ผลของปริมาณข้าวกล้องข้าวเหนียวดำงอกต่อการพัฒนาผลิตภัณฑ์โยเกิร์ตในเชิงอาหารฟังชั่น

EFFECT OF GERMINATED BLACK GLUTINOUS BROWN RICE CONTENTS ON PRODUCT DEVELOPMENT OF FUNCTIONAL YOGURT

นิจฉรา ทูลธรรม ชัยวุฒิ บัวเนี่ยว*

***Nitchara Toontom*,** Chaiwut Bourneow*

คณะสาธารณสุขศาสตร์ มหาวิทยาลัยมหาสารคาม

Faculty of Public Health, Mahasarakham University.

****Corresponding author, e-mail:** nitchara@yahoo.com*

***Received:** September 19, 2018; **Revised:** November 30, 2018; **Accepted:** December 27, 2018*

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาผลิตภัณฑ์โยเกิร์ตในเชิงอาหารฟังชั่นที่มีปริมาณกรดแกรมม่าบิวทิริค (สารกาบา) สูง โดยใช้ข้าวกล้องข้าวเหนียวดำงอก (BGBR) ผลของระยะเวลาที่แตกต่างกัน (0 24 36 และ 48 ชั่วโมง) ในการงอกข้าวกล้องข้าวเหนียวดำต่อปริมาณสารกาบา สารแอนโธไซยานิน และฤทธิ์การต้านอนุมูลอิสระ DPPH จะถูกศึกษา โดยพบว่า BGBR ที่งอกด้วยระยะเวลา 48 ชั่วโมง มีปริมาณของสารกาบาเพิ่มขึ้นและมีฤทธิ์การต้านอนุมูลอิสระ DPPH มากที่สุด ($P \leq 0.05$) BGBR ที่งอกด้วยระยะเวลา 48 ชั่วโมง ถูกนำมาผลิตเป็นผลิตภัณฑ์โยเกิร์ต จำนวน 6 สูตร และวิเคราะห์หาปริมาณสารกาบา แอนโธไซยานิน ฤทธิ์การต้านอนุมูลอิสระ DPPH และจำนวนจุลินทรีย์ผลิตกรดแลคติกที่มีชีวิต รวมไปถึงคะแนนการยอมรับของผู้บริโภค (9-point Hedonic Scale, n = 200 คน) ความสัมพันธ์ของข้อมูลระหว่างค่าคุณสมบัติเชิงหน้าที่และคะแนนการยอมรับของผู้บริโภคถูกวิเคราะห์โดยเทคนิค Principal Component Analysis (PCA) พบว่าเมื่อเติมปริมาณ BGBR งอกเพิ่มขึ้น pH ของผลิตภัณฑ์โยเกิร์ตมีค่าลดลงจาก 4.44 ถึง 3.33 ขณะที่ค่าความเป็นกรดโดยรวมเพิ่มขึ้นจาก 0.67% ถึง 0.87% ผลิตภัณฑ์โยเกิร์ตที่เติม BGBR งอกในปริมาณร้อยละ 0 ถึง 80 มีปริมาณจุลินทรีย์ผลิตกรดแลคติกมีชีวิตอยู่ในช่วง 1.56×10^{10} ถึง 2.75×10^{10} CFU/มิลลิลิตร ซึ่งเป็นปริมาณที่เหมาะสมต่อการให้ประโยชน์ทางสุขภาพและเป็นไปตามมาตรฐานสมาคมโยเกิร์ตแห่งชาติของสหรัฐอเมริกาและสมาคมนมหมักและเครื่องดื่มที่ผลิตโดยแบคทีเรียผลิตกรดแลคติกของญี่ปุ่น สูตรที่มีปริมาณ BGBR งอกร้อยละ 70 ได้รับคะแนนการยอมรับต่อคุณลักษณะทางประสาทสัมผัสทุกด้านมากที่สุด ($P \leq 0.05$) ซึ่งสูตรนี้มีปริมาณสารกาบาเท่ากับ 55.5 มิลลิกรัม/กิโลกรัม ปริมาณสารแอนโธไซยานินเท่ากับ 0.08 มิลลิกรัม/กิโลกรัม ฤทธิ์การต้านอนุมูลอิสระ DPPH ที่ร้อยละ 50 (IC_{50}) เท่ากับ 0.18 กรัม ของกรดแกลลิค/มิลลิลิตร และปริมาณจุลินทรีย์ผลิตกรดแลคติกที่มีชีวิต เท่ากับ 1.65×10^{10} CFU/มิลลิลิตร ดังนั้นจึงน่าจะเป็นสูตรที่เหมาะสมในการผลิตผลิตภัณฑ์โยเกิร์ตต้นแบบที่เติม BGBR งอกในเชิงอาหารฟังชั่น

คำสำคัญ: ข้าวกล้องข้าวเหนียวดำงอก กาบา โยเกิร์ต การทดสอบผู้บริโภค

Abstract

This study aimed to produce functional yogurt with high γ-aminobutyric acid (GABA) by using germinated black glutinous brown rice (BGBR). Effect of different germination times (0, 24, 36 and 48 h) on GABA, anthocyanin and DPPH scavenging activity was investigated. It was found that the highest GABA content and DPPH scavenging activity was observed in the samples germinated at 48 h ($P \leq 0.05$). The 48 h-germinated BGBR were used for producing 6 yogurt formulas. Some properties of the samples were investigated such as GABA, anthocyanin content, DPPH antioxidants activity and viable LAB count. Also, consumer acceptance (9-point Hedonic Scale) of the products were evaluated by consumers (n=200). Principal Component Analysis (PCA) was applied to present relationships among functional properties and consumer acceptances. The results showed that pH of yogurt products decreased from 4.44 to 3.33 with amount of germinated BGBR increased. While, total acidity of the yogurt products increased from 0.67% to 0.87%. The viable LAB counts of yogurt adding 0-80% germinated BGBR were a rage of 1.56×10^{10} to 2.75×10^{10} CFU/ml. This was in the optimal range for promoting the health benefits and considering in accordance with the National Yogurt Association of the United States and Fermented Milks and Lactic acid Bacteria Beverages Association of Japan. Formula containing 70% of germinated BGBR was rated the highest liking scores (5.78-6.16) of all sensory attributes when compared with scores of the other formulas added germinated BGBR ($P \leq 0.05$). It contained GABA 55.5 mg/kg, anthocyanin 0.08 mg/kg, IC_{50} of DPPH 0.18 g equivalent to gallic acid/ml and LAB 1.65×10^{10} CFU/ml. Therefore, it is probably appropriate formula for producing prototypes product of yogurt added with germinated BGBR, which is valuable as functional yogurt.

