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Anthocyanin Content, Physicochemical, Nutritional and Sensory Properties of Purple Sweet Potato and Riceberry Biscuits with Rice Bran Organogel

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Abstract

Consumers' awareness on healthful and functional foods has been increasing worldwide. This research aimed to evaluate the anthocyanin content, physicochemical, nutritional, and sensory properties of biscuits prepared from purple sweet potato and riceberry flours. The biscuits were evaluated for their physicochemical properties including color, water activity, moisture contents, and texture profiles. The results showed that the lightness values (L*) ranged from 21.05 to 21.52 with red (a* = 4.97−5.23) and yellow (b* = 0.74−0.84) pigments. The biscuits had the moisture content ranging from 21.72 to 23.70% and the water activity (a_w) was within the range that most microorganisms cannot survive (a_w = 0.845 to 0.883). Increasing the purple sweet potato flour content in the biscuit formulation resulted in decreased hardness, cohesiveness, springiness, gumminess, and chewiness. All biscuit formulations were analyzed for the nutritional values using the nutritional analysis program. The energies of biscuits ranged from 306.55 to 309.48 kcal. Therefore, the recommended packaging for biscuits was two servings per container. Anthocyanin contents were evaluated using the pH-differential method. The result showed that the formulation with the highest anthocyanin content was formulation P5 (90 purple sweet potato flour: 10 riceberry flour, 66.50±0.99 mg/100g of biscuit). Moreover, formulation P5 also received the highest score in the consumer sensory acceptability test (6.64±1.33). These results suggested that biscuit formulation P5 could be used as a prototype for sweet potato and riceberry flour-based products to promote healthier diet.

Introduction

Functional foods and ingredients have received a considerable attention from the food industry because of consumers' demand for more healthful foods (Childs, 2015). Among bakery products, biscuits are the most popular and versatile snack foods due to a variety of taste, availability, long shelf life, and inexpensive price (Murugkar et al., 2014). Main ingredients of biscuits generally include wheat flour and fat (Klunklin & Savage,

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2018). Gluten in wheat flour may not be suitable for consumers with celiac disease (Emami et al., 2018). Alternatives to wheat flour are flours from corn, purple sweet potato, and rice. Purple sweet potatoes (*Ipomoea batatas* (L.) Lam.) are starchy tubers that are beneficial to health. They are excellent sources of dietary fiber, minerals, vitamins, and phytochemical compounds (Li et al., 2019) and exhibit an intense purple color because of the accumulation of acylated anthocyanins as peonidin-based and cyanidin-based anthocyanins (Phomkaivon et al., 2018). Riceberry (*Oryza sativa* L.), a new valuable rice variety in Thailand, is a popularly consumed by the locals (Settapramote et al., 2018). It has become well-known for its taste and lower commodity price compared to wheat flour. In addition to containing higher levels of dietary fiber than wheat, riceberry also contains cyanidin-3-O-glucoside and peonidin-3-O-glucoside (Klunklin & Savage, 2018). These anthocyanin compounds possess various biological properties such as free radical scavenging, anticarcinogenic, and antihypertensive activities (Montilla et al., 2011).

Fats in biscuits are mainly composed of triglycerides, including monounsaturated, polyunsaturated, and saturated fatty acids. Saturated fatty acids could unfavorably affect health (Puscas et al., 2020). Recent studies have shown that trans and saturated fats are associated with cardiovascular diseases (Zhu et al., 2019). Alleviating adverse effects from oil consumption could be accomplished through modifying the structure of liquid oils into gels, hydrogel, or organogel, reducing saturated fat intake (Hwang et al., 2013; Yilmaz & Ogutcu, 2014). Several types of organogels have been developed from edible oils including rice bran oil. Rice bran oil is one of the popular edible oils because it contains several phytochemical compounds such as tocotrienols, tocopherols, phytosterols, polyphenols, squalene, and gamma-oryzanol, that are beneficial to human health (Li et al., 2017).

Products with higher phytonutrient content are preferred by consumers with diabetic and obese conditions. This study aimed to develop biscuits with higher phytonutrient. Flours from sweet potato and riceberry were particularly selected for the study because they were inexpensive raw materials containing various bioactive compounds. Furthermore, the use of rice bran oil in the form organogel could reduce the presence of trans fat in the biscuits. Consumer acceptability, anthocyanin content, physicochemical, nutritional and sensory properties of the biscuits were also evaluated.

