The effect of fermentation of germinated brown rice and KKU URL0381 cultivar black rice on GABA content and chemical composition of by-product and fermented rice juice

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Abstract

The purpose of this study was to investigate the fermentation process of rice two types (germinated brown rice and KKU URL0381 cultivar black rice) on GABA content and chemical composition (*i.e.* moisture, protein, fat, ash and crude fiber) of by-product and fermented rice juice. The results indicated that the fermented-germinated brown rice has more protein content than fermented black rice. The fermentation process increased the amount of Gamma-aminobutylic acid (GABA) in germinated brown rice to approximately 4–6 times, whereas, approx. one time in KKU URL0381 cultivar black rice. Results from this study were used for the development production of probiotic supplemental products.

Keywords: fermentation; germinated brown rice; black rice; GABA

1. Introduction

Rice is an important agricultural product for domestic consumption and export. Traditional germinated rice, Hang rice and black rice are products from local rice species which are developed and value added by processing to be Hang rice or germinated Hang rice containing rich nutrient and potent bioactivity as retard or protective effect on the degeneration symptoms. The chemopreventive potential has been related to the bioactive phytochemicals present in the rice such as ferulic acid, tricin, b-sitosterol, g-oryzanol, tocotrienols/tocopherols, and gamma-aminobutylic acid (Henderson *et al*., 2013). Germinated brown rice extracts with enhanced levels of GABA have an inhibitory action on leukemia cell proliferation and have a stimulatory action on the cancer cell apoptosis (Chan-Ho and Suk-Heung, 2004). In black rice, pigments are located in the aleurone layer, which is characterized as dark purple to black in color and probably represents a mixture of anthocyanins (Hu *et al*., 2003). *Aspergillus oryzae* is used extensively for the production of the traditional Japanese fermented foods sake (rice wine), shoyu (soy sauce),

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and miso (soybean paste) (Keietsu *et al*., 2006). The fermented brown rice by *Aspergillus oryzae* (FBRA) inhibits N-nitrosomethylbenzylamine-induced esophageal tumor development in rats possibly through inhibition of cell proliferation in the post-initiation phase, and suggest that FBRA is a promising dietary agent for prevention of human esophageal cancer (Kuno *et al.*, 2004). Therefore, the objective of this study was to evaluate the effect of fermentation of germinated brown rice and KKU URL0381 cultivar black rice on GABA content and chemical composition of by-product and fermented rice juice

2. Materials and Methods

2.1 Microbe, chemicals and reagents

Aspergillus oryzae and *Lactobacillus acidophilus* were purchased from Thailand Institute of Scientific and Technological Research. HPLC grade formic acid and acetonitrile were purchased from Acros Organics, Belgium. The GABA standard and other chemicals were purchased from Sigma Aldrich, USA.

2.2 Materials and sample preparation

The germinated brown rice was obtained from Sakon Nakhon, Thailand. The KKU URL0381 cultivar black rice was obtained from Khon Kaen, Thailand. The samples were cooked with rice cooker (rice: water; 1:2.5) and cooled to room temperature. Then, the samples were fermented with *Aspergillus oryzae* at 25ºC for 72 h.; batch pasteurization at 75ºC for 15 minutes and inoculated with *Lactobacillus acidophilus* for 72 h., separated of fermented rice juice and by-product (sludge) before packed in plastic bags (polyethylene). The samples were stored in a freezer (-20 ºC) until further analysis.

2.3 Proximate analysis

Chemical compositions including moisture, protein, fat, ash and crude fiber of the samples before and after fermentation were investigated using the method of AOAC (2000).

2.4 Gamma-aminobutyric acid (GABA) analysis

The GABA content in the samples was investigated using the method of Maisont and Narkrugsa (2010). Analysis was performed using HPLC, a LUNA C-18 column and a diode array detector. The mobile phase consisted of 0.10% formic acid (mobile phase A) and acetonitrile (mobile phase B) by gradient program elution at a flow rate of 1.0 ml/min. The absorbance was

read at 330 nm. The quantification will carry out by comparing with a GABA standard solution (External standard).

