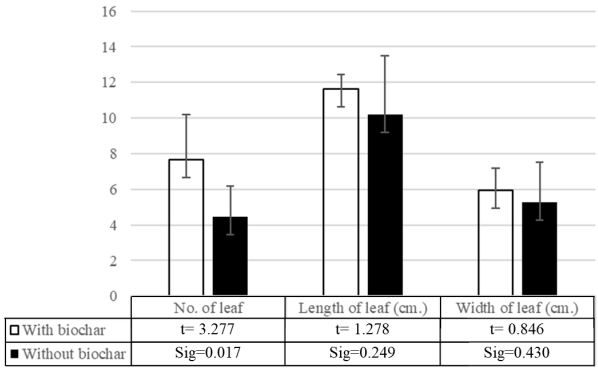


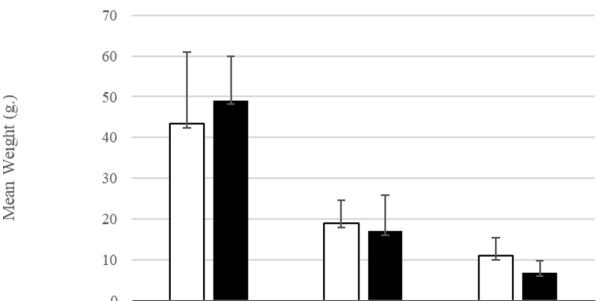
Fig. 7 Comparison of Red Oak lettuce growth from hydroponic growth media with and without biochar (n=10)



	No. of leaf	Length of leaf (cm.)	Width of leaf (cm.)
With biochar	t= 3.277	t= 1.278	t= 0.846
Without biochar	Sig=0.017	Sig=0.249	Sig=0.430

Fig. 8 Comparison of Green Oak lettuce growth from hydroponic growth media with and without biochar (n=10)

When compared the dry weight of these three hydroponic plants, it is seen that the difference of Green Oak lettuce dry weight from hydroponic growth media with and without biochar is significant ($p<0.05$). While this is disparate for Cos lettuce and Red Oak lettuce.



	Cos lettuce	Red Oak lettuce	Green Oak lettuce
With biochar	t= 1.146	t= 0.539	t= 2.420
Without biochar	Sig=0.281	Sig=0.602	Sig=0.039

Fig. 9 Comparison of the dry weight of Cos lettuce, Red Oak lettuce, and Green Oak lettuce from hydroponic growth media with and without biochar (n=10)

From the result from Fig. 6-9, the hydroponic plants which grow from the growth media with biochar grows better. Since the aliphatic carbon content with 99.27% fixed carbon and the high porosity of biochar, these have the advantage that they promote nutrition, from liquid fertilizer, to hydroponic plants (Awad et al., 2017). However, the differences in each treatment were showed to be non-significant according to result off the pH range of biochar which was in the alkaline region. These should inhibit the nutrition accessibility of plants (Nurhidayat & Mariati (2014). Furthermore, the addition of biochar might impact to reduce Nitrogen (N) and Manganese (Mn) availability but increased Phosphorus (P) availability (Atkinson et al., 2010). The biochar property for water holding capacity was not accounted in this hydroponics system which was operated under water-saturated conditions (Mukherjee & Zimmerman, 2013). However, the benefit of adding biochar on plant growth might effective when applied in the soil. As the study of Zheng et al., (2013) and Ding et al., (2016), suggested that biochar could improve the rhizosphere and microbial activity that are important to water and nutrient uptake which do not exist in the hydroponic environment.

Conclusion

The commercial corncob biochar has high aromatic carbon and carbonyl group proportion. Their surface morphology and porosity are advantages for water retention. These properties may offer several advantages for the hydroponics system such as provide resistance to microbial and better nutrient utilization. Moreover, this biochar also shows an appropriate pH and high ionic exchange capacity which should benefit the growth of the hydroponic plants. All these properties were proved benefits on the growth of the plants depending on the type of plant. The different number of leaves of Cos lettuce and dry weight of Green Oak lettuce from growth media with and without biochar is significant ($p<0.05$). It could recommend that adding some corncob biochar could endorse nutrients used in the hydroponics system. This would advantage for put forth shoots of hydroponic plants.

Acknowledgment

The authors are grateful to the Mea Fah Luang University research found for financially supporting the research project (project no.611B05007)

References

Angmanee, R., Chuangcham, K., & Homchan, U. (2017). Properties of corn waste biochar and potential for soil improvement. *VRU Research and Development Journal Science and Technology. 11*(1), 11.

Atkinson, C.J., Fitzgerald, J.D., & Hipps, N.A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. *Plant and soil, 337*(1-2), 1-18.

Awad, Y.M., Lee, S.-E., Ahmed, M.B.M., Vu, N.T., Farooq, M., Kim, I.S., Al-Wabel, M. (2017). Biochar, a potential hydroponic growth substrate, enhances the nutritional status and growth of leafy vegetables. *Journal of Cleaner Production, 156,* 581-588.

Batista, E.M., Shultz, J., Matos, T.T., Fornari, M.R., Ferreira, T.M., Szpoganicz, B., ... & Mangrich, A.S. (2018). Effect of surface and porosity of biochar on water holding capacity aiming indirectly at preservation of the Amazon biome. *Scientific Reports, 8*(1), 1-9.

Blagodatskaya, E., Blagodatsky, S., Anderson, T. H., & Kuzyakov, Y. (2014). Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. *PloS one, 9*(4), e93282.

Blok, C., Van der Salm, C., Hofland-Zijlstra, J., Streminska, M., Eveleens, B., Regelink, I., . . . Visser, R. (2017). Biochar for horticultural rooting media improvement: Evaluation of biochar from gasification and slow pyrolysis. *Agronomy, 7*(1), 6.

Centre for Agricultural Information. (2017). *Total maize: Cultivated area, area of harvest, yield and yield per area: Year 2555 - 2557.* Retrieved March 3, 2018, from http://aginfo.oae.go.th/ewtnews/maize.html

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., . . . Zheng, B. (2016). Biochar to improve soil fertility. A review. *Agronomy for sustainable development, 36*(2), 36.

Fryda, L., & Visser, R. (2015). Biochar for soil improvement: Evaluation of biochar from gasification and slow pyrolysis. *Agriculture, 5*(4), 1076-1115.

Jahromi, N.B., Walker, F., Fulcher, A., Altland, J., & Wright, W.C. (2018). Growth response, mineral nutrition, and water utilization of container-grown woody ornamentals grown in biochar-amended pine bark. *HortScience, 53*(3), 347.

Kasikorn research center. (2020). *How much does the economy affect?* Retrieved January 20, 2020, from https://kasikornresearch.com/th/analysis/k-social-media/Pages/FB-PM25-23-01-20.aspx

Khoomsab, R., Pongpian, W., Winyakul, C., & Chomchalao, P. (2015). Preparation and analysis on adsorption of activated carbon from waste corncob by chemical activation method. In *the National Conference on Research for Sustainable Development* (pp. 1393-1400). Retrieved June 14, 2019, from http://www.rdi.ssru.ac.th/researchconference/index.php/proceeding-online

Mukherjee, A., & Zimmerman, A.R. (2013). Organic carbon and nutrient release from a range of laboratory-produced biochars and biochar–soil mixtures. *Geoderma, 193-194,* 122-130.

Novak, J.M., Lima, I., Xing, B., Gaskin, J.W., Steiner, C., Das, K., . . . Busscher, W. J. (2009). Characterization of designer biochar produced at different temperatures and their effects on a loamy sand. *Annals of Environmental Science, 3,* 195-206.

Nurhidayati, N., & Mariati, M. (2014). Utilization of maize cob biochar and rice husk charcoal as soil amendment for improving acid soil fertility and productivity. *Journal of Degraded and Mining Lands Management, 2*(1), 223.

Rizwan, M., Ali, S., Qayyum, M.F., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T., & Ok, Y.S. (2016). Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: A critical review. *Environmental Science and Pollution Research, 23*(3), 2230-2248.

Ronsse, F., Van Hecke, S., Dickinson, D., & Prins, W. (2013). Production and characterization of slow pyrolysis biochar: Influence of feedstock type and pyrolysis conditions. *Gcb Bioenergy, 5*(2), 104-115.

Singh, B., Dolk, M.M., Shen, Q., & Camps-Arbestain, M. (2017). Biochar pH, electrical conductivity and liming potential. In *Biochar: A Guide to Analytical Methods,* (pp.23-38). Clayton, Australia: Csiro Publishing.

Smith, P. (2016). Soil carbon sequestration and biochar as negative emission technologies. *Global change biology, 22*(3), 1315-1324.

Tangtaweewipat, S., Pongpiachan, P., Yammuenart, S., Tapingkae, W., Banziger, S., & Kornkal, C. (2012). *Smong reduction corn village "reduction of the smog problem and the environment conservation through science and integrated agricultural technology on highland" case study Baanbonna Mae Chaem, Chiang Mai, Thailand* (Research report). Chiang Mai: Chiang Mai University.

Tansathit, T., Rotchanamekha, S., & Thepwong, R. (2016). Biochar production from stubble and rice straw for water holding in soil. In *the 1st National RMUTR Conferenc 2559 (Building Innovation 2016 : B-inno 2016)* (pp.401-410). Retrieved June 14, 2019, from http://repository.rmutr.ac.th/123456789/269

Vaughn, S.F., Eller, F.J., Evangelista, R.L., Moser, B.R., Lee, E., Wagner, R.E., & Peterson, S.C. (2015). Evaluation of biochar-anaerobic potato digestate mixtures as renewable components of horticultural potting media. *Industrial Crops and Products, 65,* 467-471.

Zheng, H., Wang, Z., Deng, X., Herbert, S., & Xing, B. (2013). Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. *Geoderma, 206,* 32-39.

Zhu, X., Chen, B., Zhu, L., & Xing, B. (2017). Effects and mechanisms of biochar-microbe interactions in soil improvement and pollution remediation: A review. *Environmental Pollution, 227,* 98-115.

JFHB Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science

Journal homepage : http://jfhb.dusit.ac.th/

Life Cycle and Larval Feeding Habits of *Macrostemum indistinctum* Banks 1911 (Trichoptera: Hydropsychidae) in the Stream Flows into Krasiow Reservoir, Thailand

Orawan Khamrak & Taeng-On Prommi*

Department of Biological Science, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

Article info

Article history:
Received : 1 November 2020
Revised : 27 November 2020
Accepted : 30 November 2020

Keywords:
Hydropsychidae, *Macrostemum indistinctum,* Gut content, Feeding, Larval stage

Abstract

The aim of this work was to determine the feeding habits of *Macrostemum indistinctum* larvae using gut content analysis. The study was carried out in the stream flows into the Krasiow dam. A total of 126 larvae were captured and had the head capsule width measured. The gut content was analyzed under microscope. Seven categories of food items were determined: arthropod fragment, blue green algae, diatoms, gastropods, green algae, protozoa and rotifers. When comparing both populations in two seasons, statistically significant differences ($P<0.05$) were found for gastropods and protozoa. The food items, arthropods, blue green algae, diatoms, green algae and rotifers showed no difference between both populations. *Macrostemum indistinctum* was found to be an omnivorous collector organism. The similarities in the seasonal trophic structure might indicate the constant availability of food.

Introduction

Aquatic insects are one of the most dominant elements in freshwater ecosystem trophic webs, participating in energy flow and nutrient cycling (Whiles & Wallace, 1997). They are also important food resources for fish (Wallace & Webster, 1996) and some insectivorous birds (Ward et al., 1995). The distribution and abundance of insects in freshwater systems is the result of complex interactions between their ecological roles and the physicochemical conditions that characterize the habitat, and food availability (Merritt & Cummins, 1996). Thus, the community structure depends on a number of factors, such as water quality, type of substrate, particle size of sediment, water flow, available of sediment organic matter, oxygen concentration as well as environmental conditions surrounding the watercourse (Ward et al., 1995; Buss et al., 2004). They reflect environmental changes, aquatic insects are often used as indicators of the effects of human activity on water systems, providing information on habitat and water quality (Woodcock & Huryn, 2007). Amongst the aquatic insects, order Trichoptera (or caddisflies) are the most widely distributed; their larvae are common in running water (Ward, 1992) and they are one of the relatively well-studied orders of aquatic insects in South East Asia (Malicky, 2010; Morse, 2017). The larvae of many species coexist in running waters and are known to have specific habitat and environmental requirements (de Moor, 2007).

* Corresponding Author
e-mail: faastop@ku.ac.th

The filter-feeding caddisflies of Hydropsychidae comprise one of the largest families of Trichoptera, with about 1,756 described adult species worldwide (Morse, 2011). Larvae of hydropsychids live in running waters and are generally collectors-filterers. Hydropsychidae larvae use nets spun with their silk glands to capture drifting food materials in stream (Merritt & Cummins, 1996). They usually construct a silken filter net at the entrance to their fixed tubular retreat (Wiggins, 1996). Larvae present a high ecological diversity and display a wide range of tolerance to different levels of pollution, which makes them very useful organisms in biological water quality monitoring programs (Resh, 1995). The species *Macrostemum indistinctum* Banks 1911 from the Thai basin constructs a tube of sand grains (Fig. 2) on the sandy bottom of streams and utilizes an extremely fine meshed net that captures food. However, it is not known on the trophic behavior of this species in the study area during a year sampled, the aim of the present paper was to determine the feeding habits of *M. indistinctum* larval stages using gut analysis from the two seasons collected in corresponding on water quality measurement.

Materials and methods

1. Study area

The study was conducted in the stream flows into the Kasiow reservoir, Dan Chang district, Suphan Buri Province, Thailand (14°56.859´N, 099°38.118´E) (Fig. 1). This stream is part of the Tha Chin River, and is 140 km long. The stream watershed is located between Khao La and Khao Yai, north of Ban Rai district, Uthai Thani Province. The study site is located at an elevation of 81 m and the stream is on average 2.0 m wide and 0.6 m deep. It presented clear water, gravel, a sandy muddy bottom, and marginal herbaceous vegetation. The collecting sites are located in the extensive areas with the sugar cane cultivation.

2. Sampling and laboratory analyses

Nine water physicochemical variables were measured once a month during January to December 2019. The physicochemical water quality parameters were recorded directly at the sampling site and included pH (measured by a pH-meter Waterproof Model Testr30), water temperature (measured by a hand-held thermometer), and dissolved oxygen (DO, measured by a HACH® Model sensION 6 DO meter), total dissolved solid (TDS) and electrical conductivity (EC) (measured by a EURECH CyberScan CON110 conductivity/TDS meter). Water samples from each collecting period were stored in polyethylene bottles (500 mL). Ammonia nitrogen (NH_3-N), sulfate (SO_4^{2-}), nitrate-nitrogen (NO_3^--N), orthophosphate (PO_4^{3-}), and turbidity were determined in accordance with standard procedures (American Public Health Association (APHA), 1992).

Aquatic insects were collected during the wet season (July to October 2019) and cold-dry season (November to April 2019), along with 50 m stretch of the stream. Samplings were collected with a D-frame kick net with 250 µm mesh size, including by hand picking. The caddisflies larvae, *M. indistinctunm* (Fig. 2) were taken together with other aquatic insects and preserved in 70% ethanol.

All the organisms were sorted and kept its in 70% ethanol. For the *M. indistinctum,* head capsule width was use to classified into the larval instar, following the method described by MacKay (1978). Head capsule width was measured using an ocular micrometer. The numbers of larval instars were determined by analyzing the frequency distributions of head capsule widths, following which the width ranges for instars were determined for each month.

For a qualitative assessment of the chief food item of *M. indistinctum,* a total of 50 larvae of the cold-dry season and 66 larvae of the wet season were dissected under a stereomicroscope (Olympus SZ51). The whole digestive tracts were removed to a glass slide with water, shredded, and examined under dissecting and compound microscopes (Olympus CX31). Gut contents were separated into arthropod fragment, blue green algae, diatoms, gastropods, green algae, protozoa, and rotifers.

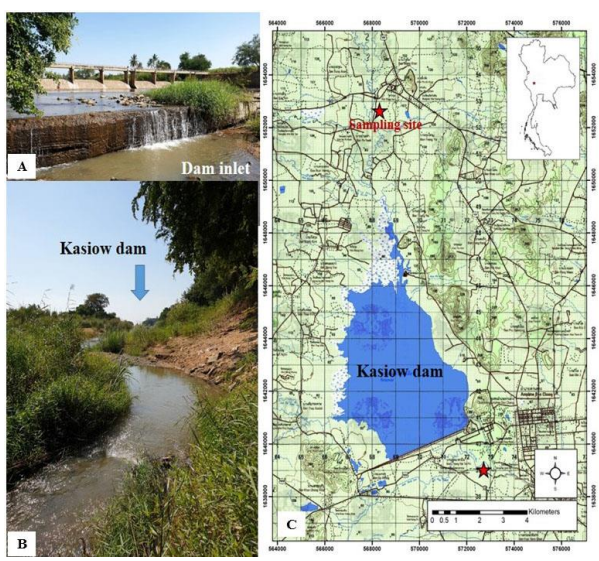


Fig. 1 Map of the Kasiow reservoir showing the sampling site (A) Dam inlet, (B) the collecting sites, and (C) Map of the Kasiow reservoir

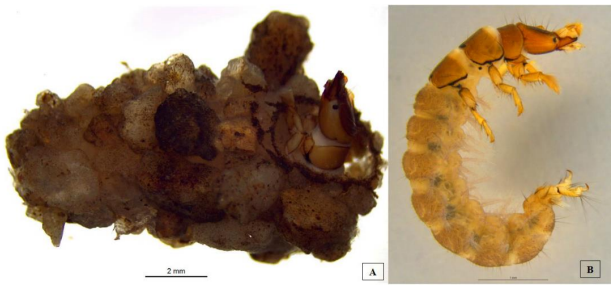


Fig. 2 The characteristics of larva with case (A) and larva of *Macrostemum indistinctum* (B)

3. Data analysis

The SPSS v. 13.0 (http://www.spss.com/) was used to perform the statistical analyses. Mean, standard deviation, maximum and minimum values of prey (as the maximum and minimum number of individuals identified) were calculated for the percentage of each item of gut content. The ontogenetic shift on diet was assessed by means of a correlation (Gamma correlation test) between larval size (measured as head width) and percentage of the different items in the larval gut. To compare the food items in gut contents between populations in cold dry and wet season, Mann-Whitney *U*-test was used.

Results and discussion

1. Physicochemical parameters of water quality

Mean values of selected physicochemical parameters of water quality at the stream flows into Kasiow dam during this study are presented in Table 1. All water parameters such as water temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS), electrical conductivity (EC), nitrate-nitrogen (NO_3^--N), ammonia-nitrogen (NH_3-N), orthophosphate (PO_4^{2-}) and turbidity varied significantly during the sampling periods ($p<0.05$), using ANOVA (Table 1).

Water temperature greatly varied from 22.37± 0.21 to 39.50±1.15°C. Temperature is one of the most important physical parameter in the biosphere, partly because it affects the movement of saturation constants of dissolved gasses in water, metabolic rate of organisms and other factors that directly or indirectly affect life on earth (Hauer & Hill, 1996). In tropical streams, the mean annual temperature generally exceed 20°C (Dudgeon, 1999). This average temperature is similar to the average result in this study.

The mean value of pH ranged from 7.47±0.15 to 9.30±0.44. The pH is parameter concerned with the concentration of carbon fractions and hydrogen ions (Goldman & Horne, 1983), which shows a similarly trend of local and seasonal difference to the previous ions mentioned (i.e. EC and TDS). However the result of pH showed significant differences.

