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The Study of GABA Content and Development of GABA-Enriched Yogurt from Germinated Red Rice (Munpu rice)

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ABSTRACT

The objectives of this study were to investigate the effect of germination time on the gamma-aminobutyric acid (GABA) content of germinated aromatic paddy and red rice and to develop yogurt with enhanced levels of GABA. Germinated red rice was prepared by soaking in water, using a rice-to-water ratio of 1:3 (w/v) for 24 h and leaving in darkness for 48 h to germinate, while collecting samples every 8 h. The GABA content of germinated grains was determined using high performance liquid chromatography (HPLC). The results indicated that the GABA content of grains after germination significantly increased compared to that of control grains and the optimum condition providing the highest content of GABA (21.32 mg/100 g DW) was at 32 h after germination. The yogurt formula was developed and 30% germinated red rice flour prepared using the optimum conditions was selected to add to the formula. The enriched yogurt contained 4.09 mg/100 g of GABA, which was significantly higher than that of the control yogurt, in which GABA was not detected.

Key words: GABA, germination, aromatic red rice, yogurt

INTRODUCTION

Rice is considered the main staple food for about half of the world's population. The production and consumption of rice are concentrated in Asia (Kakinuma, 2004). Munpu rice is one of pigmented rice varieties that contains a high amount of anthocyanin, which has higher levels of antioxidant and anticancer activity than other varieties (Min, 2004). In addition to providing other sources of nutritional benefit, such as vitamin B, E, beta-carotene and gamma oryzanol, rice germ contains a good bio-functional compound, γ-amino-butyric acid, or GABA (Mori *et al*., 1999).

GABA is a four-carbon, non-protein amino acid and widely distributed in nature. It is known to be one of the major inhibitory neurotransmitters in the sympathetic nervous system and to play an important role in cardiovascular function (Takahashi *et al*., 1955). Typically, GABA levels in plant tissues are low (ranging in fresh weight (FW) from 0.03 to 2.00 μmol/g FW) but increase several fold in response to many diverse stimuli, including heat shock, mechanical stimulation, hypoxia and phytohormones (Shelp *et al*., 1999). It has been reported that soaking brown rice in water promotes germination, and GABA is accumulated during this process (Komatsuzaki *et al*., 2005). GABA in rice grains is synthesized from glutamic acid decarboxylase (GAD), and the activity of GAD

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shows high correlation with the germination ratio (Bautista *et al*., 1964). Due to germination, there are certain changes that could occur, including the quantity and type of nutrients within the seed (Bau *et al*., 1997; Dhaliwal and Aggarwal, 1999). Therefore, the aim of this study was to evaluate the effect of germination condition on the GABA content in aromatic red rice and to develop yogurt with an enhanced level of GABA.

MATERIALS AND METHODS

Materials

Samples were collected of aromatic red rice (Munpu rice) cultivated in the north of Thailand, in paddy rice (husk remaining) and brown rice (husk removed) form. Both samples were harvested in 2006 and stored at 4°C before analysis.

Chemical reagents

Standard γ-amino-n-butyric acid was obtained from Sigma. Acetic acid, ethanol and sodium acetate (analytical grade), were purchased from Merck. Acetonitrile, of high performance liquid chromatography (HPLC) -grade, was purchased from Lab-Scan. Triethylamine and phenyliso-thiocyanate (PITC) were obtained from Unilab and Fluka, respectively.

Rice germination

Rice grains were rinsed using tap water, disinfected by soaking in 0.1% sodium hypochlorite for 30 min and rinsed twice with sufficient water. The germination process was carried out by the method of Ohtsubo *et al*. (2005), with a slight modification. The experimental plan was a 2×7 factorial design, in which there were two forms of rice grain (with and without husks), and seven different germination times. Sanitized grains were soaked in water (1:3 w/v) for 24 h and the water was drained off. Soaked grains were allowed to germinate in darkness for 48 h. Rice grains were sampled at 0, 8, 16, 24, 32, 40 and 48 h during germination. Germinated grains were placed in aluminum trays and dried to less than 13% moisture content at 50°C using a tray dryer. Dry germinated paddy rice was once milled in a rice-milling machine to remove the husks. Rice samples were then ground to rice flour, which was packed in plastic bags and stored at refrigeration temperature for GABA analysis. For a comparative study, flour was also prepared from un-germinated rice grains as the control.

