PT001

Effect of Thermal Shock by Impinging Stream Technique on Bioactive Compounds of Germinated Difference Rice Varieties

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Abstract

The bioactive compounds in rice showed an improvement after germination, i.e., α-tocopherol, γ-oryzanol, γ-aminobutyric acid (GABA) including low molecular weight antioxidants as total phenolic content (TPC). This study was performed to investigate changes in the chemical compositions of germinated difference rice Varieties after impinging stream heat treatment. The rice samples were germinated in the water bath at 35oC for 12 h and heated to 130-190oC temperatures by impinging stream dryer (ISD). Germination method had significant effect on the contents of GABA, TPC and total antioxidant capacity (TAC). In this study, the heat treatment could increase the GABA content in germinated rice (GR) both SP.1 and KDML 105 vairties approximately 17 and 20 folds compared to that of the un-germinated. TPC of GR prepared by SP.1 and KDML 105 Varieties were increased about 3.7 and 1.8 fold, respectively, compared to that of the un-germinated. In addition, TAC of GR was increased about 4.4 fold (SP.1) and 2.1 fold (KDML 105).

Keywords: Germinated rice, Impinging stream thermal treatment, GABA content, Total phenolic content, Total antioxidant capacity.

1. Introduction

γ-aminobutyric acid (GABA), a four-carbon non-proteinamino acid that is widely found in eukaryotes and prokaryotes, is produced by the α-decarboxylation of L-glutamic acid catalyzed by aglutamate decarboxylase (GAD, EC 4.1.1.15) (Bown and Shelp, 1997). GABA is functionally involved in the induction of hypotensive, diuretic, and tranquilizing effects, playing a principal inhibitory neurotransmitter in the central nervous system (Inoue et al., 2003). Rapid increases in GABA concentration occur in plants in response to extreme temperatures, anoxia, hypoxia, mechanical damage, salinity, acidosis and defence against herbivory (Allan et al. 2008). Germinated rice (GR), one of the most popular rice products, is rich in nutrients, particularly GABA (Chungcharoen et al., 2015). Many rice cultivars are grown in Thailand. Some cultivars contain relatively high contents of GABA, which was 28.3 mg/100 g (Moongngarm and Saetung, 2010) but some cultivars contain very low GABA. There are several studies paid an attention on increases GABA level in rice (Kim et al., 2015; Komatsuzaki et al., 2007; Techo et al., 2016; Thuwapanichayanan et al., 2015).

Kim et al. (2015) studied the method to increase of GABA content in rough rice (*Oryza sativa* L.) by high hydrostatic pressure treatment (HPT) and found GABA 2.92 times higher than the non-treated sample. Komatsuzaki et al. (2007) studied the germination process by soaking and gasous treatment and found that the combination of soaking and gasous treatment provided the germinated rice with 2.5 times of GABA higher than that obtained from the soaking method. Techo et al. (2016) studied the effect of heat treatment on the GABA content and found that the combination of soaking, rapid thermal treatment by impinging stream dryer (ISD) at the temperature of 150-

170°C and hypoxic state provided the higher GABA content (17.3 times of GABA obtained from the raw sample). Thuwapanichayanan et al. (2015) reported the content of GABA of the germinated rice (*Oryza sativa* L. cv. Pathum Thani 1) prepared by combination of soaking, anaerobic condition and thermal treatment using the fluidized bed dryer at 120°C and found the higher GABA content when compared with the samples rice prepared by soaking combined with anaerobic condition and the soaking alone. These studies indicated that thermal treatment is one of the considerable methods which could enhance the GABA content during germination. However, a difference in the amount of GABA content in rice depends on an important factor; that is, rice variety.

Investigation on the effect of thermal treatment on the GABA of different varieties of germinated rough rice is limited. Hence, the objective of this work was to investigate the effect of thermal treatment on chemical composition such as the GABA in GR with different varieties. In addition, the bioactive compounds in terms of Total phenolic compounds (TPC) and antioxidant activity (TAC) of germinated rice were also investigated.

2. Materials and Methods

2.1 Material.

Rough rice sample.

Rough rice samples was prepared from different cultivars namely, *O. sativa* L. cv. Khao Dawk Mali 105 (KDML105) and Supanburi 1 (SP.1). KDML105 was harvested in November 2015, obtained from the Rice Department of Nakhon Ratchasima Province, Thailand. SP.1 was harvested in March 2015, purchased from Chaijalearn Limited Partnership, Supanburi Province, Thailand.

Impinging Stream Dryer (ISD).

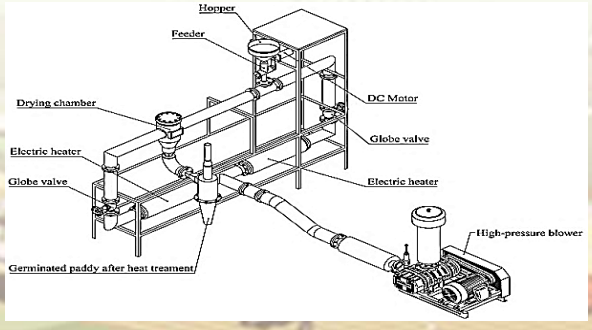

Figure 1. A schematic diagram of ISD.

