

Development of High Anthocyanin Crispy Rice Bar

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Abstract

Nowadays, consumers are increasingly interested in healthy food. This study aimed to increase the nutritional value of crispy rice by using Thai rice which presented important natural ingredients. Five formulations were developed including Thai colored indica rice (*Oryza sativa* cv. Riceberry), black sticky rice (*Oryza sativa* cv. Leum Phua), white glutinous rice (*Oryza sativa* cv. RD6), RD6 soaking in water obtained from Leum Phua sticky rice, and a combination of RD6 and Leum Phua glutinous rice. Water activity, moisture content, crispness, color, total anthocyanin content, and sensory evaluation were analyzed. The results showed that there was no significant difference in crispness and moisture content of products. The lowest free water was observed in both Riceberry and soaked RD6 formulations with water activity below 0.42. Leum Phua and Riceberry recipes had the lowest brightness. Obviously, crispy rice bar from Leum Phua sticky rice had the highest total anthocyanin content, followed by Riceberry rice. According to sensory test, the color score was high in the RD6 and soaked RD6 formulations. During the storage for 2 months, the increase of water activity and lipid peroxidation were observed. However, there was no growth of pathogenic microorganisms. The crispy rice by soaked RD6 formula was acceptable up to 2 months of storage. Therefore, it can be concluded that the RD6 sticky rice soaking with water from black glutinous rice is suitable for commercial production because it can increase the nutritional value for consumers by providing high total anthocyanin content. 3456789

Keywords: Riceberry rice, Leum Phua rice, RD6 rice, Black glutinous rice, Anthocyanin

1. Introduction

Many studies have been pointing out to the critical issue of food eating awareness related to physical illness throughout the past few decades. Eating behavior is an important factor that impacts on human health. Thus, healthier food choice is recommended for healthy consumers. In the present, human lifestyle has been completely changed to hurry in living which directly influences trend of food decision as well as their dietary pattern consumption. Food characteristics, suitable for people who living in a hurry each day, should be ready-to-eat and easy-to-carry such as sandwiches, bread and snacks. Importantly, making decision of eating food must include both healthy and safe as the priority. In present, people are facing a challenge to develop nutritious food product which fit in daily

lifestyle. Therefore, this ongoing issue causes of global food industry focusing on functional ingredients from natural plants for developing healthier products. Hence, utilization of anthocyanin-based food is needed to add the value in food products and also enriched nutrition in food with natural source.

Thailand is well known as the land of agriculture, especially rice cultivation in different strains with high quality. Thai colored indica rice (*Oryza sativa* cv. Riceberry), black glutinous rice (*Oryza sativa* cv. Leum Phua) and white glutinous rice (*Oryza sativa* cv. RD6) have been cultivated widely in Thailand. Interestingly, pigmented rice contains phytonutrient which is higher antioxidant activity and phenolic compounds than non-pigmented rice. Several varieties of pigmented rice, particularly Riceberry

rice and Leum Phua sticky rice have been exhibited as source of anthocyanin. It is water-soluble, phenolic of plant which contributes to red, purple or blue colors in rice. Anthocyanin-based food products have been developing for healthier choice. It has becoming popular in food and beverage research, such as substitution of Riceberry flour in noodle and fortification of anthocyanin from Riceberry rice in milk chocolate (Sirichokworakit, Phetkhut, & Khommoon, 2015; Ngamdee, Bunnasart, & Sonda, 2019). Moreover, effect of anthocyanin has been reported as a potent nutraceutical by its antioxidant capacity. Health benefits were reported in previous literature that mainly involved in anti-oxidative stress and anti-inflammation throughout the body. Furthermore, pharmacological effect of anthocyanin in Thai rice as anti-hyperlipidemic and anti-cancer property have been reported. (Khoo, Azlan, Tang, & Lim, 2017; Sivamaruthi, Kesika, & Chaiyasut, 2018). Glutinous rice can be modified in many kinds of food such as snack or dessert. Leum Phua and RD6 are Thai glutinous rice with pigmented and non-pigmented carrier, respectively. Leum Phua, black glutinous rice, has been reported that it exhibited high anthocyanin profile, phenolic compounds and radical scavenging activity than Black Rose, Hawm Nil, and Klam rice (Suwannalert & Rattanachittawat, 2011). White glutinous rice RD6 is one of the popular rice grown in Thailand. It is commonly modified and developed to snack or cake due to puffable property even though lower antioxidant activity is exhibited when compared to other colored Thai rice.