The mean value of dissolved oxygen ranged from 6.13±0.16 to 8.63±0.50 mg/L. Beside the ions concentration, dissolved oxygen is the most widely studied chemical in aquatic environment. Dissolved oxygen greatly affects aquatic life as well as biochemical processes. Nearly all stream organisms are sensitive to DO. Organic pollution may significantly reduce DO concentration in entire stream reaches as microbial processes consume the oxygen from the water (Hauer & Hill, 1996). The fact that DO saturation mixed well at above 80% at the unpolluted stream, which is greatly replenished from the air, supports that DO concentrations of each month showed significant differences with excessive concentration.

The mean electrical conductivity values ranged from 202.73±0.59 to 602.33±0.58 µS/cm. Within the water column, conductivity, which indicates the ability of

Table 1 Physicochemical parameters of the sampling site during January to December 2019*

Parameter/month	Jan	Feb	Mar	Apr	Jul	Aug	Sep	Oct	Nov	Dec
Water temp. (°C)	27.67±0.3^{bc}	30.13±1.18^{de}	31.30±0.53^{e}	39.50±1.15^{f}	29.13±0.06^{cd}	29.87±0.06^{de}	29.10±0.53^{cd}	31.60±1.08^{e}	22.37±0.21^{a}	26.80±0.17^{b}
DO (mg/L)	8.21±0.13^{c}	7.21±0.13^{b}	8.63±0.50^{c}	6.88±0.65^{ab}	6.13±0.16^{a}	6.65±0.07^{ab}	7.07±0.03^{b}	6.89±0.07^{ab}	6.99±0.02^{b}	7.06±0.04^{c}
pH	8.20±0.10^{bc}	7.97±0.40^{abc}	8.53±0.06^{c}	7.87±0.12^{ab}	8.03±0.06^{abc}	8.17±0.06^{bc}	7.47±0.15^{a}	8.30±0.00^{bc}	9.30±0.44^{d}	8.43±0.06^{bc}
TDS (mg/L)	228.67±0.58^{f}	111.67±11.50^{b}	134.10±0.75^{c}	95.93±1.05^{a}	136.70±3.91^{c}	162.57±0.41^{d}	175.00±0.26^{e}	245.67±2.52^{g}	385.50±0.35^{i}	359.00±1.00^{h}
EC (uS/cm)	457.00±1.00^{g}	216.00±2.00^{b}	283.33±1.53^{c}	202.73±0.59^{a}	289.00±0.00^{d}	372.67±1.53^{e}	393.33±1.53^{f}	569.33±1.53^{h}	602.33±0.58^{i}	762.33±4.04^{j}
NH_3-N (mg/L)	0.08± 0.04^{a}	0.69±0.34^{c}	0.23±0.01^{ab}	0.72±0.04^{c}	0.22±0.13^{ab}	0.20±0.03^{ab}	0.48±0.30^{bc}	0.26±0.01^{ab}	0.15±0.03^{a}	0.24±0.02^{ab}
NO_3-N (mg/L)	4.13±0.06^{bcd}	7.20±0.17^{d}	4.10±0.17^{bcd}	NA	1.77±0.15^{abc}	1.57±0.46^{ab}	5.63±4.21^{cd}	2.93±0.06^{abc}	3.70±0.00^{abcd}	4.40±0.62^{bcd}
PO_4^{3-} (mg/L)	1.18±0.08^{d}	2.25±0.15^{ef}	0.84±0.04^{cd}	0.76±0.25^{bcd}	0.26±0.27^{ab}	0.17±0.07^{a}	0.40±0.19^{abc}	0.67±0.10^{abcd}	1.82±0.39^{e}	2.43±0.06^{f}
Turbidity (NTU)	7.39±1.72^{a}	10.55±3.36^{a}	4.96±2.96^{a}	1.87±0.75^{a}	127.33±13.65^{c}	41.63±4.97^{b}	364.33±16.50^{d}	7.39±1.72^{a}	10.55±3.36^{a}	4.96±2.96^{a}

Remarks: *The heavy rains occur in the month of May and June, environmental variables were no measured. Values with different letters indicate significant mean difference following Turkey post hoc tests ($P<0.05$).

solution to carry on an electric current, is related to the water fertility (Mustow, 1997). Conductivity was found as the gradual built-up along a downstream progression, which was clearly shown in high values in stream flow in to the Kasiow reservoir. This may be explained by the dissolution of rocks and soil nearby stream, which was high up at the lower altitude. Conductivity, moreover, showed evidence of seasonal pattern, which indicated a high value in hot-dry season and less value in cold-dry season and wet season, respectively. This pattern was explained by the theory of Bishop (1973). In hot-dry season, the amount of the falling leaves or leaf litters were high. Leaves were quickly decomposed by high temperature and then, the soluble nutrients were released into the water.

The mean total dissolved solids values ranged from 95.93±1.05 mg/L to 385.50±0.35 mg/L. Total dissolved solids is another parameter, which indicates the materials that are chemically dissolved in water. This includes materials such as calcium, chloride, sodium, magnesium, silicate and carbonate. The TDS enters the stream from three natural sources; (1) atmosphere (i.e. rainfall), (2) soil and rock weathering and (3) biological process (Webster & Ehrman, 1996). Seasonal pattern of TDS indicates that a high concentration of TDS appears in hot-dry season and is less concentration in cold-dry season. This shows a similar trend to electrical conductivity parameter and can be explained by the same explanation.

The mean turbidity values ranged from 1.87±0.75 to 364.33±16.50 NTU. The high turbidity was recorded during the wet season due to heavy rainfall. Turbidity of the water is caused by suspended solids and any coloration produced by dissolved substance. The source of suspended solids and dissolved substance can be inorganic particles and organic debris from soil erosion in the agricultural areas, with high erosion during the flood (Bisson & Montgomery, 1996). As results of this study indicated the turbidity of all month were different, especially in the month of rainy season. The adverse effects of turbidity on freshwater system included; low penetration of light which then reduces primary and secondary production, high adsorption of nutrient molecules to suspended materials making the nutrients unavailable for plankton production, decreased oxygen concentration, and clogged filter-feeding apparatus, and digestive organs of planktonic organisms, which may adversely affect the production of larvae (Gupta & Gupta, 2006).

The mean dissolved nutrients, NH_3-N, NO_3^--N, and PO_4^{3-} concentrations varied from 0.08± 0.04 to 0.72±0.04 mg/L, 1.57±0.46 to 7.20±0.17 mg/L, and 0.17±0.07 to 2.43±0.06 mg/L, respectively. Nitrates are the most oxidized forms of nitrogen and the end product of aerobic decomposition of organic nitrogenous matter (Qadri et al., 2020). Nitrogen is always present in aquatic ecosystems and is as abundant as gas in the atmosphere. Relatively small quantities of nitrogen exist in the combined forms of ammonia, nitrate, nitrite, urea, and dissolved organic compounds. Nitrate is usually the most important nutrient. Natural changes in the vegetation of the drainage basin caused by fire, floods, or artificial clearing usually results in an increase of nitrate in streams. Even moderate environmental disturbances, such as sensible farming or logging without severe erosion, release a higher quantity of nitrate more than ammonia or phosphate (Goldman & Horne, 1983). Therefore nitrate concentration also reflects the range of pollution or disturbances, while ammonium concentration indicates a metabolic waste product of animals. At the time of this study nitrate concentrations were found to be slightly low. Although it should be noted that according to Goldman & Horne (1983) indicated that the concentration of most nitrogen compounds in lake and stream tended to follow regular seasonal pattern. The extreme values in this study in wet and hot-dry season, respectively, coincide with the recorded heavy rain before collecting began the same as the value of turbidity and others. The result of ammonia was also similar to nitrate concentration. Phosphate, in contrast to nitrate, is readily adsorbed to soil particles and does not move easily in groundwater. High flows of total phosphorus is due to erosion of particles from steep slopes with easily erodible soils. Agricultural, domestic and industrial wastes are the major sources of soluble phosphate. Phosphate containing detergents, for example, commonly contribute about half the phosphorus contained in domestic sewage. Here the detected phosphorus is treated as the reflection of waste within water column and hill-slope. The amounts of phosphorus concentration has significant seasonal pattern similarly to the results of the nitrate and ammonia.

2. Larval instars of *Macrostemum indistinctum*

From the specimens collected on all occasions during the wet season (July to October 2019) and cold-dry season (November to April 2019), a total of 5,583 individual of hydropsychid larvae were found. 126 larvae of *M. indistinctum* were identified and measured of head capsule width.

Larval head width ranged from 0.13 to 1.98 mm, which all developmental stages of larvae were present in our analysis (Table 2). The larvae were classified into five instars using 126 specimens. Head widths of first instars ranged from 0.13-0.18 mm (n = 8). Head widths of second instars ranged from 0.26 to 0.44 mm (n = 18). The third instars ranged from 0.50 to 0.81 mm (n = 30). The fourth instars ranged from 0.83 to 1.36 mm (n = 34) and the fifth instars ranged from 1.38 to 1.98 mm (n = 34) (Fig. 3 and Table 2).

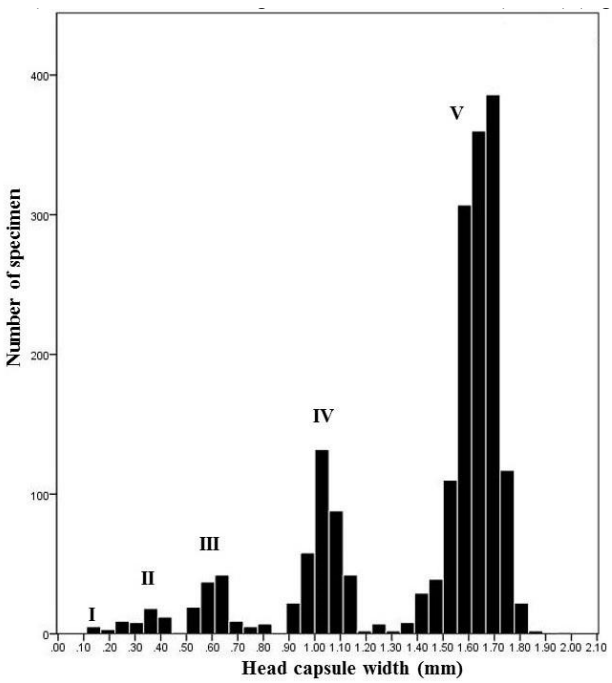


Fig. 3 The frequency distribution of larval instars of *M. indistinctum* at all sampling sites, based on head capsule width

Table 2 Median and range of head capsule width (mm) for larval instars of *M. indistinctum* during study period

Larval instar	Number measured	Mean head width (mm)	Range of head width (mm)
I	8	0.16±0.02	0.13-0.18
II	18	0.34±0.05	0.26-0.44
III	30	0.62±0.07	0.50-0.81
IV	34	1.06±0.08	0.83-1.36
V	34	1.63±0.09	1.38-1.98

3. Larval gut contents in Macrostemum indistinctum

Larval gut contents were assessed qualitatively. Gut contents were separated into arthropod fragments, blue green algae, diatoms, gastropods, green algae, protozoa and rotifers. Gut content analysis indicated that larvae are omnivorous filterers. The major resource items used by *M. indistinctum* larvae were diatoms, green algae and blue green algae (Tables 3-4 and Fig. 4).

In the cold-dry season, 50 larvae were analyzed gut content. The most abundant trophic resource was diatoms followed by green algae, blue green algae, gastropods, arthropods, rotifers and protozoa (Table 3 and Fig. 4). Significant positive correlations (P<0.05) was found between head width and percentage of arthropods (Gamma correlation = 0.707), blue green algae (Gamma correlation = 0.352), diatoms (Gamma correlation = 0.587), green algae (Gamma correlation = 0.563), and rotifers (Gamma correlation = 1.000). No significant positive correlations was found between larval stages and percentage of gastropods (Gamma correlation = –0.034) and protozoa (Gamma correlation = –1.000). The example of food items in the gut of *M. indistinctum* was shown in Fig. 5.

Table 3 Gut contents of the larvae of *Macrostemum indistinctum** in the dry season

Food item/ individual	Instar II		Instar III		Instar IV		Instar V	
	N	Mean	N	Mean	N	Mean	N	Mean
Arthropods	6	1.2±0.4 (1-2)	15	1.5± 0.9 (1-4)	14	3.9±6.8 (1-24)	15	5.6±3.3 (1-13)
Blue green algae	6	9.6±13.2 (1-39)	15	9.2± 8.5 (1-33)	14	11.6±10.2 (1-34)	15	32.6±45.2 (1-176)
Diatoms	6	45.3±65.2 (9-211)	15	64.5±11 (11-155)	14	165.9±113.1 (13-415)	15	218.5±142.9 (26-573)
Gastropods	6	15.7±16.0 (1-34)	15	9.1± 8.6 (2-29)	14	9.2±6.1 (2-20)	15	8.5±6.7 (3-20)
Green algae	6	6.7±6.5 (1-35)	15	10.0±5.2 (1-17)	14	20.4±9.8 (3-32)	15	37.4±25.2 (1-74)
Protozoas	NA	NA	15	1.3± 0.6 (1-2)	14	1.0±00 (1)	15	1.0±00 (1)
Rotifers	NA	NA	15	1.0± 0.0 (1)	14	1.0±00 (1)	15	13.6±14.0 (3-38)

Remarks: *Gut content of first instar was not dissected for qualitative assessment

In the wet season, 66 larval analyzed in this study had gut content, and they ingested mainly diatoms followed by green algae, blue green algae, gastropods, arthropods, rotifers and protozoa (Table 4 and Fig. 4). A significant positive correlation (P<0.05) was found between head width and percentage of arthropods (Gamma correlation =0.635), blue green algae (Gamma correlation = 0.660), diatoms (Gamma correlation = 0.594), green algae (Gamma correlation = 0.589), and protozoa (Gamma correlation = 0.280). No significant correlations was found between head width and percentage of gastropods (Gamma correlation = 0.103) and rotifers (Gamma correlation = –0.600).

Comparing populations in two seasons, statistically significant differences (P<0.05) were found for

gastropods (Mann-Whitney U = 0.021) and protozoa (Mann-Whitney U = 0.043). The food items, arthropods (Mann–Whitney U test, P = 0.564), blue green algae (Mann–Whitney U test, P = 0.773), diatoms (Mann–Whitney U test, P = 1.000), green algae (Mann–Whitney U test, P = 0.386) and rotifers (Mann–Whitney U test, P = 0.796) showed no difference between both populations.

Table 4 Gut contents of the larvae of *Macrostemum indistinctum** in the wet season

Food item/ individual	Instar II		Instar III		Instar IV		Instar V	
	N	Mean	N	Mean	N	Mean	N	Mean
Arthropods	12	1.0±0.0 (1)	15	1.4±0.5 (1-2)	20	1.6±1.3 (1-6)	19	4.4±4.1 (1-15)
Blue green algae	12	3.2±1.3 (1-4)	15	8.8±7.7 (1-27)	20	20.5±28.5 (2-119)	19	43.8±19.7 (10-77)
Diatoms	12	11.4 ±10.8 (1-38)	15	86.6 ±65.3 (1-223)	20	126.1±115.8 (1-386)	19	269.2±166.0 (7-659)
Gastropods	12	4.2± 3.2 (1-10)	15	6.7 ±6.1 (1-20)	20	3.0±2.9 (1-11)	19	8.1±6.3 (1-24)
Green algae	12	2.7± 2.2 (1-7)	15	28.9±25.1 (2-82)	20	52.8±68.9 (2-254)	19	113.3±102.6 (4-363)
Protozoa	NA	NA	15	2.0±1.4 (1-3)	20	2.0±1.7 (1-4)	19	3.2±2.8 (1-8)
Rotifers	NA	NA	15	1.0±00 (1)	20	2.0±00 (2)	19	1.0±00 (1)

Remarks: *Gut content of first instar was not dissected for qualitative assessment

The feeding habits of *M. indistinctum* populations in the stream flows into Kasiow reservoir widely coincided with that observed in other species of Hydropsychidae larvae populations in Thailand (Maneechan et al., 2018; Thamsenanupap & Prommi, 2020). They are mainly filtering-collectors, but detritus is also an important component of their smaller larvae diet. Among the food items, diatoms, green algae and blue green algae are also the major resources for most of the studied Hydropsychidae species (Maneechan et al., 2018; Thamsenanupap & Prommi, 2020). When analyzing changes in diet with growth, apart from a general decrease in detritus intake, they seemed to be a significant trend to ingest higher size prey (as gastropods, protozoa and rotifers) by bigger larvae. This was the result of different conditions and environmental parameters at the sampling site (Table 1). The same result was published by Gil et al. (2008). During both periods the most representative items were invertebrates and amorphous substance constituting about 70% of the diet. Hydropsychidae, *Smicridea (Rhyacophylax) dithyra* Flint, 1974 between both periods preferred amorphous material (37.4%), invertebrate remains (32.6%), filamentous algae (11.5%), leaves fragments (6.3%), inorganic matter (5.9%) and unicellular algae (4.8%). Hyphae, fine sediment and pollen were found in small percentages between 0 and 0.7%. Chaetas of oligochaeta, eggs, legs and tegument of mayflies and antennas, among others, were observed in the invertebrate remains.

Hydropsychidae larvae are described as filterer collectors (Merritt & Cummins, 1984). A filterer collector is classified by the way that they eat: those which feed on seston moved by a current, using silk nets or body parts (passive), and those which resuspension deposits which are filtered using silk nets or body parts (active) Palmer & O´Keefe (1992). Gallardo-Mayenco et al. (1998) described the feeding habits of Hydropsychidae in relation to the different net sizes which can be related to the larvae development stage.

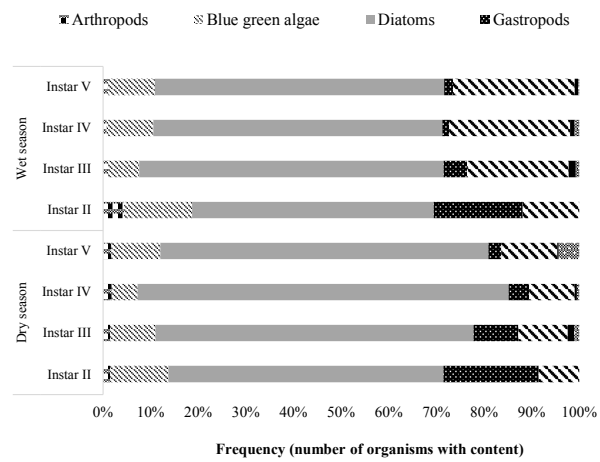


Fig. 4 Frequency of food items in the gut of *Macrostemum indistinctum*

The food item composition in larval gut observed in *M. indistinctum* obtained from the stream flows into the Kasiow reservoir was in agreement with the genus description due to the variety of ingested food items. Considering the size of the items found in the digestive tracts of *M. indistinctum* larvae, the definition given for omnivorous collectors might be extended since they have been traditionally described as processors of fine particulate organic matter (FPOM) and coarse particulate organic matter (CPOM). However, some cases, coarse particulate organic matter (CPOM), such as complete preys or fragments of big filamentous algae, were found.

The similarities found in the seasonal trophic structure might indicate the constant availability of the food resource. This possibility was reinforced by some studies carried out with coleopterans and dipterans, which showed that a change in the trophic group involved a change in the proportion of items consumed during the dry and wet seasons (Motta & Uieda, 2004).

