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

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Abstract

The purpose of this study is to develop functional yogurt product that contains high levels of lactic acid bacteria (LAB) by using germinated black glutinous brown rice (BGBR) at different germination periods (0 24 36 and 48 hours). The effects of germination periods on the contents of LAB, anthocyanins, DPPH antioxidant, and live lactic acid bacteria were studied. Results showed that BGBR germinated for 48 hours had the highest content of LAB, anthocyanins, and DPPH antioxidant (P ≤ 0.05). BGBR germinated for 48 hours was used to produce 6 types of yogurt, and the contents of LAB, anthocyanins, DPPH antioxidant, and live lactic acid bacteria were analyzed. The results showed that the pH of yogurt declined from 4.44 to 3.33, while the total lactic acid bacteria content increased from 0.67% to 0.87% with increasing germination periods. BGBR yogurt had a mean pH of 3.33, a total lactic acid bacteria content of 2.75 x 10^10 CFU/mL, and a total lactic acid bacteria content of 1.65 x 10^10 CFU/mL. Therefore, the yogurt produced from BGBR germinated for 48 hours is suitable for the production of functional yogurt.
Abstract

This study aimed to produce functional yogurt with high ɣ-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 ≤ 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 range of 1.56 x10^{10} to 2.75x10^{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 ≤ 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 1.65x10^{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 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 ± 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

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

**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):

\[
\text{Anthocyanin content (mg/l)} = \left( \frac{A \times MW \times DF \times 1000}{\varepsilon \times L} \right) \quad (1)
\]

where \( A = \left( A_{515} - A_{700} \right) \text{ pH 1.0} - \left( A_{515} - A_{700} \right) \text{ at pH 4.5} \), \( MW \) is equal to 449.2 (molecular weight of cyanidin-3-glucoside), \( DF \) is the dilution factor of sample, and \( \varepsilon \) is the molar absorptivity of cyanidin-3-glucoside equal to 26,900.

**Determination of \( \gamma \)-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):

\[
\text{Inhibition } \% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100
\]

(2)

where, \( A_{\text{blank}} \) is absorbance of control blank, and \( A_{\text{sample}} \) 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 ≤ 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 ≤ 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 ≤ 0.05) (Figure 2a–c.). The GABA, anthocyanin content and DPPH scavenging activity increased significantly with an increase in the supplement amounts (P ≤ 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 ≤ 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 ≤ 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](image)

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

![Figure 4. The number of viable LAB count in the yogurt products](image)

Note: Different letters refer to significant difference (P ≤ 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 ≤ 0.05).

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

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

Note: Different letters within a column refer to significant difference (P ≤ 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\textsubscript{50} of DPPH presented negative effects to GABA and anthocyanin contents. This explains that the product which has higher IC\textsubscript{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\textsubscript{50} received the highest liking scores on all sensory attributes (5.78–6.16) when compared with other formulas added germinated BGBR.
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

Figure 5. Principal components loading scores of the quality values (a) and 6 yogurt products (b) for components 1 and 2.
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 \((0.7\% \text{ and } 1.20\%)\) 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 3rd 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 \(1.56 \times 10^{10}\) and \(1.75 \times 10^{10}\) 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<sub>50</sub>. 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.

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References


