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Development and Study on Physical and Sensory Properties of Dark Chocolates Fortified with Anthocyanin from Broken Riceberry Rice

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Received 7 December 2019; Revised 17 May 2020; Accepted 23 July 2020

Abstract

Broken rice of Thai cross breed black rice, Riceberry, was an under-utilized agricultural by-product that contains high content of anthocyanins, the multiple health benefits flavonoid. In this study, these anthocyanins were used to develop healthy dark chocolates. The anthocyanin extract from the broken Riceberry rice was prepared into anthocyanin powder using freeze-drying technique in which maltodextrin was used as a carrier material. Healthy dark chocolates were prepared by replacing cocoa powder with anthocyanin powder by 5, 10 and 15 g/g cocoa powder, which respectively delivers DC5, DC10, and DC15 anthocyanin-fortified dark chocolate bars. The color analysis showed no blooming (undesired white color at the chocolate surface) in all chocolates. The increase the anthocyanin powder content significantly decreased the hardness of the dark chocolates (33.9-27.2 N). Total anthocyanin content (TAC) in the dark chocolates increased respectively as anthocyanin powder content increased. The health benefit of the anthocyanin-fortified dark chocolates was improved as the DPPH antioxidant activity of all treatments was 4-9% higher than that of the control DC0. Sensory evaluation revealed that DC10 received higher liking scores (6.4-7.5) than the DC0 indicating more consumer preference. The anthocyanin-fortified dark chocolate delivers an alternative strategy to improve the health property of chocolates. This application of anthocyanin from the broken Riceberry rice could help increase the value and the utilization of Thai agricultural by-product.

Keywords : Dark Chocolate; Anthocyanin; Broken Riceberry Rice**;** Sensory Evaluation

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1. Introduction

Anthocyanins are bioactive compounds that have been reported for their multiple health benefits: oxidative stress relief, prevention of cardiovascular diseases, anti-inflammatory activity, anti-carcinogenic activity, and control of diabetes [1]. In addition, anthocyanins have been proposed as a highly feasible functional ingredient to be used in food product development as they provided health-promoting benefits and considerable low cytotoxicity [1], [2]. Daily consumption of anthocyanin at 2.5 mg/kg body weight is considered as safe according to the announcement of FAO and WHO [2].

Riceberry rice, the non-glutinous cross breed black rice of Thailand, contains a high content of anthocyanins [3]. This rice has been cultivated throughout Thailand and mostly marketed in the form of milled rice. Recently, small local mills produced a considerable amount of the broken Riceberry rice as a by-product. However, the utilization of these colored broken rice was limited. Unlike the broken white rice, the broken Riceberry rice is not accepted by noodle factories due to the color contamination issue. As a result, it is sold as a cheap feed stock, which is the underutilization. For this reason, anthocyanins can be extracted from the broken Riceberry rice and used as a healthy functional ingredient in food products.

Chocolate, a worldwide confectionary product, is a promising food model for the application of the anthocyanin extract as a health promoting ingredient. The color of the regular chocolate is dark blown. For this reason, the addition of the dark purple anthocyanin extract into the chocolate would have less effect on the color of chocolates. In addition, the chocolate products are also considerable unhealthy foods as they contain a high content of saturated fat and sugar (for sweet chocolates). However, the global market value for chocolate products was as high as 103 billion US dollars per annum and is continuously growing [4]. This indicates that people will continue eating chocolates even though they are very unhealthy. To stop people from consuming chocolates, as to protect their health, may not be possible. On the other hand, fortification of health benefits ingredient into the chocolates could be a practical way to reduce the risk of consumer's health. Healthier chocolates were mostly developed via reduction or substitution (or both) the major unhealthy ingredients, saturated fat [5] and sugar [6]. However, reducing the contents of these ingredients is limited as it would basically affect the physicochemical, textural and sensory properties of the chocolate [7]. Alternatively, fortification of healthy ingredients such as bioactive compounds into the chocolate in order to improve its antioxidant property is considered as a feasible strategy.