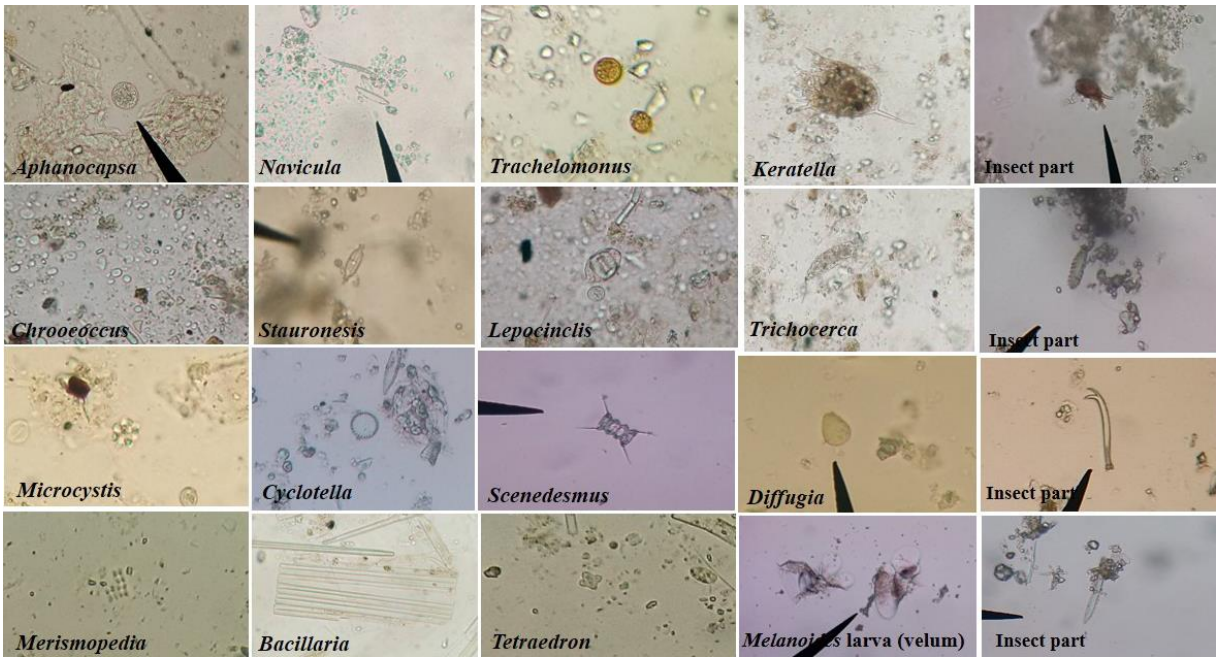


Fig. 5 Food items in the gut of *M. indistinctum* under bright field microscope (magnification x40)

Conclusion

Most of *M. indistinctum* consumed diatoms, blue green algae, and green algae as the main food source. The small larval of *M. indistinctum* consumed detritus as part of their diet, whereas the larger larval consumed larger food items. *M. indistinctum* is not exclusively herbivorous, but rather omnivorous species with flexible feeding habits. The physico-chemical of water quality parameters such as electrical conductivity, total dissolved solids, water turbidity, orthophosphate, pH, water temperature, dissolved oxygen, ammonia-nitrogen, and nitrate-nitrogen are currently affecting on the life stages of *M. indistinctum* in Kasiow reservoir, Thailand. Hydropsychidae are richly diverse in Thai streams and other species can be expected to be affected in the same way.

Acknowledgement

This research work was supported by the Faculty of Liberal Arts and Science, Kamphaeng Saen Campus, Kasetsart University.

References

American Public Health Association (APHA). (1992). *Standard methods for the examination of water and wastewater* (18th ed.). Washington, D.C.: American Public Health Association.

Bishop, J.E. (1973). *Limnology of a small Malayan river Sungai Gombak.* Canada: W. Junk, The Hague.

Bisson, P.A., & Montgomery, D.R. (1996).Valley segments, stream reaches, and channel units. In F.R. Hauer & G.A. Lamberti (Eds), *Methods in Stream Ecology.* San Diego: Academic Press.

Buss, D.F., Baptista, D.F., Nessimain, J.L., & Egler, M. (2004). Substrate specificity, environmental degradation and disturbance structuring macroinvertebrate assemblages in neotropical streams. *Hydrobiologia, 518*(1-3), 179-188.

de Moor, F.C. (2007). Regional biogeographical differences in Trichoptera diversity in South Africa: Observed patterns and processes. In J. Bueno-Soria R. Barba-Alvares & B. Armitage (Eds.), *Proceedings of the 12nd International Symposium on Trichoptera* (pp. 211-218). USA: Caddis Press.

Dudgeon, D. (1999). *Tropical Asian streams: Zoobenthos, ecology and conservation.* Hong Kong: Hong Kong University Press.

Gallardo-Mayenco, A., Prenda, J., & Toja, J., (1998). Spatio - Temporal distribution and ecological preferences of coexisting Hydropsychid species (Trichoptera) in two Mediterranean River Basins (S Spain). *International Review of Hydrobiology, 83*(2), 123–134.

Gil, M.A., Tripole, S., & Vallania, E.A. (2008). Feeding habits of *Smicridea (Rhyacophylax) dithyra* Flint, 1974 (Trichoptera: Hydropsychidae) larvae in the Los Molles stream (San Luis - Argentina). *Acta Limnologica Brasiliensia, 20*(1). 1–4.

Goldman, C.R., & Horne, A.J. (1983). *Limnology.* New York: McGraw-Hill Book Company.

Gupta, S.K., & Gupta, R.C. (2006). *General and applied ichthyology (fish and fisheries).* New Delhi: S. Chand and Company.

Hauer, F.R., & Hill, W.R. (1996). Temperature, light, and oxygen. In F.R. Hauer & G.A. Lamberti (Eds.), *Methods in stream ecology* (pp.103-110). California: Academic Press.

Mackay, R.J. (1978). Larval identification and instar association in some species of Hydropsyche and Cheumatopsyche (Trichoptera: Hydropsychidae). *Annals of the Entomological Society of America, 71*(4), 499-509.

Malicky, H. (2010). *Atlas of Southeast Asian Trichoptera.* Chiang Mai: Chiang Mai University.

Maneechan, W, Kruttha, P., & Prommi, T. (2018). The larva and pupa of *Potamyia flavata* (Banks 1934) (Trichoptera: Hydropsychidae): Description, life cycle, and notes on its biology. *Zootaxa, 4394*(3), 395–406.

Merritt, R.W., & Cummins, K.W. (1996). *An introduction to the aquatic insects of North America* (3rd ed.) Iowa: Kendall/Hunt Publishing Company.

Merritt, R.W., & Cummins, K.W. (1984). *An Introduction to the Aquatic Insects of North America* (2nd ed.) Iowa: Kendall-Hunt.

Morse, J.C. (2017). *Trichoptera World Checklist.* Retrieved December 29, 2017, from http://entweb.sites.clemson.edu/database/trichopt/

Morse, J.C. (2011). The Trichoptera world checklist. *Zoosymposia, 5,* 372–380.

Mustow, S.E. (1997). *Aquatic macroinvertebrate and environmental quality of rivers in northern Thailand* (Doctoral dissertation). London: University of London.

Motta, R.L., & Uieda, V.S. (2004). Diet and trophic groups of an aquatic insect community in a tropical stream. *Brazilian Journal of Biology, 64*(4), 809–817.

Palmer, C., & O`Keeffe, J. (1992). Feeding patterns of four macroinvertebrate taxa in the headwaters of the Buffalo River, Eastern Cape. *Hydrobiologia, 228*(2), 157–173.

Qadri, H., Bhat, R.A., Mehmood, M.A., & Dar, G.H. (2020). *Fresh water pollution dynamics and remediation.* Singapore: Springer Nature Singapore.

Resh, V.H. (1995). Freshwater macroinvertebrates and rapid assessment procedures for water quality monitoring in developing and newly industrialized countries. In W.S. Davies & T.P. Simon (Eds.), *Biological Assessement and Criteria* (pp. 167–177). Boca Raton: Lewis Publishers

Thamsenanupap, P., & Prommi, P. (2020). Influence of water quality parameters on larval stages of *Pseudoleptonema quinquefasciatum* Martynov 1935 (Trichoptera: Hydropsychidae) in streams of western Thailand. *Jordan Journal of Biological Science, 13*(3), 305–300.

Wallace, J.B., & Webster, J.R. (1996). The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology, 41,* 115–139.

Ward, J.V. (1992). *Aquatic insect ecology.* New York: John Wiley & Sons.

Ward, D., Holmes, N., & Jose, P. (1995). *The new rivers and wildlife handbook.* Bedfordshire, England: RSPB / NRA / The Wildlife Trusts.

Webster, J.R., & Ehrman, T.P. (1996). Solute dynamics. In F.R. Hauer & G.A. Lamberti (Eds.), *Methods in Stream Ecology* (pp.145-160). San Diego: Academic Press.

Wiggins, G.B. (1996). *Larvae of the North American Caddisfly Genera (Trichoptera)* (2nd ed.). Toronto, Canada: The University of Toronto Press.

Whiles, M.R., & Wallace, J.B. (1997). Leaf litter decomposition and macroinvertebrate communities in headwater streams draining pine and hardwood catchments. *Hydrobiologia, 353*(1-3), 107–119.

Woodcock, T.S., & Huryn, A. (2007). The response of macroinvertebrate production to a pollution gradient in a headwater stream. *Freshwater Biology, 52*(1), 77–196.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Cholinesterase's Enzymes Inhibition and Michaelis-Menten Kinetics Studies on Ethnomedicinally Important Plant *Chenopodium botrys*

Naveed Iqbal[a], Farhat Ullah[a], Abdul Sadiq[a], Muhammad Saeed Jan[a], Muhammad Shahid[b] & Muhammad Ayaz[a]*

[a] *Department of Pharmacy, Faculty of Biological Sciences, University of Malakand, Chakdara, 18000 Dir (L), KP, Pakistan*
[b] *Department of Pharmacy, Sarhad University of Science and Information Technology (SUIT) 10800, Peshawar, Pakistan*

Article info

Article history:
Received : 1 August 2020
Revised : 5 December 2020
Accepted : 15 December 2020

Keywords:
Chenopodium botrys, Michaelis-Menten kinetics, Alzheimer's disease, Acetylcholine, Acetylcholinesterase, Butyrylcholinesterase

Abstract

Chenopodium botrys (*C. botrys*) methanolic extract (Cb.Cr) and subsequent fractions were screened for inhibitory potentials against cholinesterase's. Acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) *in vitro* inhibitory potentials were evaluated employing Ellman's assay. Lineweaver-Burk a plot (1/v versus 1/[s]) in which v is the velocity of reaction and [*s*] is substrate concentration was sketched by Michaelis-Menten kinetics. In AChE inhibition assay, chloroform (Cb.Chf), ethyl acetate (Cb.EtAc) and crude extract (Cb.Cr) showed highest activity with 80.12±1.97, 71.79±0.67 and 69.00±1.52% inhibitions at concentration of 1 mg/mL with IC_{50} values of 50, 115 and 130 µg/mL, respectively. Similarly, Cb.Chf, Cb.EtAc and Cb.Cr showed the strongest activity against BChE causing 76.20±0.28, 70.48±0.19 and 62.75±1.79% inhibitions at 1 mg/mL with IC_{50} of 25, 55 and 195 µg/mL, respectively. For the AChE inhibition, the V_{max} and K_m values were noted as 70.08 µg/min and 55.21 µg/mL intended for Cb-Cr, 54.38 µg/min and 107.6 µg/mL for Cb-Hex, 82.65 µg/min and 51.09 µg/mL for Cb-Chf, 72.83 µg/min and 63.05 µg/mL for Cb-EtAt, and 64.4 µg/min and 82.27 µg/mL for Cb-Aq. likewise, the V_{max} and K_m values for BChE also displayed effective inhibitory potential of Cb-Cr (63.51 µg/min and 51.82 µg/mL), Cb-Hex (53.13 µg/min and 47.71 µg/mL), Cb-Chf (77.37 µg/min and 33.13 µg/mL), Cb-EtAc (72.28 µg/min and 37.84 µg/mL), and Cb-Aq (59.18 µg/min and 34.67 µg/mL), respectively. In conclusion, *C. botrys* contains bioactive components which can be effective in the curing of Alzheimer's disease (AD) and other stress associated diseases.

Introduction

Medicinal plants, especially herbs, have been used as the chief source of medicine by majority of world's population for many years. The bioactive principles derived from medicinal plants are providing huge contributions towards healthcare throughout the world due to the ubiquitous nature medicinal plants and

* Corresponding Author
e-mail: Ayazuop@gmail.com,
ORCID: https://orcid.org/0000-0002-4299-2445

multiple health benefits (Ayaz et al., 2017a). Among the bioactive principles derived from medicinal plants, some of the natural compounds have also been proven to show good results in the treatment of various diseases (Ayaz et al., 2016). Alzheimer's disease is one of them, which shows good results among patients using drugs derived from plant sources especially "galantamine". Alzheimer's disease is the main/primary cause of dementia which associated with loss of social as well as intellectual capabilities and thus may interfere the routine functioning (Ali et al., 2017). The brain region of the AD patients prove a progressive loss of cholinergic synapses which playing higher mental functions, largely in the neocortex and hippocampus (Ayaz et al., 2017b). A decrease level of Acetylcholine (ACh) neurotransmitter, In the AD patients appear to be significant aspect in progression of dementia (Henry et al., 2010) . Thus, AD, dementia and other related neurological diseases can be treated via agents that restore the acetylcholine level by inhibition of both cholinesterases enzyme like AChE and BChE which is responsible for its degradation (Ayaz et al., 2017c). Furthermore, the AChE inhibition acts a vital role not only increasing the cholinergic transmission, but also helps in the formation of the neurotoxic fibrils and to reduce the aggregation of amyloid beta peptide (Aβ) in AD (Yoo & Park, 2012; Sadiq et al., 2015). Numerous treatment approaches have been urbanized, but the most valuable alternatives approach is cholinesterase inhibitors in cure of AD.

Currently five drugs including tacrine, eserine, rivastigmine, galanthamine and donepezil have been permitted for management of the AD and other neurological diseases. Among these, galanthamine is derived from plant sources (Berkov et al., 2009), whereas, rivastigmine is a synthetic derivative of naturally occurring compound physostigmine (Russo et al., 2013). Several other natural compounds including curcumin, catechins and myricetin are reported to act at various pathological targets of the AD (Baum & Ng, 2004; Frautschy et al., 2001; Lim et al., 2001; Ono et al., 2004; Yang et al., 2005; Ayaz et al., 2019a; Rice-Evans et al., 1996). The currently available drugs have limitations for their clinical use due to its serious adverse effects with bioavailability troubles, hepatotoxicity and gastro-intestinal disorders (Ullah et al., 2016). Consequently, investigation for efficacious, safe and more potent AChE and BChE inhibitors from alternative basis like natural products is ongoing.

Chenopodium botrys belongs to *Chenopodiaceae,* a family employed in traditional medicine as anti-asthmatic, anthelmintic, anti-spasmodic, and as spice (Buchbauer et al., 1995). It is traditionally used in various neurological disorders including convulsions, headache, CNS stimulant and as neurotonic (Morteza-Semnani, 2015). The species has been reported for the presence of monoterpenes like delta-3-carene, camphor, fenchone, mentone, linalool, beta-pinene, nerol, terpineol-4, thujone, pulegone, and sesquiterpenes such as elemol, beta eudesmol, beta elemene, (de Pascual-T et al., 1981). Some flavonoids including salvigenin, hispidulin, 7-methyleupatulin, 5-methyl salvigenin, and sinensetin have also been isolated from *C. botrys* (Morteza-Semnani, 2015). It's essential oils are reported to possess elemol, ledol, germacrene and other important compounds. These essential oils are reported to possess considerable antioxidant, acetylcholinesterase and butyrylcholinesterase inhibitory potentials (Ozer et al., 2017). Essential oils secluded from the aerial parts of *C. botrys* have been revealed noteworthy anti-fungal and anti-bacterial activities (Maksimović et al., 2005). Based on the relevant traditional uses of the plant and scientific exploration of its essential oils against cholinesterase's and free radicals, the current study has been carried out to investigate AChE and BChE inhibitory potentials of crude methanolic extract as well as various fractions of *C. botrys.*

Materials and methods

1. Plant collection and identification

The aerial parts of *C. botrys* were collected in July 2013 from district Dir (L), Khyber Pakhtunkhwa, Pakistan. The plant materials was confirmed via taxonomy professor, Dr. Jehandar Shah, Shaheed Benazir Bhutto University, Sheringal, Dir (U) KPK, Pakistan and plant sample was deposited with voucher No: CB-1036 at the same University herbarium for future reference (Ullah et al., 2017).

2. Extraction and fractionation

C. botrys crude powder (6.4 kg) was soaked in 22 L methanol (80%) for 12 to 14 days with vagarious shaking and then filtered via muslin cloth. The filtrates were concentrated at 40°C with rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany) till a greenish crude methanolic extract 445 g was obtained. A total of 400 g from this greenish crude methanolic extract (Cb. Cr) was displayed for fractionation via n-hexane (Cb. Hex), chloroform (Cb.Chf), ethyl acetate (Cb.EtAc) in

triplicate and finally aqueous fraction (Cb.Aq) was collected. The dissimilar fractions acquired were sealed and hoard at 20°C until required for anti-cholinesterase evaluation (Shah et al., 2014; Shah et al., 2015). Solvents were evaporated from various fractions and the effect of solvents used for UV analysis was nullified as the analysis were *in vitro* only.

3. Anticholinesterase assays

In this assay, the enzyme AChE and BChE from *Electric eel* and equine serum respectively were used to probe the enzyme inhibitory potential of *C. botrys* via Ellman's assay (Ellman et al., 1961; Khalil et al., 2018; Ovais et al., 2018a). The Plant samples were mixed with a small amount of methanol and then dissolved in 0.1 M phosphate buffer in various concentration (125-1000 µg/mL). The enzyme AChE (518 U/mg) and BChE (7-16 U/mg) were prepared in 0.1 M phosphate buffer having pH 8.0 until the last concentrations of AChE and BChE (0.03 U/mL, 0.01 U/mL) was attained. The other solutions of this assay like ATchI (0.5 mM), DTNB (0.2273 mM), and BTchI (0.5 mM) were equipped in distilled water and transfer to eppendorf tubes in refrigerator. In each assay, the 5 µL of enzyme was taken in the cuvette pursued by 205 µL plant samples and 5 µL DTNB as indicator. The solution mixture was preserved for 15 min at 30°C in a water bath. The substrate solution (5 µL) was further added for starting the reaction. This reaction was analyzed via double beam spectrophotometer at 412 nm. The absorption and reaction time was noted for 4 minutes in this assay while Galantamine was used as standard drug. Each experiment was performed three times. Percent enzyme potential and inhibition of enzyme via tested and control sample were deliberated from absorption rate with time change.

$$V = \Delta \text{Absorbance} / \Delta \text{time}$$

as: % Enzyme inhibition = 100 - % enzyme activity, while

$$\% \text{ Enzyme activity} = 100 \times V/V_{max}$$

Where

V_{max} is enzymatic potential in absence of inhibitor.

4. Kinetic parameter estimation

The Kinetic values were applied by altering data of Lineweaver-Burk plots (1/v versus 1/[*s*]) where v is apparent velocity reaction and [*s*] is the given concentration of substrate were schemed from assays via range of extract concentrations. The V_{max} and K_m values were indomitable via Michaelis Menten kinetics (Ovais et al., 2018a).

5. Statistical data evaluation

The plant and its various concentrations given 50 % inhibition (IC_{50}) were deliberated via excel graph of percentage inhibition opposed to the extract various concentration. Two-way ANOVA were applied, followed by Bonferroni multiple comparison tests for the assessment of standard drug galantamine and tested groups. The P values less than 0.05 were considered significant statistically. IC_{50} values was calculated using SPSS programme and mean ± SEM were determined at 95 % confidence interval (Ayaz et al., 2014).