2.5 Statistical analysis

Experimental results are given as means ± the standard deviation of three replicates. The experimental design was a Completely Randomized Design (CRD). Data were analyzed by ANOVA, and differences between means were determined by the Duncan's New Multiple Range Test. The level of significance was considered at $p \leq 0.05$.

3. Results and Discussion

3.1 Chemical composition

Moisture

The germinated brown rice and KKU URL0381 cultivar black rice had moisture 9.08 and 11.42%, respectively (Table 1). The samples meet qualification according Thai Rice Standards and Thai Hom Mali Rice Standards, Access to Foreign Trade Department. It was assigned to moisture content not more than 14.00%.

Protein

The results shown that the germinated brown rice and KKU URL0381 cultivar black rice had protein content 10.50 and 8.55%, respectively ($p \leq 0.05$). Consistent with the data of Rice Gene Thresher (2013) have been reported Hang rice has protein content approx. 6.00–12.00%. The fermented-germinated brown rice sludge has more protein content than the fermented black rice sludge at 4.93 and 3.89%, respectively ($p \leq 0.05$). As well as the fermented-germinated brown rice juice and the fermented black rice juice had protein content 3.26 and 1.72%, respectively ($p \leq 0.05$) (Table 1).

Fat

The germinated brown rice and KKU URL0381 cultivar black rice had fat content 1.78 and 1.54%, respectively ($p > 0.05$). According to Nittaya (2006), the fat typically found in rice was 1.40%. The fermented-germinated brown rice sludge and fermented black rice sludge were also similar to the fat content before fermentation, because lipids were insoluble in water. The fermented-germinated brown rice juice and fermented black rice had fat content 0.24 and 0.35%, respectively ($p > 0.05$) (Table 1).

Ash

The germinated brown rice and KKU URL0381 cultivar black rice had ash content 8.48 and 8.29%, respectively (p>0.05). Ash content of the fermented-germinated brown rice sludge and fermented black rice sludge were decreased approx. one-half percent, but ash content between the two types of rice no difference (p>0.05). Therefore, some minerals were dissolved in juice. The study found that the fermented-germinated brown rice juice and fermented black rice juice had ash content 3.68 and 4.52%, respectively (Table 1).

Crude fiber

The germinated brown rice and KKU URL0381 cultivar black rice was relatively more crude fiber at 1.40 and 2.75%, respectively (p≤0.05). According to Nittaya (2006), whole wheat flour had crude fiber 2.3%, as white wheat flour had crude fiber 0.3%. The fermented-germinated brown rice sludge and germinated brown rice had crude fiber no significant difference (p>0.05), because germinated brown rice has steaming process before rice milling, so grain was sticky and soluble fiber was less. The fermented-germinated brown rice juice had crude fiber only 0.45% (Table 1).

Table 1 Chemical composition of the samples before and after fermentation.

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude fiber (%)
G	11.42^{e} ± 0.12	10.50^{a} ± 0.12	1.78^{a} ± 0.12	8.48^{a} ± 0.37	1.40^{b} ± 0.02
H	9.08^{f} ± 0.64	8.55^{b} ± 0.01	1.54^{a} ± 0.28	8.29^{a} ± 0.02	2.75^{a} ± 0.33
GS	74.46^{c} ± 0.89	4.93^{c} ± 0.10	1.16^{b} ± 0.24	4.48^{bc} ± 0.02	1.13^{b} ± 0.05
HS	69.42^{d} ± 1.25	3.89^{d} ± 0.05	1.70^{a} ± 0.26	3.94^{cd} ± 0.03	1.29^{b} ± 0.09
GL	84.69^{b} ± 1.64	3.26^{e} ± 0.05	0.24^{c} ± 0.02	3.68^{d} ± 0.44	0.45^{c} ± 0.10
HL	90.67^{a} ± 1.49	1.72^{f} ± 0.05	0.35^{c} ± 0.01	4.52^{b} ± 0.47	1.14^{b} ± 0.15

Note: X ± SD = mean ± standard deviation (n=3)

a-f = Data in a column followed by different letters are significantly different by DMRT at p≤0.05.