Recently, only a few published studies reported the fortification of plant bioactive compounds into chocolate products for the improvement of their health properties. Mangosteen pericarp

was used to enhance polyphenol content in chocolate products [8]. The encapsulated powder of anthocyanin-containing black mulberry extract was used to fortify the dark chocolate for a health improvement purpose [9]. These studies indicate a possibility of the application of the anthocyanins as a health improving ingredient in chocolates.

This study aimed to utilize the undervalued broken Riceberry rice to develop healthier dark chocolates. The anthocyanin was extracted from the broken Riceberry rice and processed into anthocyanin powder by using maltodextrin as a carrier. The powder was used to produce dark chocolates by replacing the content of cocoa powder in the regular one. Effects of the anthocyanin powder on the physical, chemical, antioxidant and sensory properties of the chocolates were analyzed.

2. Research Methodology

2.1 Material and Chemicals

The important ingredients for producing dark chocolate were cocoa powder (Tulip brand, Sino-Pacific, Thailand), cocoa butter (KLK-Kepong, Malaysia) and lecithin (Cargill, Netherland). The 99.9% ethanol used for the extraction was of commercial grade (Chemipan, Thailand). The 2,2-diphynyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, USA) was used for the antioxidant assay.

2.2 Broken Riceberry rice

The broken Riceberry rice was contributed by a local rice mill in Chon Sombun Sub-district, Nong Muang District, Lopburi Province, Thailand. The rice was harvested in November 2017. The broken rice sample was collected immediately after the milling and stored in a vacuum sealed metalized bag and kept in a refrigerator at 4 ^{o}C.

2.3 Preparation of the Anthocyanin Powder

The anthocyanin was extracted from the broken rice berry (200 g) by soaking the rice in 400 ml 70% ethanol for 90 min [10]. The soluble fraction was filtered and concentrated using a rotary evaporator (Rotavapor® R-300, Buchi, Switzerland) at 40 ^{o}C. The obtained anthocyanin-rich concentrate was diluted with distilled water to the total soluble solid (TSS) of 5 ^{o}Brix, which was measured using a refractometer (Atago 3840 PAL-α, China).

To prepare the anthocyanin powder, maltodextrin DE10 (Dong Xiao, China) was used as an encapsulating material. The maltodextrin was dissolved into the previously diluted anthocyanin solution at room temperature until the TSS value of the solution reached 20 ^{o}Brix [10]. The solution was frozen in a 500 mL round bottom flask before equipped with a freeze dryer (Martin Christ, Alpha 1-4 LDplus, Germany). The freeze-drying was performed at -80 ^{o}C and 0.1 mbar of pressure until the sample was completely dried. The obtained anthocyanin powder was stored in a vacuum sealed metalized plastic bag and kept in a desiccator.

2.4 Preparation of Anthocyanin-Fortified Dark Chocolate

The dark chocolate products supplemented with anthocyanin powder were prepared by replacing the gram content of cocoa powder used in a control chocolate with the anthocyanin powder by 5 (DC5), 10 (DC10) and 15 g/g cocoa powder (DC15). The control chocolate (DC0) was prepared as described by D. Komes et al. [11]; cocoa butter (19.5 g) was melted at 60 ºC before cocoa powder (40 g) and sugar (30 g) were added and mixed. The chocolate mixture was removed from the heater and lecithin (0.5 g) was immediately added and mixed. The chocolate mix was cooled down to 40 ºC before it was poured into rectangular (long × wide × thick; 60 × 30 × 10 mm) silicone mold. The chocolate bars were left cooling down to the room temperature (26 ± 1 ºC) and kept at 4 ºC in a refrigerator for at least 24 h before analysis.

2.5 Physical properties analysis

2.5.1 Color

The color of the chocolate was expressed in the CIE expression system. The color coordinate are L^* (0 = black and 100 = white)), a^* (-a^* = green and +a^* = red), and b^* (-b^* = blue and +b^* = yellow) [12], which were measured from triplicate samples using a colorimeter (Hunter Lab ColorFlex EZ, USA). The whiteness index (WI) was calculated according to the following equation:

$$WI = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{0.5}$$

2.5.2 Water Activity

The water activity (a_w) was measured from the homogenized chocolate (2 g) using a water activity meter (Aqualab 4TE, USA). Triplicate samples were measured and the mean value was calculated. Moisture content was determined using AOAC official method 935.29 [13].