Recently, Thai rice containing anthocyanin has been widely studied due to rise of concern in healthier eating habits. The application of natural material on Thai traditional snack is still needed. In addition, to our knowledge, the comparison of pigmented rice utilization in snack bar is rarely observed. Therefore, the objective of this study was to increase the nutritional value of crispy snack bar using colored rice, as the main ingredient. In this study, physicochemical properties of three rice varieties including Riceberry rice, Leum Phua glutinous rice, and RD6 sticky rice were compared. This study provided the development of crispy rice

bar as healthier snack recipe and suggested for further utilization in prospective commercial grade.

2. Materials and methods

2.1 Materials

Raw materials include black glutinous rice (Leum Phua), white glutinous rice (RD6 rice), Riceberry rice, sunflower seeds, pumpkin seeds, cashew nuts, black sesame, white sesame, raisins, ripe banana (cv. Kluai Kai), glucose syrup, gum arabic, honey, brown sugar, salt, vegetable oil. Chemical reagents including ethanol, acetic acid, sodium acetate, potassium chloride, sulfuric acid, sodium hydroxide and 3,5-dinitrosalicylic acid were used in this study.

2.2 Experimental design

To develop nutritious snack bar with Thai rice mixed cereals, the sample of each type of rice varieties were conducted in Thailand. There were five experimental groups in this study, consisted of crispy rice product by Riceberry rice, black glutinous rice (Leum Phua), white glutinous rice (RD6), soaked white glutinous rice in water from black glutinous rice (soaked RD6 rice), and a combination of white and black sticky rice (combined RD6 with Leum Phua). Physicochemical and sensory test were evaluated.

2.3 Crispy rice formulation development

2.3.1 Crispy rice preparation with/without oil

RD6 glutinous rice was dried at 65° C. 10 g of dried RD6 was roasted without oil on pan fried at 270° C. Crispy rice characteristics in terms of rice seed inflation, color, odor and texture were observed after roasting the rice. Another dried RD6 (10 g) was fried at 230° C for 7 to 9 sec. Characteristic descriptions of crispy rice were noted. The effect of rice preparation with/without oil on product characters were reported.

2.3.2 Cereals preparation

Pumpkin seeds were roasted at 270° C for 10 min. Cashew nuts were roasted at 150° C for 15 min. Black and white sesame were roasted at

270° C for 7 min. All roasted cereals were packed in polyethylene bag.

2.3.3 Banana syrup preparation

Ripe bananas were cleaned and peeled. Banana pulps were sliced and mixed with water. The ratio of bananas and water was 1:1. Sliced banana was heated at 100° C for 5 min. Banana pulp was filter. The clear banana juice was heated at 80° C and further concentrated to 60 to 70° Brix. Banana syrup was contained in sterile bottles and stored at 4 to 5° C.

2.4 Crispy rice formulations

Standard formulation for crispy rice mixed cereals bar includes crispy rice 200 g, glucose syrup 160 g, Kluai Kai syrup 50 g, honey 60 g, brown sugar 50 g, salt 3 g, pumpkin seeds 30 g, sunflower seeds 30 g, white sesame 10 g, black sesame 10 g and raisin 50 g. Crispy rice and cereals were mixed and rested in the bowl for a while. Subsequently, glucose syrup mixed with Kluai Kai syrup were heated for 5 min. Honey, brown sugar and salt were added to mixed syrup and stewed for 2 min until the mixture was homogeneous. The mixture was poured into the bowl and immediately mixed with crispy rice and cereals. Then, look it cool down for 5 min and cut into small pieces with equal size (2.5×5×1 cm). Crispy rice mixed cereal bar were packed in polyethylene bag and stored at room temperature for further analysis.