3.2 GABA content

The germinated brown rice and KKU URL0381 cultivar black rice were fermented with *A. oryzae* and *L. acidophilus* for 6 days, separated sludge and juice, extracted with water. Analysis GABA content by HPLC at 330 nm. Using standard spiked to indicate the retention time of GABA with external standard. The results shown in Figure 1 and Figure 2, standard GABA was separated at 15.02–15.30 minutes. For GABA in the germinated brown rice, fermented-germinated

brown rice sludge and fermented-germinated brown rice juice found that the retention time at 16.37–16.90, 16.46–16.53 and 16.46–16.49 minutes, respectively (Figure 1). The KKU URL0381 cultivar black rice, fermented black rice sludge and fermented black rice juice found that the retention time at 16.42–16.97, 16.42–16.45 and 16.40–16.44 minutes, respectively (Figure 2).

GABA content in the samples were calculated by the linear equations obtained from the standard curve (R^2=0.999). The results indicated that the germinated brown rice and KKU URL0381 cultivar black rice has GABA content at 0.33±0.07 and 0.47±0.09 mg/g, respectively. The fermented-germinated brown rice sludge and fermented-germinated brown rice juice had GABA content 1.55±0.34 and 2.25±0.17 mg/g, respectively. As the fermented black rice sludge and fermented black rice juice, GABA was found at 0.79±0.24 and 0.97±0.07 mg/g, respectively (Figure 2). This study was shown that fermentation of rice with *A. oryzae* and *L. acidophilus* was able increase to higher GABA content.