Preparation of germinated aromatic red rice for yogurt making

Germinated aromatic red rice with the highest GABA content was selected as an important ingredient for making yogurt. Briefly, 50 g of germinated red rice grains was ground in a food blender for 3 min. One hundred and fifty ml of water was added to the red rice powder and the mixture was water steamed for 20 min before leaving to cool down. The resulting germinated red rice paste was further used as a yogurt ingredient.

Development of yogurt formula

Production of yogurt with enhanced levels of GABA using germinated red rice was studied. The main ingredients for yogurt production are given in Table 1.

Table 1 The main ingredients for making yogurt.

Component	Content (g)
Milk	100
Skim milk powder	4
Sucrose	5
Starter culture (*S. thermophillus* and *L. bulgaricus*)	17

Milk was heated at 92°C for 5 min. Skim milk powder and sucrose were then added. The mixture was cooled down to 45°C, inoculated with the starter (*S. thermophillus* and *L. bulgaricus*) and fermented at room temperature. The fermentation was stopped when the pH of the yogurt reached 4.4-4.6 and the yogurt was stored at 4°C. Commercial yogurt was used as a control in this experiment. The yogurt with enhanced levels of GABA was produced by substituting milk in the formula with 30, 35 and 40% (w/w) germinated red rice paste, prepared as previously described.

Sensory evaluation of yogurt

Samples of yogurt with 30, 35 and 40% (w/w) germinated red rice substituted in the formula were subjected to evaluation of their sensory properties, including color, odor, taste, texture and overall liking, using a nine-point hedonic scale, where 1 was dislike extremely, 5 was neither like nor dislike and 9 was like extremely. In order to select the most acceptable product, differences in preferences between the products were analyzed using Duncan's multiple range test (DMRT). Moreover, a "just-about-right test" (JAR), by 40 panelists, was used to describe the attribute intensities of the product, including color, rice odor, yogurt odor, sourness, viscosity and texture, where 1 equaled "much too weak", 3 equaled "just about right" and 5 equaled "much too strong" (Lawless and Heymann, 1998).

Determination of GABA content

The GABA content of the rice and yogurt samples were analyzed using HPLC as described by Wang *et al*., (2004), with a slight modification. GABA was extracted and derivatized to phenylthiocarbamyl-GABA (PTC-GABA). After derivatization, the dry residue containing PTC-GABA was dissolved in 200 μl of the mobile phase. The supernatant was filtered using a nylon membrane filter (13mm dia. 0.45 μm) and injected into HPLC equipped with a column (Cosmosil C18, 4.6 mm I.D. × 150 mm, Nakarai Tesque, Inc., Kyoto, Japan). A mixture of 80% solution A (aqueous solution of 8.205 g sodium acetate, 0.5 ml triethylamine, 0.7 ml acetic acid and 5.0 ml acetonitrile in 1000 ml) and 20% solution B (acetonitrile-water; 60:40), adjusted to pH 5.8, was used as the mobile phase with a flow rate at 0.6 ml/min and the injection volume was 20 μl. Column temperature was 40°C and the ultraviolet detector was set at 254 nm.

Isocratic HPLC separations were performed on a Shimadzu, Class VP apparatus, consisting of a model LC-10AD VP liquid chromatograph, a model CTO-10A with a 20-μl injection loop and a model SPD-10A UV-vis detector.

