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Effects of Water Soaking on Gamma-Aminobutyric Acid (GABA) in Germ of Different Thai Rice Varieties

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ABSTRACT

This research was conducted to investigate the effects of water soaking on the content of γ- amino-n-butyric acid (GABA) in 6 Thai rice varieties; namely, Khao Dawk Mali 105, Pathum Thani 1, Chai Nat 1, Suphan Buri 1, Leuang Pratew 123 and Plai Ngahm. The result revealed that Plai Ngahm had the highest percentage of germ weight while Patum Thani 1 had the lowest. Percentage of germ weight showed no relation to the GABA content. High GABA content of germ was found in 3 rice varieties: Khao Dawk Mali 105 (186.2 mg/kg of germ) Pathum Thani 1 (154.6 mg/kg of germ) and Chai Nat 1 (144.5 mg/kg of germ). Plai Ngahm, on the other hand, contained GABA 116.9 mg/kg of germ. Water soaking can enrich GABA content in the germ of all rice varieties. The GABA accumulation differed among rice varieties and according to soaking time.

Key words: γ- amino-n-butyric acid, GABA, rice germ

INTRODUCTION

The development of products conferring a health benefit is a relatively new trend, and recognizes the growing acceptance of role of diet in disease prevention and treatment. Through these, many functional foods are developed, mostly from agricultural products. Rice is the main staple food for world populations. It has been reported that rice components have several roles in prevention of disease. Rice bran and rice germ provide as rich sources of hypoallergenic protein, oil, dietary fiber and nutrient essential for life: such as vitamin B, E, beta-carotene and gamma oryzanol, etc. (Mori *et al*., 1999).

Besides, rice germ also contain a good functional compound called "γ- amino-n-butyric acid" or "GABA". GABA is an amino acid that is produced by decarboxylation of glutamic acid in living organisms. It is known that GABA plays an important role in the central nervous system as a neurotransmitter and function by lowering the blood pressure in human brain. It can lower hypertension, promote the sleepiness and has the benefit for human health. In Japan, highly purified GABA is used as the medication for amelioration of brain blood stream (Okada *et al.*, 2000). There are many reports about anticancer of rice germ (Kawabata *et al*., 1999; Mori *et al*., 1999). The data suggested that constituents of rice germ are possible dietary preventatives for human colon cancers. Saikusa *et al*. (1994) had applied for a patent about GABA-enriched food material and method for producing GABA. According to the method, some safe solvents are used to extract GABA from rice germ.

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In this research the amount of GABA in germ of different Thai rice varieties were investigated using HPLC and enriched-rice germ in those rice varieties were also performed.

MATERIALS AND METHODS

Rice samples

Six varieties of paddy rice were purchased from National Thai Rice Research Institute. All are non-waxy Indica varieties named: aromatic rice (Khao Dawk Mali 105), Pathum Thani 1, Chai Nat 1, Suphan Buri 1, Leuang Pratew 123 and Plai Ngahm.

Reagents

Standard of ϒ-amino-n-butyric acid was purchased from Sigma Aldrich Chemical Germany. Acetic acids and sodium bicarbonate were purchased from Merck (Darmstadt, Germany). 4-Dimethyl-aminoazobenzene-4-sulfonyl chloride (DABSYL-CL) analytical grade from Fluka Chemical, and acetonitrile (HPLC grade) was obtained from BDH (UK).

Equipments

The HPLC system (Aglient 1100 series) consisted of a solvent delivery pump (G1311A Quaternary pump DE11113611, USA), a guard column (Platinum C18 100 A 5 μm, 7.5×4.6 mm I.D Alltech Associates, IL USA), and analytical column (Platinum C18 100A 5 μm, 250×4.6 mm I.D., female B, Alltech Associates, IL USA), and a UV variable wavelength detector (G1314A VWD JD92113015, Japan). Shaking water bath (Heto SBD50 Scientific promotion Co., LTD. Thailand), Centrifuge, Homoginizer (ModleT45/ NJankeKunkd KG Sttautfen Breisgau), Mixer (auto-vortex SA6, UK) and polishing machine (Ngek Seng Huat Ltd., Thailand) were used in this research.

