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Contributed Paper

Determination of GABA Content in Thai Brown Rice by an Optimized Enzyme-Based Method

Suwapat Kittibunchakul [a], Parunya Thiyajai [a], Uthaiwan Suttisansanee [a] and Chalat Santivarangkna [a]*

[a] Institute of Nutrition, Mahidol University, Nakhon Pathom 73170, Thailand.

*Author for correspondence; e-mail: chalat.san@mahidol.ac.th

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ABSTRACT

GABA content in brown rice from 32 Thai rice varieties was determined using a rapid enzyme-based method. The enzyme GABase and $NADP^+$, which were expensive reagents used in the assay, were optimized. GABA was firstly extracted from brown rice with the optimal extraction technique using 0.3% (w/v) sulfosalicylic acid and sonication. The GABA content was then indirectly determined by the absorbance measurement of NADPH derived from enzymatic reaction of GABase. Under the optimized assay system, the enzyme-based method could accurately and economically quantified GABA when GABA concentration was in the range of 0-1.0 mM. With the GABA recovery of 92.71% evaluated by the spike recovery test, GABA in rice was precisely quantified by the optimized method. Among rice varieties that were examined, Chaw Lamud contained the lowest GABA content (7.60 mg/100 g), while RD41 contained the highest GABA content (29.46 mg/100 g). Concentration of GABA in brown rice was positively correlated with size and shape of rice grains. In conclusion, the enzyme-based method optimized in this study could be applied well for the determination of GABA from rice, and may also replace the commonly-used HPLC analysis of GABA in other raw-materials. The information on GABA content in rice obtained from this study will be useful for the production of healthy rice products.

Keywords: GABA, Brown rice, GABase, Enzyme-based method, GABA analysis, Bioactive nutrient

1. INTRODUCTION

Rice is one of the most important crops that play role as a staple food in many parts of the world. In addition to its nutritional benefits, rice contains several bioactive compounds including γ-aminobutyric acid (GABA). GABA is a well-known derivative of free glutamic acid that is widely used as a dietary supplement as well as a functional ingredient in health foods. The remarkable roles of GABA in suppression of stress and treatment of various chronic diseases, such as hypertension, cardiovascular disease and diabetes have been proven by clinical studies [1-3]. In rice, GABA is accumulated in the

germ and bran layers [4]. Brown rice is well recognized as a good source of GABA, which is richer in quantity comparing to the ordinary milled rice [5].

Several methods have been demonstrated for measuring the quantity of GABA, and a high-performance liquid chromatography (HPLC) is commonly used in the analysis. Nevertheless, detecting GABA by this method has high cost and is inconvenient because the derivatization of GABA is required [6]. This is a drawback when a large number of samples must be tested.

Among the methods used for analysis of GABA, the enzyme-based method has been used successfully for the determination of GABA in liquid lactic acid bacterial growth medium [6]. By using the enzyme-based method, GABA content can be indirectly determined by the absorbance measurement of NADPH derived from a reaction catalyzed by enzyme GABase [7]. The application of enzyme-based method utilizing a 96-well microtiter plate allows the quantification of GABA from a large number of samples simultaneously. However, this method has not yet been applied to the GABA analysis of rice. Therefore, this study aimed to determine the GABA content in commercial and local Thai brown rice by the newly-developed rapid enzyme-based method. The GABA extraction from rice was optimized in this study as well.

2. MATERIALS AND METHODS

2.1 Rice and Chemicals

Brown rice grains from 32 varieties of commercial and local Thai rice (*Oryza sativa* L.) were obtained from Thai local markets and Bureau of Rice Seed, Rice department, Ministry of Agriculture and Cooperatives of Thailand. Rice varieties were Khao Dawk Mali 105, Hua Na, Hawm Jan, Maw Arun, Yah Sai, Chaw Lamud, Chiang Phatthalung, Chaw Jangwad, RD41, Pathum Thani 1, So Mali, Beu Gi, Phitsanulok 2, Suphan Buri 1, Sin Lek, Chai Nat 1, Khao Tah Haeng 17, Hawm Mali Daeng, Leuang Soi Tawng, Si Nin, Sangyod Phatthalung, Rice Berry, Hawm Daeng Sukhothai 1, Law Taek, Niaw San-pah-tawng, RD6, Saen Sabai, Gam Pleuak Khao, Gam Yai, Gam Noi, Niaw Daeng Yai and Leum Pua. Rice grains were packed under vacuum in plastic bags and stored at 4 °C throughout the experiment.

