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Development of a sweet beverage from germinated brown rice: A product of high nutritional value enriched with high bioactive compounds for promoting good health

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Abstract

To develop a value-added product from germinated brown rice (GBR), an experiment was developed to optimise the production conditions for a sweet beverage enriched with bioactive compounds (EBCSB) by optimising the *A. oryzae* spore ratio (0.3 - 0.6%), ratio of GBR to *A. oryzae* (GA), steamed GBR (SG) (1:3 to 1:9), and hydrolysis process. Results showed that GBR after cooking with water (GBR: water at 1:1.5), and when used as a medium for spore inoculation (0.4%) at 40°C for 36 h yielded the highest amylase activity in GA (2.37 UI/g). Subsequently, GA and SG at 1:5 ratio was incubated at 60°C for 6 h, and yielded the best composition of glucose, gamma-amino butyric acid, and ergothioneine at 12.18%, 883.9 mg/kg, and 210.9 mg/kg, respectively. The product was then sterilised at 121°C for 4 min corresponding to F_{value} = 7.47 (F_o = 7) to increase product safety and maintain good quality for eight weeks. Results further indicated that GBR can be used to produce EBCSB that is good for health.

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Introduction

Sweet beverage (SB) produced from the action of *Aspergillus oryzae* on rice, and called *amazake* in Japan is a traditional product divided into various types from strong to mild depending on the sugar concentration. There are also two types of SB based on the preparation method. The first type is made from rice *koji*, and the second one from *sake* lees (Saigusa and Ohba, 2007; Shinpei *et al.,* 2011). However, *koji* rice *amazake* (KA) is the most popular. It is manufactured from rice *koji* which is produced by *A. oryzae* growing on steamed rice, water, and in some cases, cooked rice. The glucose concentration of SB reaches approximately 20% due to the starch in rice *koji* being saccharified by enzymes such as α-amylase and glucoamylase, which are produced by *A. oryzae*. Owing to various ingredients in the final product such as sugars, amino acids, and organic acids, SB has been previously reported to have high nutritional value (Shikata *et al.,* 1997; Tukiyama *et al.,* 1997). There are 12 types of oligosaccharides present in SB, among them are nigerose, kojibiose, trehalose, isomaltose, gentiobiose, raffinose, panose, and isomaltotriose, which are produced at the highest levels (50 - 60°C), whereas sophorose production is maximal at 70°C (Oguro *et al.,* 2019). Thus, the oligosaccharide composition of SB is dependent on the saccharification temperature. These are useful information for improving consumers' appeal towards SB by enhancing its oligosaccharide content. The anti-obesity, anti-hypertension, and anti-amnesic effects of *sakekasu amazake* might be due to the presence of dietary fibres and peptides (Oura *et al.,* 2007). In fact, there are many functional properties beneficial to human health in SB. The intake of SB as a late evening drink improves the subjective symptoms of patients with liver cirrhosis (Nagao and Sata, 2013). This indicates that branched-chain amino acids in SB influence the local immune system of the liver in cirrhotic patients. Although the beneficial effects of SB on human health have been widely studied, its functional components are still unclear. The metabolite profile of KA and its lactic acid fermentation (LAF) product by *Lactobacillus sakei* UONUMA has been reported (Oguro *et al.,* 2017). In this study, 13 saccharides were identified, including two unknown trisaccharides, and there were no differences in these between the two

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beverages. In LAF-*amazake*, lactic acid, vitamins B_2, B_3, and B_6 significantly increased as compared to KA, whereas malate and glutamine decreased. This provided useful information for promoting functional analyses of KA and LAF-*amazake* for human health.

Germinated brown rice (GBR) is a value-added product from rice that contains various bioactive compounds (Banchuen *et al.,* 2010; Patil and Khan, 2011). This product generates many basic as well as functional ingredients during the early stage of germination from pre-GBR, depending on the soaking and germinating conditions (Reggiani *et al.,* 2000; Yang *et al.,* 2001; Komatsuzaki *et al.,* 2007). Germination requires energy and enzyme activities, and these influence germination speed of brown rice (Bewley and Black, 1994; Bewley, 1997). There is a correlation between protein and glutamic acid, as well as between glutamic acid and gamma-amino butyric acid (GABA) levels. Thus, a protein composition that is high in glutamic acid such as that of rice, brown rice, and rice bran may facilitate GABA biosynthesis (Chungcharoen *et al.,* 2014). GABA is primarily synthesised by the decarboxylation of L-glutamic acid, which is catalysed by glutamate decarboxylase (GAD) (EC 4.1.1.15) with pyridoxal phosphate 50 (PLP) as a cofactor (Roohinejad *et al.,* 2009). GABA is also one of the main inhibitory neurotransmitters in the central nervous system and plays a physiological role in many systems outside the central nervous system, including regulation of cardiovascular function, inhibition of cancer cell metastasis, and regulation of pituitary function and growth hormone production in mice. Furthermore, it has been shown that GABA or GABA-enhancing compounds, such as the amino acid L-theanine, can play an important role in stabilising mood disorders (Abdou *et al.,* 2006).

