การพัฒนาอาหารเชิงหน้าที่: ช็อกโกแลตนมเสริมแอนโทไซยานินจากปลายข้าวไรซ์เบอร์รี่

DEVELOPMENT OF A FUNCTIONAL FOOD: MILK CHOCOLATE FORTIFIED WITH ANTHOCYANIN FROM BROKEN RICEBERRY

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บทคัดย่อ

การรับประทานอาหารเชิงหน้าที่ที่มีผลดีต่อสุขภาพมากกว่าอาหารปกติ พบว่าช่วยลดความเสี่ยงต่อโรคในกลุ่มโรคไม่ติดต่อที่เป็นสาเหตุสำคัญของการเสียชีวิตจากโรคของประชากรไทย ปลายข้าวไรซ์เบอร์รี่เป็นผลิตภัณฑ์ผลพลอยได้ทางการเกษตรที่ถูกใช้ประโยชน์น้อยแต่มีปริมาณแอนโทไซยานินสูง ซึ่งจัดเป็นสารต้านอนุมูลอิสระที่ป้องกันโรคดังกล่าวได้ ในงานวิจัยนี้ใช้สารสกัดปริมาณแอนโทไซยานินสูงจากปลายข้าวไรซ์เบอร์รี่เป็นส่วนผสมเชิงหน้าที่เพื่อผลิตช็อกโกแลตนมที่ดีต่อสุขภาพมากขึ้น ซึ่งผลิตโดยแทนที่ผงโกโก้ด้วยผงแอนโทไซยานิน (แอนโทไซยานินห่อหุ้มด้วยมอลโทเด็กซ์ทริน) ในปริมาณร้อยละ 0 (MC0), 21 (MC21), 42 (MC42) และ 70 (MC70) โดยน้ำหนัก ปริมาณแอนโทไซยานินทั้งหมดใน MC21, MC42 และ MC70 มีค่าเพิ่มขึ้นเป็นลำดับ (11.1, 82.2 และ 107.1 mg cyanidin-3-glucoside equivalent/100 g) ตามปริมาณผงแอนโทไซยานิน แต่ตรวจไม่พบใน MC0 ส่งผลให้ฤทธิ์การต้านอนุมูลอิสระ DPPH ของ MC0, M21, MC42 และ MC70 เพิ่มขึ้นจากร้อยละ 70.7, 72.1, 74.3 ถึง 76.4 ตามลำดับ มอลโทเดกซ์ทรินซึ่งเป็นองค์ประกอบหลักในผงแอนโทไซยานินทำให้ความแข็งของช็อกโกแลตลดลงจาก 16.6 N (MC0) จนถึง 9.6 N (MC70) ช็อกโกแลต MC42 มีคะแนนทางประสาทสัมผัสสูงที่สุด (7.0-7.7) ต่อคุณลักษณะด้านสี กลิ่นรส รสชาติ เนื้อสัมผัส และการยอมรับโดยรวม ผลการทดลองแสดงให้เห็นว่าแอนโทไซยานินสกัดจากปลายข้าวไรซ์เบอร์รี่อาจใช้เป็นส่วนผสมเชิงหน้าที่ที่มีคุณประโยชน์ต่อสุขภาพเพื่อผลิตอาหารที่ดีต่อสุขภาพมากขึ้นได้

คำสำคัญ: แอนโทไซยานิน สารต้านอนุมูลอิสระ ช็อกโกแลต อาหารเชิงหน้าที่ ข้าวไรซ์เบอร์รี่

Abstract

The consumption of functional foods, healthier foods, could reduce the risk of non-communication diseases, the major cause of the casualty of the Thai population. Broken Riceberry, an underutilized agricultural byproduct, is rich in anthocyanins, the antioxidants that prevent the diseases. In this study, the anthocyanin-rich extract from broken Riceberry was used as a functional ingredient to produce healthier milk chocolate products. The milk chocolates were prepared by replacing the cocoa powder with the anthocyanin powder (anthocyanin was encapsulated in maltodextrin) by 0 (MC0), 21 (MC21), 42 (MC42) and 70 % w/w (MC70). The total anthocyanin content in the MC21, MC42 and MC70 chocolates increased sequentially (11.1, 83.2 and 107.1 mg cyanidin-3-glucoside equivalent/100 g) with the anthocyanin powder content while it was not detected in MC0. Consequently, the DPPH antioxidant activity of MC0, MC21, MC42, and MC70 respectively increased from 70.7, 72.1, 74.3 to 76.4 %. Maltodextrin, the major constituent in the anthocyanin powder, caused the decrease of the chocolate hardness from 16.6 N (MC0) to the lowest of 9.6 N (MC70). The MC42 received the highest sensory scores (7.0-7.7) for the color, flavor, taste, texture, and overall acceptance attributes. These results indicated that anthocyanin extracted from broken Riceberry could be used as a health benefit functional ingredient for producing healthier foods.