2.5 Product quality analyses

2.5.1 Physical property of crispy rice bar

Color and water activity (a_w) were analyzed by a spectro-photo-colorimeter and LabMaster-A_w, respectively. Color value was expressed as L*, a' and b'. Degree of whiteness or darkness were indicated by L* (0 = black, 100 = white). Degree of redness (+) or greenness (-) were expressed as a*. Yellowness (+) and blueness (-) scales were indicated as b*. Water activity was expressed as the ratio of the vapor pressure in food to the vapor pressure of pure water. A texture analyzer (TA.XT.Plus) was used to measure the crispness.

2.5.2 Chemical property of crispy rice bar

Moisture content was determined by A.O.A.C method (1995). Reducing sugar was analyzed using dinitrosalicylic acid (DNS) method as previously described by James, C. S. (1995).

2.5.3 Quantification of total anthocyanin content

Total anthocyanin content was determined by pH differential method (modified from Lee *et al.*, 2005). Ethanol solvent at 70% was used to extract the anthocyanin. Absorbance was read at 510 nm in a spectrophotometer.

2.5.4 Organoleptic evaluation

Crispy rice sensory evaluation was obtained from 24 untrained panels by 9-point hedonic scale test. Color, odor, flavor, and texture as well as the overall liking were evaluated from extremely dislike as '1 point' to extremely like as '9 point'.

2.5.5 Product quality during storage

The final formula of crispy rice was selected by the highest sensory point for product quality analysis during storage for 2 months. The selected product was evaluated for color, lipid oxidation, and water activity as well as bacterial growth. Product was observed and reported at day 0, 15, 30 and 60.

2.6 Data analysis

All analyses were carried out in triplicate. The results were expressed as mean values and standard deviation (SD). The mean of three or more groups were analyzed for the statistically significant differences using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Two-sided p -value < 0.05 were regarded as statistically significant. All statistical analyses were performed using Statistical Package for the Social Science (SPSS) 17.0 software.

3. Results and discussion

This study explored the effect of cooking process on quality characteristics of crispy rice by the comparison of frying with /without vegetable oil. The

result showed that cooking oil influenced on crispy rice quality in terms of seed swelling, color, odor and texture. Crispy rice with cooking oil represented more pleasant product quality than crispy rice without cooking oil (Table 1). Fat and oil play important roles to food product. One of oil capacity is the heat absorption which is more effective than air or water. It transfers heat to rice seed surface as well as enhances color and texture of the crispy rice. It plays important role in food product which is responsible for developing texture and enhancing flavor of fried foods. In addition, oil penetrates and replaces water in food during frying, resulting in more tenderizing. Oil also releases ingredient flavor and produces moistness feeling in the mouth. (Oke et al., 2017) Hence, heating process with vegetable oil resulted in better characteristics in terms of crispness, desirable color and pleasing smell of the fried crispy rice while another cooking process was slightly burnt and hard. The crispy rice by optimum oil usage was considerably more acceptable

Table 1. Qualitative characteristics of crispy rice by heated with/ without vegetable oil

Rice characters	Without oil	With oil
Rice seed	Little inflated	More inflated
Color	Yellow-brown	Yellow-white
Odor	Burnt smell	Pleasing scent
Texture	Crisp with hard cracked	Crisp with easily cracked