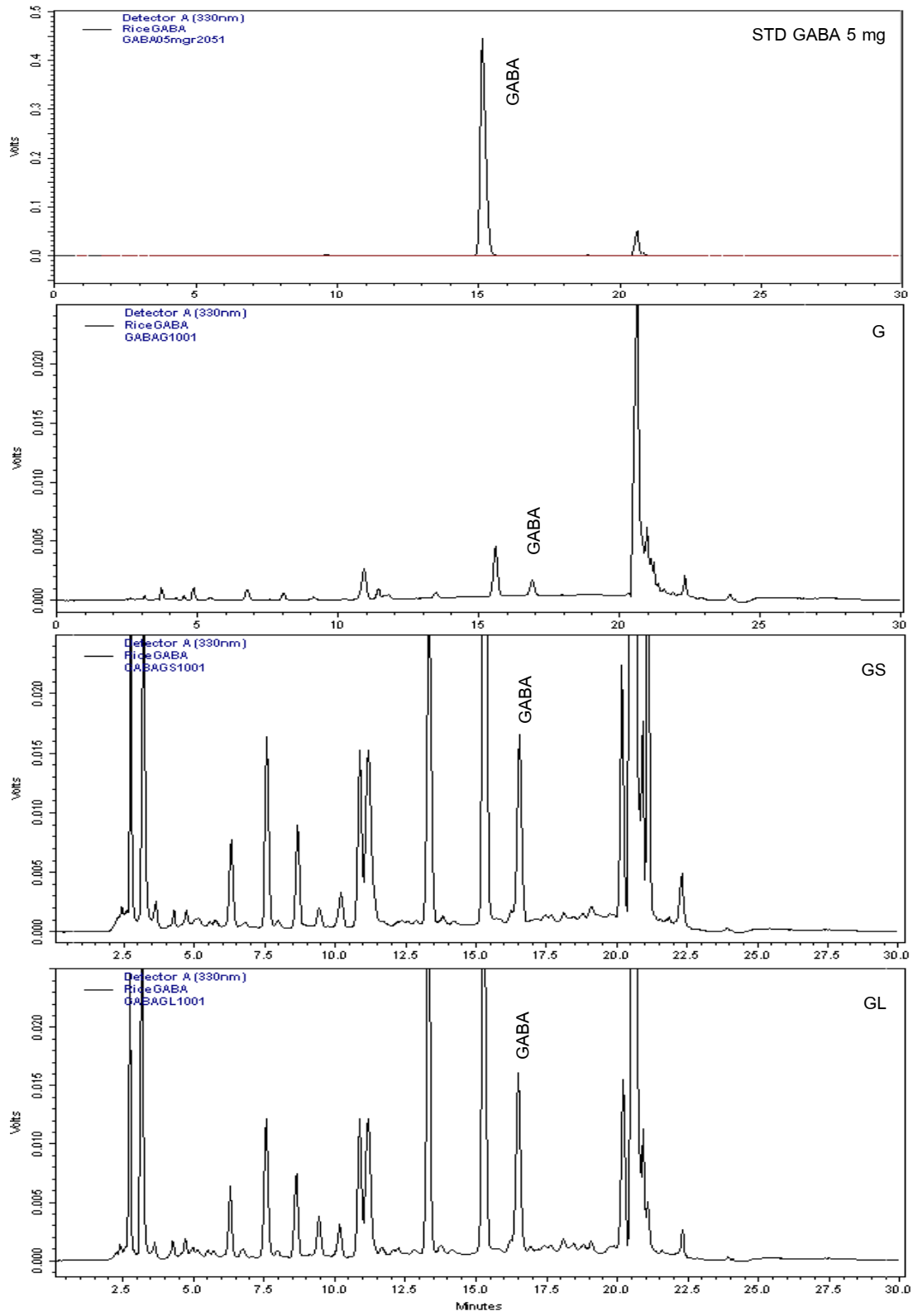

Figure 1 Chromatograms of GABA in germinated brown rice was fermented with *A. oryzae* and *L. acidophilus* for 6 days.