2.5.3 Hardness

The chocolate bars were measured for the hardness using TA.XTplus texture analyzer (Stable Micro Systems, UK). The hardness determined 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 chocolate bar (10 mm of thickness) at 20 ºC over a distance of 6 mm.

2.6 Chemical Properties Analysis

2.6.1 Preparation of Chocolate Extracts

The chocolate extracts were used for the analysis of total anthocyanin content (TAC) and antioxidant activity. The pre-freeze chocolate sample (20 g) was ground in a 250 mL Erlenmeyer flask using a small electric handheld blender. The ground chocolate was defatted with 50 mL hexane and the lipid extraction was repeated for two more times. The defatted chocolate was extracted with acidified methanol (1 M HCl:CH_3OH = 15:85 v/v), which targets anthocyanin [14], by vigorously stir using a vortex. The soluble fraction was separated from the non-extractable one using a centrifuge at 5000 rpm for 10 min

and the extraction was repeated for 2 more times with the non-extractable fraction. The supernatant fractions from 3 repeated extractions were combined and the solvents were removed at 40 ºC using a rotary evaporator (BÜCHI Rotavapor® R-100, Switzerland). The obtained crude extract was diluted with 95% ethanol to the volume of 10 mL and stored in a 15 mL glass bottle with screw-capped. All the extracts were kept in a freezer at 0 ºC for further analysis.

2.6.2 Determination of the Anthocyanin Content

The total monomeric anthocyanin content (TAC) in the broken Riceberry rice extract, the anthocyanin powder, and the chocolates was measured using the pH differential method [15]. Two aliquots of sample solutions (0.1 mL) was diluted in potassium chloride buffer pH 1.0 (3 mL) and sodium acetate buffer pH 4.5 (3 mL) and incubated for 15 min. The absorbances (A) of the incubated solutions were measured at 510 nm and 700 nm using a UV-visible spectrophotometer (Hitachi UH5300, Japan). The TAC in mg/L was calculated using the following equation:

$$TAC\ (mg/L) = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times L)}$$

Where $A = (A_{510} - A_{700})$ pH 1.0 – $(A_{510} - A_{700})$ pH 4.5), MW of cyanidin-3-glucoside (C3G) (449.2 g/mol), DF = dilution factor, ε = molar absorptivity (26,900 l/mol·cm) and L = path length (1.0 cm).

2.6.3 Determination of Antioxidant Activity

The antioxidant activity of the chocolate extracts against DPPH free radicals was analyzed as described by L. P. Leong and G. Shui [16]. Briefly, the extract (0.1 mL) was diluted in 3 mL DPPH solution (100 µM in methanol) and the mixture was incubated for 30 min for the reaction. The absorbance of the reaction solution was measured at 515 nm. The percentage DPPH scavenging activity of the extract was calculated regarding the absorbance of the control (the extract was replaced with methanol).

2.7 Sensory Evaluation

The chocolates were determined for sensory attributes: appearance, color, flavor, taste, hardness, texture, and overall acceptance using the 9-point hedonic scale method [17], in which the panelists scored liking level of a chocolate from 1 (dislike extremely) to 9 (like extremely). The untrained panelists were 30 female and 20 male who experience the taste of a chocolate in a previous one-month period. The evaluation was conducted by serving a piece of chocolate (long × wide × thick; 30 × 30 × 10 mm), which was coded with random three-digit on the container, and served along with mineral drinking water for mouth rinsing between tests.

2.8 Statistical Analysis

The mean ± standard deviation was calculated from a triplicate experiment. The statistical analysis 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).

3. Results and Discussion

3.1 Physical and Chemical Properties of the Anthocyanin Powder

According to the results in **Table 1**, the anthocyanin powder prepared from the broken Riceberry rice extract contain 9.02 C3G/100 g. This anthocyanin powder had a_w value of 0.44 indicated that the powder will not affect the a_w value of the chocolate, which is a critical property that makes chocolates long shelf life food products [18].