Keywords: Germinated Glutinous Black Brown Rice, GABA, Antioxidant, Yogurt, Consumer Test

Introduction

Black glutinous rice (BGR) is a type of *Oryza sativa* L. and contained high amount of antioxidant (anthocyanin) in the pericarp (outer part of kernel). It is becoming popular in recent years because of its high nutritive value and and antioxidative properties [1]. The pericarp (outer part) of this rice is black due to its contained anthocyanin. BGR is efficient, and exhibits 2-fold stronger with respect to antioxidant activities of blueberries [2]. It is known that the germination process can increase the amount of phytochemicals in rice, such as vitamin B1, phenolic and γ-aminobutyric acid (GABA) [3-4]. Germinated brown rice (GBR) has decrease of carbohydrate and fat contents but its protein and vitamin E increased [5-6]. In addition, it is known that GBR has 2-fold increased amount of GABA when compared with non-germinated rice [7]. Furthermore, the GBR has high amounts of bioactive with health promoting benefits such as, antidiabetic, hypocholesterolemic and antiobesity [8-9].

Although GBR has higher nutritional value than non-germinated rice, only 3% of consumers consume this product [10]. Since, GBR has a hard texture, unattractive color, hard cooked. Also, it has short shelf life (about 3-6 months) because of rancidity as well as insect destruction. One way to promote more consumption of GBR is transformation of GBR into a food product which is easier to consume, such as yogurt. Yogurt is a fermented milk product with lactic acid bacteria. Lactic acid bacteria are recognized as a safe microorganism to prolong the life of food, enhance nutritional value, and improve flavor and quality of the product [11].

Objectives

This research aimed to study effect of different germination times (0, 24, 36 and 48 h) on GABA, anthocyanin and DPPH scavenging activity. Also, the optimum amount of germinated black glutinous brown rice (BGBR) adding in yogurt was determined. A commercial yogurt was used as a starter. The study focused on content of GABA and anthocyanin, antioxidant activity and viable lactic acid bacteria count in the product in order to be an alternative food for health conscious consumers.

Methods

Chemicals

Gamma butyric acid (GABA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acids were purchased from Sigma Chemical Co. Folin- Ciocalteu's phenol reagents, sodium carbonate, and acetonitrile and ethanol (HPLC grade) were purchased from Fluka Analytical Co. Ltd. Hexane, di-sodium hydrogen phosphate, and phenyl iso-thiocynate were purchased from Merk Co. Ltd. Man rogosa sharpe agar (MRS) was purchased from Hi-media Co. Ltd. Dutchie original yoghurt (0% fat yoghurt plain) and Dutch mill (whole milk) was a commercial yogurt and pasteurized milk which purchased from a convenience store.

Rice sample

The black glutinous brown rice (*Oryza sativa* var. glutinosa) was obtained from a local market in Borrabue district, Maha Sarakham, Thailand The quality of the samples was also inspected according to the Thai Agricultural Standard (TAS 4003-2012) [12].

Germinated of Black glutinous brown rice

Germination process was modified from that of Bourneow and Santimalai [13]. One hundred grams of BGBR sample were immersed in 300 mL of tap water at ambient temperature (25 $\pm$ 2°C) for 12 h. Every four hours of immersion, the used water was replaced throughout the course to minimize the fermented odour. After that, the sample were drained off and covered with a three layers of the humid cloth sheets [13-14]. The sample were collected at 24, 36 and 48 h of incubating time in the darkly place. At the attainment time, the samples were washed with distilled water, blotted on dried cotton cloth sheets, and kept in a plastic box at 4°C until use.

Process of producing germinated BGBR yogurt

The germinated BGBR which had the highest GABA content was selected to produce yogurt. The germinated BGBR were

immersed overnight in tap water (1:2 ratio) at ambient temperature (25 ± 2°C) and then cooked by rice cooker (1.8L KS-COM, Sharp.). After that, 1 Kg of the cooked rice was blended with 2 L of tap water by using a blender (350 W. LM2211BA, Moulinex). It was mixed with pasteurized milk (whole milk) in ratios of 0:100, 100:0, 80:20, 70:30, 60:40 and 50:50 (w/w), for producing 6 yogurt formulas (Table 1). Each formula used 50 g of sugar and 2 g of gelatin. All formulas were pasteurized at 80°C for 30 min and then cooled to 43°C. After that, each formula was added with commercial plain yogurt (100 g). All formulas were incubated at 43°C for 6 h and were then removed from the incubator and cooled to 15°C and stored at 4°C. The viable lactic bacteria counts of the products were conducted on 3rd day, post-production.

Table 1. Yogurt product formulas

Formulas	Total 1000 g		Sugar (g)	Gelatin (g)	Commercial plain yogurt (g)
	Germinated BGBR (g)	Pasteurized Milk (g)			
100% milk (0% germinated BGBR :100% milk)	0	1000	50	2	100
100% g-BGBR (100% germinated BGBR : 0% milk)	1000	0	50	2	100
80% g-BGBR (80% germinated BGBR :20% milk)	800	200	50	2	100
70% g-BGBR (70% germinated BGBR :30% milk)	700	300	50	2	100
60% g-BGBR (60% germinated BGBR :40% milk)	600	400	50	2	100
50% g-BGBR (50% germinated BGBR :50% milk)	500	500	50	2	100

Chemical Analysis

Determination of pH and total acidity

pH and total acidity of the samples was determined, according to the method of A.O.A.C. [15]. Sample was measured for the pH value at ambient temperature with a pH meter (Satorious, USA) which was calibrated with pH 4.0 and 7.0. To measure total acidity, 10 g of sample was mixed with 10 mL of distilled water, and titrated to pH 8.3 with 0.1 N NaOH (factor = 1.001). The amount of NaOH was used for calculation of total acidity expressed as lactic acid.