Ergothioneine (ERG) is a naturally occurring amino acid analogue synthesised in some bacteria and fungi, but not in animals, and has received much attention as a therapeutic agent due to its antioxidant properties (Nguyen *et al.,* 2012; Kerley *et al.,* 2018). ERG exists in two forms, thiol and thione, of which the second is more preferred, originally discovered in *Claviceps purpurea*, and later identified as betaine of 2-thiol-L-histidine. Early research on ERG has reported its presence in red blood cells and mouse liver, and it was suggested that it came from plant materials (Dola *et al.,* 1991). The antioxidant activity of ERG is associated with lipid peroxidation resistance in atherosclerosis, smoking, and diabetes (Meister, 1992). Therefore, ERG is a promising, safe, and effective antioxidant to control lipid oxidation and the ensuing changes in food quality. It may also be useful for preventing various oxidative stress-related diseases. ERG is found at a high concentration in various mushrooms and moulds, including *A. oryzae* (Shin *et al.,* 2012; Nguyen *et al.,* 2013). Therefore, in the present work, in order to diversify the functional products of GBR, SB from GBR was developed to obtain a product with enriched nutritional and functional properties with high levels of GABA and ERG.

Materials and methods

Materials

The rice variety IR 50404 was purchased from Mekong Delta Rice Institute, Vietnam. *A. oryzae* spores were received from Biotechnology Institute of Research and Development (BIRDI), Can Tho University, Vietnam. GABA and ERG standards were purchased from Merck, Germany. Other chemicals and solvents for HPLC (Shimadzu, Japan) analysis were purchased from Sigma-Aldrich (USA).

GBR koji A. oryzae preparation

GBR was prepared following the method of Banchuen *et al.* (2010) with slight modifications. Briefly, an IR 50404 paddy was dehusked with a Yanmar ST50 (Yanmar Company, Tokyo, Japan), with the two rubber rollers of the machine was adjusted to a suitable distance to obtain the best pre-GBR. Subsequently, the pre-GBR was soaked in water for 6 h until saturation was reached (33 ± 0.5%). The saturated pre-GBR was then incubated at 37°C under anaerobic conditions for 24 h to obtain GBR. In order to produce GBR *koji* from *A. oryzae* (GA), 20 g GBR was cleaned and cooked in 1:1, 1:1.5, and 1:2 of GBR: water ratios, corresponding to water contents of 36, 57, and 77%, respectively, for 40 min, to produce steamed GBR (SG). Subsequently, they were inoculated with *A. oryzae* at 0.2 - 0.5% (1.6×10^7 CFU/g), and incubated for 48 h. They were mixed and incubated again at 32°C every 12 h. Considering the time interval and amylase activity, the ERG and GABA contents were measured to estimate the time needed to optimise the GA quality.

EBCSB preparation, sterilisation, and preservation of the final product

In order to determine the best EBCSB production conditions, the GA was mixed with SG in ratios of 1:3 to 1:9, and the mixture dissolved in two

volumes of water before treatment at 60°C and pH 5 for 2 -10 h of hydrolysis. Considering the time interval, the glucose, ERG, and GABA contents were measured with the time needed to optimise EBCSB quality. The best recipe of EBCSB was homogenised at 28 Megapascals (MPa), then bottled and heated to 85°C to remove the air from the bottle for 2 min. Next, the product was sterilised at 121°C for 4 - 10 min to optimise the sterilisation conditions. The final product was then preserved for two months (eight weeks), and the microbial loads were checked every two weeks during preservation.

Determination of gamma-amino butyric acid (GABA)

The GABA content was determined following the method of Banchuen *et al.* (2010). Briefly, 2 g of ground GBR was weighed and placed into a 50-mL flask, and 9 mL of demineralised water was added and shaken for 90 min to extract the sample. Then, 1 mL of sulfosalicylic acid was added to the mixture and centrifuged at 8,000 rpm for 10 min. Next, 100 µL of the supernatant was transferred to a 1.5-mL microcentrifuge tube, and 100 µL of 100 mM $NaHCO_3$ and 100 µL of 4 mM 4-dimethyl-4-sulfonyl chloride amino azobenzene solution diluted in acetonitrile were added. The mixture was shaken and heated at 70°C for 10 min. Subsequently, 500 µL of ethanol and phosphate buffer, respectively, with pH 6.8 were added to the mixture, which was then shaken well and centrifuged at 13,000 rpm for 10 min. The supernatant was then filtered through a 0.2-µm filter before analysis by HPLC. Absorption was measured at a wavelength of 465 nm. The mobile phase was ammonium acetate buffer 25 mmol/L and acetonitrile in a ratio of 55:45, with a flow rate of 1 mL/min, and a column temperature of 55°C.