RESULTS AND DISCUSSION

The GABA contents (mg/100 g DW) of germinated and un-germinated paddy rice and red rice are shown in Table 2. From statistical analysis of the 2×7 factorial design, an interaction between the rice type and germination time was observed. Table 2 shows that the germination time and rice type affected the GABA level; GABA contents in both germinated rice types continued to increase and then decreased rapidly during the last period of germination. The GABA content of un-germinated paddy rice was 0.86 mg/100g. The highest GABA content in paddy rice was found in grains germinated in darkness for 40 h (13.09 mg), which was 15.2 times higher than that of un-germinated paddy rice. However, the GABA content decreased rapidly to 5.09 mg at the last measurement period in germination (48 h).

Similar to paddy rice, the GABA content in un-germinated red rice, was relatively low (initial content 1.03 mg/100 g DW). Immediately after soaking (0 hr germination time), the GABA level increased to 2.05 mg. During the germination process (8-48 h), the GABA content increased noticeably several fold, varying from 2.55 to 21.32

Table 2 GABA content of germinated paddy and red rice of aromatic red rice at different germination times.

Rice type	Germination time (h)	GABA content (mg/100 g, DW)
Paddy rice*	0	1.53[h] ± 0.07
	8	1.50[h] ± 0.01
	16	2.4[f] ± 0.00
	24	3.72[f] ± 0.04
	32	4.97[e] ± 0.02
	40	13.09[c] ± 0.10
	48	5.09[e] ± 0.04
Red rice**	0	2.05[gh] ± 0.04
	8	2.55[f] ± 0.04
	16	4.67[e] ± 0.18
	24	7.21[d] ± 0.93
	32	21.32[a] ± 0.77
	40	17.18[b] ± 0.34
	48	5.29[e] ± 0.00

Note: Different letters in the same column are significantly different by DMRT (P<0.05).
* = GABA content 0.86 mg/100 g DW
** = GABA content 1.03 mg/100 g DW

mg. However, a significant reduction of the GABA content (P<0.05) to 17.18 and 5.29 mg/100 g was observed in the last periods of germination at 40 and 48 h, respectively. This could have been due to the shortage of glutamate, a substrate of GABA producing enzymes (glutamic acid decarboxylase (GAD)), leading to a decline of GABA in the later stages of the germination period (Saisuka *et al*., 1994). During the germination process, the germination ratio of red rice was higher than that of paddy rice. This agreed with the higher amount of GABA in germinated red rice compared to that of germinated paddy rice.

GABA in rice grains is synthesized from glutamic acid by GAD and the activity of GAD has shown a high correlation with the germination ratio (Bautista *et al*., 1964). Saikusa *et al*. (1994) reported that GABA could be increased substantially by soaking rice germ in water as due to water absorption hydrolytic enzymes are activated. In addition, storage protein is decomposed, changed into transportable amide and conveyed to the growing parts of the rice seedling (Lea *et al*., 1990). During the soaking process, GAD becomes activated and glutamic acid is converted to GABA. Furthermore, the concentration of GABA rises remarkably by several times in response to many diverse stimuli, including, hypoxia, cold shock and darkness (Servaites *et al*., 1979; Robert *et al*., 1984)

The increment and degradation of the GABA content in this study could be explained by protein turnover. Hydrolysis of seed storage proteins during germination provides amino acids for protein synthesis in the growing seedling (Singh, 1999). Moreover, it was also observed that the GABA level in germinated paddy rice tended to be lower than that of germinated red rice. This could have been due to the rate of water absorption or the differences in chemical composition, as well as the storage time after harvesting.

From the results shown in Table 2, the optimum condition for producing red rice with the highest GABA content was 32 h of germination period for red rice without husks. This sample, therefore, was selected for further use as an ingredient in the yogurt.

Development of yogurt with enhanced levels of GABA

The replacement of germinated red rice paste at 30, 35 and 40% (w/w) in yogurt to enhance the level of GABA had a considerable effect on the sensory characteristics as shown in Table 3.

The results showed that there were no significant differences in the preferences for color and texture among the samples (P<0.05). Furthermore, no change in the preferences for appearance, odor, taste and overall liking was observed between the samples with replacement of 35 and 40% germinated red rice paste, respectively. The addition of germinated red rice in the product was limited, since it caused an undesirable flavor after the fermentation process.

