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Production of germinated Red Jasmine brown rice and its physicochemical properties

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Abstract

The effects of soaking conditions and anaerobic treatment on the gamma-amino butyric acid (GABA) content in germinated Red Jasmine brown rice (GBR) were investigated in this study. Firstly, GBR was produced by soaking brown rice grains in water at different temperatures (5, 28, 35°C) and soaking times (8, 16, 24 h), then GBR was incubated under nitrogen gas for 12 h. The results showed that GABA content in GBR was highest after soaking for 24 h at 35°C and its content obviously increased when stored under anaerobic condition. The number of microorganisms in the GBR increased during germination but steaming and boiling reduced the microbial load. Germinating altered the proximate composition of GBR and caused noticeable changes in the pasting characteristics.

Introduction

Brown rice is the whole grain rice obtained from unpolished rice. It is an excellent source of nutrients such as protein, dietary fibre, fat, minerals and vitamins (Ohtsubo *et al*., 2005). Moreover, brown rice contains the phytochemicals such as tocopherol, tocotrienol, oryzanol and ferulic acid (Tian *et al*., 2004; Miller and Engel, 2006; Lai *et al*., 2009). The functional properties of brown rice seed can be improved through the process of germination. Germinated brown rice is obtained by soaking the seed of brown rice in water. After hydration, the seed has a metabolic change and reserved materials were broken down into metabolizable molecules by different enzymes. For example, endosperm starch was hydrolyzed by alpha-amylase into sugars which provide the energy for the growth of roots (Saman *et al*., 2008). Storage proteins were decomposed into amide via the action of proteolytic enzymes and used as a nitrogen source for embryo growth of rice seedling (Komatsuzaki *et al*., 2007). Moreover, proteins can breakdown into amino acids especially glutamic acid which can be changed into GABA via the glutamate decarboxylase (GAD) enzyme (Mayer *et al*., 2009). GABA is a non-protein amino acid that functions as a neurotransmitter in the brain and directly affects the personality and the capability of a person to manage stress (Shiahs and Yatham, 1998.). It can also help in the reduction of hypertension and inhibits development of cancer cells (Okada *et al*., 2000; Patil and Khan, 2011).

Several works have reported on the production of germinated brown rice. The accumulation of GABA during germination depended on the varieties of rice, pH of soaking water, soaking time and temperature (Komatsuzaki *et al*., 2007; Charoenthaikij *et al*., 2010;Watchararparpaiboon *et al*., 2010). The increase in the GABA content during soaking is due to the action of GAD that transforms glutamic acid to carbon dioxide and GABA. Moreover, under soaking condition, the respiration rate of plant tissue is reduced and some metabolisms are suppressed. Such stress may contribute to the accumulation of GABA (Rhodes *et al*., 1986). There are a number of literature reports on producing germinated rice from brown rice. However, there are few literature reports on red rice. Red aromatic rice is a variety of Thai rice (*Oryza Sativa* L.) that is red in color and has a high amount of phenolic compounds which show a high antioxidant activity (Vichapong *et al*., 2010). Most of the rice consumed worldwide is white rice, while the Red brown rice is not preferred because of its hard texture. The process of soaking and germination could be used to improve organoleptic qualities and enhance the bioactive substances of brown rice (Ohtsubo *et al*., 2005).

There is one problem that occurs in the process

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of germinating brown rice from a safety point of view and that is the microbial contamination during soaking (Bandara *et al*., 1991; Ohtsubo *et al*., 2005). To reduce the microorganisms in germinated brown rice, treatments such as heating, steaming, and drying could be used (Komatsuzaki *et al*., 2007). In this study the effect of steaming, ethanol and boiling treatment after soaking stage on the reduction in the microbial load of germinated brown rice is investigated. As these techniques are of low cost, they can be used as an alternative method for the preparation of germinated brown rice in the household and small scale production.

Germination altered the chemical composition of germinated brown rice and also affected the pasting characteristics (Saman *et al*., 2008; Mohan *et al*., 2010). Knowledge of some of the physicochemical properties and chemical contents of germinated brown rice could be useful for application in food products. In the present study, the relationship between the germination conditions and the amount of GABA and chemical contents was investigated. The microbial quality and pasting property of germinated brown rice were also examined.