Determination of anthocyanin content

Anthocyanin content of the samples was determined, according to the modified pH differential method of Giusti and Wrolstad [16]. One-hundred microliter of the sample

extract was mixed with 5 mL of potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5), separately. The mixture was vortex vigorously 5 and then was stand for 15 min before centrifugation at 10,000 x *g* at 4°C for 20 min. The clear solution was then measured at 515 and 700 nm against a blank cell filled with distilled water in a UV-visible spectrophotometer (Cecil, Aquarius 7400). All measurements should be made between 15 min and 1 h after sample preparation, since longer standing times tend to increase observed readings. The anthocyanin content was calculated using the following equation (1):

$$\text{Anthocyanin content (mg/l)} = (A \times MW \times DF \times 1000)/(\varepsilon \times L) \quad (1)$$

where $A = [(A_{515}-A_{700})$ pH 1.0 – $(A_{515}-A_{700})$ at pH 4.5], MW is equal to 449.2 (molecular weight of cyanidin-3-glucoside), DF is the dilution factor of sample, and ε is the molar absorptivity of cyanidin-3-glucosideequal to 26,900.

Determination of γ-aminobutyric acid (GABA)

GABA content was determined using high performance liquid chromatography (HPLC) by the modified methods of Lee and others [14] and Ohtsubo and others [17]. Briefly, 2.5 g of ground rice or yogurt sample was mixed with 25 ml 70% ethanol, agitated in a vortex mixer for 10 min and subsequently incubated at 4°C for 12 h. After that, samples were centrifuged at 12,000 *x g* at 4°C for 20 min. The supernatant (500 µL) was evaporated and then dissolved in deionized water 50 µL before derivatized by 20% (v/v) trimethylamine in 50% methanol (v/v) and adding 30 µL of tri-fluoroacetic acid. Thereafter, the samples were then reconstituted in 500 µL of 0.1 M ammonium acetate, pH 6.5 (mobile phase A), centrifuged at 12,000 *x g* at 4°C for 5 min and filtered through a 0.22 µm nylon filter. The sample (10 µl) and a gradient mobile phase of (1.0 ml/min) was injected into a water symmetry reverse phase analytical column (Ultra C-18, 150 x 3.9 mm, 5 µm) at 40°C. The chromatograms were developed at a flow rate of 0.7 ml/min by eluting the sample in mobile phase A (0.1 M ammonium acetate, pH 6.5), and mobile phase B (acetonitrile and methanol 4.6:1, with the ammonium acetate buffer, pH 6.5) with the isocratic flow of 100% A for 15 min, gradient flow from 100% A to 100% B for ~30 min, until finally equilibrated. The system consisted of an HPLC (Waters 2690 Alliance, U.S.A.) connected to fluorescent detectors, excitation wavelength 270 nm and emission wavelength 315 nm.

Determination of DPPH scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the samples was measured according to the modified method of Brand-Williams and others [18]. The reaction mixture contained 3 ml DPPH working solution (4.73 mg of DPPH in 100 ml ethanol) to which was

added 100 µl samples. The mixture was shaken and held for 30 min in the dark at room temperature (30±1°C). The absorbance was then read at 515 nm using a UV-visible spectrophotometer (Cecil, Aquarius 7400). The inhibition percentage of the absorbance of the DPPH solution was calculated using the following equation (2):

$$\text{Inhibition } \% = [(A_{blank} - A_{sample}) \times 100] / A_{blank} \quad (2)$$

where, A_{blank} is absorbance of control blank, and A_{sample} is absorbance of sample extract.

Then, the DPPH radical scavenging activity was then calculated in the term of IC_{50} (concentration providing 50% inhibition/ scavenging). Gallic acid was used as standard.

Determination of viable lactic acid bacteria count in yogurt products

Determination of lactic acid bacteria (LAB) was conducted according to the modified method of Kim and others [19]. Briefly, 25 milliliter of samples were mixed with 225 ml of peptone water (0.1%) and then homogenized the mixture by stomacher for 2 min, and diluted using the ten-fold dilution method to count LAB. After that, 1 mL aliquot was transferred to a plate, and MRS agar was poured into the plate, and incubated at 37°C for 48 h. The standard plate count method was used to enumerate the bacteria, wherein the number of colonies was multiplied by the dilution factor and reported as the number of colony forming units (CFU) per milliliter of sample.

Consumer acceptance test on yogurt products

Acceptance on product sensory quality was evaluated for overall liking, appearance liking, color liking, flavor liking and taste liking on a 9-point category hedonic scale (9 = "like extremely" and 1 = "dislike extremely"). Two-hundred consumers, whose age ranged from 18 to 35 years were recruited. All consumers were presented with random 3-digit coded samples. Balance first-order and carry-over-effect design [20] was applied for serving plan on 6 germinated BGBR yogurt samples, within 1 testing sessions. The samples were presented to consumer who sat individually in testing booths.

Statistical analysis

A completely randomized design was used to study the chemical quality and lactic acid bacteria of the samples. The experimental design of consumer acceptance test was a randomized complete block. Data were subjected to analysis of variance (ANOVA). Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Principal Component Analysis (PCA) was applied to present relationships among consumer liking scores, chemical qualities and viable LAB count. Statistical analyses were performed by using the using SPSS packaging program (version 20).