GABA determination

100 mature grains were milled to remove bran and germ in a Model K-1 polishing machine. All rice germs were obtained from brown rice and separated from broken rice by hand and 32 mesh screen. The germ of each rice variety was weighed and calculated as percentage of brown rice. GABA content in those rice germs was determined using HPLC. One-fifth to one-half gram (0.2-0.5 g) of rice germ powder was weighed in the plastic tube and 2 ml of deionized water was added. The sample solution was fully mixed, then centrifuged at 4500 rpm for 10 min. One ml of supernatant was pipetted and added with 200 μL of 0.4 M/L $NaHCO_3$ and 400 μL of 6 mM/L DABSYL-Cl acetonitrile solution. The reaction was performed at 70°C for 20 min. After derivatization, the sample was filtered to vial and 10 μL of sample was injected into HPLC (Cohen and Michaud, 1993).

Effects of water soaking on the GABA content of rice germ

One-fifth gram of rice germ was weighed in plastic tube. Then 2 ml of deionized water was added. The samples were incubated at 40°C while shaking (100 strokes/min, 4 cm amplitude). Each suspension was removed from the water bath at 0, 0.5, 1.0, 1.5 and 4.0 hr of incubation, thereafter it was centrifuged at 4500 rpm/min for 10 min and the supernatant was separated. Quantitative analysis of GABA content was performed by using HPLC.

RESULTS AND DISCUSSIONS

Table 1 shows the percentage weight of germ and GABA content of various rice varieties. High amylose rice varieties provided higher percentage of germ than low amylose rice. Among high amylose rice varieties, Plai Ngahm had the highest value of germ portion. While low amylose rice, Khao Dawk Mali 105 had a bigger germ compared with Pathum Thani 1. The results

revealed that the GABA content in rice germ varied according to rice varieties. Khao Dawk Mali 105 showed the highest content of GABA (186.2 mg/kg) followed by Pathum Thani 1 (154.6 mg/Kg) and Chai Nat 1 (144.5 mg/kg), respectively. Suphan Buri 1, on the other hand, showed the lowest GABA content.

Effects of water soaking on GABA content

It is reported that GABA content in *Koshihikari japonica* rice germ increased greatly during soaking in water, which is the most popular cultivar of rice in Japan for the high eating quality (Saikusa *et al*., 1994). Increasing GABA content in water soaked, *indica* long grain rice was also found in this experiment. Change in GABA content in each rice variety was observed. Soaking time and rice varieties affected the GABA content of the germs (Figure 1). GABA content in germ of all rice varieties increased during soaking in water. Pathum Thani 1, Khao Dawk Mali 105, Chai Nat 1 and Suphan Buri 1 demonstrated greatly increase.

The great GABA accumulation was observed in the germ of Pathum Thani 1, which reached 555.1 mg/kg after 4-hour incubation at 40°C. Pathum Thani 1 is an aromatic rice and could be a promising source for GABA. GABA accumulation in the germ of Suphan Buri 1, Khao

Table 1 Percentage weight of germ and GABA content in different Thai rice varieties.

Variety	Percentage weight of germ in brown rice (a)	GABA content (mg/kg of germ) (b)
Khao Dawk Mali 105	3.61	186.2
Pathum Thani 1	2.95	154.6
Chai Nat 1	3.75	144.5
Suphan Buri 1	4.08	107.5
Leuang Pratew 123	3.95	113.4
Plai Ngahm	5.01	116.9

(a) Each value was determined from 100 mature rice grains.
(b) Average are based on three measurements of each sample.