Total anthocyanin contents in Leum Phua sticky rice and Riceberry rice were compared before the heating process and after food processes by drying and frying (Table 2). The result indicated that food processing affected on anthocyanins. Total anthocyanin content was high at raw and subsequently decreased during food process. Significantly, Leum Phua glutinous rice had higher anthocyanins than Riceberry rice at stage of raw material and after drying. However, total anthocyanin contents were no different between Riceberry rice and Leum Phua sticky rice after frying process. In fact, anthocyanin are easily oxidized during process and storage by several factors such as pH, light, and oxygen, as well as

enzymes. One of the important factors to be considered is processing temperature. Anthocyanin stability was affected and degraded into various intermediate compounds by heat. (Patras *et al.*, 2010, pp. 3-11). The finding was consistent with Surh and Koh's study (2014) that presented higher anthocyanins at raw in two cultivars of black rice (*Oryza sativa* cv. Sintoheugmi and Sinnongheuchal). The large amount of anthocyanins were decreased after heat process, for example, roasting, pan-frying, steaming and boiling. As same as the study of Hiemori, Koh and Mitchell (2009) that revealed the degradation of total anthocyanins into the protocatechuic acid in cooking black rice (*Oryza sativa* L. *Japonica* var. SBR). Leum Phua and Riceberry rice showed total anthocyanins content in approximately 317.28 and 235.64 mg/100 g of rice, respectively (Table 2). The result was in range from previous study of Riceberry in different areas of Thailand which was 24.69-272.76 mg/100 g of rice (Settapramote et al., 2018).

Table 2. Comparison of total anthocyanins in Leum Phua and Riceberry rice after heat process

	Total anthocyanin content (mg/100 g of rice)		
	Raw	After drying	After frying
Leum Phua	5712.87± 1273.34 ^a	1384.15± 141.00 ^a	450.87± 28.92 ^a
Riceberry	669.81± 125.25 ^b	317.28± 130.43 ^b	235.64± 57.93 ^a

Values are shown as mean±standard deviation. Means within each column bearing different superscripts are significantly different ($p < 0.05$).

Crispy rice product were fried using vegetable oil. All formulas were developed as shown in Figure 1. The brightness was observed in RD6 crispy rice, followed by soaked RD6, combined RD6 with Leum Phua, Riceberry rice, and Leum Phua sticky rice, respectively (Table 3). The highest redness and yellowness were also detected in RD6 formula. Obviously, pigmented rice as Riceberry and Leum Phua were expressed lower scale of brightness and increased blue and green colors. According to the finding by previous reports in Thailand, Leum Phua

and Riceberry rice significantly presented the darkness color whereas RD6 sticky rice showed more brightness (Poomipak et al., 2018). It can be explained that anthocyanin accumulation in Leum Phua and Riceberry resulted in lower of brightness scale.

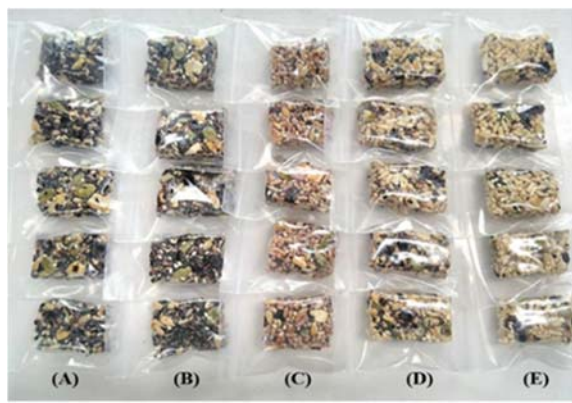


Figure 1. Crispy rice bar products (A) Leum Phua sticky rice, (B) Riceberry rice, (C) soaked RD6 in water from Leum Phua sticky rice, (D) combined RD6 with Leum Phua sticky rice, and (E) RD6 sticky rice

Table 3. Color scale of crispy rice products by different formulations

	Color scale		
	L*	a*	b*
Leum Phua	46.74±0.19 ^c	2.20±0.15 ^d	0.43±0.00 ^e
Riceberry	47.08±0.33 ^c	2.80±0.04 ^c	6.18±0.18 ^d
RD6 rice	54.69±0.80 ^a	5.12±0.18 ^a	16.64±0.31 ^a
Combined RD6 with Leum Phua	51.92±0.70 ^b	3.86±0.07 ^b	12.30±0.01 ^b
Soaked RD6 in water from Leum Phua	54.26±0.57 ^a	4.11±0.20 ^b	11.00±0.04 ^c

Values are shown as mean±standard deviation. Means within each column bearing different superscripts are significantly different ($p < 0.05$).

