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Product Development of Germinated Black Glutinous Rice Drink in a Sachet as Affected by Roasting and Brewing Time

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Abstract

Germinated black glutinous rice (GBGR) drink in a sachet was developed. The effects of roasting time (0, 5, 10 and 15 min) and brewing time (3, 5, 7, 10 and 15 min) on color, chemical compositions and antioxidant capacity were investigated. Prolonged roasting time increased L*, positive b*, C* and h° values, whereas decreased positive a* value, resulting in lighter color. Ash and carbohydrate contents were higher when longer roasting time was applied; however, a_w, moisture and protein contents were lower. GABA contents (61.04 mg/kg) retained in GBGR with 5 min-roasting time. Fat, crude fibers, total phenolic (TPC), total anthocyanin (TAC) contents and DPPH scavenging capacity did not significant change according to roasting time. Regarding brewing time with water at 98°C, L* and h° values of GBGR drink decreased. Higher TPC, TAC and DPPH scavenging capacity of GBGR drink were induced by using short brewing time 3-5 min). Therefore, roasting and brewing time of 5 and 3 min, respectively, were recommended for healthy drink production from GBGR. GABA concentration of 0.17 mg/150 mL was detected in finished product. For consumer acceptability test, GBGR drink was liked moderately and 77% of consumers would like to buy the product. Physical, chemical and microbial changes of the product prototype during storage time of 98 days were accepted following the Thai agricultural standard TAS 4404-2012 Good manufacturing practices for germinated brown rice standard.

Introduction

Rice is not only a stable food for Thai people but also an important economic crop. In Thailand, varieties of rice are cultivated, each of them possesses unique characteristics, especially pigmented rice such as riceberry, aromatic black rice (Hom-nil rice) and black glutinous rice. Pigmented rice is a kind of typical whole grain with colored pericarp. Pericarp is a part that provides protection for the seed coat and give colors to the rice such as light brown, red, purple and black (Ito & Lacerda, 2019). In the past, pigmented rice was

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demanded by consumers because of its specific characteristics including medical properties and exclusive taste (Ahuja et al., 2007). In comparison of white rice, pigmented rice has recently received increasing attention due to higher nutrients and antioxidants.

Black glutinous rice (*Oryza sativa* L.) is a well-known local pigmented rice in Thailand and characterised by dark purple pigment in the hull and pericarp and distinctive smell. The color of riceberry is determined by the different anthocyanins (Abdel-Aal et al., 2006). Anthocyanin is a flavonoid which belongs to polyphenol and responsible for antioxidant capacity. The significant roles of anthocyanins have been reported to alleviate several chronic diseases such as cardiovascular disease, hypertension and cancer, resulting from their antioxidant capacity. Black glutinous rice is commonly processed using steaming method and consumed as main dish. In addition, it has also been applied as ingredient for snacks or desserts (Loypimai et al., 2016). However, black glutinous rice is not cultivated in Thailand as an export crop; therefore, it could be processed as healthy food product in order to add value and increase commercial chances.

In this study, germinated black glutinous rice (GBGR) was used as main ingredient for healthy food development because of its higher nutritive values and larger amount of bioactive compounds, when compared to normal rice. During seed germination, the content and bioactivity of active compounds such as free amino acid, vitamins, and polyphenols increase (Chu et al., 2020). Additionally, gamma-aminobutyric acid (GABA), a natural occurring free amino acid, is greatly synthesized during germination. Several studies have been reported that a neurotransmitter GABA benefits human health by reducing blood pressure, accelerating the metabolism in the brain, inhibiting cancer cells and promoting relaxation and sleep (Klaykruayat et al., 2020). To my knowledge, application of GBGR as healthy food and beverage is very limited. Therefore, rice drink in a sachet using GBGR was developed in this study and the effects of roasting and brewing time on color, chemical compositions and antioxidant capacity were determined in order to obtain a high quality product that is desirable to consumers. The final product made from GBGR might be an alternative functional beverage which is a source of dietary bioactive compounds including phenolics, anthocyanins and GABA. Furthermore, manufacturing processes of this product was also friendly to environment and not complicated. Finally, this study could provide the efficient and suitable process of production of functional drink from GBGR in a sachet and might be useful commercially for healthy food production from black glutinous rice.

Materials and methods

1. Materials and chemicals

Black glutinous rice was purchased from local market in Bangkok in 2018. Folin-Ciocalteu's reagent, gallic acid, 2,2-diphenly-1-picrylhydrazyl (DPPH) and Trolox was purchased from Sigma-Aldrich (St. Louise, USA). Sodium carbonate, potassium chloride, sodium acetate and ethanol were purchased from Merck (Germany). All chemicals used are analytical grade. Sodium hypochlorite used is food grade.

