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Original article

Effect of incubation time, buffer type and concentration on gamma-aminobutyric acid (GABA) production using Khao Dawk Mali 105 rice bran

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ABSTRACT

Rice bran of Khao Dawk Mali 105 (KDML105) variety was selected for production of gamma-aminobutyric acid (GABA). The effect of incubation time on GABA production was studied and the maximum GABA was produced after 6 h of incubation. Different types of 50 mM buffers (containing 0.2% glutamic acid) consisting of Tris, citric acid, boric acid and phosphate buffer (pH 5.6) were used to stabilize the pH of the reaction system. The highest GABA content (5.05 mg/g of bran) was found in the phosphate buffer system. Therefore, the effect of phosphate buffer concentrations (0–200 mM) on GABA production was investigated. The results showed that rice bran with phosphate buffer at a concentration of 80 mM at pH 5.6 with a rice bran to phosphate buffer at a ratio of 1–8 (weight per volume) produced the highest GABA content ($p \leq 0.05$). GABA production was increased about 2.7 times in the phosphate buffer system compared with the control and about 11 times compared to the initial GABA content (0.58 mg/g of bran) in the rice bran. The results indicated that incubation time, buffer type and concentration significantly affect GABA production using rice bran.

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops and is grown in almost every part of Thailand (Zeigler and Barclay, 2008; Jannoey et al., 2010). Rice bran is the main by-product produced during milling of whole rice grain and rice bran is a rich source of glutamate decarboxylase (L-glutamate-1-carboxylyase, EC 4.1.1.15, GAD) (Wang et al., 2010). It also contains high levels of glutamic acid (Juliano, 1985). In rice bran, glutamate decarboxylase enzyme (GAD) is naturally present in the cytosol of the cell and plays an important role in GABA production; GAD is a pyridoxal 5'-phosphate (PLP) dependent enzyme, which catalyzes the irreversible alpha-decarboxylation of L-glutamic acid (Glu) to produce gamma-aminobutyric acid (GABA) and carbon dioxide. GAD also naturally located in the cytosol of rice bran cell (Zhang et al., 2007). GABA is widely distributed in nature and plays an important role in the central nervous system as a neurotransmitter and lowers blood pressure in the human brain (Kimura et al., 2002; Wang et al., 2010). GABA also plays an important role in nitrogen storage, plant growth, glutamic acid utilization and in the plant's defense system against phytophagous insects (Bown and Shelp, 1997).

Control of the GABA and GAD levels in the brain was found to prevent many neurological disorders such as seizures, Parkinson's disease, stiff-man syndrome, and schizophrenia (Bao et al., 1995; Adeghate and Ponery, 2002). Furthermore, consumption of GABA-enriched foods can inhibit cancer cell proliferation and improve memory and the learning abilities (Oh and Oh, 2004; Dhakal et al., 2012). Therefore, GABA has been classified as a bioactive component in foods and pharmaceuticals. The health benefits of GABA have resulted in it becoming of keen interest to researchers in their work to develop functional foods containing high levels of accumulated GABA. Methods to increase GABA concentrations in rice have been studied by various researchers.

Ohtsubo et al. (2000) used rice germ as an enzyme (GAD) source. They produced 290 mg/g of germ of GABA by adding exogenous Glu. Zhang et al. (2006) hydrolyzed germ protein with the addition of trypsin and produced Glu for GABA accumulation. They produced GABA at a rate of 22.6 mg/g of rice germ. GABA production by Zhang et al. (2006) was much lower than the GABA

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produced by Ohtsubo et al. (2000). This indicates that the GABA production level was lowered by the endogenous Glu naturally present in the rice germ and this was not sufficient for high GABA production. Karladee and Suriyong (2012) produced GABA at a level of 0.23 mg/g of grain by soaking brown rice for 24 h at 40 °C. Similarly, Jannoey et al. (2010) produced GABA at a level of 2.45 mg/g of grain by soaking germinated brown rice at a controlled temperature of 30 °C for 72 h. GABA has been produced in many other cereals by different methods. Nogata and Nagamine (2009) produced GABA from wheat bran by soaking in sodium phosphate buffer and obtained a GABA content of 1.48 mg/g of wheat bran under optimum soaking conditions (pH 5.5, 24 h, 40 °C). Iimure et al. (2009) developed a simple method of GABA production from barley bran supplemented with glutamate and found that the optimal reaction conditions for GABA production were sodium glutamate (10 mM), barley bran (150 mg:100 mL), reaction time (6 h) and reaction temperature (20 °C). As a result, 11 mM GABA could be produced using this method.

Soaking or incubating the rice or cereal bran is an effective method for GABA production at control temperatures. However, the incubation time must be optimized because GABA production is adversely affected when the incubation time exceeds the optimum (Komatsuzaki et al., 2007). In a previous experiment on rice bran, the current authors compared the GABA content when external glutamic acid was added and when it was not (data not shown). GABA content with glutamic acid addition was higher than when no glutamic acid was added. Therefore, it was decided to add glutamic acid to increase the substrate of the alpha-decarboxylation reaction and produce more GABA. Moreover, the effect of buffer type and concentration on GABA production using rice bran has not been previously investigated. Therefore, the objective of this research was to investigate the effect of the incubation time on GABA production and to produce a higher GABA content from rice bran by selecting the proper buffer type and concentration at a stabilized pH during incubation.