The chemicals used in this study including γ-aminobutyric acid (GABA) (purity ≥ 99%), tris(hydroxymethyl) aminomethane ($NH_2C(CH_2OH)_3$), dithiothreitol (DTT), nicotinamide adenine dinucleotide phosphate ($NADP^+$), α-ketoglutaric acid disodium salt dihydrate ($C_5H_4Na_2O_5 \cdot 2H_2O$), sodium sulfate ($Na_2SO_4$) and *Pseudomonas fluorescence* GABase (0.97 units/mg) were purchased from Sigma-Aldrich (St. Louis, MO, USA), while 5-sulfosalicylic acid dihydrate ($C_7H_6O_6S \cdot 2H_2O$) and sodium bicarbonate ($NaHCO_3$) were obtained from Merck (Damstadt, Germany). 4-Dimethyl-aminoazobenzene-4-sulfonyl chloride (DABSYL-CL) was obtained from Fluka Chemika-Biochamika (Buchs, Switzerland). Solvents including acetonitrile (HPLC grade) and absolute ethanol were purchased from Lab-Scan Analytical Sciences (Dublin, Ireland) and RCI Labscan (Bangkok, Thailand), respectively.

2.2 Validation of Enzyme-Based Method for Determination of GABA

The enzyme-based method was modified from the method for the determination of GABA produced by lactic acid bacteria [6]. The assay consisted of Na_2SO_4 solution (750 mM), DTT solution (10 mM), α-ketoglutarate solution (2 mM), $NADP^+$ solution (1.4 mM), and GABase

(50 µg/100 µL) in 80 mM Tris-HCl buffer (pH 9.0). To the reagents (90 µL), 0-100 mM standard GABA solution (10 µL) was added and carefully mixed in a 96-well microtiter plate. After incubation at 30 °C for 1 hr, the absorbance of the mixture was measured at 340 nm using a microplate reader (Synergy HT Multi-Mode Microplate Reader, Biotek Instruments Inc, Winooski, VT, USA). The blank value was measured in the absence of enzyme GABase. The range of GABA concentration representing a linear relationship with the absorbance was defined as the detection range of the method.

2.3 Optimization of GABase and $NADP^+$ Concentration for Determination of GABA

The enzyme GABase and $NADP^+$, which were relatively expensive reagents used in the enzyme-based assay, were optimized to reduce the analytical cost. Based on the enzyme-based method of Tsukatani *et al.* [6], various reactive concentrations of GABase, including 10, 20, 30 and 50 µg/100 µL, were mixed with $NADP^+$ (1.4 mM) to determine the minimum GABase concentration that could accomplish the enzymatic reaction with 1 mM GABA within an hour. The selected concentration of GABase was tested with four reactive concentrations of $NADP^+$, including 0.7, 1.4, 7.0 and 14.0 mM to obtain the appropriate $NADP^+$ concentration. The developed method with the optimized GABase and $NADP^+$ concentration would be applied for the further study.