Keywords: anthocyanin, antioxidant, chocolate, functional food, Riceberry

Introduction

Riceberry, Thai non-glutinous black rice, is produced by small community enterprises throughout Thailand. Broken Riceberry is a byproduct of the milling process which is, recently, underutilized. This rice is rich in anthocyanins, natural antioxidants that help prevent the non-communication diseases (e.g. cancers and cardiovascular diseases) (Yousuf et al., 2016). For this reason, the broken Riceberry extract that riches in anthocyanin could be used as a functional ingredient for producing functional foods.

Milk chocolate is an appropriate food model as its dark color will not be altered by the dark red color of the anthocyanin extract. In addition, milk chocolate lost its benefits of phytochemical compounds that were inactivated by the milk protein (Gallo et al. 2013). For this reason, fortification of anthocyanin could elevate the health benefit of the milk chocolate. Recently, fortification of anthocyanin into chocolate was solely reported by Gültekin-Özgüven et al. (2016) who used the spray dried mulberry extract to enhance the health property of a chocolate product.

This study proposed to develop healthier milk chocolate products by using anthocyanin-rich extract from broken Riceberry as a functional ingredient. The anthocyanin was prepared into powder by spray drying and was added to the milk chocolate by replacing the content of cocoa powder at different levels. The effects of anthocyanin powder on the physical (color, water activity and hardness) and chemical (total anthocyanin content and DPPH antioxidant activity) properties and sensory characteristics were analyzed.

Materials and Methods

1. Chemicals

The 2,2-diphynyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich, USA. Cocoa powder (Tulip brand, Sino-Pacific, Thailand), cocoa butter (KLK-Kepong, Malaysia) and lecithin (Cargill, Netherland) were used to prepare milk chocolates.

2. Rice samples

Broken Riceberry was obtained from a small local rice mill in Nong Don District, Saraburi, Thailand. The rice was cultivated and harvested in year 2017. The broken Riceberry was collected immediately after the milling and was kept in plastic bags and stored in a cold dry and dark place for further experiment.

3. Preparation of anthocyanin powder

The anthocyanin-rich extract was obtained from the extraction of the broken Riceberry (500 g) with distilled water (1:3 w/v). The total soluble solids (TSS) value of the extract was measured using a refractometer (Atago 3840 PAL-α, China). The anthocyanin powder was prepared using the spray-dry method as described by Laokuldilok et at. (2015). Briefly, the anthocyanin-rich extract was diluted with distilled water (TSS = 5 ^{o}Brix) and the maltodextrin DE10 (Dong Xiao, China) was dissolved into the solution to the TSS value of 10 ^{o}Brix. The mixture was dried using a spray dryer (Labphant UK SD-06, United Kingdom) (feed rate = 25 mL/min, inlet air temperature = 180 ^{o}C).

4. Preparation of milk chocolate fortified with anthocyanin powder

Milk chocolate was prepared by following the procedure described by Hannaneh & Soleiman (2009). Briefly, to produce one batch (88.8 g) of milk chocolate (MC0), cocoa butter (31 g) was melted at 60 ^{o}C then cocoa powder (16 g), icing sugar (25 g), milk powder (15.4 g) and lecithin (0.4 g) were added and mixed. To produce milk chocolate fortified with the anthocyanin, different contents of anthocyanin powder (3, 5 and 7 g to produce MC21, MC42, and MC70 chocolates, respectively) were used in replacing the content of the cocoa powder. The chocolate mixture was casted in a plastic mold (60 x 30 x 10 mm) and stored at 4 ^{o}C for overnight. The milk chocolate bars were wrapped in aluminum foils and stored at 4 ^{o}C before analysis.