Determination of ergothioneine (ERG)

The ERG content was determined following the method of Bao *et al.* (2009) with slight modifications. Briefly, 3 g of sample was extracted with 27 mL of cold 70% ethanol (containing 0.1 mmol/L 1,4-dithiothreitol) and 1 mL of 1,000 ppm 2-mercapto-1-methylimidazole as the internal standard. The mixture was homogenised at 400 rpm for 3 min. Subsequently, the suspension was kept on ice for 30 min, and centrifuged at 20,000 *g* and 4°C for 20 min. Next, the supernatant was evaporated to dryness at 50°C. Thereafter, the residue was dissolved in 1 mL of deionised water, and 1 mL of 5% basic lead acetate with 1 mL of hexane was added. Subsequently, the mixture was incubated on ice for 30 min, and centrifuged at 20,000 *g* at 4°C for 20 min. Ultimately, the supernatant was subjected to analysis on a Shimadzu HPLC system equipped with a UV-Vis two-column detector to aid in peak separation in the spectral well (column 1: Supelco C18, 250 mm × 4.6 mm × 5 µm and column 2: Lichrospher RP-C18, 250 mm × 4 mm × 5 µm), and the absorbance was detected at a wavelength of 254 nm at 40°C. The mobile phases were acetic acid (2.5% by volume) and deionised water (50: 50) at a flow rate of 0.7 mL/min.

Chemical compositions

The starch in GBR samples was determined following the method of Bertrand. The total protein content in GBR samples was determined by the Kjeldahl method. The glucose contents in GBR and EBCSB products were determined following the method of Bertrand. The peroxide index of EBCSB products, and the amylase activity of GBR and GA samples were determined following the methods of AOAC (Horwitz and Latimer, 2010).

Statistical analysis

All experiments were performed in triplicate. The results were analysed by using analysis of variance and the least significant difference test using Statgraphic Centurion 15.1 and Microsoft Excel 2010 softwares.

Results and discussion

Effect of moisture content of GBR on amylase activity, GABA, and ERG during the production of GBR koji

Table 1. Chemical composition of germinated brown rice IR 50404.

Ingredient	Content (dry basis)
Total protein	6.80 ± 0.00 (%)
Starch	77.02 ± 0.00 (%)
Total lipid	1.69 ± 0.01 (%)
Moisture content	14.04 ± 0.04 (%)
GABA	795.60 ± 0.10 (mg/kg)
Reduced sugar	0.45 ± 0.02 (%)
α-amylase activity	1.76 ± 0.14 (UI/g)

Values are mean ± standard deviation (SD) of three replicates (n = 3).

The results in Table 1 show that GBR contained a high concentration of starch and protein. GABA was also found in GBR at high level. Moreover, amylase was also found with high activity. These results indicated that GBR can be used for KA and then for the production of EBCSB.

The growth cycle of mould can be divided into three periods: (1) a period of growth, germination, and spore attachment (the first 12 h); (2) a period of rapid growth of the fibrous system (lasting from 12 - 24 h); and (3) a period of strong amylase activity (lasting from 24 - 36 h) (Oguro *et al.,* 2019). Under the action of amylase, starch is broken down into glucose and maltose. Therefore, it is necessary to optimise percentage of mould used and the incubation period. In addition, GBR plays a key role in the growth of mycelia and the amylase activity. The results in Figure 1 show a statistically significant difference in amylase activity when cooking the GBR with different ratios of mould to incubation times as well as different moisture contents of GBR. Amylase activity increased over time up to 36 h of incubation (the maximum time). SG had the highest value of amylase activity, 2.37 ± 0.14 UI/g, when the ratio of GBR:water was 1:1.5 at a mould rate of 0.4% after 36 h of incubation. After peaking at this time, the amylase activity showed signs of decrease; however, the increase and decrease in the activity showed the same trend for the three types of SG when cooked with three different water ratios, 1:1, 1:1.5, and 1:2. Therefore, the GBR:water ratio of 1:1.5 with a mould rate of 0.4% and 36 h of incubation were the conditions selected for the next experiment.

