

## COMPARISON OF HEAT AND COLD TREATMENTS ON QUALITY ATTRIBUTES OF GERMINATED BROWN RICE

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**Abstract:** Germination can improve textural property of cooked rice and enhance nutrients, especially  $\gamma$ -aminobutyric acid (GABA) and phenolic compounds. The nutrients are health benefits and have received more attention for developing functional food. The objective of this study was therefore to compare changes in the chemical compositions and textural property of germinated rice after heat and cold treatments. The GABA content of germinated rice prepared by soaking in combination with heat and hypoxic state treatment (S-ISD-H), were significantly higher than those of the germinated rice prepared by soaking in combination with cold and hypoxic state treatment (S-C-H), soaking in combination with hypoxic state treatment (S-H) and soaking method (S). The cold and heat treatments did not affect the TPC compound of GP, but the hypoxic state strongly affected the TPC. The hardness of cooked germinated rice was decreased with increasing germination time and the germination preparation methods influenced the cooked rice texture.

**Keywords:**  $\gamma$ -aminobutyric acid, Heat treatment, Cold treatment, Phenolic compound, Textural property

### INTRODUCTION

Consumers are increasingly interested in healthy foods. Among the wide varieties of such food products, germinated brown rice is of interest because it is rich in nutrients, including  $\gamma$ -aminobutyric acid (GABA), phytic acid, inositols, tocotrienols, ferulic acid, zinc, potassium, magnesium,  $\gamma$ -oryzanol, prolylendopeptidase

inhibitor (Banchuen et al., 2009) and serves as a main source of phenolic compounds which associated with antioxidant effects (Moongngarm and Saetung, 2010; Ti et al., 2014; Xia et al., 2017). Among them, GABA, a four carbon non-protein amino acid that is primarily synthesized through the decarboxylation of L-glutamic acid, which is catalyzed by glutamate decarboxylase enzyme (GAD, EC 4.1.1.15), plays a relevant role as a major inhibitory neurotransmitter in

the synapses of the central nervous systems (Choi et al., 1998; Malomouzh et al., 2015). GABA is also used for the treatment of several diseases related with sleeplessness, depression, and autonomic disorders (Okada et al., 2000). Such health benefits of GABA have received attention for developing functional foods containing high levels of accumulated GABA. The methods to increase GABA contents in seed have been studied by several researchers (Komatsuzaki et al., 2007; Youn et al., 2011; Thuwapanichayanan et al., 2015).

Komatsuzaki et al. (2007) claimed that application of soaking and hypoxic state treatment gave the higher content of GABA than the soaking alone (2.47 folds). Youn et al. (2011) observed that application of anaerobic and heat treatment by using hot air at temperature of 120-140°C flowing through the germinated wheat for 30 s could produce the higher content of GABA in the germinated wheat when compared to the soaking only. Thuwapanichayanan et al. (2015) studied the content of GABA of the germinated rice prepared by combination of soaking, anaerobic and heat treatment (SAH) using the fluidized bed dryer (FBD) at 120°C for 30 s and found the higher GABA content when compared with the samples prepared by soaking combined with anaerobic condition and the soaking method. Liao et al. (2013) pointed out that the pre-processes (soaking, germinating, and cold shock) of adzuki beans affected GABA accumulation. Among the pre-processing methods, cold shock treatment resulted in the highest GABA content (201.2 mg/100 g); a 150-fold increase compared to the non-treated adzuki beans. Under stress conditions, GABA may represent a major fraction of the free amino acid content which plays a role as an osmoprotectant in plants (Bown and Shelp, 1989).

These studies indicated that heat and cold treatment is one of the important methods which could increase the GABA during germination. In case of heat treatment, most works have applied the heat treatment by the oven method. This method is not practical to mass production since the contact between grains and heating medium in the oven is poor, resulting in the difference of seed temperatures amongst them which might directly affect the GABA content. Impinging stream dryer (ISD) is a novel drying technique. An interested feature of this drying technique was a short resident time of particles in the dryer (Prachayawarakorn et al., 2016). Nimmol and Devahastin (2010) were among the first to research the use of ISD for paddy. They found that the dryer could reduce the moisture content of paddy by 3.4-7.7% (d.b.) within a very short period of time (~2 s). Thus, ISD was selected as it provides extremely high heating and drying rates appropriate for the required stimulation. Up until now, there is no report on the use of rapidly heating technique and cold treatment

for increasing the GABA level during germination period of paddy. In addition, changes in chemical qualities i.e. total phenolic content of GP after heat and cold treatment have not well be reported.