2. Preparation of GBGR

The method was modified from Charoenthaikij et al. (2009). Black glutinous rice seeds were screened, washed with clean water. The seeds were soaked into 0.07% sodium hypochlorite for 30 min and washed with sterile water. In germination process, the seeds were soaked into sterile water with the ratio of 1:5 (rice: water) for 12 h at 35°C. After that, the soaked seeds were washed with sterile water. The seeds were put on wet cheese cloth in stainless steel tray which contained 700 mL sterile water and cover with another wet cheese cloth. Then, the soaked seeds were incubated at 35±2°C for 24 h. After germination, the germinated seeds were dried at 45°C until the moisture content was lower than 10%. The dried germinated seeds were kept at -40°C until analysis.

3. Effect of roasting time on physical and chemical properties

The effect of roasting time was determined in this study and the method was modified from Noosing et al. (2014). The germinated rice was roasted for 0, 5, 10 and 15 min at 100°C using hot-air roasting machine (Tefal, Model FZ 7072, Franch). In each sample, physical and chemical properties were investigated. The color values (L*, a*, b*, Chroma (C*) and hue (h°)) were determined as physical properties. The a_w value, proximate analysis, total phenolics, total anthocyanins, GABA and DPPH scavenging capacity were investigated for chemical properties.

4. Effect of brewing time on physical and chemical properties

The effect of brewing process was investigated by using different time. The method was modified from Noosing et al. (2014) and Burillo et al. (2018). The

roasted GBGR of 10 g were put into a sachet and sealed tightly (Fig. 1). For brewing, the roasted GBGR in the sachet was dipped into 150 mL water with temperature of 98°C at 3, 5, 7, 10 and 15 min. The sample solution was kept at room temperature until the temperature reached at 25°C and then kept at -40°C until analysis physical and chemical properties. For the physical properties, the values of L* a* b* C* and h° values were analyzed. The total soluble solids, pH, total phenolics, total anthocyanins, GABA and DPPH scavenging capacity) were investigated for the chemical properties.

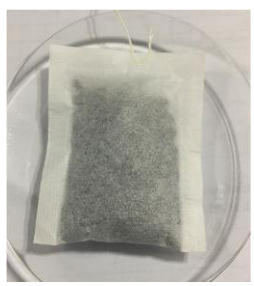

Fig. 1 Rice drink made from germinated glutinous black rice in sachet

5. Sample extraction

Roasted GBGR were extracted using the method of Kim & Lee (2002) and Wrolstad et al. (2005) with some modifications. The powder samples were prepared by grinding and sieving with 80 mesh sieve (Endecotts, England). A gram of roasted GBGR powder was mixed with 12 mL HCl in methanol (1% v/v), homogenized on ice for 2 min and sonicated at 4°C for 15 min using homogenizer (T10 basic Uitra-turrax, IKA® Staufen, Germany) and ultra-sonicator (CP 360T, CREST Ultrasonic, Malaysia), respectively. After that, the mixture was centrifuged at 11,000 rpm for 30 min at 4°C using refrigerated centrifuge (Sorvall RC 6-Plus, Thermo fisher, Thermo Scientific, Germany). The supernatant was collected and the residue was re-extracted with 13 mL HCl in methanol (1% v/v). After the second extraction, the volume of extract was adjusted to 25 mL in volumetric flask. The extract was stored at -40°C until analysis. The extracts were used in determination of total phenolics, total anthocyanins, DPPH scavenging capacity and GABA content of roasted GBGR.

6. Color measurement

Color parameters were quantified as the physical properties. The roasted GBGR and the drink from roasted GBGR after brewing were sampled as 100 g and 100 mL, respectively. Then, each sample was put into clear plastic tray before color measurement. The values of L*, a*and b* (CIE system) were generated by using colorimeter (Model WF30, U.S.A). The L* represents color lightness (0 = black and 100 = white), the a* represents the red (positive values) or green (negative values) and the b* represents yellow (positive values) and blue (negative values). The C* value represents color intensity. The h° value was expressed in 0-360 degrees with red color at 0°; yellow color at 90°; green color at 180°; blue color at 270° and magenta color at 360°. The C*and h° values were calculated using the values of a* and b* according to the equations; $C^* = (a^{*2} + b^{*2})^{1/2}$ and $h^\circ = \tan^{-1}(b^*/a^*)$, where $a^* > 0$ & $b^* \geq 0$; $h^\circ = \tan^{-1}(b^*/a^*)+360$, where $a^* > 0$ & $b^* > 0$ (McGuire, 1992). The colorimeter took 10 readings and generated an average for each sample. The experiment was done in replication with three measurements.