Materials and Methods

Thai rice (*Oryza sativa* L.), Red aromatic brown rice, was collected from the rice mill in Sa Kaew province, Thailand. The reagents used were of analytical grade.

Preparation of germinated brown rice by soaking and anaerobic incubation

Red aromatic brown rice was sterilized using 0.1% sodium hypochlorite for 0.5 h and rinsed with tap water. Then brown rice was soaked in sterile water (1:3 w/v) at different temperatures (5, 28, 35^{o}C) and times (8, 16 and 24 h) with the water being changed every 6 h. After soaking, the grains were placed on moist filter paper in covered glass dishes and kept at room temperature in the dark for 24 h. Germinated brown rice was dried with a tray dryer at 50^{o}C until moisture content was less than 13%. Dried germinated brown rice was then ground, sieved through a 100 mesh screen and kept at 4^{o}C for GABA analysis. The soaking condition that gave the highest GABA content was selected for further study.

Soaking combined with anaerobic treatment (adapted from Chung *et al*., 2009) was carried out. The brown rice was steeped in the selected soaking condition chosen from the previous step and then placed in a 250 mL flask that was purged with nitrogen gas for 2 min. The flask was covered tightly and kept in a dark room at 28 $\pm$ 2^{o}C for 12 h. Germinated brown rice was dried, ground and kept for GABA and further analysis.

Treatment of germinated brown rice by steaming, ethanol and boiling to reduce microorganisms

To reduce the microorganisms in germinated brown rice, treatment by steaming, ethanol and boiling were used (Komatsuzaki *et al*., 2007). Germinated brown rice (100 g) was steamed for 20 min and then soaked in 70% (v/v) ethanol solution for 3 min. The grains were boiled for 5 and 10 min after steaming and soaking. The samples were further analysed for total microbiological characteristics.

Microbiological analysis

The germinated brown Red aromatic rice samples were analysed for total plate count and yeast and mold count according to standard procedures (AOAC, 2000). Samples were aseptically removed and transferred to a sterile blender jar. The aerobic plate counts were determined as follows: one gram of sample was blended in 9 mL of phosphate buffer. Serial dilutions were transferred in the plate count agar (PCA) media (0.1 mL/plate). The colonies were counted after incubation at 35^{o}C for 48 h and expressed in CFU/g. Yeasts and molds were measured by the same procedure using a potato dextrose agar instead of PCA.

Proximate composition analysis

Brown rice (control) and germinated brown rice were analyzed for moisture, protein, fat, ash and crude fibre contents by the AOAC (2000) method. GABA content was determined by the method of Watchararparpaiboon *et al*. (2010). Briefly, 70% (v/v) ethanol solution (30 mL) was added to germinated brown rice powder (3.0g). The aqueous layer containing GABA was obtained through centrifugation (8000 g, 4^{o}C, 5 min). The extraction was repeated twice and the collected supernatant was evaporated under vacuum at 40^{o}C. The mixture of solution (0.2 M borate buffer, 0.2 mL: 6% phenol reagent, 1 mL) was added to the concentrated extract (0.1 mL). Then 0.4 mL of 7.5% sodium hypochlorite was added, and boiled for 10 min. The sample was immediately cooled for 5 min and measured the optical density at 630 nm. Calibration curve of standard GABA (Sigma, St. Louis, USA) were prepared at different concentration and used to determine the concentration of GABA in samples.

Pasting viscosity

The pasting viscosity of brown rice and the

germinated brown rice flours was determined using a Rapid Visco Analyzer (RVA-4D, Newport Scientific, Narrabeen, Australia). Flour slurry at a concentration of 8% (w/w) was heated from 50 to 95°C at the rate of 13°C/min, maintained at 95°C for 2.7 min, and then cooled to 50°C at the same rate.

Statistical analysis

All experiments were carried out using three samples and three replicates of each sample were analyzed. The results were subjected to analysis of variance (ANOVA). Means were compared by Duncan's multiple range test (DMRT). Difference between control and germinated brown rice was evaluated by paired t-test. A level of significance of 0.05 was used.