5. Physical property measurement

5.1 Color

The color of the chocolate was measured by using a colorimeter (Hunter Lab ColorFlex EZ, USA) with the CIE expression system L* (0 (black) to 100 (white)), a* (green (-) to red (+)), and b* (blue (-) and yellow (+)) (Aidoo et al., 2015).

5.2 Water activity

A 10 g of milk chocolate was homogenized and 2 g of the homogenized sample was used to determine the water activity (a_w) at 25 °C using a water activity meter (Aqualab 4TE, USA). Triplicate samples were measured and mean values were calculated.

5.3 Hardness

The hardness of chocolate bars was measured using the TA.XTplus texture analyzer (Stable Micro Systems, UK). The hardness was reported as the maximum force (N) required to penetrate (at a constant head speed of 1.5 mm/s) a cylindrical flat-end probe (d = 1.6 mm) into the chocolates at 20 °C over a distance of 6 mm.

6. Chemical property analysis

6.1 Chocolate extraction

The chocolate (10 g) was defatted with hexane and extracted with a mixed solvent (1 M HCl in water/methanol; 15:85 v/v) (Ngamdee et al., 2016). The extract was concentrated using a rotary evaporator (BÜCHI Rotavapor® R-100, Switzerland) at 40±2 °C and the volume was adjusted to 10 mL with methanol and stored at -20 °C.

6.2 Determination of total anthocyanin content

The anthocyanin extract was diluted with potassium chloride buffer pH 1.0 and sodium acetate buffer pH 4.5 (Giusti & Wrolstad, 2001). The absorbance (*A*) of the solutions was measured at 510 nm and 700 nm using a UV-visible spectrophotometer (Hitachi UH5300, Japan). The TAC (mg/L) was calculated using the Equation 1.

$$\text{TAC (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times L) \quad \text{...1)}$$

Where $A = (A_{510} - A_{700})\text{pH } 1.0 - (A_{510} - A_{700})\text{pH } 4.5)$, *MW* of cyanidin-3-glucoside (449.2 g/mol), *DF*= dilution factor, ε= molar absorptivity (26,900 l/mol•cm) and *L*= path length (1.0 cm).

6.3 Determination of DPPH antioxidant activity

Scavenging capacity of the chocolate extracts against DPPH radicals was analyzed as described by Leong & Shui (2002). The extract solution (50 µL) was mixed with 2 mL DPPH solution (100 µM in methanol) and measured at 515 nm after 30 min of the reaction. The antioxidant activity was expressed as percentage scavenging of the DPPH radical regarding the control (the extract was replaced with methanol).

7. Sensory evaluation

Sensory attributes of milk chocolates including color, flavor, taste, melting rate in mouth, firmness and overall acceptance were evaluated using the 9-point hedonic scale method (Kalva et al., 2014) by 30 untrained panelists (Hannaneh &

Soleiman, 2009). Briefly, chocolates were presented randomly in three-digit-coded containers along with mineral drinking water for mouth rinsing between tests.

8. Statistical analysis

Mean ± standard deviation (SD) from at least triplicate was analyzed by One-Way ANOVA followed by Duncan's multiple range test at 95% confidence level ($p \leq 0.05$) which was performed using SPSS v 16.0 (SPSS Inc., Chicago, Illinois, USA).

Results

1. Anthocyanin-rich Riceberry extract and anthocyanin powder

Table 1 shows the TSS (4.7 ^{o}Brix) and TAC values (14.6 mg C3G/L) of the anthocyanin-rich extract from Riceberry. This anthocyanin-rich extract was spray dried in the presence of maltodextrin which served as wall material for the encapsulation of the anthocyanin (Laokuldilok et at., 2015). The anthocyanin powder contains 455.5 mg C3G/100g of anthocyanin and had the a_w value of 0.50 (Table 1). The spray-dried anthocyanin powder was light red indicated by positive values of L* (66.67) and a* (8.08).