Results

GABA, anthocyanin content and antioxidant activity of germinated BGBR at the different germination times

In this study, the functional properties namely, GABA, anthocyanin content and DPPH scavenging activity of the BGBR after 24, 36 and 48 h of germination were in Figure 1. After observing throughout the time course of germination (0, 24, 36 and 48 h), GABA content in the rice was increased with germination time increased. It reached to the maximum at 48 h of germination time which was at 0.80 g/kg. From the result, there was a significant in the production and accumulation of GABA by germination time ($P \leq 0.05$).

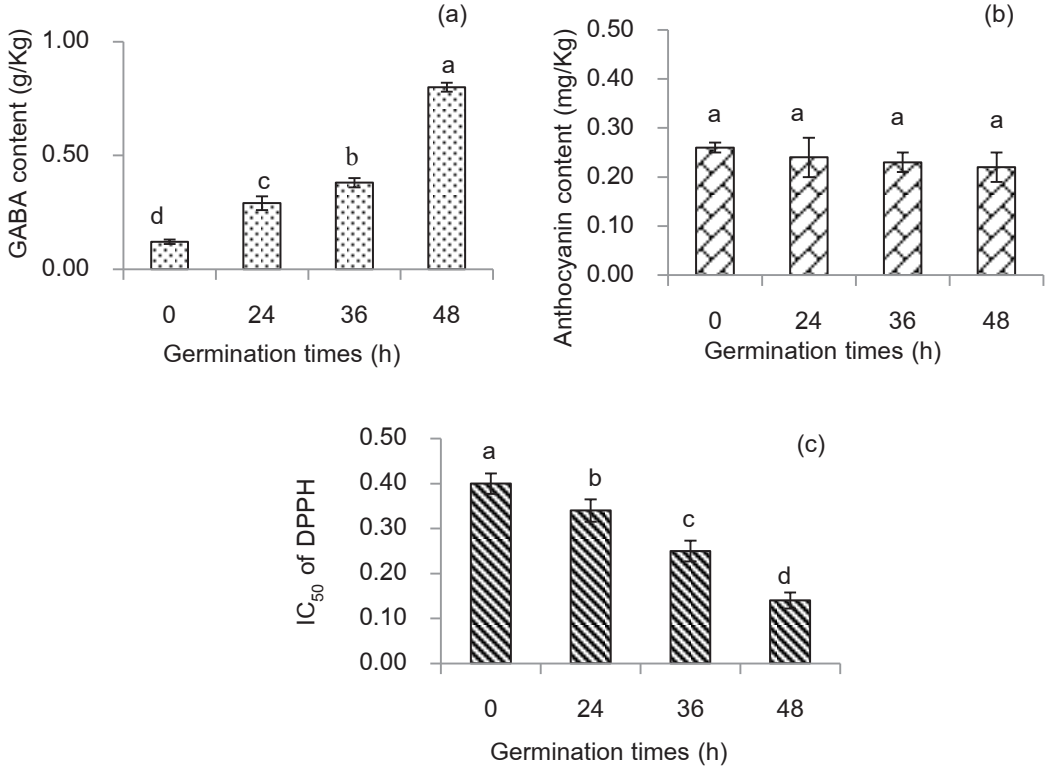


Figure 1. GABA (a), anthocyanin content (b) and IC_{50} of DPPH (c) of germinated BGBR at the different germination times.

Note: Different letters refer to significant difference ($P \leq 0.05$).

After germination, it was shown that germination time had no effect on anthocyanin ($P>0.05$) and possessed in the range of 0.22-0.26 mg/Kg (Figure 1b). As shown in Figure 1c, DPPH radical scavenging assay was used to evaluate for antioxidant activity of the rice and represented by the inhibitory concentration (IC_{50}). Germinated BGBR had IC_{50} of DPPH in a rage of 0.14-0.40 mg equivalent to gallic acid throughout the time course of germination.

Functional properties of yogurt added with different contents of germinated BGBR

In this study, the 48 h-germinated BGBR were used for producing 6 yogurt formulas in order to study the functional properties represented by GABA, anthocyanin content and DPPH scavenging activity. The results were found that the GABA, anthocyanin content and DPPH scavenging activity of yogurt products were significantly different among the samples ($P \leq 0.05$) (Figure 2a-c.). The GABA, anthocyanin content and DPPH scavenging activity increased significantly with an increase in the supplement amounts ($P \leq 0.05$). The GABA and anthocyanin contents of yogurt product without germinated BGBR (100% milk) were not detectable. Also, antioxidant activity of the yogurt product lower than that of yogurt products adding germinated BGBR. Whereas, all of yogurt products adding germinated BGBR had the GABA contents varied between 24.5 and 84.5 mg/Kg (Figure 2a.), anthocyanin content varied between 0.03 and 0.14 mg/Kg (Figure 2b) and IC_{50} of DPPH varied between 0.08 and 5.70 g equivalent to gallic acid/ml (Figure 2c.).