7. Determination of chemical properties

7.1 Water activity (a_w)

Water activity (a_w) were determined using a_w meter (Sprint Novasina, Model TH-500, Switzerland). The powdered samples were applied for a_w measurement. The experiment was done in duplication with triplicate measurements

7.2 Proximate analysis

Proximate analysis was performed using standard methods of Association of Official Analytical Chemists (AOAC, 2000). The contents of moisture, crude fat, crude protein, crude fiber and ash were determined. Carbohydrate content in sample was obtained by calculation. The experiment was done in duplication with triplicate measurements

7.3 Total phenolics

Total phenolic contents using Folin-Ciocalteu's reagent was modified from Maizura et al. (2011). The sample or standard (0.4 mL) were mixed with 2 mL of 10% (v/v) Folin-Ciocalteu's reagent and incubated at room temperature for 4 min. Then, 1.6 mL of 5% (w/v) sodium carbonate ($NaCO_3$) was added and stood for 30 min at ambient temperature. The mixture was centrifuged at 5,000 rpm for 10 min before absorbance measurement at 765 nm using UV-visible Spectrophotometer (T60, PG instrument, England). Gallic acid (0-100 µg/mL) was used as standard. The experiment was done in duplication with triplicate measurements

7.4 Total anthocyanins

The total anthocyanin contents were determined using a pH-differential method which was modified from

Giusti & Wrolstad (2005). Each 0.5 mL extract was separately mixed with 4.5 mL of 0.4 M hydrochloric acid–sodium acetate buffer (pH = 4.5) and 4.5 mL of 0.025 M hydrochloric acid–potassium chloride buffer (pH = 1). Absorbances at 510 and 700 nm were measured using a spectrophotometer (T10 basic, Uitra-turrax, IKA® Staufen, Germany). Total anthocyanins were calculated using the below equation.

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

Where A is $(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$; MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF is the dilution factor; l is the cell length (1 cm.); ε is the molar extinction coefficient (26,900 L × $cm^{-1} \times mol^{-1}$).

The total anthocyanin contents were expressed as cyaniding-3-glucoside equivalents. The experiment was done in duplication with triplicate measurements

7.5 Determination of GABA

GABA contents in samples were determined using HPLC (Agilent 1100 series, Agilent Technologies, Santa Clara, CA, U.S.A). The analysis was modified from In-house method based on Thai agricultural standard TAS 4003-2012 (2012). A reverse phase HPLC system consisted of 5 mm Symmetry® (4.6x250 mm) column (Waters, Milford, MA, U.S.A) and fluorescence detector were used in this study. The column was maintained at 25°C. The mobile phase used was 0.05% trifluoroacetic acid in water (A), acetronitrile (B) and methanol (C). The solvent isocratic elution was performed as 55% (A), 25% (B) and 20% (C). The flow rate was 1 mL/min. The injection volume of extract was 5 µL. GABA was detected using excitation and emission wavelengths at 270 and 315 nm, respectively. The GABA content of sample was calculated by comparing the standard curve of GABA standard. The GABA content of each was determined in duplicate.

8. DPPH scavenging capacity determination

DPPH is a highly stable synthetic free radical. The solution of DPPH was freshly prepared using 50% ethanol. DPPH scavenging capacity was investigated using the method of Du et al. (2009). Briefly, the extract 1 mL was mixed with 1 mL of 200 µM DPPH solution. After incubation at room temperature for 30 min, the absorbance was measured at wavelength of 515 nm. Distilled water was used as blank and Trolox (0-50 µM) was used as standard. The experiment was done in duplication with triplicate measurements.

9. Consumer acceptability test

A consumer test consisting of 100 participants, untrained panelists, was administered in the sensory facilities. The prototype of rice drink made from GBGR was prepared and presented in random order with a three-digit random number coding on a plastic cup to minimize any bias. For sample preparation, roasted GBGR in a sachet was soaked in 150 mL of water at 98°C for 3 min before presenting to the panelists. The sample was placed inside plastic cup on a plastic tray along with a glass of water and a paper ballot sheet to evaluate five attributions: color, appearance, flavor, taste and overall. Each untrained panelist tasted and rated the sample based on the degree of liking on a 9-point hedonic scale (where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = liking slightly, 7 = like moderately, 8 = like very much and 9 = like extremely).

10. Shelf life study

Roasted GBGR is a dried product which was recommended to keep at room temperature. In this study, the rice drink from roasted GBGR in a sachet was packed in aluminum foil bag and stored at 35°C (average room temperature in Thailand) for 98 days. The product prototype was investigated its physical, chemical and microbial properties for every 7-day interval. Physical and chemical properties were analyzed as color and a_w, respectively. Microbial quality of product, total plate count, yeast and mold, and *Bacillus cereus* (*B. Cereus*) were tested following U.S. Food and Drug Administration, Bacteriological Analytical Manual (2001a; 2001b; 2001c).

11. Statistical analysis

Completely randomized design (CRD) was performed to study the effects of roasting and brewing process of rice drink in a sachet from roasted GBGR. The randomized completely block design (RCBD) was used to study the consumer acceptability test. The results were expressed as average ± standard deviation (S.D.). The difference between treatments was analyzed using analysis of variance (ANOVA) with Duncan's new multiple range. The difference was considered to be statistically significant at $p \leq 0.05$.

Results and discussion

1. Effect of roasting time on physical and chemical properties

Roasting process is an important step for improving

taste, color, texture, appearance and flavor of products, especially edible seeds including rice (Bagheri et al., 2019). In this study, GBGR was roasted at 100°C for 0, 5, 10 and 15 min. Color (L*, a*, b*, C* and h° values) and chemical compositions including a_w, moisture, ash, crude fiber, crude protein, crude fat and carbohydrate, total phenolics, total anthocyanins and GABA contents were investigated in the dif ferent 4 samples. The results showed that color of GBGR was significantly affected by roasting time as showed in Table 1 ($p \leq 0.05$).