2.4 Extraction of GABA from Rice

Rice germ of Khao Dawk Mali 105 was used to evaluate the effect of extraction methods on GABA extraction capacity. The sample was firstly ground into rice powder by a high speed miller (Cyclotec 1093 Sample Mill, FOSS Ltd., Hoganas, Sweden). GABA extraction with deionized (DI) water, sulfosalicylic acid (0.3% w/v) and ethanol (75% v/v) were examined using the processes modified from Varanyanond *et al.* [8], Banchuen *et al.* [9], and Watchararparpaiboon *et al.* [10], respectively. To increase GABA extraction capacity, the additional step of sonication using a sonicator (input of 240 V. 50/50 Hz.) was applied with the reference extraction methods. The mixture of solvent and rice powder was sonicated for 10 min with mixing every 2 min. After removing rice particle, GABA in rice extract was analyzed. GABA content measured by the developed enzyme-based method was compared with that measured by HPLC. In addition, interlaboratory study of the effect of extraction methods on GABA extraction capacity was observed. The test was conducted by the Center for Scientific and Technological Equipment (CSTE), Suranaree University of Technology (Nakhon Ratchasima, Thailand) and Central laboratory Co., Ltd. (Bangkok, Thailand) using the methods modified from Varanyanond *et al.* [8] and Mustafa *et al.* [11], respectively. Moisture content of the rice powder (5 g) was measured by an infrared moisture balance (FD-720, Kett Electric Laboratory, Tokyo, Japan). Thus, dry weight of rice sample could be calculated. GABA content was reported as mg/100 g of rice, on a dry weight basis.

Effect of sieving on GABA extraction was also investigated. Rice powder of Khao Dawk Mali 105 was sieved through a 60 mesh screen (pore size of 0.25 mm). The rice particles, which passed through the sieve pores, were defined as sieved rice powder. The rice particles left on the sieve were defined as non-sieved powder. Concentrations of GABA in the extracts of sieved and non-sieved sample as determined

by the developed enzyme-based method and the HPLC were compared, and the condition provided higher GABA content was applied with the appropriate solvent for the extraction of GABA from brown rice.

2.5 Analysis of GABA by HPLC

The analysis of GABA in rice by HPLC was conducted according to the method of Banchuen *et al.* [9] with a slight modification. Rice extract prepared as shown above (50 µL) was mixed with 100 mM $NaHCO_3$ (50 µL) and 4 mM DABSYL-Cl acetonitrile solution (50 µL). GABA was then derivatized by heating the mixtures at 70 °C for 10 min. After derivatization, the sample was mixed with absolute ethanol (250 µL) and 25 mM phosphate buffer (250 µL, pH 6.8). The samples were then filtered through a 0.2 µm filter and analyzed using HPLC. The determination of GABA was carried out by an Agilent HPLC 1100 series (Agilent Technologies, USA). The HPLC was equipped with Supelcosil LC-DABS column (4.6×150 mm, 3 µm) (Supelco, Bellefonte, PA) and diode array detector (at 465 nm). The mobile phase was made up from 25 mM acetate buffer:acetonitrile in the ratio of 65:35, respectively. The system was operated at 55 °C with the flow rate of 0.5 mL/min. The concentration of GABA was calculated from the area under its own peak comparing with the calibration curve of standard GABA (purity ≥ 99%).

2.6 Accuracy Evaluation of Enzyme Based Method for Determination of GABA Content in Rice

To assess the accuracy of the optimized enzyme-based method, the spike recovery technique was used. The extract from sieved rice powder of Khao Dawk Mali 105 rice was spiked with standard GABA solution. The concentrations of standard GABA solution used for spiking were 50%, 100% and 150% of the known GABA content represented by the non-spiked sample. GABA content measured from spiked samples was computed, and accuracy of the method was accepted when the recovery was in the range of 100±10% [12].

2.7 Determination of Width, Length and Thickness of Brown Rice Grains

Twenty grains of brown rice from each rice variety were collected. Their width, length and thickness were measured by a Vernier caliper. Area of the grains was calculated by multiplying the width by the length [13]. The length describes the grain size, and the length-to-width ratio describes the grain shape. Rice was categorized by size and shape of the grain according to Dela Cruz and Khush [14]. The correlations between GABA content and some characteristics of the grains, including size, shape, width, thickness and area, were determined.

2.8 Statistical Analysis

GABA content was expressed as mean±standard deviation. Analysis of Variance (ANOVA) was used to determine the difference among mean values. When significant difference was detected, mean values was then compared using Duncan's Multiple Range Test (DMRT). Relationships between characteristics of brown rice grains and GABA content were examined using Pearson's correlation (*r*) Test. The statistical analysis were carried out at significant value ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Detection Range of Enzyme-Based Method for Determination of GABA

In this study, the enzyme GABase was used for the determination of GABA. As a result, the GABase enzymatic reaction

proceeded well under the assay system. The detection range of the method, which was presented by the range of GABA concentration corresponded to the linear part of the curve, suggested that the enzyme-based method could accurately determine GABA at concentration ranged from 0 to 1.0 mM (Figure 1). This detection range is wide enough to detect GABA content in rice. Besides, the great sensitivity with the detection limit approaching 0 mM allows the method to analyze the sample containing very low GABA concentration and to clearly define the sample without GABA.