Reducing sugar, moisture, and free water as well as texture were reported in Table 4. There was no significant difference of moisture and texture among groups. Factors affecting the textural quality of

crispy rice snack include amylose content and moisture content. Amylose molecules are aligned by hydrogen bonds and release water molecules. However, the RD6, riceberry and Leum Phua are the rice with very low amylose content (5-12%) (Boonmeejoy et al., 2019). Thus, the moisture content and crispness were not difference in this study. The higher amount of reducing sugar was found in combined RD6 with Leum Phua, followed by soaked RD6, RD6, Riceberry, and Leum Phua glutinous rice, respectively. The reducing sugar of formulations with RD6 sticky rice were likely to be high because white glutinous rice had higher starch digestibility than colored rice varieties. Therefore, increasing amount of reducing sugar can be imply to degradation of the starch during the process (Pasakawee et al., 2018). The lowest free water in product was observed in both Riceberry and soaked RD6 formulas with water activity 0.41. All formulations were within the range 0.41-0.44 which reaction rate was low for undesirable microorganism growth, browning, and enzyme activity (Sandulachi, 2003).

Table 4. Reducing sugar, moisture content, water activity and texture of crispy rice product by different formulations

	Reducing sugar (g/ml)	Moisture (%)	Free water	Crispness (N)
Leum Phua	12.91	7.08±0.30 ^a	0.43±0.00 ^a	51.54±26.02 ^a
Riceberry	13.02	7.05±0.42 ^a	0.41±0.00 ^b	63.73±33.23 ^a
RD6 rice	13.11	7.22±0.57 ^a	0.42±0.00 ^a	75.34±60.71 ^a
Combined RD6 with Leum Phua	13.56	7.07±0.25 ^a	0.42±0.00 ^a	38.05±17.39 ^a
Soaked RD6 in water from Leum Phua	13.22	6.77±0.21 ^a	0.41±0.00 ^b	61.55±21.13 ^a

Values are shown as mean±standard deviation. Means within each column bearing different superscripts are significantly different ($p < 0.05$).

Mean of total anthocyanin contents were compared by various formulations as shown in Figure 2. Total anthocyanins significantly differed

between pigmented rice and pure RD6 sticky rice. There was no significant difference among Riceberry rice, combined RD6 and soaked RD6 formulations. Additionally, Leum Phua crispy rice had the highest total anthocyanin content (371.09 ± 11.58 mg/100g), followed by Riceberry (144.72 ± 16.70 mg/100g), combined RD6 with Leum Phua (128.02 ± 25.51 mg/100g), soaked RD6 (122.46 ± 22.27 mg/100g), and RD6 sticky rice (87.21 ± 47.99), respectively. Notably, colored rice, particularly Leum Phua was represented as the rich source of anthocyanins. In this sense, the previous study additionally supported that Leum Phua and Riceberry rice had higher phenolic compounds and scavenging ability than white glutinous rice, RD6 (Poomipak et al., 2018).

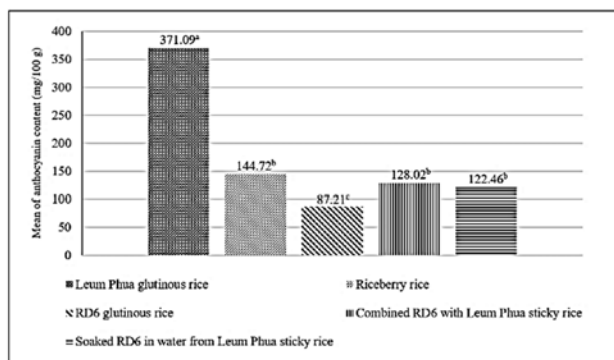


Figure 2. Mean of anthocyanins content in crispy rice products by different formulations