Table 1 Physical and chemical properties of the anthocyanin extract

Samples	Properties					
	TSS (^{o}Brix)	TAC	a_w	Color		
				L*	a*	b*
Anthocyanin-rich extract from broken Riceberry	4.7±0.5	14.6±1.7†	-	-	-	-
Anthocyanin powder		455.5±70.3‡	0.50±0.01	66.67±0.04	8.08±0.01	12.89±0.02

Remark †the value is presented in mg C3G/L. ‡ the value is presented in mg C3G/100 g.

2. Physical property of the milk chocolates

According to the results in Table 2, all color parameters of MC21 sequentially increase ($p \leq 0.05$) with the percentage of the anthocyanin fortification as compared to those of MC0. The increase of L* and a* values indicated that the color of the milk chocolate changed toward light brown color as the anthocyanin powder was added.

Table 2 Physical property of the milk chocolates

Samples	Color			a_w^{ns}	Hardness (N)
	L*	a*	b*		
MC0	20.03±0.03[d]	2.09±0.01[d]	0.50±0.01[d]	0.55±0.01	16.6±0.8[a]
MC21	20.58±0.01[c]	3.02±0.03[c]	0.98±0.04[c]	0.54±0.04	15.2±0.8[b]
MC42	21.08±0.02[b]	3.75±0.01[b]	2.13±0.05[b]	0.54±0.01	13.8±0.1[c]
MC70	26.03±0.03[a]	4.27±0.01[a]	2.72±0.03[a]	0.54±0.03	9.6±0.3[d]

Remark [a-d] indicate significant difference ($p \le 0.05$) of the means in the same column. [ns] represents an insignificant difference of mean values in the column.

The a_w value of MC0, MC21, MC42 and MC70 were not significant different (p> 0.05). The hardness of the fortified chocolates: 15.2 N (MC21), 13.8 N (MC42), and 9.6 (MC70), decreased sequentially with the increase of the anthocyanin powder content.

3. Chemical property analysis of the milk chocolates

The anthocyanin was not found in the MC0, the milk chocolate without anthocyanin powder fortification (Table 3). The TAC in the MC21, MC42 and MC70 chocolates increased sequentially (10.1, 15.6 and 23.9 mg C3G/100 g) regarding percentage replacement of the cocoa powder with the anthocyanin powder. The percentage retention of anthocyanin values in Table 3 indicates the percentage of the anthocyanin remained in the MC21, MC42, and MC70 regarding the added amounts.

Table 3 Chemical property of the milk chocolates

Chocolate	TAC (mg C3G/100 g)	% recovery of anthocyanin[†]	DPPH scavenging activity (%)
MC0	-	-	70.7±0.3[d]
MC21	10.1±2.0[c]	65.6	72.1±0.1[c]
MC42	15.6±2.4[b]	60.8	74.3±0.5[b]
MC70	23.9±3.1[a]	66.6	76.4±0.1[a]

Remark [a-d] indicate significant difference ($p \le 0.05$) of the means in the same column. [†] calculated from the TAC values of the chocolate extract regarding the TAC of the added anthocyanin powder (3, 5 and 7 g).

The DPPH scavenging activity (Table 3) increased sequentially with the content of the anthocyanin powder, in which the activity of MC21 (72.1 %), MC42 (74.3 %) and MC70 (76.4 %) were significantly higher ($p \le 0.05$) than that of MC0 (70.7).

4. Sensory evaluation

Table 4 shows that the MC21 and MC42 received higher ($p \le 0.05$) liking scores over the MC0 for the color, taste, and overall acceptance attributes. In term of flavor, melting rate in mouth, and firmness, MC21 and MC42 received equivalent scores ($p > 0.05$) to those of MC0. The MC21 and MC42 received the highest liking scores for most attributes. However, when the antioxidant activity was considered, the MC42 was considered the most desirable healthier milk chocolate product.