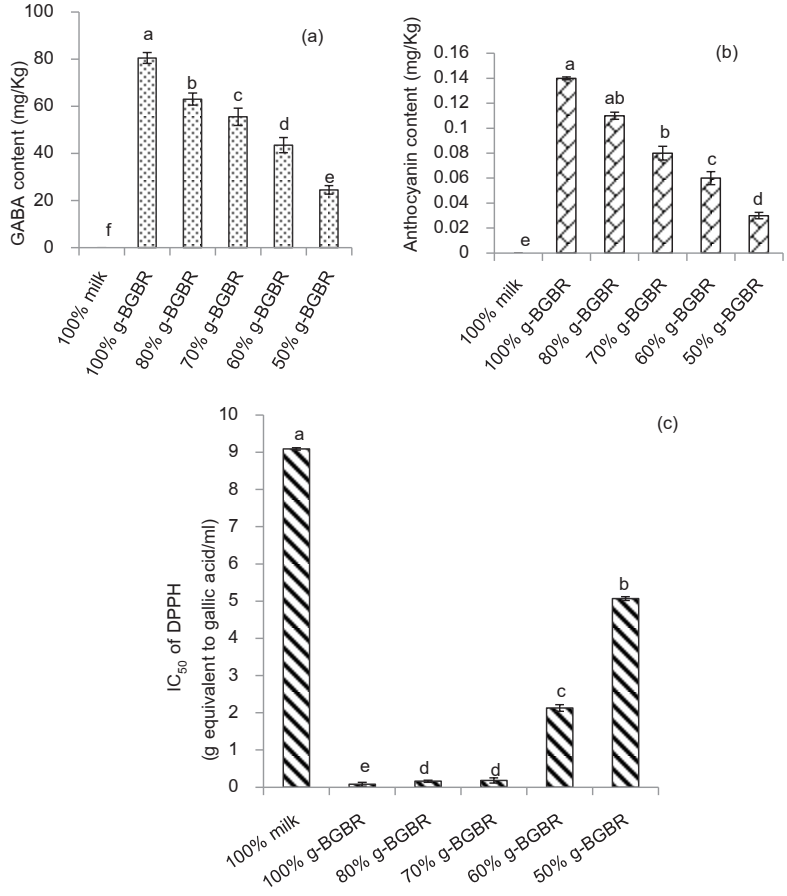


Figure 2. GABA (a), anthocyanin content (b) and IC_{50} of DPPH (c) of yogurt products adding different amounts of germinated BGBR.

Note: Different letters refer to significant difference ($P \leq 0.05$).

pH and total acidity of yogurt added with different contents of germinated BGBR

The pH values and total acidity of yogurt products were shown in Figure 3. The pH and total acidity of the yogurt products were significantly different among the samples ($P \leq 0.05$). The results that pH of yogurt products decreased with amount of germinated BGBR increased, whereas total acidity of the yogurt products increased. The pH values and total acidity of all yogurt products varied between 3.33 and 4.44. The total acidity of all yogurt products varied between 0.67 and 0.87%.

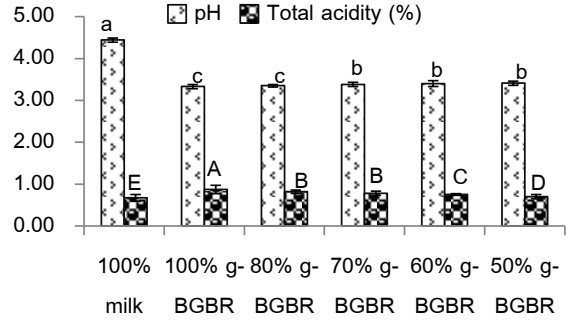


Figure 3. pH and total acidity of 6 yogurt products

Note: Different small letters within pH value refer to significant difference ($P \leq 0.05$).
Different capital letters within total acidity value refer to significant difference ($P \leq 0.05$).

Viable lactic acid bacteria (LAB) count of yogurt added with different contents of germinated BGBR

The viable LAB count of the yogurt products was shown in Figure 4. The yogurt products adding germinated BGBR showed a tendency of decrease in the viable LAB count with amount of germinated BGBR increased. While, the yogurt without germinated BGBR (100% milk) had the most of viable LAB count ($P \leq 0.05$).

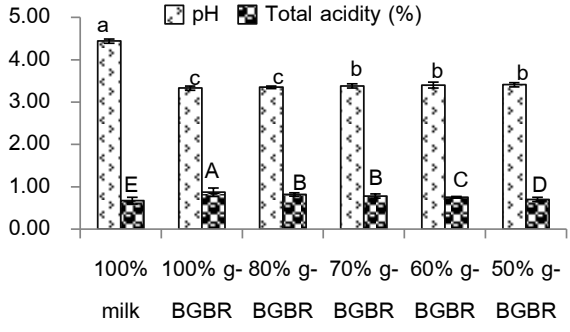


Figure 4. The number of viable LAB count in the yogurt products

Note: Different letters refer to significant difference ($P \leq 0.05$).

Consumer acceptance on yogurt added with different contents of germinated BGBR

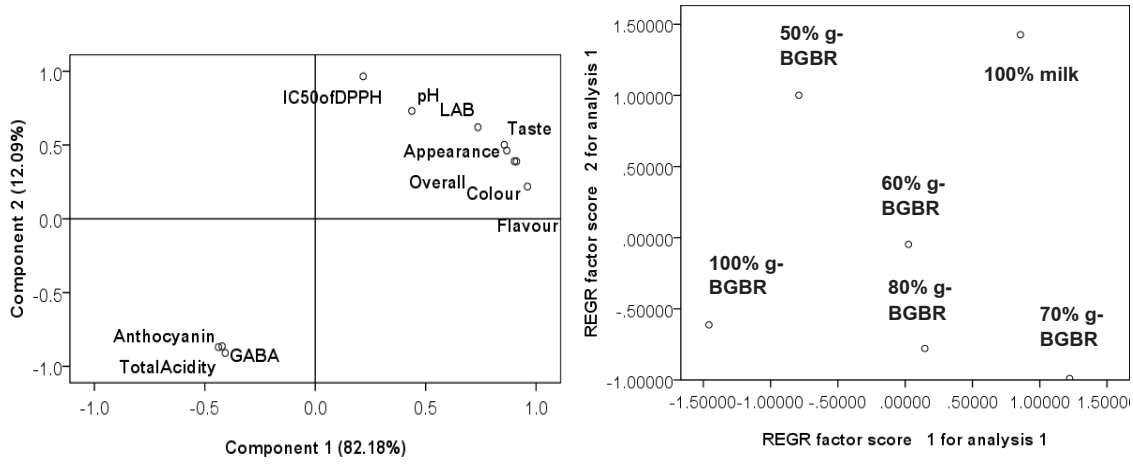
Averaged mean scores of 200 consumers on overall, appearance, color, flavor and taste likings are shown in Table 2. ANOVA and mean differences tests indicate that yogurt product without germinated BGBR (100% milk) gained the highest liking scores of all attributes, ranged moderately liking (7.00-7.88). While, all of yogurt formulas adding germinated BGBR gained liking scores of all attributes in a range of 3.40 to 6.16. Among yogurt product adding germinated BGBR, the product adding 70% germinated BGBR (70% g-BGBR) was rated the highest liking scores of all attributes when compared with other products ($P \leq 0.05$).