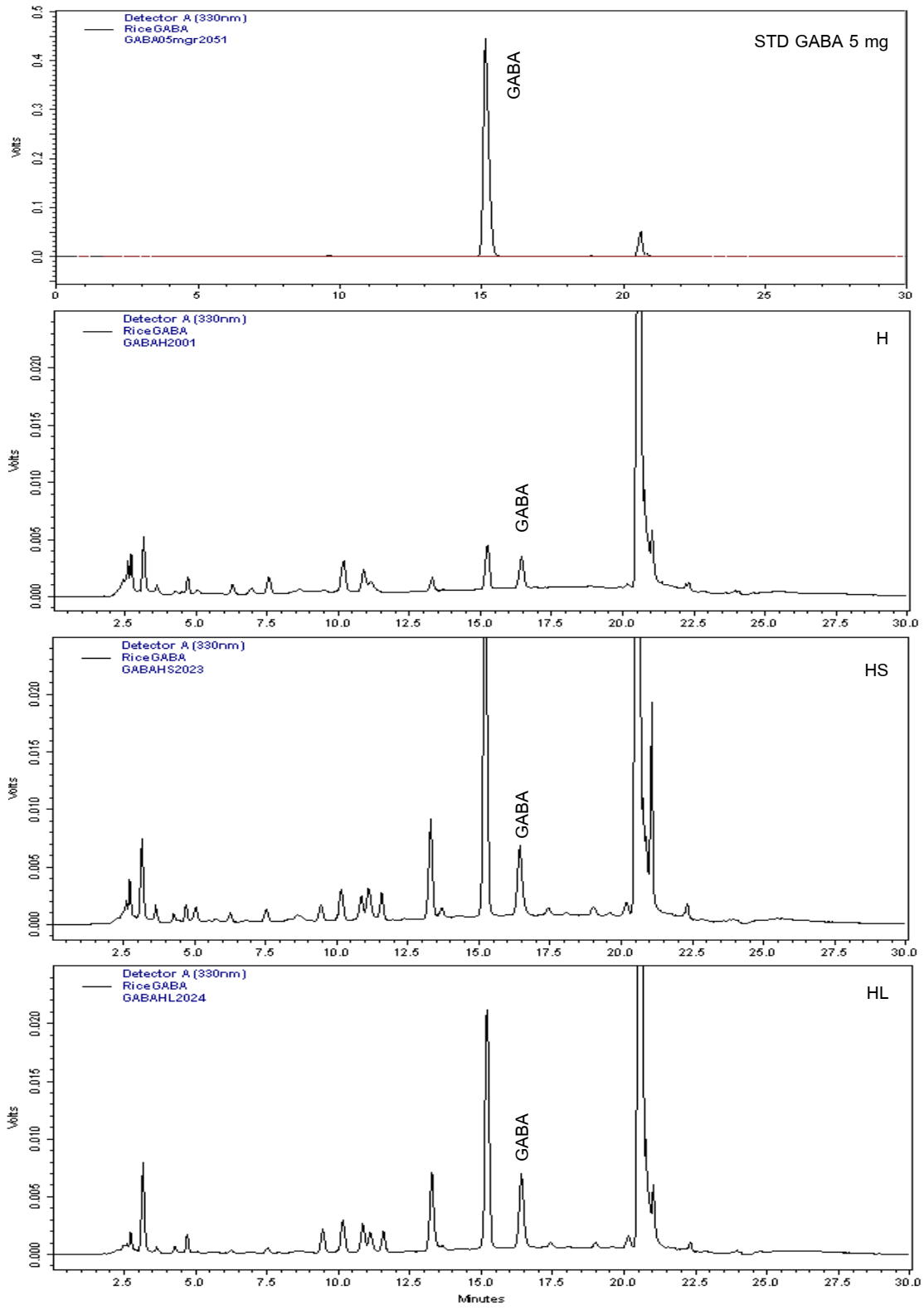

Figure 2 Chromatograms of GABA in KKU URL0381 cultivar black rice was fermented with *A. oryzae* and *L. acidophilus* for 6 days.

4. Conclusion

Protein content of the fermented-germinated brown rice sludge and fermented black rice sludge were decreased approx. one-half percent, because it was digested with metabolism of microbe into GABA. The study was found that GABA content of by-product and fermented rice juice was increased approx. 1–6 times, depending on type of rice. The fermented-germinated brown rice had more GABA content than fermented black rice (Figure 3).

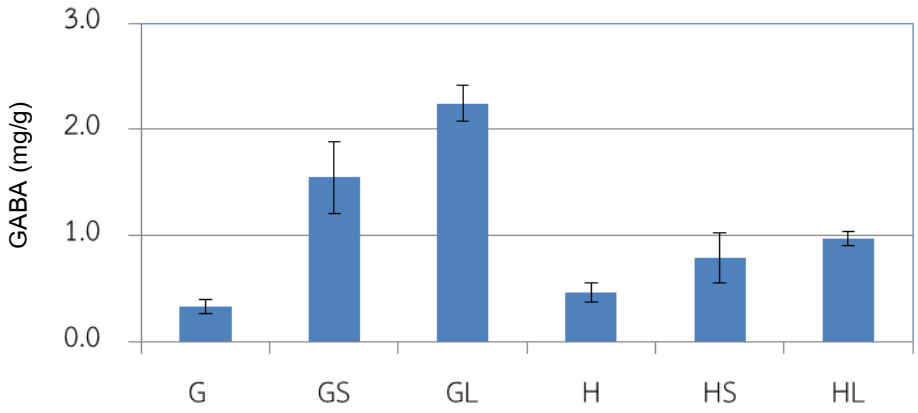

Figure 3 GABA content in germinated brown rice and KKU URL0381 cultivar black rice was fermented with *A. oryzae* and *L. acidophilus* for 6 days.

Abbreviations

G; germinated brown rice,

H; KKU URL0381 cultivar black rice,

GS; fermented-germinated brown rice sludge (by-product),

HS; fermented black rice sludge (by-product),

GL; fermented-germinated brown rice juice,

HL; fermented black rice juice

Acknowledgements

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