Results and discussion

AD is an age related persistent neurological disorder which is frequently characterised by progressive loss of cognitive ability primarily memory impairment, cognitive dysfunction and behavioural disturbances which may lead to dementia (Ovais et al., 2018b). In AD patients, it is noted that reduction in the level of Ach shows major aspect in the progression of dementia (Ayaz et al., 2020b). The chief approach in the treatment of AD entails the continuation of the enough levels of Ach at the sites of neurotransmission (Ayaz et al., 2020a; Kamal et al., 2015). Thus, the reservation of AChE and BChE stop hydrolysis of the ACh, which in turn maintains normal memory function (Ahmad et al., 2016). From the literature, it is clear that various synthetic drugs and its analogues are causing toxicity and a lot of side effects (Ahmad et al., 2020a). That's why, there has been a renewed attention worldwide, for the search of strong AChE as well as BChE inhibitory compounds from the natural sources, mainly medicinal plants (Ahmad et al., 2020b; Nair & van Staden, 2012). Medicinal plants have long been employed for the management of symptoms related to cognitive memory dysfunction (Kim et al., 2014). Presently, numerous reports are available which identify the biological activities of natural products as AChE inhibitors *in vitro* and memory enhancers *in vivo* (Ayaz et al., 2014; Mantle et al., 2000).

Results of AChE and BChE inhibitory assays are shown in Table 1. In AChE inhibitory assay, Cb.Chf, Cb. EtAc and Cb.Cr showed the highest activity with 80.12±1.97, 71.79±0.67 and 69.00±1.52 % AChE inhibitions at 1 mg/mL concentration, respectively as compared to the standard galantamine. The IC_{50} values for the strongest activity fractions were 50, 115 and 130 µg/mL correspondingly (Fig. 1). All the remaining fractions displayed inhibitory potential in dose dependent

manner. Among the tested fractions of *C. botrys,* Cb.Chf, Cb. EtAc and Cb.Cr exhibited the excellent activity against BChE with 76.20±0.28, 70.48±0.19 and 62.75±1.79 % inhibitions correspondingly at 1,000 µg/mL concentration. The other fractions showed from good to moderate inhibitory activity. BChE inhibitory potential of various fractions be in order of Cb.Chf > Cb. EtAc > Cb.Cr > Cb.Aq > Cb.Hex. BChE inhibition of the standard drug galantamine was 94.21±1.01% at 1,000 µg/mL and the IC_{50} was less than 0.1 µg/mL.

Table 1 Percent Cholinesterase inhibition by different samples of *Chenopodium botrys*

Sample	Concentration (µg/mL)	% AChE inhibition	% BChE inhibition
Cb.Cr	1000	69.00 ± 1.52 ***	62.75 ± 1.79 ***
	500	60.66 ± 1.20 ***	54.91 ± 0.85 ***
	250	55.81 ± 0.74 ***	51.86 ± 0.46 ***
	125	50.16 ± 0.72 ***	45.95 ± 0.35 ***
Cb.Hex	1000	50.33 ± 2.96 ***	51.67 ± 0.17 ***
	500	43.18 ± 0.99 ***	47.72 ± 0.45 ***
	250	37.66 ± 0.66 ***	43.84 ± 0.19 ***
	125	30.00 ± 1.00 ***	39.08 ± 0.41 ***
Cb.Chf	1000	80.12 ± 1.97 ***	76.20 ± 0.28 ***
	500	73.73 ± 1.01 ***	71.82 ± 0.18 ***
	250	67.48 ± 0.28 ***	66.82 ± 0.53 ***
	125	59.64 ± 0.67 ***	62.11 ± 0.41 ***
Cb. EtAc	1000	71.79 ± 0.67 ***	70.48 ± 0.19 ***
	500	61.62 ± 1.04 ***	66.87 ± 0.82 ***
	250	55.87 ± 1.12 ***	61.55 ± 0.75 ***
	125	50.61 ± 1.37 ***	56.23 ± 1.17 ***
Cb.Aq	1000	62.29 ± 0.49 ***	58.40 ± 0.76 ***
	500	52.23 ± 1.27 ***	54.31 ± 0.83 ***
	250	47.28 ± 1.04 ***	51.17 ± 0.77 ***
	125	40.56 ± 1.52 ***	46.97 ± 1.51 ***
Galantamine	1000	94.21 ± 1.01	96.00 ± 0.30
	500	92.28 ± 0.43	92.90 ± 0.60
	250	85.35 ± 0.83	89.45 ± 0.90
	125	83.05 ± 1.03	86.23 ± 0.22

Remark: The data were analyzed as mean ± SEM of three experiments. Values were significantly varies as compared to the positive control, asterisk shows that *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

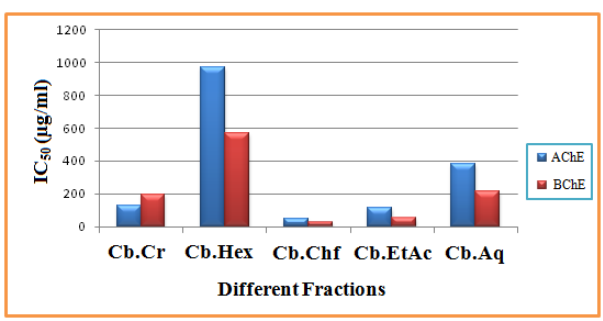


Fig. 1 Median inhibitory concentrations (IC_{50}) of different extracts of *C. botrys* against cholinesterase's enzymes

The extract and its fractions exhibited strong inhibitory potential against the acetylcholinesterase, and butyrylcholinesterase as bared from the V_{max} and K_m values which were indomitable via Michaelis-Menten kinetics and inveterate from Linewear-Burk plots for the particular enzymes (Fig. 2 and Fig. 3). For acetylcholinesterase inhibition, the V_{max} and K_m values were calculated as 70.08 µg/min and 55.21 µg/min for Cb-Cr, 54.38 µg/min and 107.6 µg/mL for Cb-Hex, 82.65 µg/min and 51.09 µg/mL for Cb-Chf, 72.83 µg/min and 63.05 µg/mL, for Cb-EtAt, 64.4 µg/min and 82.27 µg/mL for Cb-Aq. The positive control, galantamine displayed excellent inhibition of acetylcholinesterase comprises V_{max} and K_m values of 95.35 µg/min and 20.67 µg/mL, respectively. likewise, the V_{max} and K_m values for butyrylcholinesterase inhibition also exposed an excellent potential of Cb-Cr (63.51 µg/min and 51.82 µg/mL), Cb-Hex (53.13 µg/min and 47.71 µg/mL), Cb-Chf (77.37 µg/min and 33.13 µg/mL), Cb-EtAc (72.28 µg/min and 37.84 µg/mL), and Cb-Aq (59.18 µg/min and 34.67 µg/mL), correspondingly. An excellent inhibitory potential was observed for the positive control, galantamine (96.42 µg/min and 15.87 µg/mL).

Various plants extracts, essential oils and isolated compounds are reported to exhibited considerable antioxidant and enzyme inhibition properties. For instance, *Lawsonia inermis* extracts are reported to posses *in vivo* antioxidant potentials and offer neuroprotective properties in animal models (Mir et al., 2019). *Polygonum hydropiper* L. crude extracts, essential oils and isolated compounds were found active against cholinesterase's, free radicals using *in vitro* and *in vivo* analysis (Ayaz et al., 2020a; Ayaz et al., 2019b). Other medicinal plants including *Rumex hastatus* D. Don (Ahmad et al., 2015), *Nonea micrantha* Bioss. & Reut (Imran et al., 2017), *Iris germanica* var; florentina (Ullah et al., 2016), *Isodon rugosus* (Zeb et al., 2014) are reported to posses free radicals scavenging and enzymes implicated in Alzheimer's diseases. From the current study, it is accomplished that Cb.Cr and subsequent fractions of *C. botrys* possess good anticholinesterase inhibitory potential. Further isolation and characterization of the pure compounds from this plant is needed which is responsible for the anticholinesterase inhibition for their helpful consumption in the curing of Alzheimer's disease and other neurological diseases. Studies in this route are presently in progress in our laboratory.

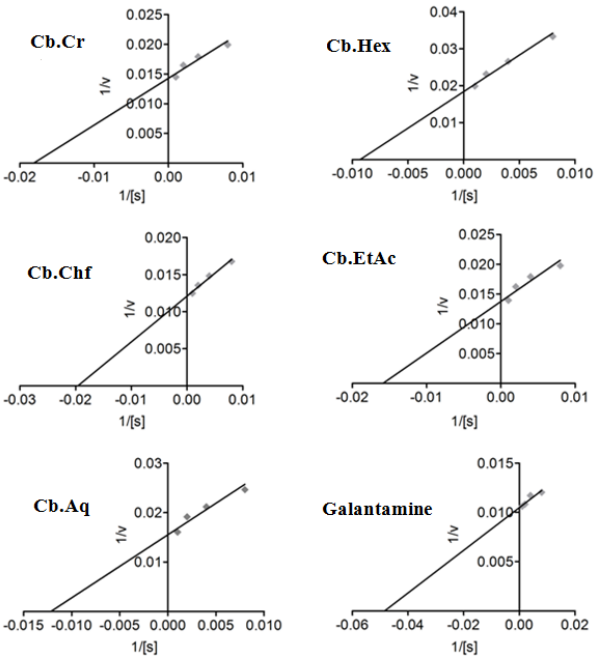


Fig. 2 Lineweaver-Burk plots showed the reciprocal of preliminary acetylcholinesterase velocity against the reciprocal of substrate concentration in existence of various concentrations of extract, its fractions and the standard drug galantamine

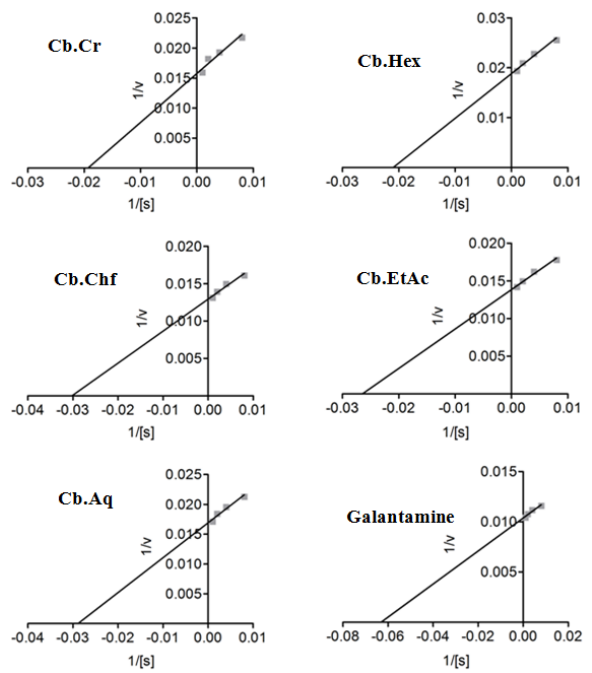


Fig. 3 Lineweaver-Burk plots showed the reciprocal of preliminary butyrylcholinesterase velocity against the reciprocal of substrate concentration in the existence of various concentrations of extract, its fractions and the standard drug galantamine

Conclusion

Results of the current study revealed that solvent extracts of *C. botrys* exhibit considerable cholinesterase inhibitory potentials. Particularly, Cb.Chf and Cb. EtAc were most potent and can be subjected to column chromatography for the isolation of pure compounds.

Acknowledgement

Authors want to thank the Department of Pharmacy, University of Malakand for the laboratory facilities to conduct the research.

References

Ahmad, S., Ullah, F., Ayaz, M., Sadiq, A., & Imran, M. (2015). Antioxidant and anticholinesterase investigations of *Rumex hastatus* D. Don: Potential effectiveness in oxidative stress and neurological disorders. *Biological research, 48*(1), 20.

Ahmad, S., Ullah, F., Sadiq, A., Ayaz, M., Imran, M., Ali, I., ... & Shah, M.R. (2016). Chemical composition, antioxidant and anticholinesterase potentials of essential oil of *Rumex hastatus* D. Don collected from the North West of Pakistan. *BMC Complementary and Alternative Medicine, 16*(1), 1.

Ahmad, A., Ullah, F., Sadiq, A., Ayaz, M., Jan, M.S., Shahid, M., ... & Sahibzada, M.U.K. (2020a). Comparative cholinesterase, α-Glucosidase inhibitory, antioxidant, molecular docking, and kinetic studies on potent succinimide derivatives. *Drug design, development and therapy, 14,* 2165.

Ahmad, S., Zeb, A., Ayaz, M., & Murkovic, M. (2020b). Characterization of phenolic compounds using UPLC–HRMS and HPLC–DAD and anti-cholinesterase and anti-oxidant activities of *Trifolium repens* L. leaves. *European Food Research and Technology, 246*(3), 485-496.

Ali, M., Muhammad, S., Shah, M.R., Khan, A., Rashid, U., Farooq, U., ... & Ahmad, M. (2017). Neurologically potent molecules from *Crataegus oxyacantha*; isolation, anticholinesterase inhibition, and molecular docking. *Frontiers in Pharmacology, 8,* 327.

Ayaz, M., Ahmad, I., Sadiq, A., Ullah, F., Ovais, M., Khalil, A.T., & Devkota, H.P. (2020a). *Persicaria hydropiper* (L.) Delarbre: A review on traditional uses, bioactive chemical constituents and pharmacological and toxicological activities. *Journal of ethnopharmacology, 251,* 112516.

Ayaz, M., Junaid, M., Ahmed, J., Ullah, F., Sadiq, A., Ahmad, S., & Imran, M. (2014). Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. *BMC complementary and alternative medicine, 14*(1), 145.

Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Ovais, M., Ahmad, W., & Zeb, A. (2016). Chemical profiling, antimicrobial and insecticidal evaluations of *Polygonum hydropiper* L. *BMC complementary and alternative medicine, 16*(1), 502.

Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Shahid, M., Ahmad, W., ... & Syed, N.I.H. (2017a). GC-MS analysis and gastroprotective evaluations of crude extracts, isolated saponins, and essential oil from *Polygonum hydropiper* L. *Frontiers in Chemistry, 5,* 58.

Ayaz, M., Junaid, M., Ullah, F., Subhan, F., Sadiq, A., Ali, G., ... & El-Shazly, M. (2017b). Anti-Alzheimer's studies on β-sitosterol isolated from *Polygonum hydropiper* L. *Frontiers in pharmacology, 8,* 697.

Ayaz, M., Ovais, M., Ahmad, I., Sadiq, A., Khalil, A. T., & Ullah, F. (2020b). Biosynthesized metal nanoparticles as potential Alzheimer's disease therapeutics. In *Metal Nanoparticles for Drug Delivery and Diagnostic Applications* (pp. 31-42). United Kingdom: Elsevier.

Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Ovais, M., Ullah, I., ... & Shahid, M. (2019a). Flavonoids as prospective neuroprotectants and their therapeutic propensity in aging associated neurological disorders. *Frontiers in Aging Neuroscience, 11,* 155.

Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Subhan, F., & Ahmed, J. (2017c). Neuroprotective and anti-aging potentials of essential oils from aromatic and medicinal plants. *Frontiers in aging neuroscience, 9,* 168.

Ayaz, M., Ullah, F., Sadiq, A., Kim, M.O., & Ali, T. (2019). Natural products-based drugs: Potential therapeutics against Alzheimer's disease and other neurological disorders. *Frontiers in pharmacology, 10,* 1417.

Baum, L., & Ng, A. (2004). Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. *Journal of Alzheimer's disease, 6*(4), 367-377.

Berkov, S., Georgieva, L., Kondakova, V., Atanassov, A., Viladomat, F., Bastida, J., & Codina, C. (2009). Plant sources of galanthamine: phytochemical and biotechnological aspects. *Biotechnology & Biotechnological Equipment, 23*(2), 1170-1176.

Buchbauer, G., Jirovetz, L., Wasicky, M., Walter, J., & Nikiforov, A. (1995). Headspace volatiles of C*henopodium botrys* (Chenopodiaceae). *Journal of Essential Oil Research, 7*(3), 305-308.

de Pascual-T,J., Gonzalez, M., Vicente, S., & Bellido, I. (1981). Flavonoids from *Chenopodium botrys. Planta medica, 41*(4), 389-391.

Ellman, G.L., Courtney, K.D., & Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology, 7*(2), 88-95.

Frautschy, S., Hu, W., Kim, P., Miller, S., Chu, T., Harris-White, M., & Cole, G. (2001). Phenolic anti-inflammatory antioxidant reversal of Aβ-induced cognitive deficits and neuropathology. *Neurobiology of aging, 22*(6), 993-1005.

Henry, W., Querfurth, H.W., & LaFerla, F.M. (2010). Mechanisms of disease Alzheimer's disease. *New Engl J. Med., 362,* 329-344

Imran, M., Ullah, F., Ayaz, M., Sadiq, A., Shah, M.R., Jan, M.S., & Ullah, F. (2017). Anticholinesterase and antioxidant potentials of *Nonea micrantha* Bioss. & Reut along with GC-MS analysis. *BMC complementary and alternative medicine, 17*(1), 499.

Kamal, Z., Ullah, F., Ayaz, M., Sadiq, A., Ahmad, S., Zeb, A., ... & Imran, M. (2015). Anticholinesterse and antioxidant investigations of crude extracts, subsequent fractions, saponins and flavonoids of *Atriplex laciniata* L.: Potential effectiveness in Alzheimer's and other neurological disorders. *Biological research, 48*(1), 21.

Khalil, A.T., Ayaz, M., Ovais, M., Wadood, A., Ali, M., Shinwari, Z.K., & Maaza, M. (2018). In vitro cholinesterase enzymes inhibitory potential and in silico molecular docking studies of biogenic metal oxides nanoparticles. *Inorganic and Nano-Metal Chemistry, 48*(9), 441-448.

Kim, M., Kim, S., & Yang, W. (2014). Mechanisms of action of phytochemicals from medicinal herbs in the treatment of Alzheimer's disease. *Planta Med, 80,* 1249–1258.

Lim, G.P., Chu, T., Yang, F., Beech, W., Frautschy, S.A., & Cole, G.M. (2001). The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *Journal of Neuroscience, 21*(21), 8370-8377.

Maksimovic, Z.A., Dordevic, S., & Mraovic, M. (2005). Antimicrobial activity of *Chenopodium botrys* essential oil. *Fitoterapia, 76*(1), 112-114.

Mantle, D., Pickering, A.T., & Perry, E.K. (2000). Medicinal plant extracts for the treatment of dementia. *CNS drugs, 13,* 201-213

Mir, N.T., Saleem, U., Anwar, F., Ahmad, B., Ullah, I., Hira, S., ... & Ayaz, M. (2019). *Lawsonia inermis* markedly improves cognitive functions in animal models and modulate oxidative stress markers in the brain. *Medicina, 55*(5), 192.

Morteza-Semnani, K. (2015). A Review on *Chenopodium botrys* L.: Traditional uses, chemical composition and biological activities. *Pharmaceutical and Biomedical Research, 1*(2), 1-9.

Nair, J.J., & van Staden, J. (2012). Acetylcholinesterase inhibition within the lycorine series of Amaryllidaceae alkaloids. *Natural product communications, 7*(7), 1934578X1200700741.

Ono, K., Hasegawa, K., Naiki, H., & Yamada, M. (2004). Curcumin has potent anti-amyloidogenic effects for Alzheimer's β-amyloid fibrils in vitro. *Journal of Neuroscience Research, 75*(6), 742-750.