A. oryzae can synthesise GABA (Ab Kadir *et al.,* 2016). The results of GABA content analysis while varying the moisture contents and mould ratios in GA production are shown in Figure 1. GABA contents varied significantly with the GBR: water ratio. The highest GABA content was 846.2 ± 0.6 mg/kg, obtained with a GBR: water ratio of 1:1.5, and the lowest was obtained with a rice:water ratio of 1:1. Cooking GBR with different ratios of water led to different cooking times; with a higher water ratio, a longer heating time led to a higher GABA content. In contrast, with a small percentage of water, the cooking time was short, and the starch in the raw material was not completely gelatinised, thus decreasing the mould growth and enzyme production. The correct amount of water will ensure the necessary moisture for mould development and extraction of highly effective compounds. On the other hand, incubation time has a significant effect on the concentration of GABA synthesised. The change in GABA content during incubation showed that, at the five time points assessed, there was a significant increase with incubation time. GABA content increased gradually during the incubation period of 0 - 48 h. In the 24-h post incubation period (36 and 48 h), GABA content showed a small increase with no significant difference. The highest GABA content was 846.2 ± 0.6 mg/kg with *koji* incubation time of 36 h, and a rice:water ratio of 1:1.5.

A. oryzae is a mould that can synthesise ERG (Takusagawa *et al.,* 2019), which are the most powerful antioxidants essential to every microorganisms. The mechanism of action of ERG is completely different from that of glutathione. Although it is a less powerful reducing agent than glutathione, ERG is described as an effective antioxidant through reduction of oxygen alone, recovering hydroxyl radicals, and inhibiting reactions with heavy metal catalysts. The results from Figure 1 show that the content of ERG produced varied with incubation times and mould rates, with significant difference ($p < 0.05$). Specifically, there was an increase in ERG content in the first phase (from 0 to 36 h), and ERG reached a maximum of 177.3 mg/kg after 36 h at a mould incubation rate of 0.4%. After this time, the content of ERG did not increase, which could be explained by the death / decline of *A. oryzae*. In this stage, *A. oryzae* declined exponentially due to exhaustion of nutrients and toxic substances generated. In addition, the increase in the percentage of additional mould (0.5%) did not increase the amount of ERG because it could not grow from the lack of nutrition.

According to Takusagawa *et al.* (2019), the humidity of the culture environment greatly affects the growth and reproduction of microorganisms. If the humidity level is not suitable, their normal physiological process will be disrupted. This is especially true in the case of mould, a microorganism that is adapted to very humid condition. In addition, moisture is one of the factors that allows microorganisms to absorb nutrients easily. Thanks to high humidity, nutrients easily penetrate the body, and hydrolytic enzymes can work. Through that process, mould can help to absorb amino acids well and help to improve biosynthesis. If the humidity is too low, a change in the state of the protoplasm occurs; such state change disrupts microbial development and biosynthetic ability. When cooking raw rice for the cultivation and production of *koji*, the ratio of GBR:water played an important role for the growth of mould. When the ratio of GBR:water was 1:1, the moisture content of the rice was 36%, so