All formulations were ranked score from 1-9 in terms of color, odor, flavor, texture, and overall liking. There was no significant difference in odor, flavor, texture, and overall liking among groups. RD6 and soaked RD6 rice were obtained higher score in color and the highest score was found in soaked RD6 formula (Table 5). The lower score was observed in product with dark color by pigmented rice as based-material. The sensory test was inconsistent with previous study by Poomipak *et al.* (2018, pp. 134-143). They showed that unpolished Leum Phua sticky rice had higher scores in color. However, in this present, color score of developed sticky rice was high in RD6 and soaked RD6 formula. This may be explained by difference of age duration of panelists between the studies. Thus, the desirable formula of crispy rice product for

consumers was made from soaked RD6 rice in Leum Phua's water sticky rice. Subsequently, the selected product was analyzed for proximate composition and quality during storage life. Moisture, protein, fat, ash and carbohydrate were 6.77, 681, 16.05, 0.73, and 69.64%, respectively (data not shown).

Table 5. Means sensory scores of crispy rice products by different formulations

	Leum Phua	Rice berry	RD6 rice	Combined RD6 with Leum Phua	Soaked RD6 in water from Leum Phua
Color	6.25±1.73 ^b	6.83±1.63 ^b	7.21±1.02 ^a	6.46±1.59 ^b	7.42±0.93 ^a
Odor ^{ns}	6.38±1.38	6.08±1.38	6.13±1.26	6.42±1.53	6.46±1.18
Flavor ^{ns}	6.30±1.55	6.46±1.35	6.33±1.34	6.42±1.25	6.58±1.21
Texture ^{ns}	5.38±1.93	6.13±1.62	6.08±1.79	5.88±1.78	6.08±1.50
Overall liking ^{ns}	6.17±1.46	6.42±1.50	6.33±1.27	6.58±1.21	6.46±1.30

Values are shown as mean±standard deviation. Means within each column bearing different superscripts are significantly different ($p < 0.05$). ns means no significant difference.

Table 6. Color analysis of crispy rice (soaked RD6 rice formula) during storage for 2 months

	Color scale		
	L*	a*	b*
Day 0	51.92±1.73 ^a	3.86±0.08 ^a	12.30±0.32 ^a
Day 15	51.80±2.82 ^a	3.82±0.03 ^a	12.70±0.12 ^a
Day 30	49.67±2.08 ^a	3.43±0.11 ^b	17.04±0.23 ^b
Day 60	48.25±2.76 ^a	3.04±0.06 ^c	17.19±0.37 ^b

Values are shown as mean±standard deviation. Means within each column bearing different superscripts are significantly different ($p < 0.05$).

Product quality was measured after storage for 2 months at day 0, 15, 30, and 60. The scale of product color had been changing during storage at room temperature. Yellowness was increase while brightness and redness were decrease (Table 6). This phenomenon can be explained by browning reaction due to the increase of available water and lipid oxidation during storage. Non-enzymatic browning

is occurred by the interaction of reducing sugars and amino acids. This reaction further to amadori compounds and form dark pigments. It causes of the quality change in food product due to darkening of light colored product (Singh & Anderson, 2004).

Malondialdehyde and thiobitric acid (TBA) reactivity was used as an index of lipid peroxidation. Lipid peroxidation and water activity were observed during the storage for 2 months (Figure 3). The increase of water activity and lipid peroxidation were detected. Water activity was not significant difference among periods. Noticeably, at day 60, the TBA value was significantly higher than day 0 and 15. Lipid oxidation is affected by light, oxygen, water activity and temperature as well as material composition. The snack formula included cereals as the main ingredient and using cooking oil for food process. Lipid reactions are catalyzed by both of non-enzymatic and enzymatic involvement which presenting in cereal components such as its membrane structure. Additionally, water content in mature grain and food processing also affected the lipid mobility and promoted reactions between lipids and other ingredients. Moreover, free fatty acids in rice and subsequently lipid oxidation products is partially attributed to storage under warm and humid conditions even the lipid content of rice is slightly low. Thus, crispy rice stored in laminated bags containing CO₂ or under refrigerated conditions were likely to be more stable than stored at ambient temperature (Lehtinen, 2003; Angelo et al., 2009). Similar results were reported by previous study (Thaweeseang et al., 2017) that showed ongoing increase of TBA value and water activity in nutritious cereal bar during the 30 days storage. However, in this study, the TBA value was in range 0.34-0.53 within 2 months that was lower than acceptance limit for rancidity (1.0 mg/kg).