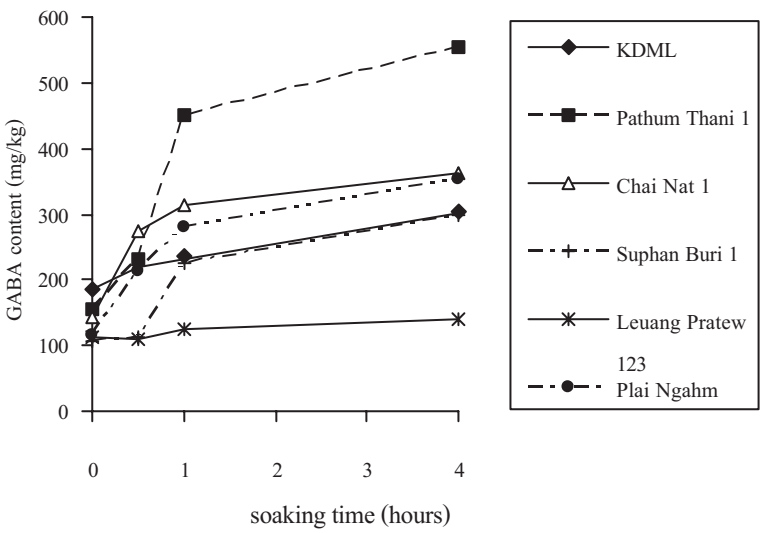

Figure 1 Accumulation of GABA content in rice germ during water soaking at 40°C.

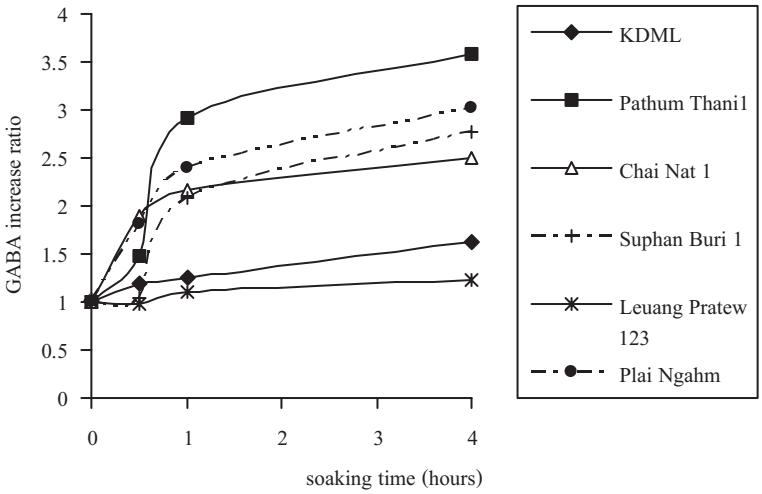

Figure 2 GABA increasing ratio during water soaking at 40°C.

Dawk Mali 105, Pathum Thani 1 and Leuang Pratew 123 was very low at the first half-hour. GABA accumulation in the germ of Chai Nat 1 and Plai Ngahm increased rapidly during 0.5-hour of incubation. The accumulation of GABA content in the germ of Leuang Pratew 123 was negligible.

However, most of rice germs from different rice varieties revealed high quantity of GABA content within four hours of incubation. As shown in Figure 2, the amount of GABA content in soaked germ was more than 2 times compared with GABA content of the unsoaked germ. It was noticed that GABA accumulation in rice germ proceeded rapidly at an early stage of incubation, accompanied by the parallel loss of glutamate concentration. It suggested that a supply of glutamate would help to accumulate more GABA during rice germ soaking in water (Saikusa *et al*. 1994). Besides, air was reported to be important during incubation: more air entered into the procedure of GABA formulation, higher accumulation of GABA will be attained.

In the experiment, six *indica*, long grain rice varieties were investigated for percentage of weight of germ as well as GABA content. The results showed that, those values were varied according to rice varieties. Percentage weight of germ had no relation to the GABA content existed in rice germ. Water soaking can enrich the GABA content in the germ of all rice varieties. The GABA content increased as the soaking time was prolonged and the amount of change were different in patterns. Among these rice varieties, germ of Pathum Thani 1 had the greatest change of GABA content as compared with un-soaked rice germ. However, the enrich GABA condition should be studied more in details as well as the use of enrich GABA rice germ for some foods products preparation.

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