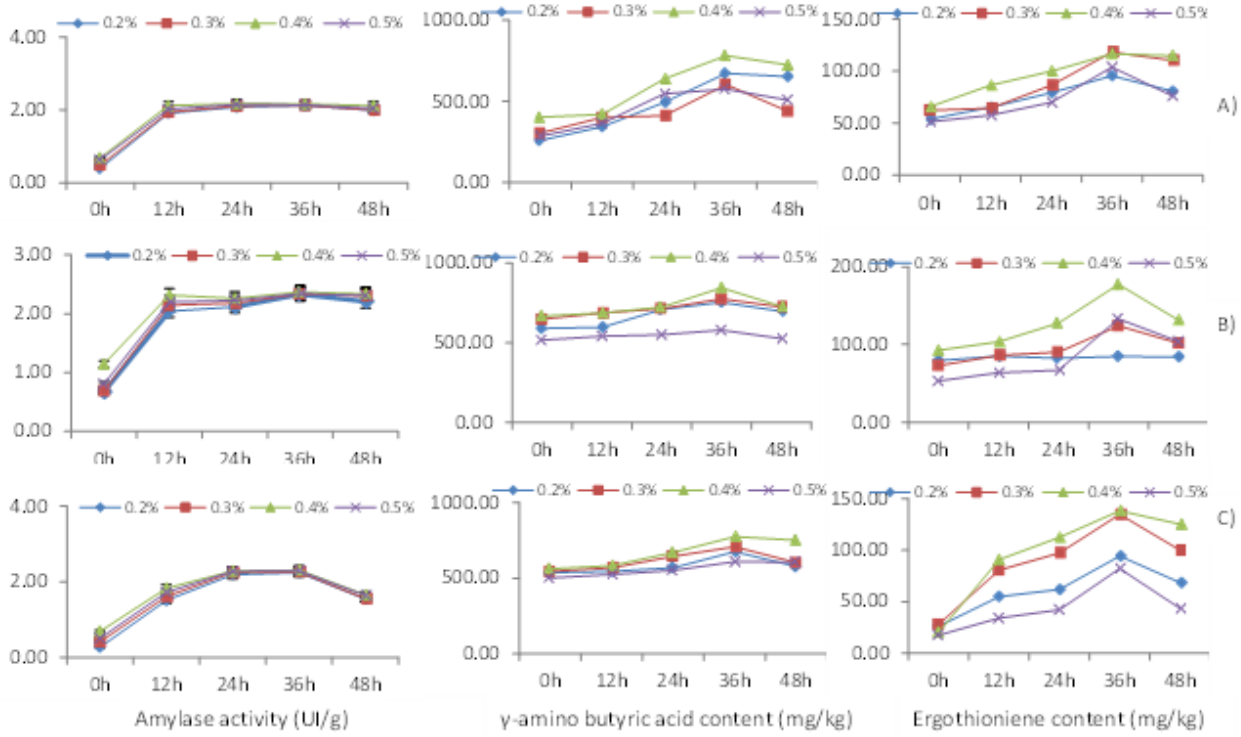

Figure 1. Changes in amylase activity (UI/g), GABA (mg/kg), and ERG (mg/kg) with different moisture contents and mould ratios in the production of GBR *koji* from *A. oryzae* (GA). 1:1.1 (A); 1:1.5 (B); and 1:2 (C). GABA = gamma aminobutyric acid; ERG = ergothioneine; and GBR = germinated brown rice.

the mould grew poorly, leading to the highest and lowest content of ERG (177.3 ± 0.9 and 118.3 ± 5.2 mg/kg, respectively) in rice when the ratio of GBR:water was 1:1.5 and the humidity was 57%, which was suitable for mould growth. According to Nguyen *et al.* (2013), the suitable humidity level for the culture medium for *A. oryzae* in *koji* production is usually approximately 45 - 55%. For rice cooked in a 1:2 ratio of rice germ:water, the moisture content after cooking was extremely high at 77%, and also caused mould to grow poorly, leading to mould contamination and a lower content of ERG as compared to that of rice cooked in a ratio of 1:1.5 (138.1 ± 5.4 mg/kg). Thus, a 1:1.5 ratio of rice:water, a 0.4% mould rate, and an incubation period of 36 h were the conditions selected for the next experiment.

Changes in chemical composition during the hydrolysis process of EBCSB production

The results in Figure 2 show that there was a significant difference when the hydrolysis increased initially, and decreased as the incubation time progress, as evidenced by an increase in glucose contents from 2 to 6 h, and a decrease from 8 to 10 h. GA consisted of many enzymes produced during the culture of GBR with *A. oryzae*, including intracellular enzymes involved in glucose synthesis and breakdown (Oguro *et al.,* 2019). Under the optimal hydrolysis conditions of amylase at 60°C and pH 5 to make EBCSB, the sugar content increased gradually up to 6 h. However, after this time, amylase activity may decrease while the activity of some enzymes that convert glucose into smaller compounds was still active, thus causing them to lose some of the glucose. When more glucose was lost than was formed, the glucose content from 6 to 10 h decreased. So, the longer the EBCSB incubation period, the greater the decrease in glucose.

In parallel with the hydrolysis time, the ratio of rice *koji* had a significant effect on the hydrolysis of starch that was gelatinised in additional germinated rice. In Figure 2, it is shown that, when EBCSB was incubated with different ratios of *koji*:GBR, the glucose content obtained after saccharification also had statistically significant differences. When the ratio of *koji*:GBR was 1:5, the highest content was obtained after 6 h of incubation (12.18%), and the lowest was obtained at the ratio of 1:9 (9.46%). This may by explained that when increasing the ratio of *koji* rice, the chance of contact between the substrate and the enzyme was higher, thus increasing the rate of hydrolysis of starch into

reducing sugars. However, the presence of a lower substrate amount led to a slower hydrolysis rate. The relationship between the substrate and enzyme concentration is limited to a certain concentration, *i.e.,* if the substrate concentration continues to increase, the enzyme activity will be inhibited.