Table 2. Consumer liking averaged scores on sensory attributes of 6 yogurt products

Formulas	Overall	Appearance	Color	Flavor	Taste
100% milk	7.50 ± 1.50^{a}	7.82 ± 1.33^{a}	7.34 ± 1.75^{a}	7.00 ± 1.74^{a}	7.88 ± 1.25^{a}
100% g-BGBR	4.00 ± 1.87^{e}	4.40 ± 1.80^{e}	3.40 ± 1.57^{d}	4.00 ± 1.69^{e}	4.46 ± 2.02^{d}
80% g-BGBR	4.88 ± 1.59^{d}	5.62 ± 1.41^{c}	4.48 ± 1.93^{c}	5.14 ± 1.64^{c}	5.62 ± 1.51^{c}
70% g-BGBR	6.16 ± 1.55^{b}	6.06 ± 1.51^{b}	5.78 ± 1.62^{b}	5.88 ± 1.75^{b}	6.02 ± 1.49^{b}
60% g-BGBR	5.22 ± 1.75^{c}	5.36 ± 1.72^{d}	4.86 ± 1.88^{c}	5.34 ± 1.65^{c}	5.58 ± 1.56^{c}
50% g-BGBR	5.08 ± 1.77^{d}	5.44 ± 1.66^{d}	4.50 ± 1.86^{c}	4.78 ± 1.81^{d}	5.48 ± 1.59^{c}

Note: Different letters within a column refer to significant difference ($P \leq 0.05$).

PCA was used to illustrate relationships among all attribute variables and grouping of the products accordingly. The bi-plots of quality values-products PCA shown in Figure 5a-b here are composed from 2 Principal Components (PC) which explain 94.26% of the data variability. GABA and anthocyanin contents presented negative effects to sensory likings. Also, IC_{50} of DPPH presented negative effects to GABA and anthocyanin contents. This explains that the product which has higher IC_{50} of DPPH, it has lower contents of GABA and anthocyanin and plays lower antioxidant activity. Whereas, pH, total acidity (%) presented positive effects to LAB (viable LAB count). The PCA characterizes the high amount of germinated BGBR with high GABA, anthocyanin contents and antioxidant activity. The amount of germinated BGBR in the yogurt product seems to be crucial for consumer acceptance, considering their ranges applied in this experiment. Consumers preferred products without germinated BGBR content (100% milk) as shown on the right quarters of the product plot (Figure 5b). Whereas, formulas contained 100% germinated BGBR content (100% g-BGBR) are located in opposite direction to product liking vectors. Formulas contained 70% germinated BGBR content (70% g-BGBR) with 55.5 mg/Kg of GABA, 13.92 mg/Kg of anthocyanin and 0.18 mg/ml of DPPH IC_{50}, received the highest liking scores on all sensory attributes (5.78-6.16) when compared with other formulas added germinated BGBR.



(a) (b)

Figure 5. Principal components loading scores of the quality values (a) and 6 yogurt products (b) for components 1 and 2.

Conclusions and Discussion

Germination markedly increased the GABA and DPPH scavenging activity in BGBR samples. However, germination time had no effect on total anthocyanin. Karladee and Suriyong [21] mentioned that the GABA content of purple rice may steadily 4-5 times increased to the range of 0.14-0.24 g/kg dry matter within 24 h of germination. Also, increasing the germination time was able to increase DPPH scavenging activity. It might be caused by the endogenous synthesis of other antioxidants in the rice germ cells that accumulated for the differentiation to form apical and roots [22-26]. Generally, brown rice contains beneficial compounds which also act as antioxidants such as γ-oryzanol and tocopherol [27], phytic acid and tocotrienol [28]. The results illustrate that germination for 48 h can be a useful method in enhancing the GABA and DPPH antioxidant activities of BGBR.

Likewise, GABA, anthocyanin content and DPPH scavenging activity were investigated for observing the functional properties of yogurt added with germinated BGBR. The GABA, anthocyanin content and DPPH scavenging activity increased significantly with an increase in the supplement amounts. It may be due to GABA and anthocyanin contents originating from germinated BGBR and also affecting on antioxidant activity. This result agreed with Kim and others [19] who studied effects of germinated brown rice addition on the flavor and functionality of yogurt and found that GABA content in the yogurt products depended on the amount of germinated brown rice added. Therefore, yogurt added with germinated BGBR is valuable as functional yogurt.

The pH of all yogurt products were in a rage of the optimum pH range of yogurt (3.27 and 4.53) [29-31]. As Figure 3, the product added 100% germinated BGBR

showed lower pH than other samples at the end of fermentation ($P \leq 0.05$). This result was in agreement with the report of Yim and others [32] and Kim and others [19], who reported a lower pH of yogurt supplemented with GABA than that of control. However, total acidity of the yogurt products increased with amount of germinated BGBR increased. The total acidity of the product added 100% germinated BGBR was higher than that of other samples ($P \leq 0.05$). This may be due to GABA and other organic acids which contained in germinated BGBR. In this study, the total acidity was found to be in a range from 0.67 to 0.87%. This agreed with Kim and others [18] who found that yogurt products adding GABA and control had between 0.79% and 0.90%. In addition, it was in a rage of normal yogurt products (0.7% and 1.20%) which reported by Davis [33].