Table 1 Physical and chemical properties of the anthocyanin powder

Properties		Anthocyanin powder
TAC (mg C3G/100 g)		9.02 ± 0.01
a_w		0.44 ± 0.01
Moisture content (%)		5.33 ± 0.57
Color	L*	53.95 ± 0.10
	a*	3.02 ± 0.02
	b*	-3.96 ± 0.03

The moisture content of the powder was 5.33%. The anthocyanin powder had positively high L^* value of 53.95 and positive a^* value indicated that the powder color was white with slightly red color.

Table 2 Physical property of the dark chocolates

Samples	Color			*WI*	a_w^{ns}	Hardness (N)
	*L**	*a**	*b**			
DC0	$18.2^{c} \pm 0.07$	$4.66^{a} \pm 0.14$	$3.21^{a} \pm 0.24$	18.0^{c}	0.45 ± 0.02	$42.2^{a} \pm 2.8$
DC5	$21.7^{b} \pm 0.27$	$3.29^{b} \pm 0.25$	$1.64^{c} \pm 0.47$	21.6^{b}	0.43 ± 0.01	$33.9^{b} \pm 2.1$
DC10	$21.4^{b} \pm 0.10$	$3.56^{b} \pm 0.10$	$2.38^{c} \pm 0.13$	21.3^{b}	0.42 ± 0.02	$31.9^{c} \pm 1.9$
DC15	$23.4^{a} \pm 0.32$	$4.25^{a} \pm 0.11$	$2.78^{b} \pm 0.10$	23.2^{a}	0.43 ± 0.02	$27.2^{d} \pm 1.5$

$^{a-d}$ indicate significant difference ($p \leq 0.05$) of the means in the same column. ^{ns} represents an insignificant difference of mean values in the column.

3.2 Physical Properties of Dark Chocolates

The physical properties of the control dark chocolate and the dark chocolates fortified with different anthocyanin powder content are presented in **Table 2**. The color coordinate (L^*, a^*, and b^*) values indicated that the chocolate products were dark in color. The L^* parameter dominated the color of the chocolate due to the high positive values (L^* values ranged between 18.2-23.4), which was also found in previous report [19]. The added anthocyanin powder changed the appearance of the chocolate toward white color. The L^* value of the DC0 (18.2) was significantly lower ($p \leq 0.05$) than those of DC5, DC10, and DC15. Among the anthocyanin-containing chocolates, the whiteness of the DC5 and DC10 was insignificant difference ($p > 0.05$). The whiteness of the DC15 was significantly higher than those of the DC5 and DC10.

The increase of the L^* values of the DC5, DC10, and DC15 was in accordance with the high L^* value of the anthocyanin powder.

The whiteness index (*WI*) determines the whiteness of chocolate surface, which is used to evaluate the migration of sugar and/or fat to the chocolate surface (so-called bloomed chocolate) [20]. The bloomed chocolate is determined by the change of the chocolate surface color from glossy brown to grayish white, as well as from small individual white dots to large white spots on the chocolate [20]. The close values between *WI* and L^* parameters of the same chocolate indicated that no chocolate bloom occurred [19].

The a_w value determines the shelf life of chocolate products. All chocolates, included the control chocolate, had insignificant different a_w values, which ranged between 0.42-0.45. This indicated that these chocolates were not easily spoiled by microbial [18]. The a_w values of the chocolates produced in this study were higher than that of the previous report of H. Farzanmehr and S. Abbasi [21] (a_w = 0.33) who also produced the chocolates that contain maltodextrin. The variation of a_w values depending largely on the a_w and moisture values of the raw material [18].

The hardness is a critical property of chocolate bar products [18]. According to **Table 2**, the hardness of the DC5 (33.9 N), DC10 (31.9 N), and DC15 (27.2 N) decreased significantly with the increase of the anthocyanin powder replacement. The anthocyanin powder consisted of maltodextrin as a major component (approximately 99% regarding the TAC value). The content of the maltodextrin caused a decrease of chocolate hardness was reported by H. Farzanmehr and S. Abbasi [21]. They suggested that the bulky structure of the maltodextrin particle could obstruct the crystallization of the cocoa butter fatty acid chains resulting in softer chocolate than the control one. The hardness of chocolates is controlled by polymorphism of cocoa butter and this is also correlated with the type of the second major solid component e.g. sugar and supplement sweeteners [22].