An impinging stream drying technique was used for heating germinated rough rice during the germination step. The ISD used hot air as the drying medium. A schematic diagram of the dryer and associated units is shown in Fig. 1. The impinging distance of 0.05 m, inlet superficial air velocity of 30 m/s and rough rice feed rate of 80 $kg_{dry\_paddy/h}$ were used.

2.2 Method.

Preparation of germinated rough rice.

Soaking (S).

The cleaned rough rice sample was soaked in water bath at 35°C until its embryo begins to bud, preferably to a length of 1–2 mm. The water was changed every 4 h. For germination, the percentage of germination was about 95%, which was a maximum value in this study. The soaked SP.1 and KDML 105 were germinated for 48 h and 60 h, respectively. The experiment was done in duplicate.

Combination of soaking and hypoxic state treatment (S-H).

After soaking cleaned-rough rice in water bath at 35°C for 12 h, the samples were placed into the hermetically sealed glass jar under hypoxic state with a ratio of rough rice to glass jar of 3:8 (v/v). The samples were kept in the oven at a temperature of 35°C and germinated up to 48 h (SP.1) and 60 h (KDML 105). The experiment was done in duplicate.

Combination of soaking, rapid thermal treatment and hypoxic state (S-ISD-H)

After soaking the cleaned rough rice in water bath at 35°C for 12 h was thermally shocked by the ISD at the temperatures of 130, 140, 150, 160, 170, 180 and 190°C after which the SP.1 and KDML 105 samples were germinated under the abovementioned hypoxic state for 36 h and 48 h, respectively. The experiment was done in duplicate.

GABA content determination.

The GABA content of the dried germinated rice prepared by S, S-H and S-ISD-H was determined by using HPLC, following the procedure of Thuwapanichayananet al. (2014) with a minor modification.

Total phenolics content (TPC) and total antioxidant capacity (TAC) determination

TPC of the dried germinated rice was analyzed using Folin–Ciocalteu reagent with method of Techo et al. (2016). TAC of GR during germination was evaluated based on the free radical scavenging effect of 2, 2-diphenyl-2-picryl-hydrazyl (DPPH). The DPPH scavenging activity of samples was determined using method of Techo et al (2016).

3. Results and Discussion

Effect of stress condition on GABA content.

The percentages of germination of rough rice prepared by SP.1 and KDML 105 Varieties were increased to the value of 95% at 48 h and 60 h, respectively. The percentage of germination did not change when germination time was longer than 48 h for SP.1 and 60 h for KDML 105. The GABA content of rough rice prepared by SP.1 and KDML 105 Varieties before germination were 2.88 mg/100 g DW and 5.05 mg/100 g DW, respectively. It was increased with increasing germination time for all treated samples. GR prepared by S-ISD-H method had higher GABA content than that prepared by S-H and S alone except for the S-ISD180-H and S-ISD190-H methods which became lower. The treatment temperature in a range of 150-170°C provided the highest GABA content for SP.1, yielding a GABA content about 50 mg/100 g DW, whilst the highest GABA content was found at the treatment temperature of 170°C for KDML 105 variety, with a content of about 100 mg/100 g DW. From these results, it indicated that the KDML 105 was rather more sensitive to heat than the SP 1. The increase of GABA was increased by 25 times for KDML 105 and 15.6 times for SP.1 as compared to the raw sample. The difference of GABA content for the samples prepared by S-ISD-H at each temperature might be due to the difference in their grain temperatures which is related to GAD enzymatic activity. Zhang et al. (2007) reported that the GAD activity from rice was highest in temperature range about 40°C and the corresponding highest GABA content was achieved. As measured from the experiments, the average grain temperatures at the ISD outlet was 40.5°C at the heated temperature of 170°C. Thus, the GP prepared by ISD at temperature 170°C had the highest GABA content. When the grain temperature was 43°C, corresponding to the treatment temperature of 190°C, the GABA content was dropped by a half of the sample treated at temperature of 170°C. As shown in Figure 2, the germinated rice prepared from KDML 105 varity had higher GABA content than that prepared by using SP.1 varity. Jannoey et al (2010) studied that the different amounts of GABA content found among the rice varieties are mainly caused by their genetic constitution.

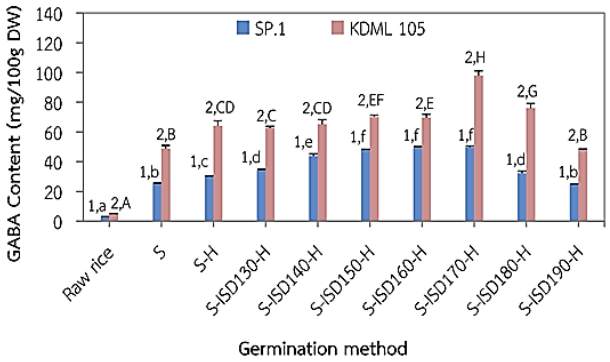

Figure 2. GABA contents in GR prepared by S, S-H and S-ISD-H methods. The different over scripts over bar presented significant difference at $p<0.05$.