The viable LAB count decreased in yogurt added with germinated BGBR. This result agreed with Kim and others [19] who found a decrease in the viable LAB count of the fermented milk supplemented with germinated brown rice. Similarly, de Silva and others [34] who studied in quantification of LAB in goat milk based yogurts with added water-soluble soy extract (WSSE) and found that yogurts with added WSSE presented lower viable LAB count than yogurts without WSSE. A decrease in viable LAB count of yogurt containing germinated BGBR could be partly due to diluting effect on c-source from milk (lactose). Another important reason of the result may be due to effect of phenolic compounds from germinated BGBR. Stead [35] mentioned that phenolic compounds can inhibit the growth of some lactic acid bacteria. Tabasco and others [36] reported that the phenolic compounds effected on growth and survival of lactic acid bacteria, such as *Streptococcus thermophilus*, *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Lactobacillus vaginalis* strains. In addition, the excess others nutrients originating from germinated BGBR, such as lipids may can inhibit microbial growth. Kozloski [37] reported that the unsaturated fatty acids had toxic effect to bacterial cells namely, change of the lipid composition and the physiochemical properties of the bacterial cell membranes. The results of the determination of the viable LAB count at 3th day of storage were found that the yogurt with added 100% germinated BGBR was not detected the viable LAB count. While, the yogurt with added germinated BGBR extract between 50% and 80% meet a count between 1.56×10^{10} and 1.75×10^{10} CFU/ml.

The National Yogurt Association of the United States specifies the criteria of fermented milk that the fermented milk should contain 10^8 CFU/ml of LAB at the time of manufacture [38]. In addition, Fermented Milks and Lactic acid Bacteria Beverages Association of Japan requires that a minimum of 10^7 LAB CFU/ml to be present in fermented milk [16]. Moreover, Chinabhark [39] mentioned that the total count of viable LAB count should be at least 10^7 CFU/ml in the final product for promoting the health benefits of the consumer, such as

lactose digestion, adjusting the balance of microorganisms in the intestine, anti-cancer, improving immunity and reducing cholesterol levels in the blood. According to these criteria, viable cell count of the yogurt products in this study was in the optimal range. This study showed that the viable cell count in the yogurts was satisfactory in aspect for promoting the health benefits and considering in accordance with the current law.

The PCA illustrated the amount of germinated BGBR in the yogurt product seems to be crucial for consumer acceptance, considering their ranges applied in this experiment. Consumers preferred formulas contained 70% germinated BGBR content (70% g-BGBR) with 55.5 mg/Kg of GABA, 13.92 mg/Kg of anthocyanin and 0.18 mg/ml of DPPH IC_{50}. There is no directly and depth reported information on the effect of germinated BGBR content on consumer likings. However, this may be due to the presence of too little or much germinated BGBR, providing unwanted-color and-flavor attributes of the yogurt products which interfere sensory perception of consumers, observing theirs color and flavor score ranges shown in this experiment. While, the consumers rated the highest liking scores of all sensory attributes of product without germinated BGBR content. This may be due to familiarity of similar recipe of the product commonly produced and sold in general market. Heath and others [40] stated that the familiarity with taste, odour and flavor of food played role in consumers' liking. This is supported by Pliner [41] who used unfamiliar tropical fruit juices (e.g. guava and mango juices) and presented them to participants 0, 5, 10, or 20 times. This research was reported that an increased familiarity led to increased liking. This may be a well-known psychological effect that people express liking for things merely because they are familiar with them [42].

As the results, consumers preferred 70% germinated BGBR formula rather than other formulas added with BGBR. The formula is probably the prototypes product of germinated BGBR yogurt to be an alternative product for health conscious consumers.

Acknowledgement

This research was carried out with the financially support of Faculty of Public Health, Mahasarakham University, Thailand.

References

[1] Kushwaha UKS. (2016). Black Rice Anthocyanin Content Increases with Increase in Altitude of its Plantation. *Advances in Plants and Agriculture Research*. 5(1): 1-4.

[2] Ichikawa H, Ichiyanagi T, Xu B, Yoshii Y, Nakajima M.; & Konishi T. (2001). Antioxidant activity of anthocyanin extract from purple black rice. *Journal of Medicinal Food*. 4(4): 211-218.

[3] Ngyuen JS.; & Ooraikul B. (2008). The physic-chemical, eating and sensorial properties of germinated brown rice. *Journal of food, agriculture and environment*. 6(2): 119-124.

[4] Watcharaparpaiboon W, Laohakunjit N.; & Kerdchoechuen O. (2010). An improve process for high quality and nutrition of brown rice production. *Food Science and Technology International*. 16: 147-158.

[5] Capanzana MV.; & Buckle KA. (1997). Optimization of germination conditions by response surface methodology of a high amylose rice (*Oryza sativa*) cultivar. *LWT-Food Science and Technology*. 30(2): 155-163.

[6] Moongngarm A.; & Saetung N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chemistry*. 122(3): 782-788.

[7] Komatsuzaki N, Tsukahara K, Toyoshima H, Suzuki T, Shimizu N.; & Kimura T. (2007). Effect of soaking and gaseous treatment on GABA content in germinated brown rice. *Journal of Food Engineering*. 78(2): 556-560.

[8] Patil SB.; & Khan KM. (2011). Germinated brown rice as a value added rice product: A review. *Journal of Food Science and Technology*. 48(6): 661-667.

[9] Imam MU, Azmi NH, Bhanger MI, Ismail N.; & Ismail M. (2012). Antidiabetic Properties of Germinated Brown Rice: A Systematic Review. *Evidence-Based Complementary* and *Alternative Medicine*. 1-12.

[10] Chokeprasert P.; & Punya S. (2011). The optimization and development of mixed germinated brown rice. In *The Proceeding of Maejo university conference*, pp. 169-179. Chiang Mai, Thailand.

[11] Tanganurat P, Yodkum S, Soiyodthong N.; & Mangkit P. 2014. Development of Drinking Yogurt-like Product from Germinated Hom Nil Rice. *Agricultural Science Journal*. 45(2)(Issue with supplement): 565-568.

[12] TAS 4003-2012. (2012). *Thai Agricultural Standard of Germinated Brown Rice*. National Bureau of Agricultural Commodity and Food Standards. Ministry of Agriculture and Cooperatives.

[13] Bourneow C.; & Santimalai S. (2014). An pulsing and annealing electrical system for production of germinated berry rice. In *The Proceeding of Food Innovative Asia Conference*, pp. 277-284. Bangkok, Thailand.

[14] Lee S, Yoon BE, Berglund K, Oh SJ, Park H, Shin HS, Augustine GJ.; & Lee CJ. (2010). Channel-mediated tonic GABA release from glia. *Science*. 330: 790-796.