Table 1 Color values of germinated black glutinous rice after roasting at different times

Color	Roasting time (min)			
	0	5	10	15
L*	$21.07^{a} \pm 0.39$	$15.49^{b} \pm 0.21$	$20.73^{a} \pm 0.75$	$21.11^{a} \pm 0.87$
a*	$2.77^{b} \pm 0.06$	$3.27^{a} \pm 0.34$	$1.63^{c} \pm 0.10$	$1.21^{c} \pm 0.05$
b*	$3.51^{b} \pm 0.15$	$4.30^{a} \pm 0.06$	$4.72^{a} \pm 0.52$	$4.92^{a} \pm 0.01$
C*	$4.60^{d} \pm 0.08$	$5.41^{c} \pm 0.17$	$7.00^{b} \pm 0.13$	$8.25^{a} \pm 0.13$
h°	$51.47^{d} \pm 1.92$	$67.93^{c} \pm 0.86$	$71.62^{b} \pm 0.69$	$78.61^{a} \pm 0.79$

Remark: The results were expressed as average ± standard deviation. The difference letters among different roasting times represented significant difference at $p \leq 0.05$.

The L*values of the 10 and 15 min-roasted samples were higher than those of the 5 min-roasted sample. However, the L* value of the 10 and 15 min-roasted samples were not significantly different from control (no roasting). The 5-min roasted sample possessed the highest positive value of a* which represented intense red color. The yellowness of samples was expressed as positive b* values and these values increased when the roasting time increased, similarly to C* and h° values of samples. The results interpreted that lighter and more yellowness induced by longer period of roasting, similar to the results of Bagheri et al. (2019) and Zeng et al. (2017). It might be due to thermal oxidation and degradation of polyphenols, especially anthocyanin. Furthermore, brown pigment from Maillard reaction might also be generated. In the other hand, the sample with 5 min-roasting time exposed darker and more redness. Possibly, the color of the sample was darker due to color of anthocyanin pigment in pericarp of GBGR. However, degradation of anthocyanin and generation of brown pigment from Maillard reaction might also slightly occur.

Table 2 showed changes of chemical compositions and DPPH scavenging capacity of GBGR after roasting. Roasting time significantly affected on a_w, moisture, crude protein, ash and carbohydrate contents in samples

Table 2 Chemical compositions of germinated black glutinous rice after roasting at different times

Chemical composition	Roasting time (min)			
	0	5	10	15
a_w	$0.66^{a} \pm 0.04$	$0.23^{b} \pm 0.02$	$0.14^{c} \pm 0.03$	$0.08^{d} \pm 0.00$
Moisture content (%)	$12.49^{a} \pm 0.18$	$4.59^{b} \pm 0.20$	$3.63^{c} \pm 0.04$	$2.06^{d} \pm 0.05$
Crude fat^{ns} (%)	3.31 ± 0.03	3.33 ± 0.26	3.66 ± 0.24	3.51 ± 0.12
Crude protein (%)	$17.84^{a} \pm 0.13$	$16.55^{b} \pm 0.09$	$16.54^{b} \pm 0.02$	$16.47^{b} \pm 0.05$
Ash (%)	$1.07b \pm 0.01$	$1.11b \pm 0.01$	$1.12b \pm 0.04$	$1.19^{a} \pm 0.01$
Crude fiber^{ns} (%)	2.34 ± 0.22	2.20 ± 0.10	2.40 ± 0.13	2.47 ± 0.02
Carbohydrate (%, by difference)	$65.29^{c} \pm 0.34$	$74.42^{b} \pm 0.28$	$75.05^{b} \pm 0.05$	$76.06^{a} \pm 0.11$
Total phenolics^{ns} (mg gallic acid equivalent/g dry weight)	1.48 ± 0.10	1.39 ± 0.04	1.48 ± 0.01	1.38 ± 0.04
Total anthocyanins^{ns} (mg cyanidin-3-glucoside equivalent/g dry weight)	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
GABA (mg/kg)	$99.01^{a} \pm 2.05$	$61.04^{b} \pm 2.87$	$60.76^{b} \pm 2.04$	$40.42^{c} \pm 0.28$
DPPH scavenging capacity^{ns} (mg Trolox equivalent/g dry weight)	124.65 ± 0.78	127.59 ± 1.63	126.31 ± 0.27	124.31 ± 1.32

Remark: The results were expressed as average ± standard deviation. The difference letters among roasting times represented significant difference at $p \leq 0.05$. ns: not significant at $p > 0.05$.