Effect of stress condition on TPC and TAC

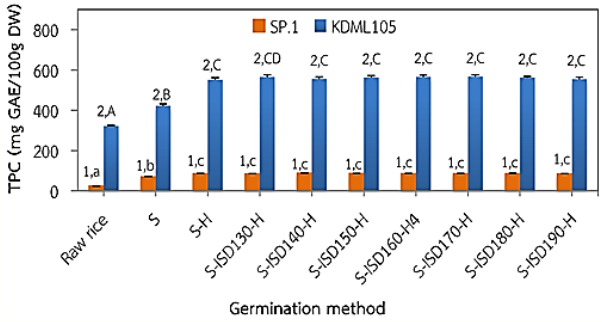

Figure 3. Total phenolic content of GP prepared by S, S-H and S-ISD-H methods. The different over scripts over bar presented significant difference at $p<0.05$.

The TPC of GR prepared by S, S-H, S-ISD-H method were shown in Fig. 3. The TPC of un-germinated brown rice were 23.7 and 321.8 mg TE/100 g DW for SP.1 and KDML105, respectively. After germination process, the TPC in all GR samples were increased. An increase in the TPC during germination process was correlated with the activation of many enzymes in the phenylpropanoid pathway and degradation of the cell wall of polysaccharides and protein, resulting in release of bound phenolics (Caceres et al., 2014). Results from this work was in agreement with the study of Caceres et al. (2014) who

reported that germination process could enhance total phenolic content in Ecuadorian brown rice. Besides the germination periods, the germination method had remarkably influenced on the TPC. The S-H method gave higher TPC in GR than that prepared by S method (Fig. 3). This result could be due to protective mechanisms under anoxic stress of plant cells against the reactive oxygen species by the action of enzymatic and non-enzymatic system including low molecular weight antioxidants such as phenolic compounds, tocopherols, ascorbate and glutathione (Shen et al., 2015). However, the TPC of GR prepared by S-ISD-H was not significantly different from those prepared by S-H as seen in Fig. 3. It was shown that thermal shock could not enhance the TPC. In all germination method used, it was found that GR prepared from KDML 105 had much higher in the TPC than that prepared from SP.1 (Fig. 3). This was probably due to differences in the varieties of rice.

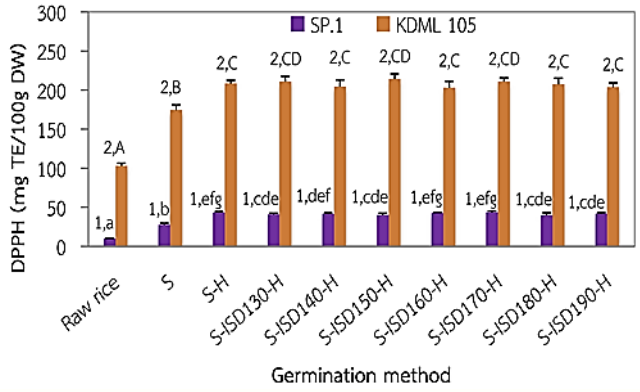

Figure 4. The DPPH scavenging activity of samples prepared by different method. The different over scripts over bar presented significant difference at $p<0.05$.

The DPPH assay is based on electron transfer (ET), which is a test to determine the capacity of an antioxidant in the reduction of an oxidant (DPPH free radical) by changing color when reduced (Uzelac et al., 2007). As presented in Fig. 4, the DPPH values of un-germinated SP.1 and KDML105 rice were 10.1 and 103.1 mg TE/100 g DW. At the end of germination process, the TAC in SP.1 and KDML105 rice were increased. Thus, it could be said that germination process improved antioxidant activity of GR. Furthermore, KDML105 GR gave the higher TAC than that of SP.1 in all germination treatments.

The antioxidant activity was increased when the germination time was proceeded, which was similar to the TPC. Moreover, the antioxidant activities of the GR in both varieties that prepared by S-H and S-ISD-H methods were insignificantly different but were higher than those of the GR prepared by S method. Changes in the antioxidant activity were related to those of the TPC as reported above. This might be because the phenolic compounds are main contributors to antioxidant activity acting as electron donor in the DPPH assay.

4. Conclusions

The GABA content, TPC and TAC were different among rice cultivars used in this study. GR prepared by KDML 105 contained higher amount of these bioactive compounds than SP.1. Germination method had significant effect on the contents of GABA, TPC and TAC. Among the germination methods used, the heat shock treatment, at 170oC, resulted in the highest GABA content (about 50-100 mg/100 g DW); an appoxiamtely 18.5 fold increase compared to the un-germinated paddy, except for those samples heat-treated at 180-190oC. The heat treatment was not influenced on TPC in GR, whereas hypoxic state treatment could improve the phenolic content. These results suggest that a combination of heat treatment and germination are efficient method for enhancement of functionality in rice, and clarify the influence of thermal treatment conditions on the GABA content, TPC and TAC in GR.

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