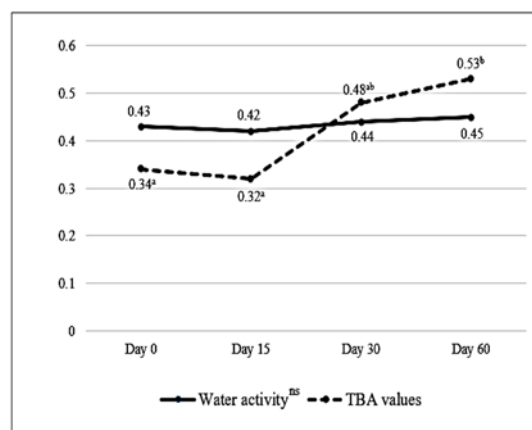


Figure 3. Means of water activity and TBA values (mg MDA/kg) in crispy rice product (the soaked RD6 formula) during storage for 2 months. The values with different superscript letters mean significantly different ($p < 0.05$). ns means no significant difference.

Table 7. Bacterial count of crispy rice product (soaked RD6 formula) stored at 25° C for 2 months

	Day 0	Day 15	Day 30	Day 60
Total bacterial count (CFU/g)	<10	<10	2.5×10	1.26×10^2
Yeast and moulds (CFU/g)	<10	<10	<10	<10
Coliform bacteria (MPN/g)	ND	ND	ND	ND
Staphylococcus aureus (MPN/g)	ND	ND	ND	ND
Escherichia coli (MPN/g)	ND	ND	ND	ND

ND: Not detected

The crispy rice products of the RD6 soaking with water from black glutinous rice were examined for microbiology count on days 0, 15, 30, and 60. Total bacterial count was below 10 at day 0 and 15. The spoilage were observed at the 30th and 60th storage day but not beyond the standard range. This result was supported by the increase of water activity during the storage period. Yeast and mould were lower than 10 CFU/g through the study. In addition, there was no growth of Coliform bacteria, Staphylococcus aureus, and E. coli during 2 months

of storage (Table 7). Hence, the product was microbiologically safe within 2 months of storage period at room temperature.

4. Conclusion

In this study, cooking process with vegetable oil resulted in the desirable quality of the crispy rice product. According to this result, five formulations, including black glutinous rice (Leum Phua), Riceberry rice, white glutinous rice (RD6), RD6 soaking in water from Leum Phua sticky rice, and combined RD6 with Leum phua sticky rice were developed to high anthocyanin snack bar by frying method. The anthocyanin from black rice was high at raw and subsequently decreased during the heating process. Obviously, Leum Phua crispy rice had the highest anthocyanin, followed by Riceberry rice. However, the color of pigmented rice affected the sensory scores. Low brightness of product was associated with lower score. The RD6 soaking in water from Leum Phua represented as the final product formula of crispy rice bar due to high anthocyanin and acceptable sensory score. Therefore, it can be concluded that crispy rice bar from glutinous rice and glutinous rice RD6 soaking with black glutinous rice are suitable for commercial production because it can increase the nutrition value for consumers by providing high anthocyanin content. Moreover, the RD6 crispy rice soaking in water from black glutinous rice was able to store up to 2 months of storage.

5. Acknowledgement

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6. Publication Ethic

Submitted manuscripts must not have been previously published by or be under review by another print or online journal or source.

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