Ovais, M., Ayaz, M., Khalil, A.T., Shah, S.A., Jan, M. S., Raza, A., ... & Shinwari, Z.K. (2018a). HPLC-DAD finger printing, antioxidant, cholinesterase, and α-glucosidase inhibitory potentials of a novel plant *Olax nana. BMC Complementary and Alternative Medicine, 18*(1), 1.

Ovais, M., Zia, N., Ahmad, I., Khalil, A. T., Raza, A., Ayaz, M., ... & Shinwari, Z.K. (2018b). Phyto-therapeutic and nanomedicinal approaches to cure Alzheimer's disease: Present status and future opportunities. *Frontiers in aging neuroscience, 10,* 284.

Ozer, M.S., Sarikurkcu, C., Ceylan, O., Akdeniz, I., & Tepe, B. (2017). A comprehensive study on chemical composition, antioxidant and enzyme inhibition activities of the essential oils of *Chenopodium botrys* collected from three different parts of Turkey. *Industrial Crops and Products, 107,* 326-331.

Rice-Evans, C.A., Miller, N.J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*(7), 933-956.

Russo, P., Frustaci, A., Del Bufalo, A., Fini, M., & Cesario, A. (2013). Multitarget drugs of plants origin acting on Alzheimer's disease. *Current medicinal chemistry, 20*(13), 1686-1693.

Sadiq, A., Mahmood, F., Ullah, F., Ayaz, M., Ahmad, S., Haq, F.U., ... & Jan, M.S. (2015). Synthesis, anticholinesterase and antioxidant potentials of ketoesters derivatives of succinimides: a possible role in the management of Alzheimer's. *Chemistry Central Journal, 9*(1), 31.

Shah, S.M.M., Sadiq, A., Shah, S.M.H., & Khan, S. (2014). Extraction of saponins and toxicological profile of *Teucrium stocksianum* Bioss extracts collected from district Swat, Pakistan. *Biological Research, 47*,(1), 1-5.

Shah, S. M., Ayaz, M., Khan, A.U., Ullah, F., Farhan, Shah, A.U.H.A., ... & Hussain, S. (2015). 1, 1-Diphenyl, 2-picrylhydrazyl free radical scavenging, bactericidal, fungicidal and leishmanicidal properties of *Teucrium stocksianum. Toxicology and Industrial Health, 31*(11), 1037-1043.

Ullah, F., Ayaz, M., Sadiq, A., Hussain, A., Ahmad, S., Imran, M., & Zeb, A. (2016). Phenolic, flavonoid contents, anticholinesterase and antioxidant evaluation of *Iris germanica* Var; florentina. *Natural Product Research, 30*(12), 1440-1444.

Ullah, F., Iqbal, N., Ayaz, M., Sadiq, A., Ullah, I., Ahmad, S., & Imran, M. (2017). DPPH, ABTS free radical scavenging, antibacterial and phytochemical evaluation of crude methanolic extract and subsequent fractions of *Chenopodium botrys* aerial parts. *Pakistan Journal of Pharmaceutical Sciences, 30*(3), 761-766.

Yang, F., Lim, G.P., Begum, A.N., Ubeda, O.J., Simmons, M.R., Ambegaokar, S.S., ... & Cole, G.M. (2005). Curcumin inhibits formation of amyloid β oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *Journal of Biological Chemistry, 280*(7), 5892-5901.

Yoo, K.Y., & Park, S.Y. (2012). Terpenoids as potential anti-Alzheimer's disease therapeutics. *Molecules, 17*(3), 3524-3538.

Zeb, A., Sadiq, A., Ullah, F., Ahmad, S., & Ayaz, M. (2014). Investigations of anticholinestrase and antioxidant potentials of methanolic extract, subsequent fractions, crude saponins and flavonoids isolated from *Isodon rugosus. Biological research, 47*(1), 1-10.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Rice Bran Oil Emulsion Organogels as Fat Baking for Brownies

Sakunta Manakla[a]*, Pattamaporn Jareonnon[a] & Supathra Lilitchan[b]

[a] *Department of Nutrition and Dietetics, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University, Pathum Thani 13180, Thailand*
[b] *Department of Nutrition, Faculty of Public Health, Mahidol University, Bangkok 10400, Thailand*

Article info

Article history:
Received : 1 November 2020
Revised : 25 December 2020
Accepted : 28 December 2020

Keywords:
Organogel, Rice bran oil, Brownies, Baking fat

Abstract

Rice bran oil emulsion organogels with different organogelators including triglyceride and policosanol are prepared and utilized as a bakery fat for making brownies. Texture profiles, percent of height gain, percent of weight loss, color, fatty acid composition, and sensory properties of the organogel brownies are investigated and compared with those of brownies made with rice bran oil and commercial margarine. The organogel brownies exhibit similar percent weight loss, percent height gain, hardness, gumminess and chewiness when compared to the commercial margarine brownies. Hardness, gumminess and chewiness in the organogel brownies are lower than rice bran oil brownie. The fatty acid compositions of both organogel brownies are similar to the rice bran oil brownie. Brownies prepared with the organogels exhibit considerable reduction in saturated fatty acid contents from 50.47% to 30.25% when compared with brownie prepared with commercial margarine. Policosanol and triglyceride organogels are potentially effective for producing nutritionally superior brownies with comparable quality attributes when compared to the margarine brownies.

Introduction

Solid fats containing high saturated fats have been extensively utilized in the bakery industry. Solid fats play an important roles in quality attributes of baked goods for improving palatability, air-incorporation, moisture barrier, shelf-life, and the tender texture and mouthfeel of the final product by preventing the cohesion of gluten strands (Cheong et al., 2011; Pyler & Gorton, 2008). However, consumption of oils rich in saturated fats could also cause health problems. Saturated fats are known to associate with increasing risk of heart disease. Reducing fat intake, particularly saturated and trans-fat, leaded to a dramatic decline in coronary heart disease mortality (Andy, 2004; Aranceta & Pérez-Rodrigo, 2012; Mozaffarian & Ludwig, 2015). More importantly, consumption of unsaturated fatty acids is highly recommended due to their health benefits (Micha & Mozaffarian, 2010; Rodriguez-Leyva et al., 2010).

In recent years, different attempts have been carried out to find alternative ways to produce solid fat with a low amount of saturated fatty acid (SFA). One of the effective techniques is using organogelation. Liquid oil can be entrapped in a thermo-reversible gel network

* Corresponding Author
e-mail: sakunta@vru.ac.th

with an assistance of various organogelators, resulting in organogels with semi-solid properties. These organogels did not alter the fatty acid composition of the entrapped liquid oil and no trans fats were generated (Marangoni, 2012; Stortz et al., 2012). The organogels are interesting products which are recently being used in structuring vegetable oils for emulsion-based products, margarine, and shortening-like products. Especially, the organogels have been utilized in substitution to solid fats in bakery products to reduce saturated fats. Various edible oils and organogelators have been evaluated for different bakery products. Cookies and biscuits prepared with organogels had similar quality parameters with those prepared with shortening (Devi & Khatkar, 2016; Hwang et al., 2016; Onacik-Gür & Żbikowska, 2019). Cakes and muffin produced with organogels had lower levels of saturated fatty acids without quality loss when they were replaced with shortening (Amoah et al., 2017; Pehlivanoglu et al., 2018; Willett & Akoh, 2019; Zhou et al., 2011). Baking fat affects dough structure and the desired final product attributes. Therefore, the replacement of conventional shortening or margarine poses tremendous challenge in the bakery production (Demirkesen & Mert, 2020).

In this study, rice bran oil emulsion with two organogelators, i.e. triglyceride and policosanol, are employed to produce solid-like organogels. Effects of the organogels on the quality attributes of the organogel brownies are then compared with commercial margarine and rice bran oil brownies in terms of texture profile, color, percent weight lost and height gain, fatty acid composition and sensory characteristics.

Materials and methods

1. Materials

Rice bran oil, triglyceride (Palsgaard 6111®, Palsgaard, Morris Plains, NJ, USA) policosanol, butter flavor, β-carotene, and citric acid were used to make the organogels margarines. Rice bran oil, commercial margarine, commercial all-purpose wheat flour, white sugar, sodium bicarbonate, sea salt, and cacao powder were purchased from a local grocery store.

2. Organogel preparation

Triglyceride organogel (organogel-1) and policosanol organogels (organogel-2) were prepared by the reported method with some modifications (Hwang et al., 2013). The water in oil emulsion organogels were prepared by dissolving 4.0% (w/w) organogelators in rice bran oil at 75°C. Water phase was prepared by mixing water with 1% (w/w) salt, 0.5% (w/w) butter flavor, 0.05% (w/w) β-carotene, and 0.03% (w/w) citric acid. The water phase was subsequently poured into the oil phase while homogenizing (T-25 basic Ultra Turrax®, Janke and Kunkel IKA, Germany) for 5 minutes. The water-in-oil emulsion was then cooled to 4°C for 1 hour. The formation of organogels was performed by placing the emulsion at −18°C for 4 hours. Once the gelation was completed, the samples were stored in a refrigerator at 4°C before baking the brownies.

3. Brownie preparation

The brownie samples were prepared by the reported method of Uruakpa & Fleischer (2016). Firstly, solid fats were melted using a double boiler method and cooled down to room temperature. Then, rice bran oil or melted fat, eggs, white sugar, and vanilla extract were mixed in a stainless-steel bowl. The mixture was then combined with the cooled chocolate mixture. All-purpose wheat flour, cacao powder, salt, and baking powder were then added into the mixture. The final mixture was placed in a baking tin (10 cm × 10 cm × 4 cm) and was oven-baked at 175°C for 15 minutes. After baking, the sample was removed from the baking tin and left to cool for 1 hour at room temperature. Cooled brownies were sealed in plastic wraps and placed in zip-lock plastic bag and stored at 4°C prior to further chemical analysis. Physical properties and sensory analysis were performed on cooled brownies. Four formulation of brownies presented in Table 1.

Table 1 Brownies formulation

Ingredients	Brownies formulation		
	Rice bran oil	Organogels	Conventional margarine
Sugar (g)	158.00	158.00	158.00
Salt (g)	1.78	1.78	1.78
Vanilla extract (g)	1.78	1.78	1.78
Eggs (g)	126	126	126
Wheat flour (g)	106.00	106.00	106.00
Cocoa powder (g)	43.00	43.00	43.00
Baking powder (g)	3.55	3.55	3.55
Rice bran oil (g)	64.00	0	0
Organogels (g)	0	64.00	0
Conventional margarine (g)	0	0	64.00

4. Texture profile analysis

Texture profiles of cooled brownies with dimensions of 3 cm × 3 cm × 3 cm were analyzed after 24 hours of baking with a texture analyzer (TA. XT2i Texture Analyzer, Texture Technologies Corp, Ltd. Hamilton, MA) under a two-cycle compression. A 75-mm

compression plate (P/75) probe was used. The strain 50 % at 10 mm with a force of 5.0 g were employed. Hardness, cohesiveness, springiness, chewiness, and gumminess of each brownie were averaged from 10 replicates.

5. Weight loss and height gain of brownies

Heights of the brownies were measured using a Vernier caliper (Model No. CD-800CSX, Mitutoyo Corp., Kawasaki, Japan). Height was measured from the highest point of the brownie to the bottom. Brownies were weighed and measured 5 times, that is before and after baking and cooling. The difference in weight and height was calculated as weight loss and height gain.

6. Color of brownies

Color of all the brownie samples were evaluated by a colorimeter (Chroma Meter CR-410, Konica Minolta, Inc., Osaka, Japan). The measuring head was placed at the center of each brownie. Color values were measured using CIE scale in 3 replicates and average values were reported as L* = lightness (0 = black, 100 = white), a* (-a*= greenness, +a* = redness) and b* (-b* = blueness, +b* = yellowness).

7. Fatty acid composition analysis

Fatty acid composition of all brownies sample was analysis using Gas chromatography technique in 3 replicates. Oil extracted from brownies with a mass of 10 mg were mixed with 3 mL of toluene in a screw-capped glass tube. Then, 1 mL of 5% (w/v) methanolic NaOH was added. The mixture was vortexed for 3 minutes and 1 mL of glacial acetic acid was added. The toluene phase was washed several times with distilled water and dried over anhydrous sodium sulfate before analysis with gas chromatography (Kaewkool et al., 2009).

Gas chromatography analysis were performed with a model 2010 gas chromatograph equipped with a flame ionization detector (Shimadzu, Tokyo, Japan), electronic pneumatic control, and a split/splitless injector. The detector time constant was set at 100 ms. A 1-µL sample was injected with a split ratio of 50:1 by an autoinjector. The injector switch was set at split throughout the analysis. Data acquisition and analysis were performed with a CBM 102 data processor. The injector and detector temperatures were set at 250°C. Helium was used as the carrier gas at a flow rate of 0.5–1.0 mL/min. The nitrogen makeup gas flow rate was 30 mL/min (Kaewkool et al., 2009).

8. Sensory evaluation

Hedonic test was utilized to determine the degree of overall preference for brownies. Fifty panelists were given with four random samples (2 cm × 2 cm × 2 cm) from the midsection of the brownies that had been held at room temperature for 24 hours. The brownies were evaluated for preference of appearance, color, texture, flavor and overall acceptability based on a nine-point hedonic scale (1 = extremely dislike, 9 = extremely like). All samples labeled with 3-digit random code numbers were randomly served to panelists. Samples were presented according to a Latin Square. The sensory test was performed in separate booths at room temperature. The mouth was rinsed with water to minimize any residual effects between each sample. Sensory evaluation was performed after the approval from the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand (Reference number: 31/2561).

9. Statistical analysis

The Completely Randomized Design or CRD was applied to create the different formulations of brownies for physicochemical properties study. The Randomized complete block design (RCBD) was applied for sensory evaluation. Three replications were performed for each sample. The mean values recorded for each test were compared using analysis of variance (ANOVA). Tukey's test was applied to detect the differences among the samples ($P < 0.05$).

Results and discussion

1. Texture profiles of brownies

The texture profiles of the brownies prepared with organogels, rice bran oil, and commercial margarine were compared as shown in Table 2. Hardness of the rice bran oil brownie (4681.72±12.31) was significantly higher than the organogel-1 brownie (2843.20±15.78), the organogel-2 brownie (2822.13±10.48), and commercial margarine brownie (2666.67±16.04). The highest hardness was observed in the rice bran oil brownies. The brownies prepared with organogels and margarine exhibited a softer texture. The reason for this observation could be attributed to the type of baking fat. Commonly, margarine plays an important role in the stabilization of air bubbles during creaming process. Air cells are incorporated into the fat phase during the mixing stage and then released into the aqueous phase when the shortening is melted during baking, giving rise to a foam structure or a homogenous crumb structure (Campbell & Mougeot, 1999). Utilization of rice bran oil led to less incorporation of air cells into the batters (Fig. 1). Furthermore, the decreasing hardness observed in margarine and both organogel brownies might be due to

Table 2 Texture profile of brownies

Formulation	Parameters				
	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness
Rice bran oil	4681.72±12.31[a]	0.57±0.01[a]	0.28±0.01[b]	1318.57±17.22[a]	525.61±26.69[a]
Organogel 1	2843.20±15.78[b]	0.59±0.05[a]	0.34±0.03[a]	805.79±35.37[c]	476.21±29.92[a]
Organogel 2	2822.13±10.48[b]	0.58±0.03[a]	0.34±0.01[a]	943.65±14.39[b]	552.13±13.23[a]
Conventional margarine	2666.67±16.04[c]	0.45±0.05[b]	0.28±0.02[b]	744.00±15.45[c]	332.76±26.40[b]

Remark: Different letters in the same column are significantly different (P <0.05)

the presence of emulsifiers playing a critical role in the stabilization of air bubbles during creaming process. Pyler & Gorton (2008) reported that baking fat contain emulsifier help in the dispersion of the fat in the batter system resulted bakery product have tender and softer textures as compared to rice bran oil. Visual observation of the internal texture revealed that brownies made of commercial margarine had well-distributed air cells as compared to other samples. Rice bran oil brownies contained less air cells that are not homogenously distributed (Fig. 1). This might be due to rice bran oil are non-emulsifiers baking fat dispersed upon mixing throughout the batter in the form of globules that are less effective in their shortening and aerating actions (Hartnett & Thalheimer, 1979).

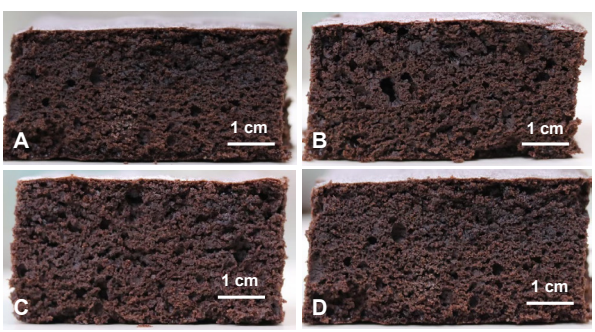


Fig. 1 Photographs of brownies prepared with rice bran oil (A), organogel with 4% of triglyceride (B), organogel with 4% of policosanol (C) and commercial margarine (D)

The springiness of organogel-1 brownies (0.59±0.05) organogel-2 brownies (0.58±0.03) and rice bran oil brownies (0.57±0.01) were significantly higher than those of commercial margarine brownies (0.45±0.05). The higher springiness values of the organogels compared to the margarine brownies due to increasing elasticity. The elasticity of the organogel brownies is reflected in the higher values of chewiness as a result of the higher level of protein crosslinking in the batter (Patel et al., 2014).

Cohesiveness relates to crumbliness or perceptions of denseness. Cohesiveness of organogels-1 brownies (0.34±0.03) and organogel-2 brownies (0.34±0.01) exhibited the highest values, followed by commercial margarine brownies (0.28±0.02) and rice bran oil brownies (0.28±0.01). There were significant differences in the cohesiveness of brownies with organogels and rice bran oil. This might be due to the compact and dense cell structure of the brownies leaded to chewy in texture of the organogels brownies.

Gumminess was calculated by hardness and cohesiveness value of food product, whereas chewiness, defined as the energy required to chew solid food to a state of readiness for swallowing (Karaoğlu & Kotancilar 2009). Chewiness and gumminess values in brownies were similar trend with the hardness values. The lowest gumminess and chewiness were observed in the commercial margarine brownie.

2. Weight loss and height gain of brownies

Percent weight loss and height gain of various brownies are shown in Table 3. Percent height gains for the brownies prepared with rice bran oil, organogel-1, and organogels-2 were 75.22, 75.81, and 76.38%, respectively. Brownie prepared with commercial margarine had the maximum height gain of 79.23. Percent height gain of brownies prepared with organogels and rice bran oil were lower than those of margarine brownies (P > 0.05). The reason for this observation could be attributed to the number of air bubbles (Patel et al., 2014). Commercial margarine had the most gained height of brownies due to during creaming process, dispersed gas could move freely to coalesce into bubbles and float rapidly to the surface and air that can be retained into the batter during the baking process (Matsakidou et al., 2010).

Table 3 Percent of height gain and weight loss of brownies after baking

Formulation	Weight loss	Height gain
Rice bran oil	5.87±0.01[a]	75.22±0.11[b]
Organogel 1	5.80±0.02[a]	75.81±0.11[b]
Organogel 2	5.52±0.03[a]	76.38±0.11[b]
Commercial margarine	3.73±0.01[b]	79.23±0.10[a]

Remark: Different letters in the same column are significantly different (P <0.05)

Percent weight loss for the brownies prepared with rice bran oil, organogel-1, and organogel-2 were 5.87, 5.80 and 5.52%, respectively. The lowest weight loss was observed in the commercial margarine brownies (3.73%). Brownies contained organogels and rice bran oil displayed a nonsignificant weight loss after baking ($P > 0.05$). The high weight loss during baking could be related to the fact that there was not a developed structure that would retain its shape and its components to any external change. Therefore, the lack of structural integrity in rice bran oil could allow easier water evaporation during baking, even if water were already hydrating the matrix (Rodríguez-García et al., 2013). Organogel brownies have performed inferior weight loss and height gain when compare to the commercial margarine brownies.