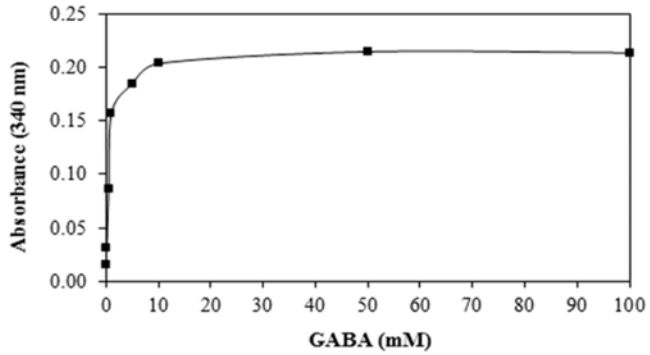

Figure 1. Correlation between concentration of GABA in the range of 0-100 mM and absorbance at 340 nm. The absorbance was measured after 60 min of GABase enzymatic reaction at 30 °C.

3.2 Optimized GABase and $NADP^+$ Concentration for Determination of GABA

The optimization of the enzyme suggested that the reaction of 30 µg/100 µL of GABase and 1.4 mM of $NADP^+$ with 1 mM of GABA reached the equilibrium at around 40 min. This was similar to the time required for the concentration of GABase at 50 µg/100 µL (Figure 2). On the contrary, at the GABase concentration lower than 30 µg/100 µL, the reaction required longer than 60 min to reach the end point. Because the concentration of 30 µg/100 µL was the minimal concentration of GABase that completed the reaction within the stipulated time, it was the optimal GABase concentration that was used in this study.

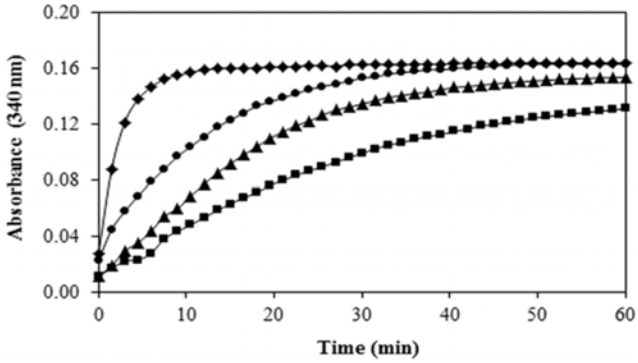

Figure 2. Effect of concentrations of GABase on the increase in absorbance during enzymatic reaction. GABase concentration (µg/100 µL): 10 (■), 20 (▲), 30 (●), 50 (◆). The reactions were performed in 80 mM Tris-HCl buffer (pH 9.0) containing 750 mM Na_2SO_4, 10 mM DTT, 2 mM α-ketoglutarate, 1.4 mM $NADP^+$ and 1 mM GABA at 30 °C.

With the optimal GABase concentration, it was founded that $NADP^+$ at the concentration of 1.4, 7.0 and 14.0 mM yielded the maximum absorbance (Figure 3). At 0.7 mM of $NADP^+$, however, the maximum absorbance was lower than those from the other concentrations. For this reason, the reactive $NADP^+$ concentration of 1.4 mM was chosen for the GABA analysis. The optimized $NADP^+$, concentration was 10 times lower than that had been suggested by Tsukatani *et al.* [6].Under the optimized condition, the results obtained from 10 separate experiments showed that the enzyme-based method developed in this study had high linearity and repeatability in GABA concentration range of 0-1.0 mM (data not shown). The developed method for the determination of GABA with the microplate reader allowed the analysis of 96 samples within 60 min, whereas the traditional HPLC takes around 40 min for one sample [15]. As calculated per unit sample, the method provided a cheaper analysis of GABA with high simplicity and convenience than using the HPLC. The expense needed for the HPLC analysis is ca. 40 USD per one sample, while the estimated analysis costs of the enzyme-based method per samples are ca. 7-10 USD depending on the number of samples per an analysis. With regards to the environmental concern, the optimized enzyme-based method could be considered as an environmentally friendly method because the volume of the assay is very small, and chemicals and materials used in the analysis are not hazardous. Since the enzyme-based method for the determination of GABA developed in this study applied well with the standard GABA, it had potential to apply with the determination of GABA from rice.