($p \leq 0.05$). As roasting time increased, a_w values and moisture content decreased. The reduction of aw value and moisture content in roasted GBGR was caused by evaporation. When heat is transferred to food with enough temperature and time, a difference of vapor pressures between surface and inside of food sample was induced, resulting evaporation of water (Yuenyongputtakal et al., 2017). Protein content in GBGR also declined when roasting time increased because protein denaturation and oxidation of some amino acids occurred during roasting (Yuenyongputtakal et al., 2017). Similar result was also found by Yenrina et al. (2019), who reported that roasting process (100 – 120°C for 30 min) diminished protein content in black glutinous rice. However, ash and carbohydrate contents enhanced. Increased ash with roasting time could be caused by decreasing in moisture and some antioxidants, as also found in Robusta coffee (Saloko et al., 2019). Similar results also found in cocoa (Djikeng et al., 2018) but that was not found in asparagus bean flour (Shah et al., 2015). Possibly, retained content of ash in food after processing might be associated with type and amount of mineral present in Oboh et al. (2010) and Nzewi & Egbuonu (2011). For carbohydrate content, the content was obtained by calculation and this value depended on fat, protein, moisture and ash contents in sample. In the other hand, fat and crude fiber contents did not depend on roasting time ($p > 0.05$). Roasting temperature and time used in this study was not significantly affected on TPC and TAC in roasted GBGR. The results were similarly to Ferreira et al. (2016), who

reported that the TPC of peanut with black testa were steady after roasting process using temperature of 175°C for 60 min. Yan et al. (2014) also found that some phenolics showed very minor decomposition at 200°C for 40 min. The degradation of anthocyanins in potato was induced by the rupture of glycosidic moiety and the formation of chalcones, when was subjected to temperature higher than 100°C (Nayak et al., 2011). However, temperature and time were not only caused by the change of anthocyanin molecules during processing, food matrix also associated. DPPH scavenging capacity of all samples was also not influenced by roasting time. GABA was also determined in the roasted GBGR and the representative chromatogram of GABA was showed in Fig. 2. As showed in Table 2, higher content of GABA remained in samples with lower duration time of roasting, similar to the results of Suppavorasatit et al. (2015) and Prommakool et al. (2014). They also found that reduction of GABA contents was induced by heat processes with long time duration. Reduction of GABA content in sample could be destroyed by heat in food processing (Tumpanuvatr et al., 2018). Therefore, the 5 min-roasted GBGR was chosen for further study due to highest GABA content and short processing time.

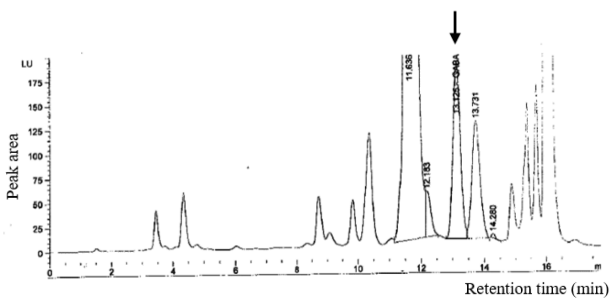

Fig. 2 Representative chromatogram of GABA in germinated black sticky rice. The GABA peak was marked with an arrow at retention time at 13.1 min.

2. Effect of brewing time on physical and chemical properties

For sample preparation in this study, GBGR was roasted at 100°C for 5 min. Ten grams of roasted sample was packed into a sachet (Fig. 1) before brewing at different times (3-15 min). During brewing, the temperature of water used was 98°C. After cooling, the color of the obtained rice drink was determined and reported as L*, a*, b*, C* and h° values. The results showed that brewing time significantly affected on L* and h° values ($p \leq 0.05$), whereas the a*, b* and C* values were not significantly different among samples ($p > 0.05$) (Fig. 3 and Table 3).

The GBGR drink possessed the highest value of L* when the sample was brewed at 7 and 10 min whereas, the GBGR drink with 15 min-brewing time showed darker color (the lowest L* value). The h° values were within the range of 270-360°, representing color shade of blue and red. This product color was possibly due to anthocyanin. Table 4 showed changes of chemical compositions of rice drink from GBGR at different brewing times (3-15 min). The pH values of rice drink were higher when brewing time increased ($p \leq 0.05$).

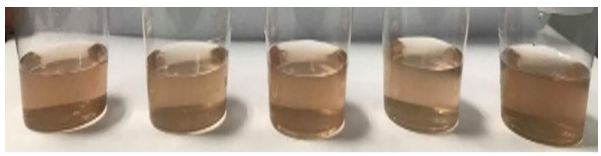

Fig. 3 Rice drink from germinated glutinous black rice after roasting at 100 ºC, 5 min and brewing at 3 min (a), 5 min (b), 7 min (c), 10 min (d) and 15 min (e)

Table 3 Color values of rice drink from germinated black glutinous rice (5 min roasting) at different brewing times