Fermentation is the most promising method to enhance GABA content in food system. Thus, effective start-up development, *i.e.,* selecting the mycelium that is capable of producing large amounts of GABA, is of utmost importance. *A. oryzae* is one of the first moulds to be recognised for its beneficial effects in commercial production of foods such as

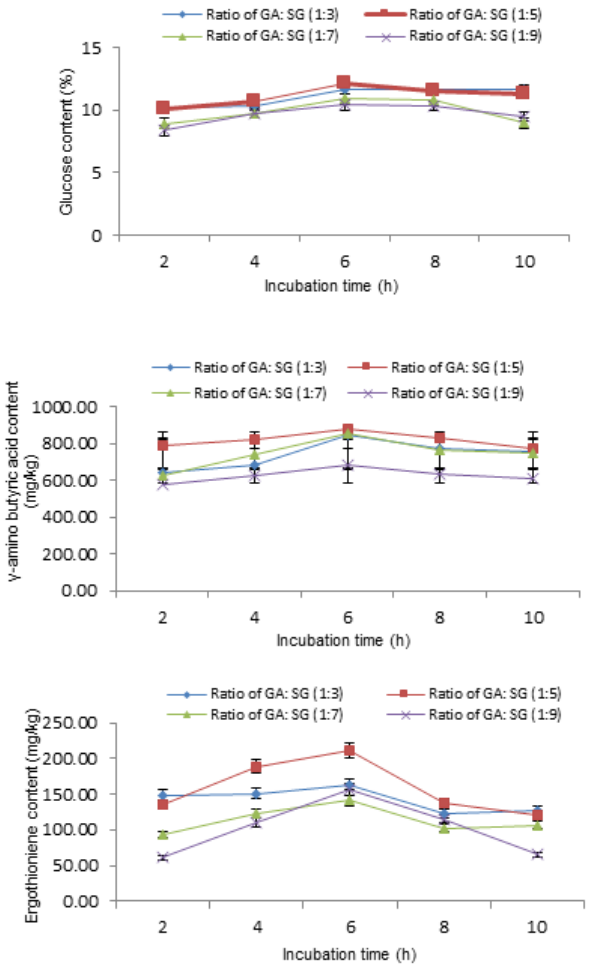

Figure 2. Changes in glucose (A), GABA (B), and ERG (C) contents during the production a sweet beverage enriched with bioactive compounds. GA = germinated brown rice *koji* with *A. oryzae* (the ratio of GBR to water was 1:1.5, inoculated with 0.4% *A. oryzae* spore (1.6×10^7 CFU/g) for 36 h); SG = steamed germinated brown rice (the ratio of GBR to water was 1:1.5). The mixture was then mixed with two volumes of water before incubation at 60°C, pH 5 for 2 - 10 h. GA = *Aspergillus oryzae*; GBR = germinated brown rice; SG = steamed GBR; and CFU = colony forming units.

miso, hamanatto, shoyu (soy sauce), and *tempeh*; and under favourable conditions, will produce a lot of GAD (Ab Kadir *et al.,* 2016). Figure 2 shows that there was a significant difference between GABA contents at different incubation times; the time with the highest GABA content was 6 h (883.9 mg/kg) with a GA:SG ratio of 1:5. Therefore, different incubation times could have a great influence on GABA production. GABA content varied with the ratio of additional *koji* rice, and showed an increasing trend with incubation time. During fermentation, the substrates of GABA formation are glutamic acid and glucose by the action of GAD (Ab Kadir *et al.,* 2016). Furthermore, GAD is the only enzyme that catalyses the irreversible conversion of L-glutamic acid to GABA and carbon dioxide through the α-decarboxylation pathway. During GABA production, the pH becomes more acidic, and GABA is still produced even after the pH increases. An increase in pH occurs due to the production of ammonia. Moreover, according to Dhakal *et al.* (2012), tempering temperature is also a major factor affecting the maximum GABA yield during fermentation due to the influence on the activity and stability of biological matter. GABA is formed by the activity of the enzyme GAD. Under the hydrolysis conditions to produce EBCSB at 60°C and pH 5, the increase in GABA content was most likely due to GAD still being active, leading to an increase in GABA in 6 h. However, after this time, the activity of GAD may decrease when exposed to high temperatures, while the activity of GABA deamination enzymes such as GABA transaminase was still active, which caused loss of GABA. *Gat1*, encoding GABA transaminase, is strongly expressed during fungal growth (Solomon and Oliver, 2002). GABA transaminase activity has also been detected and purified from several fungi, including *Aspergillus*. This enzyme could deaminise GABA to succinic semialdehyde using either pyruvate or α-ketoglutarate (Kumar and Punekar, 1997). Therefore, when the amount of GABA lost was greater than the amount of GABA formed, GABA content after 6 h decreased gradually.