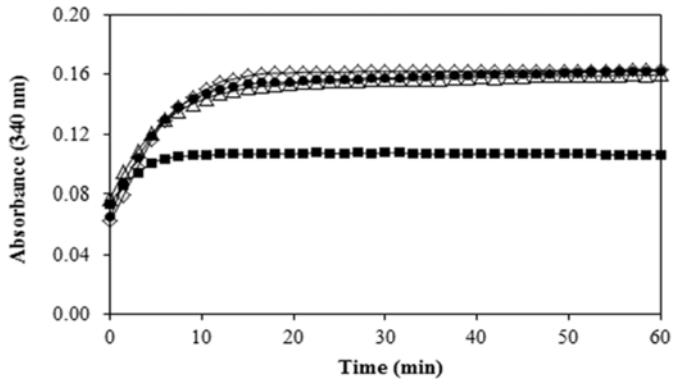

Figure 3. Effect of concentrations of $NADP^+$ on the increase in absorbance during enzymatic reaction. $NADP^+$ concentration (mM): 0.7 (■), 1.4 (△), 7.0 (●), 14 (◆). The reactions were performed in 80 mM Tris-HCl buffer (pH 9.0) containing 750 mM Na_2SO_4, 10 mM DTT, 2 mM α-ketoglutarate, GABase (30 μg/100 μL) and 1 mM GABA at 30 °C.

3.3 Optimized GABA Extraction from Rice

GABA content in rice germ of Khao Dawk Mali 105 (moisture content of 11.3%) extracted by different extraction methods is presented in Table 1. As determined by the developed enzyme-based method, extraction with 0.3% (w/v) sulfosalicylic acid yielded the highest GABA concentration of 14.29 mg/100 g in comparison of the extraction with DI water (3.19 mg/100 g) and 75% (v/v) ethanol (0.35 mg/100 g). Sulfosalicylic acid is generally used for the protein removal in the amino acid analysis [16]. In the presence of

sulfosalicylic acid, proteins in rice were precipitated, and thus GABA in the rice matrix could be released easier. For each solvent, GABA content in rice extract determined by HPLC was comparable to that determined by the enzyme-based method. These finding indicated that the newly optimized method has the equivalent performance in determining GABA to the HPLC, and GABA content extracted from rice was clearly influenced by extraction solvent and the process. In addition, it was found that degree of GABA extraction increased with the sonication. The sonication enhances extraction of GABA by passing ultrasound radiation to the rice sample, hence increasing mass transfer and induces penetration of the solvent into rice [17]. Without the sonication, GABA extracted by DI water was lower than the detection limit (4.0 mg/100 g) of the analytical method used by CSTE. Its expected value of 0.95 mg/100 g was comparable to the result of extraction with 50% (v/v) ethanol (0.83 mg/100 g) as being measured by GC-MS.

Table 1. GABA content in rice germ of Khao Dawk Mali 105 extracted by different extraction methods. GABA content was determined by the enzyme-based method (n=7-8), HPLC (n=1) and GC-MS (n=1).