3.3 Chemical Properties of the Dark Chocolates

The TAC of the dark chocolates is presented in **Fig. 1A**. The control dark chocolate DC0 showed no anthocyanin content. The theoretical TAC of DC5, DC10, and DC15 (0.46, 0.92 and 1.38 mg C3G/100 g, respectively) obtained from the calculation for the TAC that should be presented in the chocolate regarding the added amount, which was calculated as follow: [(TAC in the anthocyanin powder (9.02 mg C3G/100 g))/100] × g of the anthocyanin powder added. The apparent TAC obtained from the laboratory analysis using the spectrometric method.

The TAC in DC5, DC10, and DC15 (0.31, 0.66 and 1.01 mg C3G/100 g, respectively) increased consecutively with the content of the anthocyanin powder. However, by comparing to the theoretical TAC of each corresponding

treatments, the apparent TAC was lower by approximate 27-33%. The reasons could be due to the degradation of the anthocyanin by high thermal and neutral pH during the chocolate production process as the anthocyanins are molecules that susceptible to heat, pH, and light [23]. This result agreed with the report of K. Anukulwattana et al. [24] who reported that approximately 42% of the TAC of the cooked Riceberry rice was lost after a cooking as compared to the raw rice. They reported the lower lost value compared to that of this study as the initial TAC of the whole grain Riceberry rice was higher than that in the dark chocolates.

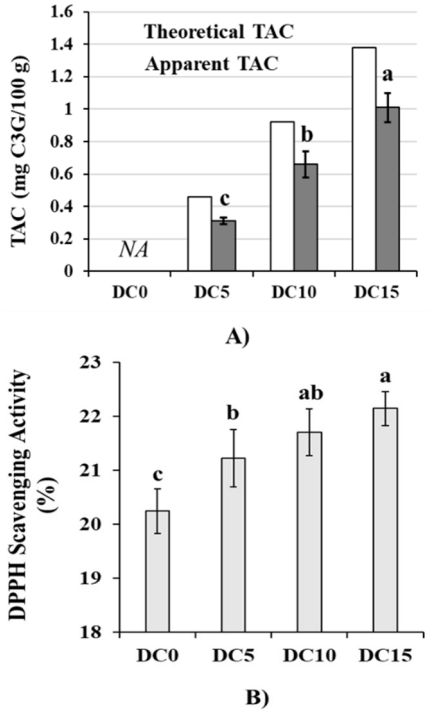

Fig. 1 A) Total monomeric anthocyanin content and B) DPPH scavenging activity of the chocolates. *NA* = not applicable as the TAC was not detected in the DC0. [a-c] indicate significant difference ($p \leq 0.05$).

The percentage of DPPH scavenging activity results in **Fig. 1B)** show that DC5 (21.2 %), DC10 (21.7 %), and DC15 (22.1 %) had significantly higher ($p \leq 0.05$) antioxidant capacity than the control chocolate DC0 (20.2 %). However, the antioxidant property of the anthocyanin-fortified dark chocolates was found to increase by only 4-9% compared to that of the DC0. This could be the result of the replacement of the cocoa powder with the anthocyanin powder. The cocoa powder is a source of flavonoids (mainly catechin and epicatechin) [25], which are considered as antioxidants. In contrast, the maltodextrin, which accounted for approximately 99% of the anthocyanin powder, is not an antioxidant.

The dilution effect of the maltodextrin on the antioxidant activity was also reported by S. Chuaychan and S. Benjakul [26]. They found that the antioxidant activities of the gelatin hydrolysate decreased when the maltodextrin was used as a carrier material. Although the antioxidant activity of the anthocyanin-fortified dark chocolate was increased in a small degree, this chocolate still had the health benefits of the anthocyanin. Anthocyanins had more favorable health-promoting property for using as a functional food ingredient over other flavonoids as they are a considerable low cytotoxicity compound [2], [27], which means that it prevents the cells from harmful substances without damaging the cells. In addition, the content of the anthocyanin at 2.5 mg/kg body weight

could be daily intake by human [2]. Regarding the anthocyanin content in the maximum anthocyanin-fortified D15, this chocolate could provide as much as per 1.01 mg anthocyanins per 100 g serving size, which is much less than the anthocyanin consumption recommendation. Due to this aspect, further increase of the anthocyanin content in the chocolate is required. The increase of chocolate consumption for the health benefit of the anthocyanin must be avoided due to overconsumption of fat and sugar. On the other hand, the stability and bioavailability of the anthocyanin fortified into the chocolate should be investigated.