During the incubation to produce SB, *A. oryzae* will depend on nutritional sources in the environment to release enzymes to hydrolyse nutrients such as amino acids to serve as a food source and at the same time to synthesise the necessary compounds for cell survival and development. The humidity of the culture medium greatly influences the growth and reproduction of microorganisms. If the humidity level is not suitable, it will disturb their normal physiological process,

especially that of mould, a microorganism that thrives in high humidity. In addition, moisture is one of the factors that allows microorganisms to accept food easily. It is thanks to high humidity that nutrients are easily absorbed into the body, which allows the hydrolytic enzyme systems to work. Through that process, mould helps to utilise amino acids effectively and contributes to the process of generating the necessary compounds. If the humidity is too low, the phenomenon of changing the state of the protoplasm occurs; when such a state change leads to the development of microorganisms, the ability to produce the necessary compounds decreases (Oguro *et al.,* 2019). The results in Figure 2 indicate that the content of ERG increased gradually when the incubation period was increased from 2 to 6 h, because *A. oryzae* continued to synthesise ERG. When the incubation period was long, the content of ERG decreased gradually; the reason is that the environment depleted the nutrients, so biosynthesis gradually decreased. Moreover, ERG was in the environment involved in fat oxidation. Therefore, it would decrease when the composting time was long. The ratio of GA:SG differed, leading to ERG content being increased and decreased in different ways during EBCSB incubation; at a ratio of 1:5, the highest ERG content was 210.9 mg/kg.

The sugar content of EBCSB products was only about 10%, which was much lower than that of yogurt-like products (13 - 14%) and *koji amazake* (22 - 23%) (Oguro *et al.,* 2017; Cáceres *et al.,* 2019). This may be because the dilution ratio in LAF-*amazake* processing was 1:3 while that in the present work was 1:5. Therefore, the sugar content of LAF-*amazake* products was doubled when compared with that in the present work. The results from Cáceres *et al.* (2019) also showed that GABA content was quite low, only approximately 0.05 mg/kg of a yogurt-like product, whereas GABA content determined in the present work reached more than 800 mg/kg, which was superior to that of yogurt-like products produced from GBR. Although there was still a partial increase in GABA during yogurt fermentation, it was actually negligible when compared with the EBCSB that was made from *Aspergillus koji*. Further Oguro *et al.* (2017) reported that in *amazake* and LAF-*amazake koji* products, GABA content was determined to be only approximately 167 ± 1 to 170 ± 2 mg/kg, respectively, quite small when compared with GABA content determined in this EBCSB product (more than 800 mg/kg). This indicated superior GABA content in *amazake* when prepared from GBR. Similarly, Oguro *et al.* (2017) also showed that ERG was detected at 818.6 ± 1.1 µg/100 mL in *koji amazake* and 470.1 ± 54.1 µg/100 mL in LAF-*amazake*. This was significantly lower than that in EBCSB of about 200 mg/kg. Thus, when using GBR as raw material for SB processing, the product would yield superior ERG and GABA contents when compared with the corresponding products. Long-term consumption of GABA-enriched foods, such as soaked or germinated BR, could prevent hypertension and hypercholesterolemia (Kawakami *et al.,* 2018). The present work indicated that EBCSB is a potential nutritional and functional food products.

Sterilisation and preservation of the EBCSB product

Thermal processing is designed to reduce microorganisms to a safe level. Optimising thermal processing is possible due to the higher temperature dependence of the inactivation rate of microorganisms as compared to the rate of decline in sensory and nutritional values. In the present work, EBCSB from fermented germ rice had a pH of 6.2 > 4.6, so EBCSB belonged to the group of low-acid, moderate heat treatment products that can be used for the purpose of killing *Clostridium botulinum* under conditions incorporating other factors to prevent damage. Pasteurisation is applied to low-acid foods, organised for distribution and consumption in room temperature environments. EBCSB products were sterilised at a temperature of 121°C for durations of 3, 4, 5 and 10 min, and the reference values are F_0 = 7 min, z = 10°C, and T_{ref} = 121.1°C. The results in Figure 3 show that, under the same sterilisation conditions with the same temperature of 121°C, the time taken for the product centre to reach the required temperature was almost identical. To ensure the required heat retention time, maintaining the heating environment temperature in the appliance is crucial in a closed device. The heat sterilisation process will inhibit the life and growth of microorganisms in the product because the sterilisation temperature is greater than the optimum growth temperature of microorganisms. At high temperatures, microbial proteins will coagulate and denature. Protein coagulation is not reversible, so destroyed microorganisms do not recover even after the product cools down.