Results and Discussion

GABA content of Red Jasmine brown rice

GABA contents of germinated Red aromatic brown rice soaked at different conditions are presented in Table 1. The GABA content of brown rice grain was 6.05 mg/100 g and it ranged from 6.32 to 41.02 mg/100 g after soaking at different conditions. This result indicated that soaking condition contributes to the increase in GABA content. Similar results have been reported for germinated rice grains with different varieties (Saikusa *et al.*, 1994; Komatsusaki *et al*., 2007; Watchararparpaiboon *et al*., 2010). The accumulation of GABA during water soaking may be due to the action of glutamate decarboxylase (GAD), which was gradually increasing during water soaking and converts glutamate to GABA (Lui *et al*., 2005; Komatsusaki *et al*., 2007).

The GABA content slightly increased after 8 and 16 h of germination and it significantly increased after 24 h soaking (Table 1). The highest GABA content was 41.02 mg/100g after 24 h soaking at 35°C, which was about 7 times higher than that of native brown rice. A similar result was observed by Watchararparpaiboon *et al*. (2010) who reported that GABA contents of brown Thai rice (Chainat 1 and Khao Dawk Mali 105) were highest after soaking rice grains in water at temperature of 35°C for 24 h.

Few researchers have studies the effect of soaking temperatures on GABA content of cereals (Chung *et al*., 2009; Watchararparpaiboon *et al*., 2010). In this study, the experiment was carried out at temperatures of 5, 28 and 35°C. GABA content was affected by the soaking temperature. Higher soaking temperature had a more pronounced effect on GABA content when compared to lower soaking temperature. Soaking at low temperature (5°C) did not significantly increased

Table 1. GABA content of brown rice grains prepared from various soaking conditions

Soaking temp. (°C) / time (h)	GABA[a] (mg/100g)
Brown rice (control)	6.05 ±0.88[g]
GBR[b], 5/8	6.32 ±1.68[g]
GBR, 5/16	6.36 ±0.35[g]
GBR, 5/24	6.67 ±0.23[fg]
GBR, 28/8	10.78 ±0.88[c]
GBR, 28/16	7.91±0.48[ef]
GBR, 28/24	19.16±0.75[b]
GBR, 35/8	9.16±0.35[de]
GBR, 35/16	8.15±0.88[ef]
GBR, 35/24	41.02±0.75[a]

[a]Each value is the average of three replicates. Mean values followed by the same letter in the same column are not significantly different (p > 0.05)
[b]GRB = germinated brown rice

Table 2. GABA content of brown rice grains with and without anaerobic treatment

Germinated brown rice	GABA[a] (mg/100g)
24h soaking, 35°C	41.73±0.75[a]
24h soaking, 35°C + N_2 gas	81.19±1.90[b]

[a]Mean values ± SD of triplicate measurements. Mean values followed by the same letter in the same column are not significantly different (p > 0.05)

GABA content (p > 0.05) with soaking time. Chung *et al*. (2009) reported that low temperature (5°C) was optimal temperature to produce GABA in barley grains, while Watchararparpaiboon *et al*. (2010) suggested that optimal soaking temperature of rice grains was 35°C. This could be due to the different grain types. As rice GAD has an optimum temperature at 40°C (Zhang *et al*., 2007), soaking at 35oC temperature would enhance its activity and result in more GABA production.

The effect of anaerobic treatment on the GABA content in germinated brown rice is shown in Table 2. The GABA content was increased after incubation under anaerobic conditions. This result is similar to that reported for Japonica brown rice grains and barley grains (Komatsuzaki *et al*., 2007; Chung *et al*., 2009). GABA accumulation in plant tissues is induced in response to certain forms of stress such as heat shock (Mayer *et al*., 1990), water stress (Rhodes *et al*., 1986), cold shock, darkness and mechanical manipulation (Wallace *et al*., 1984) and hypoxia (Aurisano *et al*., 1995). In this experiment, the increase in GABA during anaerobic treatment may be a result of hypoxic stress. In the absence of oxygen, the intracellular pH reduced and thus stimulating GAD activity and elevated the GABA level (Crawford *et al*., 1994).