Table 3 Sensory characteristics of the anthocyanin-fortified dark chocolates

Chocolate	Sensory attribute (score)						
	Appearance	Color	Flavor	Taste	Hardness	Texture	Overall acceptance
DC0	$6.7^{c} \pm 0.7$	$6.0^{b} \pm 0.8$	$5.7^{b} \pm 0.6$	$5.7^{c} \pm 0.6$	$6.1^{c} \pm 0.7$	$5.5^{c} \pm 0.8$	$5.9^{c} \pm 0.6$
DC5	$6.7^{c} \pm 0.7$	$7.3^{a} \pm 0.5$	$6.1^{ab} \pm 0.7$	$5.6^{c} \pm 0.9$	$6.1^{c} \pm 0.9$	$5.7^{c} \pm 0.9$	$6.0^{c} \pm 0.9$
DC10	$7.5^{a} \pm 0.9$	$7.5^{a} \pm 0.5$	$6.4^{a} \pm 0.7$	$7.4^{a} \pm 0.9$	$7.3^{a} \pm 0.6$	$7.2^{a} \pm 0.8$	$7.4^{a} \pm 0.7$
DC15	$7.1^{b} \pm 0.9$	$7.4^{a} \pm 0.8$	$6.1^{ab} \pm 0.8$	$6.3^{b} \pm 1.2$	$6.8^{b} \pm 0.9$	$6.6^{b} \pm 0.9$	$6.5^{b} \pm 0.8$

$^{a-c}$ indicate significant difference ($p \leq 0.05$) of the means in the same column.
9-point hedonic score: 1 = dislike extremely, 9 = like extremely.

3.4 Sensory Property of the Dark Chocolate

The acceptance of consumers on the chocolate fortified with the anthocyanin powder was evaluated over sensory attributes using 9-point hedonic score and the results are shown in **Table 3**. The DC5 was not different ($p > 0.05$) from the control chocolate DC0 in term of appearance, flavor, taste, hardness, texture, and overall acceptance attributes. The DC10 and DC15 generally received higher liking scores (6.3 – 7.5) than the DC0 (5.5 – 6.7) for all attributes with the exception of the flavor attribute, in which the DC15 (6.1) had equivalent liking score to the DC0 (6.1). The DC10 received the highest liking scores (6.4-7.5) for all attributes. This indicated that anthocyanin-fortified dark chocolate is a feasible alternative healthier chocolate for marketing.

4. Conclusion

Anthocyanin-fortified dark chocolates were prepared using the broken Riceberry rice anthocyanin, which was freeze-dried into anthocyanin powder with using maltodextrin as an encapsulating material. The fortification of the anthocyanin powder to the dark chocolate by replacing the cocoa powder generally caused the increase of the lightness of the products while reduce the hardness of ones. The anthocyanin contents in all final chocolate products were lower than the added due to the degradation of the anthocyanin during thermal processing. The health property of the anthocyanin-fortified chocolate

was slightly improved due to the replacement of the cocoa powder caused the loss of some phenolic antioxidants. The consumer preferred dark chocolate with the highest degree of anthocyanin fortification over the regular one. These results indicated that the utilization of anthocyanin from broken Riceberry rice to develop healthy chocolate products was possible. Nevertheless, the increase of anthocyanin fortification content and its bioavailability should be studied before to summarize the health-improving power of the anthocyanin-fortified chocolate.

5. Acknowledgement

The financial support of this work was provided by Thepsatri Rajabhat University (the Fiscal year 2017) through the Research and Development Institute. The authors are greatly appreciated.

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