[15] A.O.A.C. (2000). *Official Method of Analysis of Association of Official Analysis Chemist*. 17th ed. Virginia: The Association of Official Analytical Chemists, Inc.

[16] MM. Guisti.; & RE. Wrolstad. (2001). *Characterization and measurement of anthocyanins by UV-Visible spectroscopy. Current Protocols in Food Analytical Chemistry (F1.21-F1.2.13)*. New York: John Wiley & Sons.

[17] Ohtsubo K, Suzuki K, Yasui Y.; & Kasumi T. (2005). Bio-functional components in the processed pregerminated brown rice by a twin-screw extruder. *Journal of Food Composition and Analysis*. 18(4): 303-316.

[18] Brand-Williams W., Cuvelier ME.; & Berset C. (1995). Use of a Free Radical Method to Evaluate Antioxidant. *LWT-Food Science and Technology*. 28: 25-30.

[19] Kim MH, Ahn SI, Lim, CM, Jhoo JW.; & Kim GY. (2016). Effects of Germinated Brown Rice Addition on the Flavor and Functionality of Yogurt. *Korean Journal for Food Science of Animal Resources*. 36(4): 508-515.

[20] Macfie HJ, Bratchell N, Greenhoff K.; & Vallis LV. (1989). Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *Journal of Sensory Study*. 4: 129-148.

[21] Karladee D.; & Suriyong S. (2012). Gamma-aminobutyric acid (GABA) content in different varieties of brown rice during germination. *Science Asia*. 38: 13-17.

[22] RF. Bornstein; & C. Crave-Lemley. (2004). *Cognitive illusions: a handbook on fallacies and biases in thinking, judgement and memory*. UK: Psychology Press.

[23] Jannoey P, Niamsap H, Lumyong S, Tajima, S, Nomura M.; & Chairote G. (2010). Gamma aminobutyric acid (GABA) accumulations in rice during germination. *Chiang Mai Journal of Science*. 37(1): 124-133.

[24] Umnajkitikorn K, Faiyue B.; & Saengnil K. (2013). Enhancing antioxidant properties of germinated Thai rice (*Oryza sativa* L.) cv. Kum Doi Saket with salinity. *Journal of Rice Research*. 1: 1-8.

[25] Zhang Q, Xiang J, Zhang L, Zhu X., Evers J, van der Werf W.; & Duan L. (2014). Optimizing soaking and germination conditions to improve gamma-amino butyric acid content in japonica and indica germinated brown rice. *Journal of Functional Foods*. 10: 283-291.

[26] Ding J, Yang T, Feng H, Dong M, Slavin M, Xiong S.; & Zhao S. (2016). Enhancing contents of γ-Aminobutyric acid (GABA) and other micronutrients in dehulled rice during germination under normoxic and hypoxic conditions. *Journal of Agricultural and Food Chemistry*. 64: 1094-1102.

[27] Jirapa K, Jarae Y, Phanee R.; & Jirasak K. (2016). Changes of bioactive components in germinated paddy rice (*Oryza sativa* L.). *International Food Research Journal.* 23(1): 229-236.

[28] Jiamyangyuen S.; & Ooraikul B. (2009). Effect of germination on antioxidants, textural, and sensorial property of germinated. *FDA Journal.* 16(1): 25-33.

[29] Chun SI, Lo LMP.; & Kang MY. (2016). Effect of germination on the antioxidant capacity of pigmented rice (*Oryza sativa* L. cv. Superjami and Superhongmi). *Food Science* and *Technology Research*. 22(3): 387-394.

[30] Duitschaever CL, Arnott DR.; & Bullock DH. (1972). Quality evaluation of yogurt produced commercially in Ontario. *Journal of Milk and Food Technology*. 35: 173-175.

[31] Kroger M.; & Weaver JC. (1973). Confusion about yogurt compositional and otherwise. *Journal of Milk and Food Technology*. 36: 388-394.

[32] Yim SI, Park JH, Nam SH, Song JH, Cho YS.; & Lee HC. (2012). Properties characterization of tea functional component GABA-containing fermented milks. *Journal of the Korean Tea Society*. 18: 56-68.

[33] Davis JG. (1970). Laboratory control of yogurt. *Dairy Industries*. 35: 139-144.

[34] de Silva DCG, Brugnera DF.; & de Abreu LR. (2013). Quantification of lactic acid bacteria and bifidobacteria in goat milk based yogurts with added water-soluble soy extract. *African Journal of Food Science*. 7(10): 392-398.

[35] Stead D. (1993). The effect of hydroxycinnamic acids on the growth of wine-spoilage lactic acid bacteria. *Journal of Applied Bacteriology*. 75: 135-141.

[36] Tabasco R, Sanchez-Patan F, Monagas M, Bartolome B, Moreno-Arribas MV, Pelaez C.; & Requena T. (2011). Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism. *Food Microbiology*. 28: 1345-1352.

[37] GV. Kozloski. (2002). *Biochemistry of ruminants*. Santa Maria: UFSM.

[38] Kailasapathy K.; & Rybka S. (1997). *L. acidophilus* and *Bifidobacterium* spp.--their therapeutic potential and survival in yogurt. *Australian Journal of Dairy Technology*. 52: 28-35.

[39] K. Chinabhark (2012). *Product Development of Germinated Chaiya Brown Rice Yogurt*. Food Science and Technology Program. Science and Technology Faculty. Suratthani: Suratthani Rajabhat University at Thailand.

[40] Heath P, Houston-Price C.; & Kennedy OB. (2011). Increasing food familiarity without the tears. A role for visual exposure?. *Appetite*. 57: 832-838.

[41] Pliner P. (1982). The effects of mere exposure on liking for edible substances. *Appetite*. 3: 283-290.

[42] RF. Bornstein; & C. Crave-Lemley. (2004). *Cognitive illusions: a handbook on fallacies and biases in thinking, judgement and memory*. UK: Psychology Press.