Materials and methods

1. Raw materials

The purple sweet potato flour (Doi Ang Khang, Banpangsak community), riceberry flour (Fair D, Grace Bio Co., Ltd), baking powder (Imperial, KCG corporation), eggs (Betagro, Betagro Public Co.,Ltd), brown sugar (Mitr Phol, Mitr Phol Group), and iodized salt (Prung Thip, Thai Refined Salt Co.,Ltd), were purchased from a local market in Thailand. All ingredients were food grade.

The margarine from rice-bran-oil organogel was provided by Mahidol University, Bangkok, Thailand. The method for preparing the organogel was modified from Hwang et al. (2013). Briefly, the oil phase was prepared by dissolving organogelator (4.0% (w/w)) in rice bran oil at 75°C. The 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, and 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 organogels were formed by placing the emulsion at −18°C for 4 hours. Once the gelation was complete, the organogels were stored in a refrigerator at 4°C until use.

2. Biscuit preparation

The biscuit formulations were developed by varying the ratio between purple sweet potato and riceberry flours (Table 1). The two types of flour were blended with 0.7 g of baking powder and 0.1 g of salt. Then, 25.8 g of egg white and 4.2 g of brown sugar were added and blended using an electric hand mixer (Electrolux, EHM3407, China) for 2 min. After the mixture attained a spongy consistency, 14.0 g of egg yolk and 8.4 g of rice-bran-oil organogel were slowly poured into it. Then, the mixture was threshed until homogeneous. The dough was rolled out with 0.3 cm of thickness and cut to 0.8 cm of width x 2.5 cm of length. The biscuits were put on a baking tray covered with a baking paper. After applying margarine on top, the biscuits were baked in an oven (Electrolux, EOT2805K, China) at 100°C for 15 min.

Table 1 Biscuit formulations

Ingredients (per 100 grams)	Formulations (purple sweet potato flour : riceberry flour)				
	P1 (50:50)	P2 (60:40)	P3 (70:30)	P4 (80:20)	P5 (90:10)
Purple sweet potato flour	23.4	28.1	32.8	37.4	42.1
Riceberry flour	23.4	18.7	14.0	9.4	4.7

3. Physicochemical characterizations of biscuits

3.1 Color analysis

The color of the biscuits was analyzed using the Chroma Meter CR-400 colorimeter (Konica Minolta, Japan). The color meter was calibrated using a white plate CR-A43 (y = 85.70, x = 0.3177 and y = 24 0.3340) and assessed using the DP mode. The analyzed color parameters lightness (L*) (0 = black to 100 = white), a* (greenness (-) to redness (+)), and b* (blueness (-) to yellowness (+)). The measurement was performed twice.

3.2 Moisture content analysis

The moisture content measurement was performed using a moisture analyzer MA37 (Sartorius, Germany). The condition was set at 105°C using the fully automatic mode. Ten grams of ground sample was approximately weighed on the moisture analyzer. The measurement was duplicately carried out and reported as % moisture.

3.3 Water activity analysis

Water activity was measured by the auto start mode using water activity meter LabSwift-aw (Novasina, Switzerland). The ground sample was added to a sample dish and then put into the water activity meter. The measurement was performed twice.

3.4 Texture profile analysis

Texture analysis of biscuits with a diameter of 30 mm was performed using a texture analyzer CT3 (Ametek Brookfield, USA) equipped with the TA-AACC36 AACC spec probe. The program was set to the test speed at 1 mm/s. Texture characteristics including hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess, and chewiness were obtained. Duplicate measurements were carried out.

4. Nutritional values of biscuits

Nutritional values including energy, carbohydrate, protein, and fat of the biscuits were acquired using the nutritional analysis program (INMUCAL-Nutritions version 4.0, Institute of Nutrition, Mahidol University). The obtained data sets were reported as per 100 g of biscuit sample.