However, in this experiment, the replacement of 30% germinated red rice paste resulted in the most preferable and acceptable yogurt, with the sensory scores ranging from 5.88 to 7.35. In order to obtain yogurt that most satisfies the consumer, attributes of the product, including color, yogurt odor, rice odor, sourness, thickness and texture were developed using the just-about-right test. The frequencies of each attribute evaluated by the 40 panelists are shown in Figure 1.

Table 3 Mean scores for preferences on yogurt properties using the nine-point hedonic scale.

Germinated red rice content	Preference scores (Mean ± SD)					
	Appearance	Color^{ns}	Odor	Taste	Texture^{ns}	Overall liking
30 %	7.35^{a*}±1.22	6.88±1.36	6.82^{a}±1.51	5.88^{a}±1.70	5.90±1.60	6.29^{a}±1.65
35 %	6.18^{ab}±1.91	5.94±1.92	5.00^{b}±1.84	4.35^{b}±1.41	5.20±1.40	4.82^{b}±1.78
40 %	5.88^{b}±2.06	5.88±2.03	5.12^{b}±2.12	4.65^{b}±2.06	5.10±1.45	4.76^{b}±1.48

Note: Different letters in the same column are significantly different by DMRT (P<0.05).
ns = not significant.

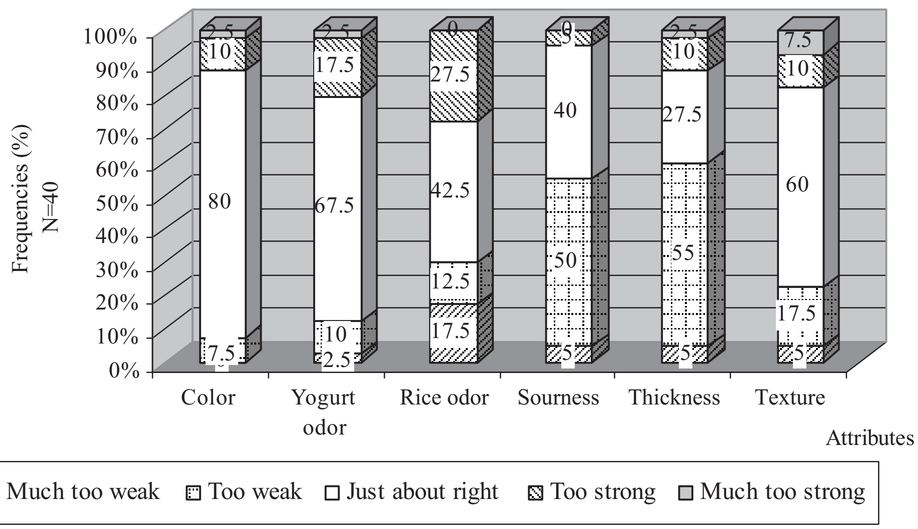

Figure 1 Attributes diagnosed and JAR percentages before formula modification.

The highest percentage of ratings by participants using the JAR scale, revealed that the intensities of color, yogurt odor, rice odor and texture of the product were rated as "just-about-right". On the contrary, the product was most rated as "too weak" for sourness and thickness strength.

Subsequently, to improve the sourness and thickness of the product, the level of sucrose was lessened to 2% (w/w) combined with an extension of the fermentation time, while more skim milk powder was added to increase the thickness. The response on the frequency of each attribute after the modification is shown in Figure 2.

Figure 2 shows that the opinions on most attributes were rated as "just-about-right" with the highest percentage of respondents ranging from 55 to 75%. The final yogurt formula was subsequently analyzed for GABA content.

GABA Content in yogurt

Results of the GABA content in the product are shown in Table 4. From Table 4, the GABA levels of yogurt containing 30%(w/w) germinated red rice paste was 4.09 mg/100 g, which was remarkably higher than that in control yogurt (reported as not detected). It has been reported that the levels of GABA in commercial yogurts were normally low and have rarely been detected (Park and Oh, 2006).