Extraction method	GABA (mg/100 g dry weight)	
	Enzyme-based method	HPLC
Extraction with water and sonication		
Extraction with water[1]	3.19 ± 0.57	2.77
Extraction with 0.3% (w/v) sulfosalicylic acid and sonication	ND[3]	<4.00*(1.0)
	14.29 ± 1.59	13.82
Extraction with 75% (v/v) ethanol and sonication	0.35 ± 0.18	0.52
Extraction with 50% (v/v) ethanol[2]	ND	0.83 (GC-MS)**

[1]GABA was analyzed by CSTE according to the method of Varanyanond *et al.* [8].
[2]GABA was analyzed by Central laboratory Co., Ltd., Thailand according to the method of Mustafa *et al.* [11].
[3]ND: Not determined
*GABA content in the extract was lower than the detection limit (4 mg/100 g) of analytical method. The expected value of 0.95 mg/100 g was derived by extension of the calibration curve.
**GABA content was determined by Gas Chromatography-Mass Spectrometry by Central laboratory Co., Ltd., Thailand.

Effect of sieving through a 60 mesh screen on degree of GABA extraction from the rice powder of Khao Dawk Mali 105 with sulfosalicylic acid (0.3% w/v) is shown in Table 2. As analyzed by the developed enzyme-based method, GABA content in sieved rice extract was 22.89 mg/100 g, greater than that found in non-sieved rice extract (14.29 mg/100 g). The results obtained from the enzymatic analysis and the HPLC were comparable. The sieving removes large rice particles and retains the fine rice particles, thus increasing the surface-area-to-volume ratio and the specific surface area exposed to the solvent. Therefore, the application of sieving with the GABA extraction using 0.3%

(w/v) sulfosalicylic acid and sonication results in the great extraction yield, and was selected as the optimal extraction method for extracting GABA from rice. The optimized GABA extraction method is shown in Figure 4. Due to the very low concentration of the sulfosalicylic acid used and the buffer system of the developed enzyme-based method, the elimination of sulfosalicylic acid before the GABA analysis is unnecessary. In addition, sulfosalicylic acid is colorless, so its remaining does not disturb the colorimetric assay [18].

Table 2. Effect of sieving through 60 mesh screen on GABA content in rice germ extract of Khao Dawk Mali 105. GABA content was determined by the enzyme-based method (n=8) and HPLC (n=2).

Sample	GABA (mg/100 g dry weight)	
	Enzyme-based method	HPLC
Sieved rice germ	22.89 ± 1.88	21.54 ± 1.82
Non-sieved rice germ	14.29 ± 1.59	13.18 ± 0.91

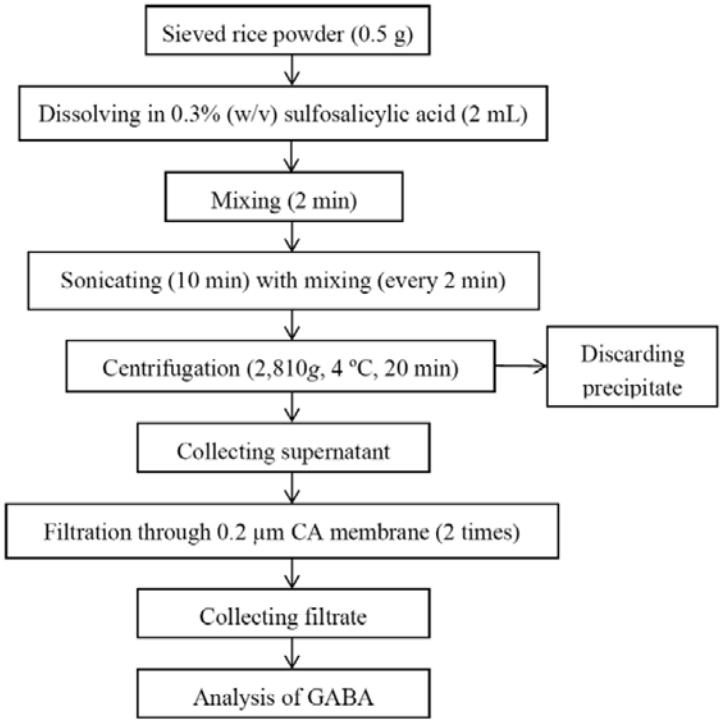

Figure 4. Process of GABA extraction with 0.3% (w/v) sulfosalicylic acid and sonication.