Materials and methods

Materials and chemicals

Rice bran was prepared from milling rice grain from different cultivars of Thailand namely, *O. sativa* L. cv. Khao Dawk Mali 105 (KDML105), Supanburi 1 (SP1), Chainat 1 (CN1), Phitsanulok 2 (PS 2) and Pathumthani 1 (PT1). These varieties were selected because of their high production levels in the country. The rice bran was passed through a 30-mesh screen and stored at −4 °C until use. Prior to incubation, the rice bran was ground to break cells so the GAD could be released from the cells and react with glutamic acid (the substrate of GABA synthesis) that was added to the buffer solution for GABA synthesis.

A standard of gamma-aminobutyric acid (purity 99%) was purchased from Sigma Aldrich Chemicals (MO, USA). Sodium acetate trihydrate, sodium bicarbonate and L-Glutamic acid (99% purity) were purchased from Sigma Aldrich Chemicals (Japan). 4-Dimethyl-aminoazobenzene-4-sulfonyl chloride (DABSYL-Cl) analytical grade was purchased from Sigma Aldrich Chemicals (Switzerland), and acetonitrile (HPLC grade) was obtained from Mallinckrodt chemicals (MO, USA).

Effect of incubation time on gamma-aminobutyric acid production

This method has been modified from Iimure et al. (2009). Glutamic acid solution (0.2% weight per volume; w/v) was added to rice bran at a rice bran to glutamic acid solution ratio of 1–8 (w/v). The mixture was then incubated at 40 °C to accumulate GABA and centrifuged. The supernatant obtained from centrifugation was assayed for its GABA content.

Effect of buffer type on gamma-aminobutyric acid production

This method has been modified from Iimure et al. (2009). Different types of buffers (containing 0.2% glutamic acid) consisting of 50 mM of Tris, citric acid, boric acid and phosphate buffers (pH 5.6) were added to rice bran with the rice bran to buffer at a ratio of 1–8 (w/v). The mixture was incubated at 40 °C for 6 h to accumulate GABA and centrifuged. The supernatant obtained from centrifugation was assayed its GABA content.

Effect of buffer concentration on gamma-aminobutyric acid production

This method has been modified from Iimure et al. (2009). Proper buffer (containing 0.2% glutamic acid) pH 5.6 was added to rice bran with the rice bran to buffer at a ratio of 1–8 (w/v). The concentrations of buffer were varied from 0 to 100 mM. Then, the mixture was incubated at 40 °C for 6 h to accumulate GABA. The mixture was centrifuged and the supernatant obtained from centrifugation was assayed for its GABA content.

Determination of gamma-aminobutyric acid content

This method was modified from Varanyanond et al. (2005). One-fifth to one-half a gram of rice bran was weighed or one-fifth to one-half a milliliter of rice bran solution (supernatant) was pipetted into a plastic tube with 1.8 mL of deionized water and 200 μL of added 3% sulfosalicylic acid. The sample solution was shaken at room temperature for 1.5 h, then centrifuged at 4500 × *g* for 10 min a sample of 50 μL of supernatant was pipetted and added to 50 μL of 100 mM $NaHCO_3$ and 50 μL of 4 mM DABSYL-Cl acetonitrile solution. The reaction was performed at 70 °C for 20 min. After derivatization, 250 μL of absolute ethanol and 250 μL of 25 mM phosphate buffer (pH 6.8) were added. The sample was filtered into a vial and 5 μL of sample was injected into a high performance liquid chromatography (HPLC) system (Series 1100; Hewlett Packard; CA, USA) with a Supelcosil LC-DABS column, 4.6 × 150 mm, 2 μm (Supelco; PA, USA). The HPLC was equipped with an ultraviolet–Vis photodiode array detector set at 465 nm wavelength. The mobile phase was 25 mM acetate buffer and acetonitrile (80:20 volume per volume; v/v) adjusted at a flow rate of 1.0 mL/min at 35 °C. GABA was used as a calibration standard.

Statistical analysis

All the data were statistically analyzed using a completely randomized design by ANOVA with a least significant difference test at the 95% confidence level.

Results and discussion

Gamma-aminobutyric acid contents of rice bran

The GABA contents of the five different cultivars of rice bran are shown in Fig. 1. The initial GABA content of KDML105 (0.58 mg/g of bran) was not significantly different from those of PSL2 (0.48 mg/g of bran), PTT1 (0.52 mg/g of bran) and SP1 (0.44 mg/g of bran). However, production using KDML105 was the highest among the various rice cultivars. It was reported that 2 million t of KDML105 were exported in 2012 which is about 28% of total exported Thai rice and in addition, approximately 2 × 10^6 t of KDML105 rice bran per year were produced as a major by-product from the rice milling