Effect of steaming, ethanol and boiling treatment on microbial load of germinated brown rice

The aerobic total plate count (TPC) as well as yeast and mold of germinated brown rice were evaluated in order to confirm the safety of germinated brown rice. The effectiveness of disinfection by steaming, ethanol and boiling was investigated and the results

Table 3. Microbiological quality of GBRTa treated by various treatments

Treatment	Total plant count (CFU/g)	Yeast and Mold (CFU/g)
Brown rice (control)	$2.8x10^5$	ND[b]
GBRT	$5.5x10^6$	ND
GBRT-steamed 20 min + ethanol	$4.2x10^4$	ND
GBRT-steamed 20 min + ethanol + boiled 5 min	$1.3x10^4$	ND
GBRT-steamed 20 min + ethanol + boiled 10 min	$1.5x10^3$	ND

[a]GBRT = germinated brown rice treated with anaerobic condition
[b]ND = No molds or yeasts were detected

Table 4. Proximate compositions of brown rice and GBRT

Compositions (%)	Brown rice (control)	GBRT*
Moisture	12.91 ± 0.10^a	12.44 ± 0.14^a
Protein	7.98 ± 0.06^a	8.34 ± 0.04^b
Fat	2.82 ± 0.28^a	2.08 ± 0.03^b
Carbohydrate	72.35 ± 0.00^a	70.69 ± 0.76^a
Ash	1.86 ± 0.04^a	1.88 ± 0.06^a
Crude fiber	2.07 ± 0.00^a	4.55 ± 1.55^b

*GBRT = germinated brown rice treated with anaerobic condition
[a,b] Mean values followed by the same letter in the same row are not significantly different ($p > 0.05$)

are shown in Table 3. TPC increased above 106 CFU/g after soaking at 35°C for 24 h and incubated in anaerobic treatment for 12 h. After steaming for 20 min and soaking in ethanol for 3 min, TPC decreased from 106 to 104 CFU/g. Boiling for 5 to 10 min reduced the microbial content from 104 to 103 CFU/g. Yeast and mold were not detected in brown rice and germinated brown rice samples.

A combination of steaming (20 min) and ethanol treatment completely eliminated viable organisms in Japonica germinated brown rice (Komatsuzaki *et al*., 2007). However, the microorganism found in the present study did not exceed permitted limits established by Thai Community Product Standard (TCPS) guidelines set by Thai Industrial Standards Institute, Thailand which provided the acceptable limit of less than 1x104 and 100 CFU/g of TPC and yeasts and molds, respectively (TCPS, 1068/2548). It is suggested that aseptic process and sterile treatment should be applied in order to improve the microbiological quality of germinated brown rice.

Proximate composition

Proximate composition of germinated Red aromatic brown rice is shown in Table 4. It was found that the germination process caused an increase in protein and crude fiber contents but a decrease in carbohydrate, fat and ash contents of brown rice. During germination, the decrease in total protein content is simultaneous with increase in amino acid content caused by the proteolysis (Veluppillai *et al*., 2009, Mohan *et al*., 2010). However, in this study we found that protein content was increased after germination. The increase in total protein content during germination may be due to the synthesis of enzyme proteins which rapidly transform free amino acids to form new protein compounds (Bau *et al*., 1997).

Fat content was decreased in germinated brown rice. Similar results were obtained by Mohan *et al*. (2010), Moongngarm and Saetung (2010). This could be explained by fat being hydrolyzed during germination to produce the energy for seed growth. The same trends were found in the level of carbohydrate content. The decrease of carbohydrate in germinated brown rice is due to the starch degradation presumably being involved in the action of several enzymes such as α-amylase, β-amylase and invertase (Ohtsubo *et al*., 2005; Mohan *et al*., 2010). These enzymes hydrolyze starch into smaller molecules such as dextrin, glucose and fructose to provide energy for seed growth and also produced a sweet flavor in germinated brown rice (Ohtsubo *et al*., 2005)

Ash content was slightly increased in germinated brown rice but did not significantly different ($p > 0.05$). Ash content in this study was close to those reported by Mohan *et al*. (2010) and Moongngarm and Saetung, (2010), who found values of ash of brown rice (Indica) ranging from 1.60 to 2.06%. Those authors also reported the same finding that ash content increased in germinated brown rice. It is of interest that crude fiber in germinated brown rice increased from 2.07 to 4.55%. This result was similar to that of Jung *et al*. (2005), Ohtsubo *et al*. (2005) and Lee *et al*. (2007) which reported that the increase of fiber may result from the formation of primary cell walls, through an increase in pectic substance in the middle lamella.