5. Anthocyanin content determination

The total anthocyanin content was determined by the pH-differential method (Sutharut & Sudarat, 2012). Anthocyanin pigments undergo reversible structural transformations with a change in pH, resulting in different absorption spectra. The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form prevails at pH 4.5. A ground biscuit sample with a mass of 5 g was mixed with 10 mL of distilled water. After centrifugation, each 0.5 mL of sample solution was mixed with 0.5 mL of potassium chloride (0.025 M of KCl; Ajax Finechem Pty.Ltd, Autralia) buffer, pH 1.0 and 0.5 mL of sodium acetate (0.4 M of CH_3 COONa; Loba Chemie Pvt.Ltd, India) buffer, pH 4.5, respectively. The absorbance of each dilution was measured at 520 and 700 nm using a microplate reader (Synergy HTX BioTek instruments, USA). The absorbance of the diluted sample (A) was calculated as follows:

$$A = (A_{520} - A_{700})\text{pH } 1.0 - (A_{520} - A_{700})\text{pH } 4.5$$

The monomeric anthocyanin pigment concentration (cyanidin-3-glucoside) in each original sample was calculated using the following formula:

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

where MW is the molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor, ε is the molar absorptivity (26,900 $M^{-1}cm^{-1}$). The value was converted to mg of total anthocyanin content /100 g sample.

6. Sensory characteristics of biscuits

The sensory scores of five formulations were evaluated by 50 volunteers that did not possess sweet potato and rice allergy. Volunteers were students of Valaya Alongkorn Rajabhat University under the Royal Patronage who consented to join this study. The served biscuit samples were labeled with a randomized three letters-digit. After each sample tasting, participants were required to rinse the mouth with water. The appearance, color, texture, flavor, taste, and overall preference of biscuit samples were assessed using the 9-point hedonic scale (1: extremely dislike to 9: extremely like). The study was approved by the research ethics committee (Ref. no. 0002/62).

7. Data analysis

Statistical data were analyzed using ANOVA. The LSD's test was applied to detect the statistical differences among the biscuit samples ($p \leq 0.05$). A completely randomized design (CRD) was used in physicochemical properties of biscuits and a randomized complete block design (RCBD) was applied in the sensory evaluation.

Results and discussion

1. Physicochemical properties

The color, water activity, and moisture contents of five biscuit formulations are shown in Table 2. There was no significant difference in lightness (L*), where the values ranged from 21.05 to 21.52. The a* (4.97 - 5.23) and b* (0.74 - 0.84) values showed redness and yellowness pigments, respectively. The red pigment of biscuits was possibly caused by thermally degraded anthocyanin pigments in purple sweet potato and riceberry (Wiriyawattana et al., 2018). The yellow pigment was likely caused by anthocyanins in the high chalcone (yellow) form (Xiu-li et al., 2015). The moisture content and the water activity of biscuit samples were also evaluated. These values displayed a direct association with the shelf life of baked products, stability, and susceptibility to microbial contamination (Mahloko et al., 2019). Even though the biscuits had a relatively high moisture content ranging from 21.72 to 23.70%, the water activity (a_w) exhibited a rather low value ranging from 0.845 to 0.883. Most microorganisms cannot propagate within the reported water activity, including *Staphylococcus aureus*, many yeasts (*Candida, Torulopsis, Hansenula, Micrococcus*), most molds (*mycotoxigenic penicillia*), most *Saccharomyces* (bailii) spp., and *Debaryomyces* (Tapia et al., 2020).

Table 2 The color, water activity, and moisture contents of biscuits

Biscuit formulations	Color			Water activity (a_w)	Moisture (%)
	L*^{ns}	a*	b*		
P1	21.41±0.11	5.14±0.02^b	0.82±0.01^a	0.882±0.00^a	21.72±1.03^c
P2	21.20±0.06	5.23±0.01^a	0.74±0.02^b	0.879±0.00^{ab}	22.13±0.19^{bc}
P3	21.05±0.11	4.97±0.02^c	0.83±0.01^a	0.868±0.02^{ab}	23.70±0.23^a
P4	21.33±0.49	5.22±0.04^a	0.84±0.01^{ac}	0.883±0.01^a	23.61±0.04^a
P5	21.52±0.09	5.10±0.01^b	0.80±0.02^{ad}	0.845±0.02^b	23.20±0.20^{ab}

Remark: $^{a-d}$ different letters in the same column indicate values that are significantly different (p≤0.05).
^{ns} in the same column indicate values that are not significantly different (p>0.05).