3. Color

Effects of different fats on the colors of baked brownies are presented in Table 4. The lightness values, i.e. L*, of the crumb and crust exhibited the lowest value when organogel-1 and commercial margarine were used, indicating a darker color. Brownies with rice bran oil and organogel-2 displayed the highest L* values. The rice bran oil and organogels brownies exhibited higher b* values. The crumb and crust color became yellowness for brownies prepared with organogels. The values of redness, i.e. a*, became lower for commercial margarine (crust), suggesting that the brownies contained organogels gave lower redness values. The result indicated that brownies showed significant differences in color measurement. However, dark brown colored brownies were visually seen as having very similar. This could be observed in the visual appearance of brownies in Fig. 1.

4. Fatty acid composition

The fatty acid compositions of brownies made with various baking fats are presented in Table 5. Commercial margarine brownie contained the highest amount of total SFA (50.47 %) and the lowest amounts of poly unsaturated fatty acid (PUFA) (9.51 %) compared to other brownies. Percentage of monounsaturated fatty acid (MUFA) was highest in margarine brownies (36.6 %) followed by rice bran oil (40–41 %) and organogels brownies (40–41 %). Organogels and rice bran oil brownies had similar amount of SFA (30–33 %) and had high content of unsaturated fatty acid (USFA). The major SFA in all of brownies sample were palmitic acid (C16:0) and stearic acid (C:18). The major MUFA and PUFA in the brownie samples was oleic (C18:1c9) and linoleic acid (C18:2), respectively. Utilization organogels for baking fat resulted in 27 % increase in USFA content of brownies. The total PUFA content in the organogels brownies increased by 64% when compared to the commercial margarine brownie MUFA s. Furthermore, the total SFA content in the organogels brownies decreased by 40% when compared to the commercial margarine brownies. Replacing the baking fat with organogels could decrease in SFA content, while increasing PUFA content, i.e. linoleic acid, in brownie products.

Table 5 Fatty acid composition of brownies

Fatty acid composition	Formulations			
	Rice bran oil	Organogel 1	Organogel 2	Commercial margarine
Lauric acid (C12:0)	1.45	0.47	0.46	0.79
Myristic acid (C14:0)	0.90	0.50	0.50	1.16
Palmitic acid (C16:0)	23.29	21.08	21.49	41.26
Stearic acid (C18:0)	4.85	6.85	5.90	6.87
Arachidic acid (C20:0)	0.76	1.15	0.90	0.39
Behenic acid (C22:0)	0.23	2.44	0.91	-
Lignoceric acid (C24:0)	0.33	0.34	0.34	-
Cerotic acid (C26:0)	-	-	-	-
Total SFA	**31.81**	**32.83**	**30.50**	**50.47**
Palmitoleic acid (C16:1)	0.33	0.39	0.40	0.37
Eicosenoic acid (C20:1)	0.45	0.42	0.45	-
Oleic acid (C18:1c9)	38.98	39.55	39.90	38.86
Oleic acid (C18:1c11)	0.80	0.17	0.92	0.80
Total MUFA	**40.56**	**40.53**	**41.67**	**40.03**
Linoleic acid (C18:2)	26.79	25.82	26.96	9.51
Linolenic acid (C18:3)	0.85	0.82	0.86	-
Total PUFA	**27.64**	**26.64**	**27.82**	**9.51**
Total USFA	**68.20**	**67.17**	**69.49**	**49.54**

Remark: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, poly unsaturated fatty acid; USFA, unsaturated fatty acid

Table 4 Color of brownies

Formulation	Lightness (L*)		Redness (a*)		Yellowness (b*)	
	Crumb	Crust	Crumb	Crust	Crumb	Crust
Rice bran oil	26.39 ± 0.58^{a}	19.58 ± 0.71^{a}	4.28 ± 0.36^{a}	3.78 ± 0.48^{a}	12.09 ± 0.42^{b}	14.88 ± 0.33^{ab}
Organogel 1	25.56 ± 0.60^{b}	18.58 ± 0.52^{b}	3.38 ± 0.60^{b}	2.63 ± 0.74^{b}	12.19 ± 0.60^{a}	15.03 ± 0.56^{a}
Organogel 2	26.31 ± 0.63^{ab}	18.11 ± 0.75^{b}	3.81 ± 0.64^{ab}	2.45 ± 0.45^{bc}	12.14 ± 0.72^{a}	14.62 ± 0.42^{b}
Commercial margarine	25.68 ± 0.97^{b}	18.07 ± 0.59^{b}	3.68 ± 0.92^{b}	2.22 ± 0.92^{c}	12.16 ± 0.67^{ab}	14.22 ± 0.45^{c}

Remark: Different letters in the same column are significantly different ($P < 0.05$)

High amount of SFA and low PUFA of were found in the margarine brownies because commercial margarine is prepared by palm oil. Rice bran oil and organogels brownies had similarly fatty acid composition because organogelation did not alter their fatty acid composition (Stortz et al., 2012). Brownies prepared with organogels instead of commercial bakery fats potentially showed the nutritional superiority to the margarine brownie. The organogels and rice bran oil brownies had higher levels of unsaturated fatty acids compared to the commercial margarine. Therefore, they could be utilized as healthier alternatives for bakery fats. Moreover, organogel-2 was prepared with policosanol, long chain alcohols extracted from rice bran wax. It is used as a dietary supplement for lowering blood cholesterols (Weerawatanakorn et al., 2019). Therefore, policosanol organogel brownie not only had lower SFA but also presence healthy functional compound (4% by weight).

5. Sensory characteristics

Sensory properties of brownies including appearance, odor, texture, taste and the overall acceptability are important factors determining consumer acceptability. The preference mean sensory scores of various brownies are shown in Table 6. No significant differences were observed in the preference of the evaluated appearance and odor in all the brownie samples. Preference texture scores of rice bran oil, organogel-1, organogel-2 and commercial margarine brownies were 6.72±0.99, 7.08±0.90, 7.36±0.22, and 7.72±0.33, respectively. The brownies using organogel-2 showed high preferences in texture followed by commercial margarine and organogel-1 brownie. Rice bran oil brownies had the lowest scores ($P<0.05$). Mean scores of texture preference for organogel-1, organogel-2 and commercial margarine brownies were insignificantly different. In the taste evaluation, there was insignificant differences between organogel-2 and commercial margarine brownies. Mean score of organogel-1 and rice bran oil brownies had significantly lower than other sample for taste preference. Rice bran oil, organogel-1, organogel-2 and commercial margarine brownies had overall acceptability scores of 6.04±0.61, 7.80±0.62, 7.26±0.87, and 7.14±0.28, respectively. No significant differences were observed between organogel-1 and organogel-2 brownies. These results indicated that both of organogel brownies and commercial margarine brownies were comparable in overall acceptability characteristic.

Conclusion

Rice bran oil was structured with policosanol and triglyceride to form organogels with semi-solid properties. The organogels were utilized for brownie preparation. Organogel brownies with performed similarly to the commercial margarine brownies in terms of texture, while providing the good sensory properties. Reduction in the SFA content of the baked product by completely replacing commercial margarine with organogels were the most effective in producing nutritionally superior brownies with comparable quality attributes to the margarine brownies.

Acknowledgment

This research was supported by Agricultural Research Development Agency (Public Organization) Thailand (Project code CRP6205030580).

References

Andy, N. (2004). Diet, nutrition and the prevention of chronic diseases. WHO technical report series 916. Report of a joint WHO/FSA expert consultation. *International Journal of Epidemiology, 3*3(4), 914–915.

Amoah, C., Lim, J., Jeong, S., & Lee, S. (2017). Assessing the effectiveness of wax-based sunflower oil oleogels in cakes as a shortening replacer. *LWT-Food Sci Technol, 86,* 430-437.

Aranceta, J. & Pérez-Rodrigo, C. (2012). Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: A systematic review. *BJN, 107*(S2), S8-S22.

Campbell, G.M., & Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends Food Sci Technol, 10*(9), 283-296.

Table 6 Sensory characteristic of brownies

Brownies formulation	Sensory characteristic				
	Appearances	Odor	Texture	Taste	Overall acceptability
Rice bran oil	7.92±0.81[a]	8.60±0.62[a]	6.72±0.99[b]	6.64±0.77[c]	6.04±0.61[b]
Organogel 1	7.76±0.46[a]	8.64±0.80[a]	7.08±0.90[ab]	7.30±0.69[b]	7.80±0.62[a]
Organogel 2	7.64±0.77[a]	8.78±0.98[a]	7.36±0.22[a]	7.94±0.79[a]	7.26±0.87[a]
Commercial margarine	7.30±0.97[a]	8.82±0.08a[a]	7.72±0.33[a]	7.96±0.84[a]	7.14±0.28[a]

Remark: Different letters in the same column are significantly different ($P<0.05$)

Cheong, L.Z., Tan, C.P., Long, K., Idris, N.A., Yusoff, M.S.A., & Lai, O.M. (2011). Baking performance of palm diacylglycerol bakery fats and sensory evaluation of baked products. *Eur J Lipid Sci Technol., 113*(2), 253-261.

Demirkesen, I., & Mert, B. (2020). Recent developments of oleogel utilizations in bakery products. *Critical reviews in food science and nutrition, 60*(14), 2460-2479.

Devi, A., & Khatkar, B. (2016). Physicochemical, rheological and functional properties of fats and oils in relation to cookie quality: A review. *JFST., 53*(10), 3633-3641.

Hartnett, D.I., & Thalheimer, W.G. (1979). Use of oil in baked products-Part II: Sweet goods and cakes. *Journal of the American Oil Chemists' Society, 56*(12), 948-952.

Hwang, H.S., Singh, M., Bakota, E.L., Winkler-Moser, J.K., Kim, S., & Liu, S.X. (2013). Margarine from organogels of plant wax and soybean oil. *Journal of the American Oil Chemists' Society, 90*(11), 1705-1712.

Hwang, H.S., Singh, M., & Lee, S. (2016). Properties of cookies made with natural wax–vegetable oil organogels. *J Food Sci., 81*(5), C1045-C1054.

Kaewkool, P., Kittiratanapiboon, K., Aryusuk, K., & Krisnangkura, K. (2009). Micro-reactor for transesterification of plant seed oils. *Eur J Lipid Sci Technol., 111*(5), 474-480.

Karaoğlu, M.M., & Kotancilar, H.G. (2009). Quality and textural behaviour of par-baked and rebaked cake during prolonged storage. *Int J Food Sci Tech., 44*(1), 93-99.

Marangoni, A.G. (2012). Organogels: An alternative edible oil-structuring method. *Journal of the American Oil Chemists' Society, 89*(5), 749-780.

Matsakidou, A., Blekas, G., & Paraskevopoulou, A. (2010). Aroma and physical characteristics of cakes prepared by replacing margarine with extra virgin olive oil. *LWT-Food Sci Technol., 43*(6), 949-957.

Micha, R., & Mozaffarian, D. (2010). Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: A fresh look at the evidence. *Lipids, 45*(10), 893-905.

Mozaffarian, D., & Ludwig, D.S. (2015). The 2015 US dietary guidelines: lifting the ban on total dietary fat. *Jama, 313*(24), 2421-2422.

Onacik-Gür, S., & Żbikowska, A. (2019). Effect of high-oleic rapeseed oil oleogels on the quality of short-dough biscuits and fat migration. *Journal of Food Science and Technology, 57*(10), 1-10.

Patel, A.R., Rajarethinem, P.S., Grędowska, A., Turhan, O., Lesaffer, A., De Vos, W.H., & Dewettinck, K. (2014). Edible applications of shellac oleogels: Spreads, chocolate paste and cakes. *Food & function, 5*(4), 645-652.

Pehlivanoglu, H., Ozulku, G., Yildirim, R.M., Demirci, M., Toker, O.S., & Sagdic, O. (2018). Investigating the usage of unsaturated fatty acid-rich and low-calorie oleogels as a shortening mimetics in cake. *J Food Process Pres, 42*(6), e13621.

Pyler, E.J., & Gorton, L.A. (2008). Baking science & technology: (Volume I): Fundamentals & ingredients. Kansas, USA.: Sosland Publishing.

Rodriguez-Leyva, D., Bassett, C.M., McCullough, R., & Pierce, G.N. (2010). The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. *CJC, 26*(9), 489-496.

Rodríguez-García, J., Laguna, L., Puig, A., Salvador, A., & Hernando, I. (2013). Effect of fat replacement by inulin on textural and structural properties of short dough biscuits. *Food Bioprocess Tech., 6*(10), 2739-2750.

Stortz, T.A., Zetzl, A.K., Barbut, S., Cattaruzza, A., & Marangoni, A.G. (2012). Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technol., 24*(7), 151-154.

Uruakpa, F.O., & Fleischer, A.M. (2016). Sensory and nutritional attributes of black bean brownies. *AJFSN, 3*(3), 27-36.

Weerawatanakorn, M., Meerod, K., Wongwaiwech, D., & Ho, C.T. (2019). Policosanols: Chemistry, occurrence, and health effects. *Current Pharmacology Reports, 5*(3), 131-149.

Willett, S.A., & Akoh, C.C. (2019). Physicochemical characterization of yellow cake prepared with structured lipid oleogels. *Journal of Food Science, 84*(6), 1390-1399.

Zhou, J., Faubion, J.M., & Walker, C.E. (2011). Evaluation of different types of fats for use in high-ratio layer cakes. *LWT-Food Sci Technol., 44*(8), 1802-1808.

JFHB Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science

Journal homepage : http://jfhb.dusit.ac.th/

Aphrodisiac Food Ingredients from the Twelve Thai Ancient Formulary Books of Police Captain Bhiam Bunyachot

Vadhana Jayathavaj[a]* & Ubol Chuensumran[b]

[a] *Thai Traditional Medicine Program, Pathumthani University, Pathumthani 10200, Thailand*
[b] *Department of Food Processing Technology, School of Culinary Arts, Suan Dusit University, Bangkok 10700, Thailand*

Article info

Article history:
Received : 20 June 2020
Revised : 9 November 2020
Accepted : 13 November 2020

Keywords:
Aphrodisiac, Food, Ingredient, Bunyachot

Abstract

An aphrodisiac is defined as any food or drug that arouses the sexual instinct, induces venereal desire and increases pleasure and performance. There are many commercial advertisements related to herbal supplements that claim to have an aphrodisiac property. But the claims almost always come from the long-standing belief or traditional wisdom (Chinese or Ayurveda) with no scientific support. To support "food is medicine", the aphrodisiac herbs from Thai ancient drugs were explored from private formulary books. The properties of aphrodisiac formulas in the formulary texts were classified into four groups as follows: 1) anti-aging, 2) physical strength and power, 3) healthy sex, and 4) relief from erectile dysfunction. The herbs that appears in Thai foods in everyday life with claimed medication results from the formulary textbooks were identified. They were 49 food ingredients from 143 herbs presented in 62 aphrodisiac formulas. The herbs with herbal aphrodisiac groups (1 and 2 and 3 and 4) were ranked by the number of times they appeared thusly; Pepper, Galingale (Finger root), Ginger, Long pepper, and Nutmeg. The paper correlates the herbal foods traditionally used aphrodisiacs with recent scientific validation for the management of sexual health.

Introduction

Sexual health, physical health, mental health, and overall well-being are all positively associated with sexual satisfaction, sexual self-esteem, and sexual pleasure (Anderson, 2013). Sexual well-being is presented as part of "Subjective Well-Being (SWB)", SWB concerns peoples' self-reported assessment of their own well-being (Hicks, 2011; ONS, 2010). The nationally representative panel survey of community-dwelling men and women aged 50 years and older in England (Steptoe et al., 2013) of which the 7,079 (67%) completing and returning the paper-based Sexual Relationships and Activities Questionnaire (SRA-Q), men and women who reported either infrequent/no sexual activity, or were sexually active but reported sexual problems, generally had lower SWB than those individuals identified in Class 1 (Class 1 consists of those with frequent levels of sexual desire, intercourse, and other partnered sexual activity, a low frequency of

* Corresponding Author
e-mail: vadhana.j@ptu.ac.th

masturbation, and no problems with erectile function or reaching orgasm). Poorer SWB in men was more strongly associated with sexual function difficulties, whereas in women desire and frequency of partnered activities appeared more important in relation to SWB (Lee et al., 2016).

An aphrodisiac is defined as any food or drug that arouses the sexual instinct, induces venereal desire and increases pleasure and performance. To overcome sexual dysfunction, the seeking of the aphrodisiac substances from plants, animals or minerals since time immemorial to serve the passion of man (Yakubu et al., 2005). A lot of natural substances have historically been known as aphrodisiacs in Africa and Europe, like yohimbine and the mandrake plant, as well as ground rhinoceros horn in the Chinese culture and "Spanish fly" which is actually toxic (Ang et al., 1997; Evans, 1969). Even in today's culture, there are certain foods that are used as aphrodisiacs, including strawberries and raw oysters. Chocolate, coffee, and honey are also believed to have aphrodisiac potential. Although these natural items are claimed as aphrodisiacs, there is no or little scientific confirmation supporting those assertions (Kotta et al., 2013).

Thai Traditional Medicine Theory (TTM), starting from King Narai the Great's Medicinal Textbook (1917), the core theory is the equilibrium of the four elements through body elements, seasons of the year, age, time period in a day, and habitat; the tastes of drugs and the 82 drug formulas were presented without how to formulate them. Until Ratanakosin Kingdom, the Bhatsart Songkroh Medicinal Book has the scriptures specific to the disease symptoms with the drug formulas to solve those symptoms, also without how to formulate them (Thai Language Institute, 2000). The properties of each part of the herb was also first established in King RAMA II period by His Royal Highness Prince Wongsa Dhiraj Snid (1808-1871 AD), without any biochemical laboratory, but from his experience and investigations (Auttakaweewajok, 1919; Suntravej, 1932). The periodic table was first established by the Russian chemist Mendeleev in 1869 (Royal Society of Chemistry, 2020). In ancient Thai medicine, they are many official medicinal texts which are not directly mentioned about improving sexual health. The private Thai herbal formulary collections are more straightforward in that they claim the formula's property in improving sexual activities.

Police Captain Bhiam Bunyachot (Pol. Capt. Bhiam) was born in Nakhon Si Thammarat, studied astrology since he was a child. He was also known Thai drugs from his home that selling herbs starting from 10 years old. He had an experienced in healing his own hemorrhoids, two eye blindness, and prong feet sweating until normal. At 14 years old, he experienced conjunctivitis and went blind, the Muslim tradition from Narathiwat suggested the medicine should consist of one handful of the gourd leaf ("Bai Tam Lung" in Thai), one handful of the orange banyan treetop ("Tsai Som" in Thai), pound together filling a banana leaf bowl (krathong) exposed to the dew for one night, wrapped with white cloth and squeezed with water added if there was not enough liquid, and mixed with the python bile in the amount of 3 heads of matches, and used as eye drops 1-5 times a day. Within 3 weeks his eyes were reported to be normal again (Bunyachot, 1972). After he was ordained he moved to Bangkok. He operated a drug store in 1937 but was forced out of business because of his political activities. From borrowing and copying many herbal drug formulas, he decided to publish them between 1971 to 1982 (Bunyachot, 1982), they were 12 books with total 4,682 pages (The number of pages per book are average 390.16, minimum 312, and maximum 455). Pol. Capt. Bhiam's formulary texts have the highest number of formulas among the formula collections.