The process of raising the temperature so that the product's central temperature reaches 121°C is quite long because of poor heat transfer in the glass packaging. Heat and cooling are also prolonged. Moreover, the longer the heat retention time, the greater the F value obtained and the safer the product. However, the longer heat retention time will reduce product quality and the cost of heating,

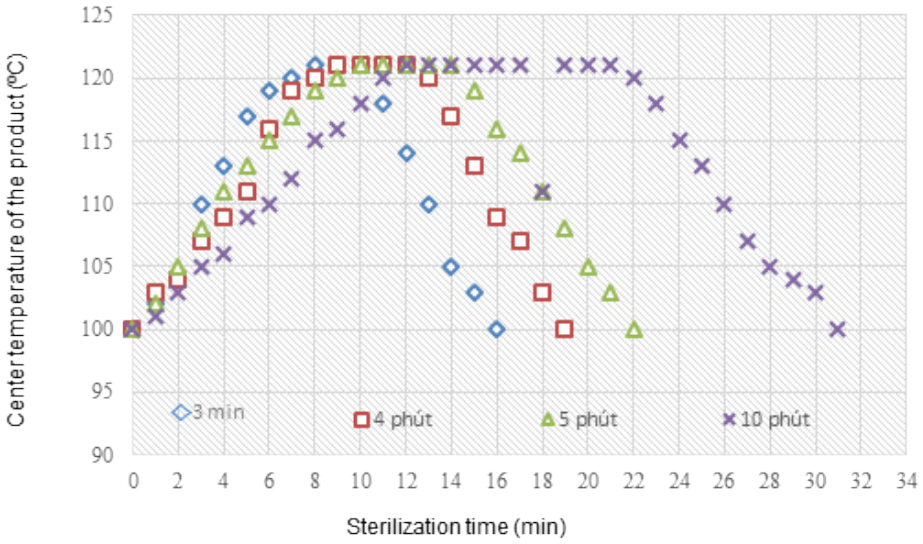

Figure 3. Changes in central temperature during sterilisation of sweet beverage enriched with bioactive compounds.

Table 2. Changes in GABA, ERG, and microbial loads of EBCSB after eight weeks of preservation at room temperature (30 ± 2°C).

Parameter	0 week	2 weeks	4 weeks	6 weeks	8 weeks
GABA (mg/kg)	815.73 ± 7.85^{a}	733.56 ± 7.35^{b}	727.96 ± 9.72^{b}	698.69 ± 2.16^{bc}	682.07 ± 4.17^{bc}
ERG (mg/kg)	201.62 ± 2.52^{a}	185.88 ± 7.28^{ab}	128.23 ± 0.40^{c}	119.74 ± 2.78^{d}	109.85 ± 1.96^{de}
Peroxide (mEq/kg lipid)	1.62 ± 0.38^{a}	1.76 ± 0.32^{b}	1.84 ± 0.26^{cd}	1.95 ± 0.10^{de}	1.99 ± 0.05^{e}
Total aerobic microorganism (CFU/g)	-	-	-	-	-
Escherichia coli (MPN/g)	-	-	-	-	-
Staphylococcus aureus (CFU/g)	-	-	-	-	-
Coliform (CFU/g)	-	-	-	-	-
Clostridium pefringens (CFU/g)	-	-	-	-	-
Total yeast and mould (CFU/g)	-	-	-	-	-

Microbial analyses followed AOAC (2010); - = not detected; CFU = colony forming unit; and MPN = most probable number.

thus suggesting that the most appropriate *F* sterilisation value selected is 7.47 under sterilisation conditions with a temperature of 121°C at 4 min. The change in GABA and ERG contents during eight weeks of storage is shown in Table 2. GABA content in the first week of storage was 815.7 mg/kg, and then decreased to 733.6 mg/kg after two weeks of storage; these were significantly different ($p < 0.05$). From the second week to the eighth week, GABA content decreased with no statistical significance. This change was due to GABA content in the product, which was affected by oxidation during storage (Parnsakhorn and Lankapin, 2013; Jongyingcharoen and Cheevitsopon, 2016). Similarity, ERG content decreased with storage time. After two weeks of storage, ERG content decreased, and similar for several weeks (4, 6 and 8). However, when the storage time was from week 2 to 4, the content of ERG decreased rapidly from 185.9 to 128.2 mg/kg. According to Akanmu *et al.* (1991),

during preservation, ERG participates in lipid oxidation, which gradually decreases its content during the storage time. Besides, with peroxide, despite a slow increase during eight weeks, it was still good enough. This may be due to the presence of ERG as a strong antioxidant. In addition, the microbiological analysis results in Table 2 show that the product was safe after at least eight weeks of storage. The result of preservation finally indicated that EBCSB was still stable for at least eight weeks of preservation at room temperature.

Conclusion

Overall, it is entirely possible to use GBR in producing EBCSB to ensure food safety and hygiene. The present work demonstrated that SB from GBR contained high levels of GABA and ERG that would protect consumers' health. This is an outstanding feature of this product.

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