3.4 Accuracy of Enzyme-Based Method for Determination of GABA Content in Rice

Accuracy of the developed enzyme-based method was evaluated by the percent recovery of the spike. The recovery of GABA in rice extracts spiked with standard GABA solution is presented in Table 3. For each level of the spike, the percent recovery was in the acceptable range of 100±10%. The satisfactory percent recovery of the spike reveals that the analyte detection is not interfered by the matrix of the sample [12]. Additionally, the percent coefficient of variation calculated from the recovery values was in the range that has been generally accepted (<10%). Therefore, the enzyme-based method developed in this study could apply for determining GABA content in rice accurately and selectively. The method is applicable and useful for the screening of GABA sources and may also replace the HPLC analysis of GABA in other raw-materials. However, the sample solution should be clarified, and the absence of an enzyme inhibitor in the sample must be ensured.

Table 3. Recovery of GABA content in rice germ extract of Khao Dawk Mali 105 spiked with standard GABA solution.

Present		Spike	Added	Calculated	Found	Recovery
mg/100 g dry weight	mM	(%)	(mM)	(mM)	(mM)	(%)
22.89	0.49	50	0.25	0.74	0.72	91.48
22.89	0.49	100	0.50	0.99	0.96	92.33
22.89	0.49	150	0.75	1.24	1.2	94.32
Mean						92.71
CV* (%)						1.57

*CV: Coefficient of variation

3.5 GABA Content in Thai Brown Rice

GABA contents in brown rice from 32 Thai rice varieties are presented in Table 4. The moisture content of brown rice was in the range of 10.1-12.7%, and GABA content in brown rice was in the range of 7.60-29.46 mg/100 g. Among the varieties of Thai rice that were tested in this study, Chaw Lamud contained the lowest GABA content, while RD41 contained the highest GABA content. RD41 was the non-glutinous, non-color rice with the highest GABA content, whereas Hawm Daeng Sukhothai 1 (22.64 mg/100 g) was the non-glutinous color rice with the highest GABA content. For glutinous non-color rice and glutinous color rice, the rice varieties with the highest GABA content were Niaw San-pah-tawng (21.84 mg/100 g) and Niaw Daeng Yai (16.66 mg/100 g), respectively. Obviously, GABA content varied considerably among rice varieties. This variation might be due to the variation in activity of GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) [19], ability of rice to metabolize protein to glutamic acid, mechanisms involving GABA synthesis in rice [20] and purity of rice varieties [21]. Furthermore, GABA content in plant is also influenced by growing environment as well as the post-harvest system [22, 23]. With the optimized extraction method using 0.3% (w/v) sulfosalicylic acid as a solvent, GABA content in some rice varieties such as Pathum Thani 1, Chai Nat 1

and Phitsanulok 2, GABA content was higher than that has been reported in some germinated brown rice [24].

As a whole, non-glutinous rice (20.41 mg/100 g dry weight) had higher GABA content than glutinous rice (16.02 mg/100 g dry weight), and brown rice (20.05 mg/100 g dry weight) had higher GABA content than color rice (17.28 mg/100 dry weight), significantly (Table 5). Since GABA in rice is mainly accumulated in the germ portion, size of rice germ directly affects GABA content in rice. According to Tungtrakul *et al.* [25], non-glutinous rice varieties have remarkably larger germ than glutinous rice varieties. This report corresponds to the result obtained in this study that the average GABA content in non-glutinous rice was higher than the average GABA content in glutinous rice.

Table 4. GABA content in brown rice from 32 Thai rice varieties.[1,2]