The texture profile analysis of biscuit samples was evaluated. Hardness, cohesiveness, springiness, gumminess, and chewiness of the samples are shown in Table 3. The P1 formulation had the highest hardness (1175.00 ± 1.41 g). Increasing purple sweet potato flour content resulted in decreased hardness. Addition of purple sweet potato flour also contributed to decreasing cohesiveness (ranging from 0.86-0.57), springiness (ranging from 0.91-0.76 mm), gumminess (ranging from 1065-55.00 g), and chewiness (ranging from 9.69-0.42 mJ). This was possibly because of the high dietary fiber in purple sweet potato, which has the ability to swell and resulted in soft biscuit (Dhingra et al., 2012). Texture profiles could be employed for predicting consumer acceptability. Softer biscuits are preferred by consumers (Klunklin & Savage, 2018). This suggested that the addition of purple sweet potato flour might increase consumer acceptance.

Table 3 Texture profiles of biscuits

Characteristics	Biscuit formulations				
	P1	P2	P3	P4	P5
Hardness (g)	1175.00± 1.41^a	936.00±2.83^b	918.00±1.41^c	414.00±2.83^d	98.00±141^e
Cohesiveness	0.85±0.01^a	0.83±0.01^a	0.73±0.02^b	0.68±0.01^c	0.57±0.02^d
Springiness (mm)	0.91±0.02^a	0.88±0.01^a	0.88±0.01^a	0.82±0.01^b	0.76±0.01^c
Gumminess (g)	1065.50±2.12^a	805.00±2.83^b	661.50±2.12^c	404.00±1.41^d	55.00±1.41^e
Chewiness (mJ)	9.69±0.10^a	7.08±0.09^b	5.70±0.03^c	3.29±0.04^d	0.42±0.02^e

Remark: $^{a-e}$ different letters in the same row indicate values that are significantly different (p≤0.05).

2. Nutritional properties of biscuits

The nutritional properties of biscuits are presented in Table 4. Most of the energy of biscuits came from carbohydrate (42.86–44.83 g). Five formulations of biscuits did not show a statistically significant difference in energy distribution from carbohydrate, which was in the range of 55.39–58.49%. The energy from these formulations was in accordance with the recommendation of acceptable macronutrient distribution ranges (AMDRs), which provided the guideline for energy intake from carbohydrate to be approximately 35–70% (Lee et al., 2015). The sugar content was in the range of 4.27 to 4.90 g. The values did not exceed the recommended guideline daily amounts (GDA) of 90 g per a day. The increased purple sweet potato in the biscuit resulted in increased carbohydrates and decreased sugar content, indicating that carbohydrates could be in the form of dietary fiber. The fatty acid energy distribution was in the range from 35.07 to 36.50% (11.95–12.55 g). Moreover, the biscuits contained low saturated fatty acid (1.48–1.49 g) and cholesterol (0.18 g). The World Health Organization (WHO) recommended the amount of saturated fatty acid to be less than 10% of the total energy intake. The lower saturated fatty acid and cholesterol is more preferred by consumers who are more conscious of the risk of degenerative disease such as cardiovascular diseases and obesity-related type 2 diabetes (Gershuni, 2018). The lower level of saturated fatty acid of the biscuits might be attributed to the utilization of rice-bran-oil organogel in the formulation. Furthermore, the biscuits were high in protein (5.33-6.27 g, 6.63–8.11% of energy distribution). Purple sweet potatoes were reported to be lower in protein and fat than riceberry (Kurnianingsih et al., 2020; Settapramote et al., 2018). Therefore, higher ratio of purple sweet potato in the biscuit decreased fat and protein. The energy of biscuits was in the range of 306.55 to 309.48 kcal. According to the recommendation by the Thai dietary reference intakes (Thai DRIs), Ministry of Public Health, the biscuits was suitable for 2 servings per container.

Table 4 Nutritional properties of biscuits (100 g)

Nutritional properties	Biscuit formulation				
	P1	P2	P3	P4	P5
Energy (kcal)	309.48	308.74	310.57	307.29	306.55
Carbohydrate (g)	42.86	43.35	44.38	44.33	44.83
Energy distribution from carbohydrate (%)	55.39	56.16	57.16	57.71	58.49
Protein (g)	6.27	5.97	5.72	5.38	5.33
Energy distribution from protein (%)	8.11	7.74	7.34	7.00	6.63
Fat (g)	12.55	12.40	12.30	12.10	11.95
Energy distribution from fat (%)	36.50	36.14	35.60	35.43	35.07
Saturated fat (g)	1.48	1.48	1.49	1.49	1.49
Cholesterol (g)	0.18	0.18	0.18	0.18	0.18
Sugar (g)	4.90	4.74	4.61	4.43	4.27