In these 12 books, there are many Thai ancient herbal drug formulas, their medicinal properties cover a broad range of diseases in Thai life, including aphrodisiac activities for anti-aging, more strength, more powerful sex, and recovery from erectile dysfunction. They are many "Iron flagpole" formulas that claimed penile erection activity (stand up like iron flagpole). Flagpole is implied the male sex organ. The word "Iron flagpole" ("Soa Thong Lhek" in Thai) came from the story that many people asked Pol.Capt. Bhiam which items they should offer to make merit and they will always have a powerful sex organ. By that time, Siamrath Newspaper answered that it should be "Iron flagpole" (Bunyachot, 1971b).

To support "food is medicine", the aphrodisiac herbs presented in Thai ancient drugs of which recorded in the formulary books were explored. Each formula was analyzed with the aphrodisiac criteria and the herbs in that formula were classified by their claimed medical results. The herbs that appears in Thai foods in everyday life with claimed medication results from the formulary textbooks were identified.

Data sources

The twelve Thai ancient formulary books of Pol. Capt. Bhiam during 1971 to 1982 were the sources of input data (Bunyachot, 1971a, 1971b, n.d.a, n.d.b, 1975, n.d.c, n.d.d, 1979, n.d.e, n.d.f, 1982). The herbal drug formula was included in data processing when the formula mentioned about their properties, claims, and stories related to the 4 aphrodisiac properties; Group 1: anti-aging; become younger-looking, glowing skin, good looking, and more youthful appearance, Group 2: more strength, more power, Group 3: good sex, power and performance, and Group 4: relief from erectile dysfunction. Apart from Group 1, the rest 3 groups are the sexual health formula for men were included. An aphrodisiac drug formula composed of one or many herbs, each herb presented in the formula should contribute their aphrodisiac properties to the formula.

In the original official Thai traditional medical texts, the texts has the formulas attach to the disease symptoms, this means that the herbs in that formula have to transfer some properties to the formula claimed properties. The first book of herbal properties had only 166 herbal items, the textbook was written by His Royal Highness Krom Luang Wongsa Dhiraj Snid, and presented in National Library in 1915 (B.E. 2458) (Division of protection and promotion of Thai traditional medicine and indigenous medicine, 2016). There are 1,390 herbs in the 3 Thai drugs compilation books (The school of ancient medicine Wat Pra Chetuphon, 1994; 1978a;1978b), and the tailoring the drug formula from the properties of each herb to match the symptom had a broad guideline (Bureau of Sanatorium and Art of Healing, 1998). In search of the properties of each aphrodisiac herb, this study identified the herb properties from the property of the formulas that the herb was in.

Data Processing

To perform data processing in deriving the properties of the herb from the formula property of which that herb was in, the data records were organized in two formats; the format of drug formula consisted of the formula identification code, book number, page number, formula name, the number of herbs in a formula and formula properties (Group 1 or Group 2 or Group 3 or group 4), and the format of herb presented in each formula consisted of the formula identification code, herb name, and the property of the formula that the herb was in. The spreadsheet program was used to process both the drug formula data, and the herb data that belong to each formula, the frequency count, percentage, and basic statistics were used to describe the findings.

Remark: The classification of both drug formulas and herbs was dependent on the data shown in the aphrodisiac delineations from the twelve Thai ancient formulary books that were recorded based on knowledge or texts passed down over generations by Pol. Capt. Bhiam only.

Foods Ingredients in the aphrodisiac drug formulas

The 62 aphrodisiac drug formulas were founded, and classified into four drug formula aphrodisiac group; Group 1 (Anti-aging), Group 2 (More strength), Group 3 (Healthy sex), and Group 4 (Relief from erectile dysfunction).

Review of the twelve books showed that the aphrodisiac formulas appeared in only seven books, the formulas classified by the book number and the number of herbs in each formula was shown in Table 1. The number of herbs in the formula ranged from 1 to 17, average 6.63, with standard deviation 4.00.

Table 1 The aphrodisiac formula classified by book number and number of herbs in formula

Unit in number of drug formulas

Book No.	Number of herbs in each formula (No duplicated formulas)														
	1	2	3	4	5	6	7	8	9	10	11	12	16	17	Total
1						1						1			2
2	1	3	5	4	3	2	1	1	3	1	1	1	2	1	29
3	1	2	2	1		3	2	1						1	13
5			1				1		1						3
8			1			1		1	1		1				5
10		1			3	1		2	1	1					9
12				1											1
Total	**2**	**6**	**9**	**5**	**4**	**9**	**6**	**3**	**7**	**2**	**3**	**1**	**3**	**2**	**62**

There was no criteria or standard related to number of herbs in one formula; for example, the Yahom Intajak has 58 herbs. To make the table compact, the formula group size by the number of herbs was a small 1-5, a medium 6-10, and a large 11-17 (excepted 13-15). The herbs in the small group may have efficacy straight to the formula claimed properties, while the group of herbs is bigger, each herb in the bigger group made less contribution to the properties of the formula or may be added to another support properties. The number of formulas classified by the drug formula aphrodisiac Group 1 (Anti-aging) and Group 2 (More strength) had 6-10 herbs at 50 % each group, Group 3 (Healthy sex) had 1-5, 6-10, and 11-17 herbs at 31 %, 38 %, and 31 %, respectively, and Group 4 (Relief from erectile dysfunction) had 1-5 herbs at 67 %, as shown in Table 2.

Table 2 The aphrodisiac formula classified by number of herbs in formula and their properties

Unit in number of drug formulas

Number of herbs in the formula	Drug Formula Aphrodisiac Groups				
	Group 1 (Anti-aging)	**Group 2** (More strength)	**Group 3** (Healthy sex)	**Group 4** (Relief from erectile dysfunction)	**Total**
1 - 5	11	2	5	8	26
6 - 10	14	3	6	4	27
11 - 17	3	1	5	0	9
Total	28	6	16	12	62
Percent	45%	10%	26%	19%	100%
1 - 5	39%	33%	31%	67%	42%
6 - 10	50%	50%	38%	33%	44%
11 - 17	11%	17%	31%	0%	15%
Total	**100%**	**100%**	**100%**	**100%**	**100%**

The 143 herbs were presented in 62 aphrodisiac formulas, and the number of herbs classified by the combination of the drug formula aphrodisiac group (the herbal aphrodisiac group code) was shown in Table 3. The 49 of 143 plants from the formulary texts are in our everyday Thai food culture. Eight herbs had properties that cover the four aphrodisiac groups (Group 1 and Group 2 and Group 3 and Group 4 or Group Code 1234) and the top 5 of them by the number of formulas they appeared, were spicy food ingredients; they were Pepper, Galingale (Finger root), Ginger, Long pepper, and Nutmeg, as shown in Table 4.

The plant data base websites from of Plant Genetic Conservation Project Office (n.d.), the Botanical Garden Organization (2011) and Faculty of Pharmaceutical Sciences, Ubon Ratchathani University (2010) were studied to find the scientific name of the 49 plants in Table 4.

Table 3 The herbs in aphrodisiac drug formulas classified by the herbal aphrodisiac group code and the number of herbs use as food

The herbal aphrodisiac group code	Description	Total number of herbs [a]	Percent	The number of herbs use as food [b]	Percent [b/a]
1	Anti-aging	35	24%	14	40%
2	More strength	1	1%		
3	Healthy sex	28	20%	13	46%
4	Relive from erectile dysfunction	19	13%	4	21%
12	1 and 2	3	2%	2	67%
13	1 and 3	13	9%	5	38%
14	1 and 4	1	1%		
23	2 and 3	5	3%	1	20%
34	3 and 4	13	9%	1	8%
123	1 and 2 and 3	12	8%	2	17%
124	1 and 2 and 4	1	1%		
134	1 and 3 and 4	2	1%	2	100%
234	2 and 3 and 4	2	1%		
1234	1 and 2 and 3 and 4	8	6%	5	63%
	Total	**143**	**100%**	**49**	**34%**

The academic articles on the top five herbs from Pol. Cap. Bhiam's formulary books are as follows; (1) Pepper or Black pepper (*Piper nigrum* L.) have testosterone 5α-reductase inhibitory effects due to its capability to catalyze the conversion of testosterone (Hirata et al., 2007). The fruit extract of black pepper potentially affects sexual drive in male mice, observing 4 groups of male mice that were given pellets with and without black pepper extract. Male mice fed on aqueous as well as ethanol extract of black pepper significantly showed a shorter courtship latency ($p<0.05$) (Sutyarso & Rosa, 2015); (2) Galingale or Fingerroot (*Boesenbergia rotunda* L.) is a daily food ingredient and traditional medicinal plant in Southeast Asia and Indo-China. It has been shown to possess anti-allergic, antibacterial, anticancer, anti-inflammatory, antioxidant, antiulcer activities and wound healing. Its phytochemical components include alkaloids, essential oils, flavonoids, and phenolics, and also rich in boesenbergin, krachaizin, panduratin, and pinostrobin, all of which has been reported its remedial properties including aphrodisiac property (Ongwisespaiboon & Jiraungkoorskul, 2017); (3) Ginger (*Zingiber officinale* Rosc. red clone) has the oleoresin compound that reported to function as an aphrodisiac. It is traditionally used to solve problems related to sexual dysfunction. (Anandita et al., 2012); (4) Long pepper or Pippali (*Piper longum* L.) is a rejuvenating herb with a warming, stimulating and kapha reducing action in Indian Ayurveda. Its oily nature prevents it from drying making it suitable for vata and its pleasant post digestive effect makes it more calming to pitta than other hot spices and herbs. It aids blood flow to the reproductive tissues when taken in combination with Ashwagandha (Chauhan et al., 2014; Dass, 2007). Pippali is one portion among 8 herbs in the Phala Ghrita (PG) which reported to be useful in improving fertility. Virechana (therapeutic purgation) is largely indicated for cases having a semen abnormality (shukra dushti). Administration of PG after performing virechana provided statistically highly significant improvement on various seminal parameters related to male infertility including oligozoospermia (Varsakiya et al., 2019); and (5) Nutmeg (*Myristica fragrans* Houtt.) has been mentioned in Unani medicine to be of value in the management of male sexual disorders. The extracts (50 % ethanolic) of nutmeg and clove enhanced the sexual behavior of male mice (Tajuddin et al., 2003).

Even the herbs with aphrodisiac properties are in the everyday food table, but the more consuming with

Table 4 The aphrodisiac food ingredients and the drug formula aphrodisiac group

Unit in number of drug formulas

The herbal aphrodisiac group code	Common name	Scientific name	Drug formula aphrodisiac group				
			Group 1	Group 2	Group 3	Group 4	Total
1234	Pepper	*Piper nigrum* L.	19	4	12	2	37
	Galingale (Finger root)	*Boesenbergia rotunda* (L.) Mansf.	12	1	11	1	25
	Ginger	*Zingiber officinale* Roscoe	7	2	5	1	15
	Long pepper	*Piper retrofractum* Vahl	7	1	2	2	12
	Nutmeg	*Myristica fragrans* Houtt.	4	1	4	1	10
134	Salt	Sodium chloride	3		2	1	6
	Wild betal, Leaf bush	*Piper sarmentosum* Roxb.	3		2	1	6
123	Caraway seed	*Cuminum cyminum* L.	1	1	1		3
	Blood leaf	*Iresine herbstii* Hook.	1	1	1		3
34	Bottle gourd	*Lagenaria siceraria* (Molina.) Standl.			1	1	2
23	Hen's egg *(not a plant)*	*Gallus domesticus*		1	1		2
13	Garlic	*Allium sativum* L.	4		1		5
	Shampoo ginger, Wild ginger	*Zingiber zerumbet* (L.) Smith.	3		2		5
	Cardamom	*Wurfbainia testacea* (Ridl.) Skornick. & A. D. Poulsen	2		1		3
	Honey	*Apis mellifera* L.	1		2		3
	Cultivated banana	*Musa sapientum* L.	1		1		2
12	Leech lime	*Citrus hystrix* DC.	2	1			3
	Cobra (snake)	*Naja Kaouthia*	1	1			2
4	Lemongrass	*Cymbopogon citratus* Stapf.				1	1
	Ma khuea chae khruea	*Securidaca inappendiculata* Hassk.				1	1
	Coconut	*Cocos nucifera* L. var. nucifera				1	1
	Rattan	*Calamus caesius* Blume				1	1
3	Bael fruit, riped	*Aegle marmelos* (L.) Correa ex Roxb.			2		2
	Cow milk	*Bos primigenius*			2		2
	Ginseng	*Panax ginseng* C.A.Mey.			1		1
	Korean Ginseng	*Panax ginseng* C.A.Mey.			1		1
	Water Chestnut	*Trapa bicornis* Osbeck.			1		1
	Paddy Rice	*Oryza sativa* L. var *indica*			1		1
	Red cotton tree	*Bombax ceiba* L.			1		1
	Winged Bean	*Psophocarpus tetragonolobus* (L.) DC.			1		1
	Water lily	*Nymphaea nouchali* Burm.f.			1		1
	Lotus	*Nymphaea lotus* L. var. *pubescens* Hook.f. & Th.			1		1
	Tamarind	*Tamarindus indica* L.			1		1
	Cinnamon	*Cinnamomum* spp.			1		1
	Butter *(not a plant)*	made from cow's milk			1		1
1	Cock roach berry (Dutch egg plant or India night shade)	*Solanum aculeatissimum* Jacq.	3				3
	Solanum trilobatum L.	*Solanum trilobatum* L.	3				3
	Bush tomato (Engl.) Indian night shade (Engl.), Poison berry (Engl.)	*Solanum indicum* L.	3				3
	Indian Mulberry	*Morinda citrifolia* L.	3				3
	Clove	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	2				2
	Licorice	*Glycyrrhiza glabra* L.	2				2
	Galangal	*Alpinia galanga* (L.) Willd.	1				1
	Black glutinous rice	*Oryza sativa* var. *glutinosa*	1				1
	Safflower	*Carthamus tinctorius* L.	1				1
	Gotu kola	*Centella asiatica* Urban.	1				1
	Fang	*Caesalpinia sappan* L.	1				1
	Indian Gooseberr	*Phyllanthus emblica* L.	1				1
	Lime	*Citrus aurantifolia* (Christm.) Swingle.	1				1
	Bitter orange	*Citrus medica* L.	1				1

more expectations will face over dose or the food and drug interactions (FDI) for the person who take medication regularly. FDI can significantly affect the outcome of patients' health. Certain foods and specific nutrients in foods, may affect the overall bioavailability, pharmacokinetics, pharmacodynamics and therapeutic efficacy of medications. FDI occurs due to extension of drug action or due to interaction between the drug and herbal medicines (HDI) as well as dietary supplements and food products, some HDI also showed their potential to make the drugs ineffective, others posed a dangerous risk of toxicity (Benni et al., 2012; Ladd, 2018). The physicians and pharmacists prescribe drugs cautiously with only suitable food supplement to get maximum benefit for the patients with minimum interactions between different foods and drugs (Bushra et al., 2011). The safety and effectiveness of the ancient aphrodisiac herbal drug formula would be prescribed by the registered Thai traditional medical professions.

Conclusion

From the twelve Thai ancient formulary books of Pol. Capt. Bhiam during 1971 to 1982, all aphrodisiac properties were selected including (1) anti-aging, become younger, glowing skin and youthful looks, (2) more strength, more power, (3) good sex power and performances and (4) relief from erectile dysfunction. The 49 food ingredients out of 143 herbs from 62 aphrodisiac formulas were food in our everyday life, the 8 herbs had properties that covered all the drug formula aphrodisiac groups (Group 1 and Group 2 and Group 3 and Group 4 or Herbal Aphrodisiac Group Code 1234) and the top 5 of them were spicy food ingredients; they were Pepper, Galingale (Finger root), Ginger, Long pepper and Nutmeg. However, there are many commercial advertisements related to the herbal supplements with aphrodisiac properties by long-standing beliefs or traditional wisdom (Chinese or Ayurveda) with no scientific support. To have more confidence in sexual health activity of the herbs from Pol. Capt. Bhiam's formulary texts, self- observation after consumed will be appropriate to find the right one.

Acknowledgment

Pol.Capt. Bhiam Bunyachot, who published his collecting efforts - the twelve books of ancient medicine formulas. At this present time, the formulary texts out of the store of "Kasembunnakij", but some of them are available in the old books for sales in social networks.

References

Anandita, D.W., Nurlaila, N., & Pramono, S. (2012). Aphrodisiac effects of red ginger (*Zingiber officinale* Rosc. red clone) essential oil and essential oil of free etanolic extract in male rats. *Majalah Obat Tradisional, 17*(1), 8 – 14.

Anderson, R.M. (2013). Positive sexuality and its impact on overall well-being. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz, 56*(2), 208–214.

Auttakaweewajok, P. (1919). *Textbook of medicinal properties of His Royal Highness Prince Wongsa Dhiraj Snid.* Phra Nakhǭn: Rongphim Sophonphiphatthanakon.

Ang, H.H., Chan, K.L., Gan, E.K., & Yuen, K.H. (1997). Enhancement of sexual motivation in sexually naive male mice by *Eurycoma longifolia* jack. *Int J Pharmacogn, 35,* 144–146.

Benni, J.M., Jayanthi, M.K., Tubaki, B.R., & Renuka, M. (2012). Knowledge and awareness of food and drug interactions (FDI): A survey among health care professionals. *Int J Pharmacol and Clin Sci., 2012*(1), 97-105.

Bunyachot, Bh. (1971a). *Ancient Thai formulary text about the diseases of children and lady* Book 1. Bangkok: Kasembunnakij.

Bunyachot, Bh. (1971b). *Ancient Thai formulary text about various diseases* Book 2. Bangkok: Kasembunnakij.

Bunyachot, Bh. (n.d.a). *Ancient Thai formulary text about various diseases* Book 3. Bangkok: Kasembunnakij.

Bunyachot, Bh. (1972). *Ancient Thai formulary text about various diseases* Book 4. Bangkok: Kasembunnakij.

Bunyachot, Bh. (n.d.b). *Ancient Thai formulary text* Book 5. Bangkok: Kasembunnakij.

Bunyachot, Bh. (1975). *Ancient Thai formulary text* Book 6. Bangkok: Kasembunnakij.

Bunyachot, Bh. (n.d.c). *Ancient Thai formulary text about various diseases* Book 7. Bangkok: Kasembunnakij.

Bunyachot, Bh. (n.d.d). *Ancient Thai formulary text* Book 8. Bangkok: Bangkok: Kasembunnakij.

Bunyachot, Bh. (1979). *Ancient Thai formulary text about various diseases* Book 9. Bangkok: Kasembunnakij.

Bunyachot, Bh. (n.d.e). *Ancient medicine powder formulary text* Book 10. Bangkok: Kasembunnakij.

Bunyachot, Bh. (n.d.f). *Ancient Thai formulary text* Book 11. Bangkok: Kasembunnakij.

Bunyachot, Bh. (1982). *Ancient Thai formulary text* Book 12. Bangkok: Kasembunnakij.

Bureau of Sanatorium and Art of Healing. (1998). *The general Thai ancient medicine: Pharmacy Branch.* Bangkok: The cooperative of Thailand printing.

Bushra, R., Aslam, N., & Khan, A.Y. (2011). Food-drug interactions. *Oman medical journal, 26*(2),77–83.

Chauhan, N.S., Sharma, V., Dixit, V.K., & Thakur, M. (2014). A review on plants used for improvement of sexual performance and virility. *Biomed Res Int., 2014,* 868062.