Type of rice	Rice variety	Moisture content (%)	GABA (mg/100 g dry weight)
Non-glutinous rice			
Non-color rice	Khao Dawk Mali 105	11.8	17.48 ± 1.47^{defg}
	Hua Na	11.9	14.93 ± 2.74^{cd}
	Hawm Jan	11.4	20.50 ± 3.59^{hijk}
	Maw Arun	11.9	15.46 ± 2.68^{cde}
	Yah Sai	10.9	10.71 ± 2.24^{b}
	Chaw Lamud	11.6	7.60 ± 2.55^{a}
	Chiang Phatthalung	11.0	19.00 ± 2.14^{ghi}
	Chaw Jangwad	11.8	17.89 ± 3.11^{efgh}
	RD41	11.2	29.46 ± 2.04^{o}
	Pathum Thani 1	10.9	23.78 ± 3.33^{lm}
	So Mali	11.3	25.24 ± 1.28^{mn}
	Beu Gi	12.3	25.18 ± 2.22^{mn}
	Phitsanulok 2	11.5	29.04 ± 2.14^{o}
	Suphan Buri 1	11.5	27.08 ± 3.18^{no}
	Sin Lek	10.9	21.80 ± 2.07^{jkl}
	Chai Nat 1	11.2	27.39 ± 2.05^{no}
	Khao Tah Haeng 17	10.1	28.05 ± 2.64^{o}
Color rice	Hawm Mali Daeng	11.8	19.19 ± 1.99^{ghij}
	Leuang Soi Tawng	12.7	22.44 ± 1.88^{kl}
	Si Nin	11.3	21.67 ± 1.57^{jkl}
	Sangyod Phatthalung	11.1	22.50 ± 1.46^{kl}
	Rice Berry	11.7	8.59 ± 1.51^{ab}
	Hawm Daeng Sukhothai 1	11.4	22.64 ± 2.37^{kl}
Glutinous rice			
Non-color rice	Law Taek	11.5	10.06 ± 1.65^{ab}
	Niaw San-pah-tawng	11.5	21.84 ± 2.59^{jkl}
	RD6	12.3	20.97 ± 1.79^{ijk}
	Saen Sabai	12.6	18.50 ± 1.48^{fghi}
Color rice	Gam Pleuak Khao	11.6	8.62 ± 1.90^{ab}
	Gam Yai	12.4	16.21 ± 1.52^{cdef}
	Gam Noi	12.2	15.78 ± 1.77^{cde}
	Niaw Daeng Yai	11.2	16.66 ± 1.55^{cdefg}
	Leum Pua	11.2	14.04 ± 1.30^{c}

[1]GABA contents were obtained from six to seven of replicate analyzes (n=6-7).

[2]Values with different superscripts within the same column were significantly different ($p \leq 0.05$).

Table 5. Average GABA content in brown rice as categorized by type of rice and color of rice.[1]

Rice	Moisture content (%)	GABA(mg/100 g dry weight)
Type of rice		
Non-glutinous rice	11.4	20.41 ± 6.47[a]
Glutinous rice	11.8	16.02 ± 4.59[b]
Color of rice		
Non-color rice	11.5	20.05 ± 6.60[a]
Color rice	11.7	17.28 ± 5.18[b]

[1]Values with different superscripts within the same column of each rice category were significantly different ($p \leq 0.05$).

3.6 Correlations between GABA Content and Characteristics of Brown Rice Grains

The statistical analysis of the relationship between GABA content and some characteristics of brown rice grains, including size, shape, width, thickness and area, showed that GABA content in brown rice positively correlated with size (p=0.003) and shape (p=0.016) of the grains. The correlation between GABA content and size of the grains was moderate with a correlation coefficient (r) of 0.511, whereas the correlation between GABA content and shape of the grains was low (r=0.421). Nevertheless, GABA content in brown rice did not correlate with width (p=0.133), thickness (p=0.938) and area (p=0.764) of the grains. The finding of relationships between GABA content and size, and GABA content and shape of brown rice grain in this study similar to the study of Panyanak *et al.* [13], which reported that rice grains with a slender shape and large germ tended to contain higher GABA level than those with others shape.

4. CONCLUSIONS

The enzyme-based method for the determination of GABA optimized in this study could accurately, rapidly and economically quantify GABA in rice. With the appropriate method for extracting GABA from rice using 0.3% (w/v) sulfosalicylic acid and sonication, the great extraction yield could be achieved in this study. GABA-rich brown rice was mostly non-glutinous rice with long grain and slender shape. Among the examined rice samples, RD41 with the highest GABA content can be used as the key ingredients of rice-based functional food products. Furthermore, the information on GABA content of other rice varieties will be useful for the production of healthy rice products. However, stability of GABA in rice during the household cooking and industrial processing still needs to be evaluated.

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