The results of this study in addition to the findings of increased GABA content may also suggest that germinated brown rice is beneficial to people who suffer from chronic diseases and obesity problems. For example, a decrease in carbohydrates is good for diabetes patients, while an increase in fiber and decrease in fat content is good for people with cardiovascular disease.

Pasting characteristics

Viscosity change during pasting could be useful for the prediction of food product quality. The pasting characteristics of the both native (control) and germinated brown rice flours (GBRF) during heating, cooking and cooling were investigated and results are shown in Figure 1. Pasting profiles of germinated brown rice flours were considerably different from the control. Although pasting temperature of control and GBRF were unaffected by germination (Table 5),

Table 5. Pasting properties of brown rice and GBRF

RVA parameters	Brown rice (control)	GBRF*
Pasting Temperature (°C)	74.10±1.01[a]	75.50±0.59
Maximum Viscosity (RVU)	84.16±0.24	30.83±0.47
Breakdown (RVU)	5.91±0.35	0.92±0.59
Final Viscosity (RVU)	131.75±0.65	55.50±0.77
Setback (RVU)	53.50±0.65	25.59±0.89

* GBRF = germinated brown rice flour treated with anaerobic condition
[a] Mean values ± SD of triplicate measurements

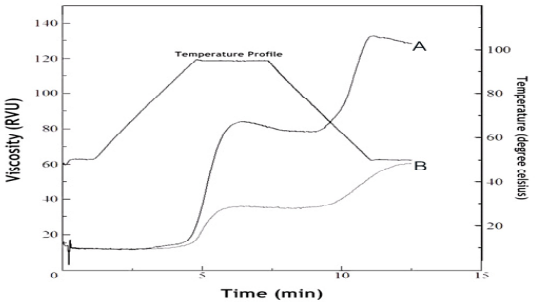

Figure 1. Pasting profiles of native brown Red Jasmine rice (A) and germinated brown Red Jasmine rice (B) samples recorded in Rapid Visco Analyzer

GBRF had a lower peak viscosity (30.83 RVU) than the control (84.16 RVU). Likewise, the breakdown viscosity, final viscosity and set back were also lower for GBRF than control. Similar changes in pasting profile by germination have been reported (Mohan *et al*., 2010; Watcharaparpaiboon *et al*., 2010). A reduction in pasting viscosity of GBRF is because the endogenous enzymes such as α-amylase, β-amylase, limit dextrinase and α-glucosinase in rice are activated during germination and convert starch into smaller molecules (Mohan *et al*., 2010). The breakdown viscosity is related to the rigidity of swollen granules to damage when the starch paste is heated and stirred. The low breakdown viscosity of GBRF indicated the instability of viscosity during the heat processing of starch. The setback and final viscosity are indicative of the retrogradation tendency that related to the structure of amylose and amylopectin. The low set back of GBRF may be attributed to the low value of amylose content during germination, where the hydrolysis of starch occurred. GBRF could be used to retard staling of bakery products, especially bread due to the low setback property (Watanabe *et al*., 2004).

The overall pasting profile of GBRF indicates that during germination the properties of starch altered to some extent and thus it will affect the quality of food products. The study by Charoenthaikij *et al*. (2010) demonstrated that it is feasible to substitute wheat flour with up to 30% GBRF in bread formulation but the bread had lower loaf volume and greater hardness than the wheat bread. In addition, substitution of GBRF for wheat flour decreased the cooking and textural quality of cooked noodles (Chung *et al*., 2012). GBRF could be used to prepare more nutritious food products but their formulation should be optimized to ensure desirable qualities and sensory acceptability.

Conclusions

Germination caused considerable changes in GABA, chemical compositions and pasting property in germinated Red aromatic brown rice. GABA content increased with higher temperatures and longer soaking time. The GABA content was highest when soaking brown rice at 35°C for 24 h. Moreover, anaerobic treatment after soaking also resulted in an increase in GABA content. Heating and ethanol treatment reduced the number of microorganisms which appeared during germination. Germinated brown rice could be used as a nutritional ingredient in a healthy diet as not only is it lower in carbohydrate and fat content but it also has a higher fibre content than native brown rice. The starch pasting viscosity and set back of germinated brown rice was lower than native brown rice which may affect the quality of food product.

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