However, the functional and nutritive values could be increased by applying microorganisms, since yogurt is a fermented food containing some strains of lactic acid bacteria with

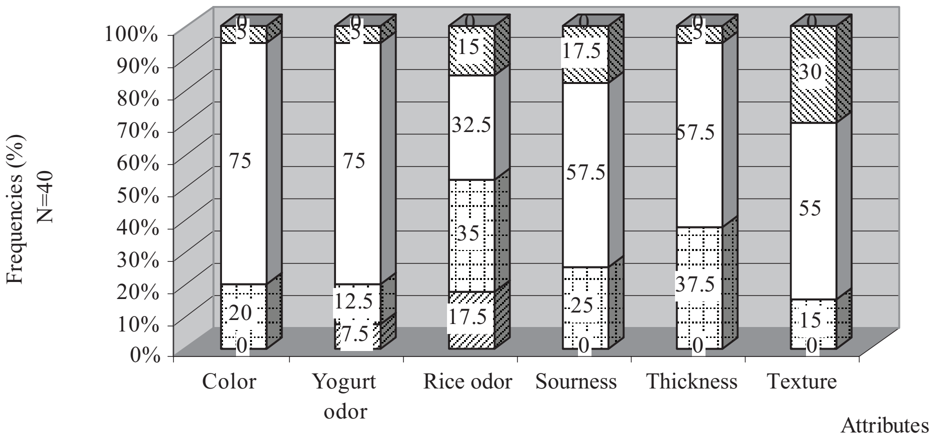

Figure 2 Attributes diagnosed and JAR percentages after formula modification.

Table 4 Changes in the levels of GABA in different steps of yogurt production.

Samples	GABA content* (mg/100 g)
Germinated red rice	$19.46^{a} \pm 0.70$
Germinated red rice paste	$17.87^{a} \pm 0.53$
GABA-Yogurt	$4.09^{b} \pm 0.22$
Control yogurt	Not detected

Note: * = Mean± SD

Different letters in the same column are significantly different by DMRT (P<0.05).

Table 5 Relationship between purchasing intent and effect of information given to panelists.

Purchasing intent	Before providing nutritional data	After providing nutritional data	χ^2
Yes	100	137	28.6*
No	9	0	
Not sure	41	13	
Total	150	150	

Note: * = Pearson Chi-square at · = 0.05, df = 2.

a high GABA-producing ability. Lactic acid bacteria are largely used in a variety of fermented foods, especially for the manufacture of dairy products with functional and probiotic properties (Leroy and Vuyst, 2004). Moreover, yogurt with a high GABA content in this study could be explained by its ingredients. Germinated red rice is a very good source of GABA that could not be destroyed by water steaming. Likewise, Komatsuzuki *et al.* (2005) reported that there was no change in the GABA content in germinated brown rice after pasteurization by water steaming for 10, 20 and 30 min.

Consumer test for final product

The final yogurt formula was subjected to a purchase decision test carried out by 150 panelists, with variation in sex, age, education, occupation and salary. Before and after information relating to the benefits of GABA was given, panelists were asked whether they would buy the product and then the frequency of each purchase decision (Yes, No, and Not sure) was counted as shown in Table 5.

The relationship between purchasing intent and the effect of information given was expressed using Chi-square (χ^2). From Table 5, the number of respondents who decided to buy the product increased after they were informed about benefits of GABA. The results of the Chi-square test revealed that the purchasing decision could be significantly influenced by the information given to panelists.

CONCLUSIONS

From the present investigation, it was found that the GABA content of germinated red rice was higher than that of un-germinated paddy and red rice. Soaked red rice grains germinated in darkness for 32 h contained the highest GABA content (21.32 mg/100 g). The inclusion of 30% germinated red rice flour (w/w) was selected for the yogurt formula. The enriched yogurt contained 4.09 mg/100 g of GABA, which was significantly higher than that of commercial yogurt, where GABA was not detected. This study revealed that germination could add more value to red rice. Product development of enriched GABA yogurt was possible using germinated red rice as an ingredient. The nutritious product is beneficial to general customers as well as those who are health-conscious.

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