Table 4 Sensory characteristics of the anthocyanin fortified milk chocolates

Chocolate	Sensory attribute (score)					
	Color	Flavor	Taste	Melting rate in mouth	Firmness	Overall acceptance
MC0	6.6 ± 1.4^{b}	6.8 ± 2.1^{ab}	6.7 ± 2.0^{b}	7.3 ± 0.9^{a}	6.7 ± 1.8^{a}	6.7 ± 1.9^{b}
MC21	7.5 ± 1.0^{a}	7.6 ± 1.3^{a}	7.5 ± 1.6^{ab}	7.0 ± 1.6^{ab}	6.2 ± 2.2^{a}	7.4 ± 1.3^{ab}
MC42	7.6 ± 0.8^{a}	7.7 ± 1.0^{a}	7.6 ± 1.2^{a}	6.3 ± 1.2^{bc}	7.0 ± 1.6^{a}	7.6 ± 0.9^{a}
MC70	7.1 ± 1.8^{ab}	6.1 ± 1.9^{b}	5.6 ± 1.7^{c}	5.4 ± 1.9^{c}	5.2 ± 1.6^{b}	5.7 ± 1.9^{c}

Remark $^{a\text{-}c}$ indicate significant difference ($p \leq 0.05$) of the means in the same column.

Discussions

The constant a_w value of the chocolate prepared with different content of encapsulation materials, maltodextrin and inulin, were also found in the studies of Hannaneh et al. (2009) and Konar (2013). Heat and pH were the major factors that caused the decrease of anthocyanin content (Ekici et al., 2014). As a result, the percentage recovery of the anthocyanin was decreased in all milk chocolate samples.

The hardness of the MC chocolates in this study was slightly higher than the values reported by Hannaneh et al. (2009). They reported that the hardness of the chocolates prepared by replacing sugar with different ratios of inulin (14-31%), polydextrose (71-84%) and maltodextrin (67-77%) ranged between 10.1-15.0 N. However, they also found that the hardness of these chocolates tended to decrease with the increase of maltodextrin content. It is the fact that the hardness of a chocolate is controlled by the crystal structure and polymorphism of cocoa butter and also correlated with the type of sugar (Afoakwa et al., 2008; Hou et al., 2013). The bulky structure of the maltodextrin particle probably obstructs the crystallization of the cocoa butter fatty chain resulting in low harness milk chocolate (Hannaneh et al., 2009).

A study on the development of chocolate containing anthocyanin was reported (Gültekin-Özgüven et al., 2016). However, different plant sources are known to provide different anthocyanin derivatives that also have different stabilities against thermal and pH (Castañeda-Ovando et al., 2009). In this study, TAC values of the chocolates increased by approximately 54% compared to the values of the next lower one (e.g. the TAC value of MC42 was 54% ([(15.6-10.1)/10.1]x100) higher than that of the MC21). However, the DPPH activity values increased by only approximately 2-3%

(e.g. MC70 had 3% ([(74.3-72.1)/72.1]x100) higher DPPH scavenging activity than the MC42). This was caused by the loss of phenolic compound content in the cocoa powder that was replaced by the anthocyanin powder, which consisted of as much as 99% of the non-antioxidant maltodextrin. A fraction of the added anthocyanin lost via binding to milk proteins. Alternatively, this indicated that anthocyanin from the broken Riceberry could compensate for the loss of the antioxidant compounds in the milk chocolates yielding healthier chocolates with higher antioxidant activity compared to the regular one.

The liking scores of the MC21 and MC42 were higher (or tended to be higher) than that of the MC0 in most attributes: color, flavor, taste, firmness and overall acceptance. However, the melting in mouth score of the MC21, MC42 and MC70 tended to sequentially decrease with the content of the anthocyanin powder. This decrease caused by the sticky characteristic of the maltodextrin (the major constituent in the anthocyanin powder) (Hannaneh et al., 2009). It probably formed a sort of thin-layer on the surface of the tongue and mouth hole resulting in perceptible low melting rate and high mouth coating properties which are unfavored property for the chocolate bar products (Hannaneh et al., 2009).

Conclusions

This content of the anthocyanin powder changed the color of the milk chocolates toward white and red color and reduces their hardness. These anthocyanin fortified milk chocolates were healthier than the regular ones indicated by higher anthocyanin content and antioxidant activity. The milk chocolate that the cocoa powder was replaced with 42 % anthocyanin powder was selected as the most desirable healthier milk chocolate with the highest overall liking scores. Additional studies are needed in order to improve the hardness and sensory properties (e.g. melting rate in mouth) and to increase the anthocyanin fortification content of the milk chocolate. This further improvement could deliver the most desirable healthier milk chocolate product for possible commercialization and, as a consequence, increases the utilization of the broken Riceberry.

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