Color value	Brewing time (min)				
	3	5	7	10	15
L*	$42.17^{b} \pm 0.09$	$42.35^{b} \pm 0.50$	$44.79^{a} \pm 0.37$	$44.87^{a} \pm 0.49$	$40.29^{c} \pm 0.60$
a* ns	0.67 ± 0.02	0.83 ± 0.14	0.82 ± 0.16	0.87 ± 0.09	0.86 ± 0.01
b* ns	-1.45 ± 0.28	-1.33 ± 0.03	-1.36 ± 0.09	-1.33 ± 0.07	-2.22 ± 0.30
C* ns	1.77 ± 0.07	1.58 ± 0.04	1.58 ± 0.25	2.29 ± 0.30	1.34 ± 0.11
hº	$292.72^{bc} \pm 3.70$	$299.32^{b} \pm 3.70$	$306.07^{ab} \pm 1.21$	$314.41^{a} \pm 11.21$	$281.43^{c} \pm 2.21$

Remark: The results were expressed as average ± standard deviation. The difference letters among roasting times represented significant difference at $p \leq 0.05$. ns: not significant at $p > 0.05$

Table 4 Chemical compositions and antioxidant capacity of rice drink from germinated black glutinous rice (5 min-roasting time) at different brewing times

Chemical composition	Brewing time (min)				
	3	5	7	10	15
Total soluble solids (°Brix)	<1±0.00	<1±0.00	<1±0.45	<1±0.00	<1±0.00
pH	$6.28^{c} \pm 0.01$	$6.38^{b} \pm 0.05$	$6.29^{c} \pm 0.02$	$6.45^{b} \pm 0.01$	$6.55^{a} \pm 0.05$
Total phenolics (mg gallic acid equivalent/150 mL)	$4.36^{a} \pm 0.31$	$3.64^{bc} \pm 0.11$	$3.29^{c} \pm 0.19$	$3.51^{c} \pm 0.06$	$4.07^{ab} \pm 0.12$
Total anthocyanins (mg cyaniding-3-glucoside equivalent/150 mL)	$0.07^{a} \pm 0.01$	$0.07^{a} \pm 0.01$	$0.04^{c} \pm 0.00$	$0.04^{bc} \pm 0.01$	$0.05^{b} \pm 0.01$
DPPH scavenging capacity (mg Trolox equivalent/150 mL)	$803.16^{a} \pm 9.54$	$780.88^{a} \pm 12.29$	$708.95^{b} \pm 20.4$	$715.79^{b} \pm 37.4$	$662.63^{b} \pm 7.45$

Remark: The results were expressed as average ± standard deviation. The difference letters among roasting times represented significant difference at $p \leq 0.05$.

The value of pH was in range of 6.28-6.55. The color of anthocyanin is depended on pH. In acidic condition (pH 4-5), anthocyanins appear as red but turn blue when the pH increases (Laleh et al., 2006; Khoo et al., 2017). In acidic condition, flavylium cation (red color) is formed and stable at low pH. At increasing pH condition, anthocyanin is transformed as carbinol base and chalcone structures, followed by formation of anionic quinonoidal species (blue-purple color). Therefore, for rice drink from GBGR, the carbinol base and charcones might increase, while flavylium decreased, resulting in a slightly reddish brown color (as shown in Fig. 3). Brewing time with 3-15 min did not change total soluble solids ($p>0.05$). TPC and TAC also significantly affected by brewing time ($p\leq0.05$). The 3 and 5 min-brewing rice drink contained the highest amount of TAC (0.07 mg cyaniding-3-glucoside equivalent/150 mL). TAC in the product was in the range of 0.04-0.07 mg cyanidin-3-glucoside equivalent/150 mL (0.004-0.007 mg/g) which was similar to the amounts found in boiled black rice (*Oryza sativa* L. *Japonica*). Noorlaila et al. (2018) reported that boiled rice (*Oryza sativa* L. *Japonica*) contained total anthocyanins as 0.006 - 0.008 mg/g, whereas the anthocyanin content in raw rice was 0.02 mg/g. Cooked black rice incurred 60-70% loss of total anthocyanin. Difference contents of anthocyanin in food samples depended on type of anthocyanin, food condition, temperature, oxygen and analysis method. The GBGR drink with 3-min brewing time also contained the highest TPC (4.36 mg gallic acid equivalent/150 mL).The results of this study were similar to Braud et al. (2015) who found that short duration time (5-7 min) of brewing contained more TPC and TAC than longer time (15-30 min). On the other hand, Burilllo et al. (2018) reported that tea phenolics (gallic acid, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate) increased as brewing time increased, resulting in enhancement of tea astringent. DPPH scavenging capacity was investigated in this study in order to determine antioxidant capacity of the rice drink.The results revealed that antioxidant capacity decreased as brewing time increased (Table 4). The highest values of DPPH scavenging capacity was showed in the sample with 3 and 5 min-brewing time. The antioxidant capacity of the rice drink might be due to its total phenolics and total anthocyanins contained in. Total phenolic content and total anthocyanins were lower since the phenolic compounds were obviously decomposed at high temperature and longer time, responsible to lessen DPPH scavenging capacity. As the results, GBGR drink which brewed with 150 mL of water with 98°C for 3 min was suggested in this study because this condition induced the highest total phenolics, total anthocyanins and strongest capacity of DPPH scavenging. GABA contents was determined in the GBGR drink with 3 min-brewing time and the drink contained 0.17±0.01 mg/150 mL.