Dass, V. (2007). *Ayurvedic herbs for male reproductive problems.* Retrieved October 8, 2020, from http://www.bluelotusayurveda.com/oldsite/male-herbs-art.html.

Division of protection and promotion of Thai traditional medicine and indigenous medicine. (2016). *Textbook of medicinal properties*. Suan Sunadha Rajabhat University: Kaewchaochom Media Center and Printings.

Evans, W.O. (1969). Chemical aphrodisiacs. *Psychopharmacol Bull, 5*(2), 10–17.

Faculty of Pharmaceutical Sciences, Ubon Ratchathani University. (2010). *Thaicrudedrug.com.* Retrieved from September 21, 2020, http://www.thaicrudedrug.com/main.php

Hicks, S. (2011). *The measurement of Subjective Well-being: paper for the measuring national well-being Technical Advisory Group.* Retrieved October 8, 2020, from www.ons.gov.uk/well-being/the-measurement-of-subjective-well-being.pdf

Hirata, N., Tokunaga, M., Naruto, S., Iinuma, M., & Matsuda, H. (2007). Testosterone 5α-reductase inhibitory active constituents of *Piper nigrum* leaf. *Biological and Pharmaceutical Bulletin, 30*(12), 2402-2405.

King Narai the Great's Medicinal Textbook. (1917). *King Narai the Great's Medicinal Textbook.* Bangkok: Rongphim Sophonphiphatthanakon.

Kotta, S., Ansari, S.H., & Ali, J. (2013). Exploring scientifically proven herbal aphrodisiacs. *Pharmacogn Rev., 7*(13), 1–10.

Ladd, J. (2018). Review uncovers dangerous herb–drug interactions. *Pharmacy Today, 24*(4), 32-33.

Lee, D. M., Vanhoutte, B., Nazroo, J., & Pendleton, N. (2016). Sexual health and positive subjective well-being in partnered older men and women. *Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 71*(4), 698-710.

Ongwisespaiboon, O., & Jiraungkoorskul, W. (2017). Fingerroot, boesenbergia rotunda and its aphrodisiac activity. *Pharmacognosy reviews, 11*(21), 27–30.

ONS. (2010). Spotlight on Subjective well-being. Retrieved September 21, 2020, from www.statistics.gov.uk/articles/social_trends/ spotlight -on-subjective-well-being.pdf

Plant Genetic Conservation Project Office. (n.d.). *Plants Data. Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highest Princess Maha Chakri Sirindhorn.* Retrieved September 21, 2020, from http://www.rspg.or.th/plants_data/herbs/

Royal Society of Chemistry. (2020). *Periodic table: History: Development of the periodic table.* Retrieved October 8, 2020, from https://www.rsc.org/periodic-table/history/about

Steptoe, A., Breeze, E., Banks, J., & Nazroo, J. (2013). Cohort profile: The English Longitudinal Study of Ageing. *International Journal of Epidemiology, 42,* 1640–1648.

Suntravej, S. (Vice Chamberlain Prince Phatayapongsa). (1922). *Textbook of medicinal properties of His Royal Highness Prince Wongsa Dhiraj Snid.* Phra Nakhon: Rongphim Sophonphiphatthanakon.

Suntravej, S. (Vice Chamberlain Prince Phatayapongsa). (1932). *phæt tambon lem nưng rūangkhlō̜t but tō̜n thī sām tamrā sapphakhun yā Thai [District Doctor Book 1 Childbirth Subject, Section 3 Thai Drugs Properties Textbook].* Phra Nakhō̜n: Rōngphim Augsorn Niti.

Sutyarso, K.M., & Rosa, E. (2015). Effects of black pepper (*Piper nigrum* Linn.) extract on sexual drive in male mice. *Research Journal of Medicinal Plant, 9,* 42-47.

Tajuddin, S.A., Latif, A., & Qasmi, I.A. (2003). Aphrodisiac activity of 50% ethanolic extracts of *Myristica fragrans* Houtt. (Nutmeg) and *Syzygium aromaticum* (L.) Merr. & Perry. (clove) in male mice: A comparative study. *BMC Complementary and Alternative Medicine, 3,* 6.

Thai Language Institute. (2000). *Medical Assistant: Thai Medical Wisdom and National Literature Heritage.* Bangkok: The Teachers' Council of Thailand Printing Ladphrao.

The Botanical Garden Organization. (2011). *BGO Plant Databases.* Retrieved September 21, 2020, from http://www.qsbg.org/Database/Botanic Book%20full%20option/search page.asp

The school of ancient medicine Wat Pra Chetuphon. (1994). *The Thai drugs compilation (part one) related to herbs, materials, and animals.* Bangkok: Borpit Karnpimp.

The school of ancient medicine Wat Pra Chetuphon. (1978a). The Thai drugs compilation (part two) related to herbs, materials, and animals. Bangkok: Num Aksorn Karnpimp.

The school of ancient medicine Wat Pra Chetuphon. (1978b). *The Thai drugs compilation (part three) related to herbs, materials, and animals.* Bangkok: Num Aksorn Karnpimp.

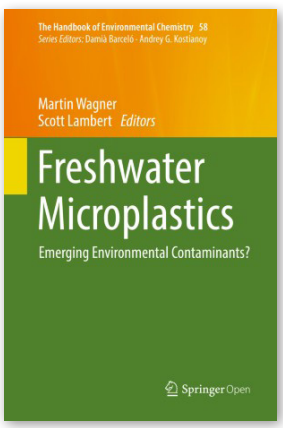
Varsakiya, J., Goyal, M., Thakar, A., Donga, S., & Kathad, D. (2019). Clinical study to evaluate role of Ayurvedic Virechana (therapeutic purgation) and oral Phala Ghrita intake in the management of oligozoospermia. *Annals of Ayurvedic Medicine, 8,* 114-25.

Yakubu, M.T., Akanji, M.A., & Oladiji, A.T. (2005). Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. *Asian J Androl, 7,* 399-404.

Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Book Review

Penkhae Thamsenanupap



Book Name:	The Handbook of Environmental Chemistry Volume 58
Book Series Title:	Freshwater Microplastics, Emerging Environmental Contaminants?
Volume Editors:	Wagner, M., & Lambert, S.
Published:	Springer Nature, 2018
Paper Back:	303 pages
Language:	English
ISBN:	978-3-319-61614-8 /
ISBN:	978-3-319-61615-5 (eBook)
DOI:	10.1007/978-3-319-61615-5

Currently, more than 5,300 grades of synthetic polymers have been produced for our convenience and have unfortunately become the source of emerging environmental pollutants. Small fragments of plastic debris are called microplastics and the knowledge of its contamination in freshwater ecosystems was very scarce in contrast to the inordinate amount of plastic being discarded. This book gave a brief overview on what plastics were, their origin, and where they end up in the environment. The authors also present interesting conflicts regarding microplastic and nanoparticle science and make an effort to learn from past experiences. The gaps in knowledge and further research topics including major advances and challenges are proposed in an interesting manner. However, many issues such as the effects on freshwater biota and ecosystem were not scrutinized due to its sensitive nature. This book also contains case studies from Europe, Asia, and Africa. The last part of the book focuses on the interaction of microplastics and the society including sociological perspectives on the risk perception, media and politics associated with microplastics. Finally, the authors conclude the book with an outlook providing solutions to the environmental plastic enigma. This book is recommended for students, environmentalists, academics and researchers who want to increase their knowledge and aid their search for "need of the hour" research topics. The book can also benefit the general public to gain understanding of current environmental situations.

Reviewer

Penkhae Thamsenanupap
Faculty of Environment and Resource Studies, Mahasarakham University, Khamriang Sub-District, Kantarawichai District, Maha Sarakham, 44150, Thailand
e-mail: penkhae.t@msu.ac.th

Guidelines for Writing and Submitting Original Manuscripts for Publication in Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science is an academic publication that aims to publish manuscripts such as original articles, review article, and book review in the fields of food, health, biological and environmental disciplines and other related fields.

The journal is published thrice annually. All manuscripts accepted for publication are copyrighted by Suan Dusit University; reproduction, in whole or in part, requires written approval from Suan Dusit Unversity. Excluding errors incurred during the printing process, all content contained within articles is the author's responsibility.

Publication Process

1. The journal accepts original manuscripts for consideration, from January to December.
2. The editorial board adjourns to consider the merits or submitted manuscripts and the scope of the journal. During this phase the integrity and accuracy of the manuscripts content is assessed.
3. An editorial letter is issued to the author for manuscripts that the editorial board deems inappropriate for publication. If the editorial board approves the manuscripts, an editorial letter will be sent to the author and the article will be subjected to peer review.
4. Articles that are deemed appropriate for publication are subjected to peer review by a panel of three experts in the appropriate field. In order to be deemed appropriate for publication, an article must be by recommended two of the three experts via the double-blinded review system.
5. The qualitative assessments of the expert panel returned by the manuscript's author. The author is expected to make the appropriate alterations indicated by the experts' feedback.
6. The author returns the edited document; the editorial staff examines the changes to make sure they are congruent with the experts' recommendations as well as the journal format.
7. The revised version is granted the University's recognition of "Accepted" for publication status with the Journal of Food Health and Bioenvironmental Science Stamp on every page. Information regarding publication status (Accepted) is located on the journal's website (http/ research dusit ac.th/new/e-Journal)
8. The editorial tearm conducts an accuracy check for all articles before sending the manuscripts to the printer to create a draft journal issue.
9. The editorial board conducts a review of the draft journal issue before publication on the journal's website (http://research.dusit.ac.th/new/e-Journal). Suan Dusit University will place their official seal of approval on each page of the manuscript and to verify before formal publication.
10. Upon approval by each author, the final version of the journal will be published as a physical journal and online publication, accessible on website (http://research.dusit.ac.th/new/e-Journal). Together with sending a physical journal to peer reviews, authors and involved sectors.

Publication Criteria

1. The original manuscript is concise and interesting to the academic community.
2. The content of the manuscript represents quality and theory of the discipline and also possesses knowledge with practical applications.
3. The manuscript's content is consistent with the aim and scope of the journal.

4. Manuscripts submitted to Journal of Food Health and Bioenvironmental Science must not have been published previously in or actively involved in the publication process of another journal.

5. All content within the manuscript must be the product of the author himself. Any use of intellectual property within must be appropriately credited to its original authors.

6. The author must comply with the writing style established by Journal of Food Health and Bioenvironmental Science.

7. There are four levels of assessments given to reviewed manuscripts:

7.1 Requires minor or no revisions prior to publication.

7.2 Requires moderate revisions prior to publication.

7.3 Requires intensive editing and revisions followed by a future evaluation. 7.4 Unsuitable for publication

In order to be assigned the "Accepted" status, an article must be assessed as “Requires minor or no modification prior to publication” by two of the three experts from the peer review process.

Formatting Guidelines

It is the author's responsibility to format manuscripts to the standards of Journal of Food Health and Bioenvironmental Science. The details of format style are contained herein,

1. Format

1.1 Single page printing on A4 paper with a width of 19 cm and height of 26.5 cm. The vertical and horizontal spacing from the margins must be 3.5 cm and 2.5 cm, respectively.

1.2 Typefaces and layout: English must be typed using Time New Roman using Microsoft word. Specific font format guidelines are as follows.

1.2.1 The header contains the page number, aligned on the right side, in 12 pt. font.

1.2.2 The title in English languages must be 12 pt. font, bolded, and center aligned. The title should not exceed two lines of text.

1.2.3 The author's name in English language must be typed 9.5 pt. font and centered below the title. Asterisks (*) should proceed the authors' names which is correspond to the appropriate author.

1.2.4 Affiliations should match each author with their appropriate affiliated institutions and organizations. In case of different affiliations, superscript numbers should follow the surname a and affiliation a.

1.2.5 A footnote must be placed on the first page of the article with the text “*Corresponding Author”, and the next line of text should contain “e-mail”.

1.2.6 “Abstract” in English must be 9.5 pt. font, bolded, left aligned, and placed below the Thai keywords section. Abstract text must be 9 pt. font, with 1 tab indentation from left and right margins.

1.2.7 “Keywords:” should appear in English language in 9.5 pt. font, placed beneath the English abstract text and be aligned with the left margin. English keywords must be 9 pt. font, and should not exceed four words. Each keyword should be separated by a comma (,) and space.

1.2.8 Regardless of language choice, the main text headings used throughout the paper must be 9.5 pt. font, bolded, and aligned with the left margin.

1.2.9 Bulleted items must appear as 9 pt. font, bolded, and be indented 1.5 tabs from the left margin.

1.2.10 Body text must appear as 9 pt. normal font, and be indented 1 tab from the left and right margins.

1.2.11 “References” must be 9.5 pt. font, bolded, and be aligned with the left margin. Individual entries must be 9 pt. font and should follow American Psychological Association (APA) formatting guidelines. Any lines of text for a single entry that exceed the first line should use a “hanging indent” of 1.5 tabs from the left margin.

1.3 An appropriate page length for publication in the Journal is approximately 15 pages.

2. Citing

Should follow American Psychological Association (APA) formatting guidelines. Click http://jfhb.dusit.ac.th/flie/Ref%20Guidelines. pdf to see the example.

3. Ordering of Titles in Journal of Food Health and Bioenvironmental Science

The written manuscript may contain only English. The content should be easy to understand and clear. If the author uses abbreviation, full word must appear before any abbreviation.

3.1 The title should be brief, the length should not exceed 100 characters.

3.2 The authors if there are more than six authors only the first author is listed, followed by "et al."

3.3 Affiliated entities associated with the author should appear in English languages.

3.4 The abstract must be written in English language. The abstract should briefly summarize the research and not exceed 250 words or 15 lines of text.

3.5 The "Keywords" section must contain no more than four keywords that allow for appropriate searching and selection based upon the article's topic.

3.6 The "Introduction" section should provide background information relevant to the research, provide information regarding the manuscript's content and state the objectives of the work.

3.7 The "Materials and methods" section delineates the procedures, how the research was conducted, sampling method (i.e. simple random samples) and population, and the creation and development of research tools used for data collection and analysis.

3.8 The "Results" section or "Results and Discussion" presents data obtained during the research and may be displayed as tables, graphs, illustrations, and accompanying explanations. Tables should be not have left and right borders and are normally black and white printed. No more than five tables should be present in the "Results" section. Pictures within the section should be clear and use simple black and white coloring with an accompanying caption, the author wishes to use colors for any item they may do so; however, the author will be responsible for the additional costs of color printing.

3.9 The "Discussion" section or "Result and Discussion" should explore the significance of the results of the work and address whether or not the data support the research hypothesis and compare research findings to other similar research works.

3.10 The "Conclusions" section should summary of the main topic covered or a re – statement of the research problem.

3.11 The "Acknowledgements" (if any) section should provide help during the research (e.g., providing materials, laboratory, equipment, etc.) and funding.

Sending Original manuscript

1. Compose the manuscript using the format of the Journal of Food Health and Bioenvironmental Science.

2. Send the manuscript via ScholarOne website https://mc03.manuscriptcentral.com/jfhb

Journal of Food Health and Bioenvironmental Science Publication Ethics

Editorial Regulations

- It is the duty of the Editors to consider the submitted manuscripts related to field of food, health, biological and environmental disciplines and other related fields. The consideration will be based solely on the content. The ethnicity, country of origin, gender, sexual orientation, political affiliation, or religious belief of authors does not influence the editor's decision.
- Throughout the submission, the editors must not share the information about the submissions to anyone except the authors, reviewers and JFHB staffs.
- Editors must make sure the manuscript has no substantial vested interests authors or affiliated organizations.
- The editorial staff have to assure that the manuscript has been peer-reviewed by at least two reviewers in the field of Food, Health, biological and environmental disciplines or other related field appropriate for each manuscript. The editorial staffs also have to be careful about the copyright Infringement, falsification of data, and plagiarisms. If there is an offense according to the said regulations, the editor must investigate and seek for evidence before consider reject the manuscript.
- In case that the Editors suspect that the manuscript might have been published elsewhere, the editor should investigate and consider reject.
- In case of unethical publishing practices that are later uncovered, the action will still be taken seriously.

Reviewer Regulations

- Reviewers are expect to give constructive and professional comments. Improper criticism must be avoided.
- If the manuscript given is lies beyond area of expertise, the reviewers should inform the staff immediately.
- Reviewers must keep the manuscript confidential. Do not share any information of the manuscript to anyone other than the editorial staff.
- In case that the reviewers find that the other works contained in the manuscript are not well credited, reviewers are required to inform the editorial staff.
- If there are conflicts of interests, reviewers should inform the editorial staff. Editors will decide whether the reviewer is appropriate for the manuscript or not.

Author Regulations

- The authors should write the manuscript related to the theme of Food, Health, biological and environmental disciplines. The research manuscript should contained relevant background information, efficient methodology, APA style citation, accurate results, and reasonable discussion.
- The authors should follow the journal guidelines strictly.
- Any opinion or perspective made in the manuscript must be explicitly highlighted as "opinion" or "perspective"
- The authors must be careful and aware that fraudulent information and omission of important information are unethical author behaviors.
- The authors must be able to provide research data if the Editor see needed.
- Authors must reference other works properly. Any work involved in the manuscript also must be well credited.
- The authors must make sure that the manuscript has not been published elsewhere before and is not currently in the publication process in other journals.

- The person must have made significant contributions to the manuscript, participate and give important efficient content during revisions and provide approval for publication in order to be listed as an author. Researchers who do not meet the above criteria should be listed in the Acknowledgements section.
- Author should identify any conflicts of interest that might have influenced the data and/or interpretations of data.
- To make the efficient revision, the authors should respond to all the given critiques and suggestions during the revision.
- If the authors find errors in their works that need to be correct, the author should inform the editors immediately.

Journal of Food Health and Bioenvironmental Science

Vol. 13 No. 3 September – December 2020

Original Articles

The Study of Citrus's Fibers Production for Power Bar and the Effect on Consumer's Acceptance 1
Nathamon Somrajlichinda

Free Radical Scavenging Activity of Riceberry and Pathum Thani Rice Extracts for Developing Face Powder 9
Watchara Senajuk, Sasamol Phasuk, Poonyanuch Nilsang & Pannraphat Takolpuckdee

Effects of Components on Qualities of Crispy Bamboo Shoot Snack 16
Narin Charoenphun & Kanokporn Pakeechai

The Improvement of Hydroponics Growth Media by Using the Corncob Biochar 26
Pannipha Dokmaingam, Sitang Kongkratoke, Amornrat Anuwatnonthakate & Sarayut Yongprapat

Life Cycle and Larval Feeding Habits of *Macrostemum indistinctum* Banks 1911 (Trichoptera: Hydropsychidae) in the Stream Flows into Krasiow Reservoir, Thailand 32
Orawan Khamrak & Taeng-On Prommi

Cholinesterase's Enzymes Inhibition and Michaelis-Menten Kinetics Studies on Ethnomedicinally Important Plant *Chenopodium botrys* 40
Naveed Iqbal, Farhat Ullah, Abdul Sadiq, Muhammad Saeed Jan, Muhammad Shahid & Muhammad Ayaz

Rice Bran Oil Emulsion Organogels as Fat Baking for Brownies 47
Sakunta Manakla, Pattamaporn Jareonnon & Supathra Lilitchan

Review article

Aphrodisiac Food Ingredients from the Twelve Thai Ancient Formulary Books of Police Captain Bhiam Bunyachot 54
Vadhana Jayathavaj & Ubol Chuensumran

Book Review

The Handbook of Environmental Chemistry Volume 58 61
Author Wagner, M., & Lambert, S.
Penkhae Thamsenanupap

RDI
Research and Development Institute
Tel: 0-2244-5280-2 Fax: 0-2668-7460
Website: http://research.dusit.ac.th