3. Anthocyanin content of biscuits

Anthocyanin contents of the biscuits are shown in Table 5. The flours of purple sweet potato and riceberry at a ratio of 50:50 had the lowest anthocyanin content. The P5 formulation displayed the highest anthocyanin content (66.50 ± 0.99 mg/100g of biscuit), but it did not significantly differ from the P4 formulation (62.93±0.29 mg/100g of biscuit). The result indicated that the addition of purple sweet potato increased the level of anthocyanins, which include peonidin and cyanidin with 3-sophoroside-5-glucoside acylated with p-hydroxybenzoic acid, ferulic acid and caffeic acid (Li et al., 2019). The anthocyanins of purple sweet potato have been reported for their stability after heating and ultraviolet irradiation because of their complex chemical structures (Kano et al., 2005). In addition, anthocyanins of purple sweet potato could contribute to the anti-arteriosclerosis activity (Kano et al., 2005), suggesting that the biscuit formulation with higher ratio of purple sweet potato flour is likely to be healthier.

Table 5 Anthocyanin content of biscuits

Formulations	Total anthocyanin content (mg/100g of biscuit)
P1	33.81±1.92^{d}
P2	41.18±1.31^{c}
P3	49.89±1.22^{b}
P4	63.93±1.29^{a}
P5	66.50±0.99^{a}

Remark: $^{a-d}$ different letters in the same column indicate values that are significantly different ($p \leq 0.05$).

4. Sensory properties of the biscuits

The sensory characteristics of the biscuits are shown in Table 6. Sensory evaluation is considered a valuable tool in determining consumer acceptability of the developed products. The results showed that the appearance (5.11-5.57) and color (5.13-5.55) of all biscuits were not significantly different (p≤0.5). This was possibly due to the similar dark purple color of both purple sweet potato and riceberry flours. The biscuit formulation P5 had the highest score in the texture property, i.e. 6.55±1.52, followed by formulations P4, P2, P3, and P1. This result suggested that consumers preferred softer biscuits. Moreover, the formulations with higher purple sweet potato flour were more popular in flavor possibly due to the presence of sweet-scented volatile oil in the sweet potato flour. The main components of volatile oil from purple sweet potato were hexadecanoic acid, phenylacetaldehyde, guaiacol, and *p*-vinylguaiacol (Nakamura et al., 2013). Regarding the taste, the P5 formulation exhibited the highest score. Nevertheless, it was not significantly different from the P4 formulation. The overall acceptance of the P5 formulation 5 displayed the highest values among all formulations. Therefore, the P5 formulation was a potential candidate for a healthy biscuit.

Table 6 Sensory properties of biscuits

Sensory properties	Biscuit formulation				
	P1	P2	P3	P4	P5
Appearance^{ns}	5.11±1.17	5.32±1.20	5.36±1.19	5.43±1.35	5.57±1.21
Color^{ns}	5.13±1.21	5.23±1.31	5.32±1.19	5.45±1.32	5.55±1.44
Texture	4.86±1.81^{d}	5.77±1.48^{c}	5.75±1.51^{b}	6.21±1.41^{abc}	6.55±1.52^{a}
Flavor	5.45±1.33^{bc}	5.40±1.38^{abc}	5.61±1.45^{abc}	5.68±1.37^{ab}	6.00±1.55^{a}
Taste	4.79±1.33^{d}	5.53±1.61^{c}	5.75±1.48^{bc}	6.13±1.61^{abc}	6.62±1.54^{a}
Overall acceptance	5.19±1.35^{d}	5.77±1.24^{c}	5.83±1.40^{bc}	6.28±1.39^{abc}	6.64±1.33^{a}

Remark: $^{a-d}$ different letters in the same row indicate values that are significantly different ($p \leq 0.05$).
^{ns} in the same row indicate values that are not significantly different ($p > 0.05$).

Conclusion

Biscuit formulations based on purple sweet potato and riceberry flours were successfully developed. The texture of the biscuits could be modified by varying the sweet potato flour content. The biscuit formulations with the highest sweet potato flour content exhibited good nutritional characteristics, low water activity, desirable sensory properties, and favorable texture characteristics. The nutritional profiles the biscuit formulation suggested that the biscuits were suitable for 2 servings per container. The results from our studies set forth a utilization of anthocyanin-rich ingredients for producing a healthier and nutritionally-rich daily snacks.

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