3. Consumer acceptance

Sensory evaluation of the product prototype obtained from 100 questionnaire respondents and shown in Table 5. The prototype was the rice drink from GBGR with 5-min roasting time and brewed with 98°C water for 3 min. Most of respondents were female, accounting for 80%. Half of them (50%) were 18-30 years old and almost 60% were students. The sensory evaluation of liking score was based on 9-point hedonic scale. The respondents rated all attributes, including appearance, color, odor, flavor and overall preference, were almost 7 which indicated that the finished product was moderately liked. If the rice drink made from GBGR will be available in the market, 77% of respondents will buy.

Table 5 Mean liking scores of rice drink made from germinated black glutinous rice

Sensory attribute	Mean±S.D.
Appearance	6.95±0.64
Color	6.83±0.67
Odor	6.95±0.63
Flavor	6.89±0.47
Overall preference	6.84±0.65

4. Shelf life of product prototype

The product prototypes of roasted rice drink in a sachet made from GBGR were tightly packed in aluminum foil bag and stored at 35°C for 98 days. In every 7-day interval, the product prototypes were sampled in order to determine physical, chemical and microbiological properties. The results of physical properties of the rice drink were showed in Table 6 as color values in CIE systems. Significant change of L* value was not detected along with storage time of 98 days when stored at 35°C ($p>0.05$), whereas a*, b*, C* and h° values were significantly different ($p\leq0.05$). The positive values of a* (redness) and the negative values of b* (blueness) increased, indicating that the sample showed more redness and blueness. The highest values were shown at day 63 for a* value and in between day 63 and day 70 for b* value. The h° values were in range of 270°-360°. The results indicated that the drink samples expressed

dark color during storage time of 98 days at 35°C. Attribution of darker color of anthocyanin-rich samples during storage was caused by oxidation of double bond in anthocyanin molecule, depending on anthocyanin type, temperature and oxygen content (Stintzing et al., 2002).

Table 6 Color (L*, a*, b*, C* and h° values) of rice drink in a sachet made from germinated black glutinous rice stored at 35°C for 98 days

Day	Color value				
	L*^{ns}	a*	b*	C*	h°
0	39.15 ± 0.51	0.61^{bc} ± 0.07	-3.04^{fg} ± 0.17	3.07^{a} ± 0.17	281.26^{de} ± 1.38
7	39.36 ± 0.37	0.42^{c} ± 0.11	-2.82^{e} ± 0.07	3.15^{a} ± 0.13	278.47^{ef} ± 2.28
14	39.23 ± 0.31	0.65^{b} ± 0.17	-2.56^{bc} ± 0.14	3.15^{a} ± 0.13	284.44^{c} ± 4.40
21	39.44 ± 0.70	0.49^{de} ± 0.11	-2.90^{ef} ± 016	2.86^{bc} ± 0.06	279.58^{e} ± 2.25
28	38.81 ± 0.24	0.41^{e} ± 0.07	-2.76^{de} ± 0.10	2.65^{de} ± 0.10	278.56^{ef} ± 1.54
35	38.71 ± 0.37	0.30^{f} ± 0.15	-2.74^{de} ± 0.14	2.92^{b} ± 0.12	276.33^{f} ± 3.06
42	39.03 ± 0.26	0.53^{cde} ± 0.08	-2.65^{cd} ± 0.12	2.77^{cd} ± 0.07	292.40^{a} ± 1.08
49	38.38 ± 0.29	0.52^{cde} ± 0.18	-3.10^{g} ± 0.14	2.77^{cd} ± 0.14	279.64^{e} ± 3.47
56	38.33 ± 0.40	0.60^{bcd} ± 0.13	-2.65^{cd} ± 0.12	2.70^{de} ± 0.11	282.82^{cd} ± 2.58
63	40.21 ± 0.98	1.08^{a} ± 0.09	-2.12^{a} ± 0.38	2.72^{de} ± 0.12	276.52^{f} ± 3.79
70	39.42 ± 0.83	0.67^{b} ± 0.06	-2.09^{a} ± 0.17	2.53^{f} ± 0.20	285.09^{bc} ± 1.66
77	39.11 ± 0.36	0.69^{b} ± 0.08	-2.49^{bc} ± 0.12	2.58^{ef} ± 0.11	276.42^{f} ± 3.68
84	39.04 ± 0.45	0.58^{bcd} ± 0.08	-2.62^{cd} ± 0.17	2.62^{ef} ± 0.10	283.47^{cd} ± 2.37
91	38.38 ± 0.29	0.52^{cde} ± 0.18	-2.45^{b} ± 0.18	2.52^{f} ± 0.17	279.70^{e} ± 3.56
98	38.88 ± 0.54	0.67^{b} ± 0.55	-3.10^{g} ± 0.14	2.30^{g} ± 0.25	287.57^{b} ± 3.24

Remark: The results were expressed as average ± S.D. (n=2)
The small letters (a-g) indicted significantly difference among samples at p≤0.05
ns: no significant difference (p>0.05)

Chemical changes of rice drink were shown in Table 7 and the results showed that moisture content and a_w value were significant enhanced when storage time was extended (p≤0.05). The increasing of moisture content and a_w during storage can either depended on moisture permeability between sample and surrounding environment or absorption or chemical or biological reactions in materials (Razak et al., 2018; Östbring et al., 2020). However, the changes of water in roasted GBGR drink in a sachet still in an acceptable range (within 12% moisture content; $a_w < 0.6$) (Cervenka et al., 2006). GBGR drink in a sachet is a dried product which possessed a_w and moisture content in the range of 0.28-0.40 and 5.54-7.92%, respectively. For dried and starchy foods, yeast and mold can grow well at temperatures in the range 25-37ºC and *Bacillus sp.* can grow in the temperature range of 10-42ºC (Rachtanapun & Tangnonthaphat, 2011; Pexara & Govaris, 2010). Therefore, yeast and mold as well as *B. Cereus* can be used as indicator for food safety of GBGR drink in a sachet when stored at 35°C. The microbial results showed that total plate count, yeast and mold and *B. cereus* in the sample stored at 35°C for 98 days were in the range of $2.0x10^5$–$6.0x10^5$ CFU/g, less than 10 CFU/g and in range of $1.0x10^2$-$3.4x10^2$ CFU/g, respectively (Table 8) which were under the Thai agricultural standard TAS 4404-2012 (2012) Good manufacturing practices for germinated brown rice standard. Regarding the standard, total microorganisms, yeasts and molds as well as *B. cereas* should not exceed $1x10^6$, 500 and $1x10^3$ colony/g sample, respectively. Thus, it could be interpreted that roasted GBGR drink in a sachet which was packed in aluminum foil bag and stored at 35°C for 98 days was safe for consumers.

Table 7 Moisture content and aw value of rice drink in a sachet made from germinated black glutinous rice stored at 35°C for 98 days

Day	Moisture content (%)	a_w
0	5.54^{e} ± 0.75	0.28^{g} ± 0.10
7	6.18^{de} ± 0.06	0.31^{f} ± 0.01
14	6.23^{de} ± 0.31	0.32^{ef} ± 0.01
21	6.40^{cde} ± 0.29	0.34^{def} ± 0.00
28	6.45^{cde} ± 0.12	0.34^{def} ± 0.00
35	6.48^{cde} ± 0.17	0.34^{def} ± 0.01
42	6.52^{cde} ± 0.07	0.35^{cd} ± 0.01
49	6.53^{cde} ± 0.06	0.35^{cd} ± 0.03
56	6.82^{bcd} ± 0.70	0.37^{abc} ± 0.02
63	6.95^{bcd} ± 0.53	0.37^{abc} ± 0.02
70	7.01^{abcd} ± 0.57	0.37^{abc} ± 0.01
77	7.30^{abc} ± 0.02	0.37^{abc} ± 0.02
84	7.30^{abc} ± 0.48	0.38^{ab} ± 0.02
91	7.67^{ab} ± 0.35	0.40^{a} ± 0.04
98	7.92^{a} ± 0.73	0.40^{a} ± 0.02

Remark: The results were expressed as average ± S.D. (n=2)
The small letters (a-g) indicted significantly difference among samples at p≤0.05

Table 8 Total plate count, yeast and mold and Bacillus Cereus found in rice drink in a sachet made from germinated black glutinous rice during stored at 35°C for 98 days

Day	Microbiological test		
	Total plate count (CFU/g)	Yeast and mold (CFU/g)	*Bacillus cereus* (CFU/g)
0	$2.0x10^5$	<10	$2.0x10^2$
7	$2.6x10^5$	<10	$3.0x10^2$
14	$2.7x10^5$	<10	$1.0x10^2$
21	$2.5x10^5$	<10	$2.2x10^2$
28	$2.8x10^5$	<10	$1.8x10^2$
35	$3.8x10^5$	<10	$1.5x10^2$
42	$3.2x10^5$	<10	$1.8x10^2$
49	$4.0x10^5$	<10	$2.4x10^2$
56	$2.9x10^5$	<10	$1.8x10^2$
63	$3.2x10^5$	<10	$1.2x10^2$
70	$4.5x10^5$	<10	$2.2x10^2$
77	$3.7x10^5$	<10	$3.0x10^2$
84	$6.0x10^5$	<10	$1.7x10^2$
91	$3.0x10^5$	<10	$1.5x10^2$
98	$4.2x10^5$	<10	$3.4x10^2$

Remark: CFU: Colony forming unit

Conclusion

Roasting and brewing times significantly affected on color, chemical compositions and antioxidant capacity of rice drink made from GBGR. From this study, roasting time of 5 min and brewing time of 3 min were suggested in GBGR drink production since this condition induced higher levels of proteins, TPC, TAC, GABA and DPPH scavenging capacity as well as the product was also preffered by consumers. Physical, chemical and microbial changes of the product prototype during storage time of 98 days were accepted following the Thai agricultural standard TAS 4404-2012 Good manufacturing practices for germinated brown rice standard.

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