Therefore, the purpose of this research was to study the effects of heat and cold treatments on the quality of the germinated brown rice in terms of textural property and other chemical properties such as GABA content and TPC.

## MATERIALS&METHOD

### *Raw paddy*

Suphanburi 1 paddy variety selected for this study was obtained from Chaijalearn Limited Partnership; Supanburi Province, Thailand, and had already been stored for 1 month before germination study. The initial moisture content of the paddy as received was approximately 13-14% on a dry basis (db).

### *Impinging Stream Dryer (ISD)*

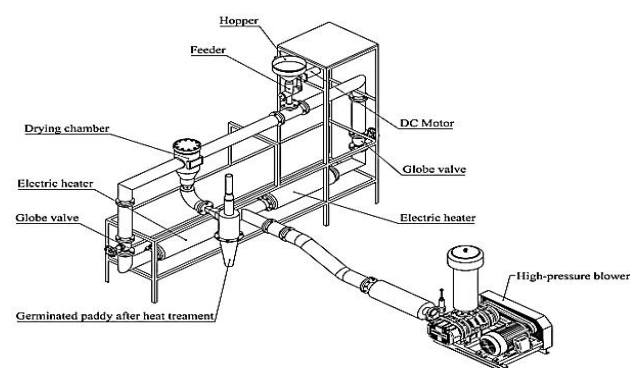


Fig. 1. A schematic diagram of ISD

An impinging stream drying technique was used for heat-stressed germinated paddy during the germination process. A schematic diagram of the ISD and associated units are shown in Fig. 1. The impinging distance of 0.05 m and inlet superficial air velocity of 30 m/s were used. A DC electric motor, rated at 117 W and controlled by a voltage regulator, was used to drive a star feeder, for feeding the germinated rice into a feeding pipe, which has an internal diameter of 3.8 cm. The germinated paddy feed rate was fixed at 80 kg dry paddy/h. One kilogram of germinated rice was heat-stressed in each experiment.

### *Soaking Method (S)*

Four-kg cleaned paddy was immersed in the water bath at 35°C for 12, 24, 36, and 48 h to promote germination, using grain-to-water ratio of 1:2 (w/v).

At each immersing time the paddy was sampled to determine the germination percentage and GABA content. During germination, the water was changed every 4 h and drained off at the end of immersion as suggested by Srisang et al (2011). To obtain 95% germination percentage, it required 48 h of the germination time; at this time, the length of germs from embryo was about 1-2 mm. When the germination time was extended from 48 to 60 h, the germination percentage and GABA content did not change. Chungcharoen et al. (2012) reported that the percentage of germination could not increase more than 95% because some of the rice kernels were imperfect.

#### *Soaking method combined with hypoxic state (S-H)*

After immersing the cleaned paddy in a water bath at 35°C for 12 h, samples were placed into the hermetically sealed glass jar under hypoxic state with a ratio of paddy to glass jar of 3:8 (v/v). The samples were kept in the oven controlled temperature of 35°C and germinated for 48 h.

#### *Soaking method combined with heat treatment and hypoxic state (S-ISD-H)*

After immersing cleaned-paddy in a water bath at 35°C for 12 h, the water was drained off and the GP was shocked thermally by the ISD at temperatures of 130, 140, 150, 160, 170, 180 and 190°C. After heat treatment, the GP was cooled down to ambient temperature by immersing it in water for 1 h after which the sample was germinated under the aforementioned hypoxic state for 12, 24 and 36 h. At the predetermined germination time, it was sampled to determine GABA content.

#### *Soaking method combined with cold treatment and hypoxic state treatment (S-C-H)*

After immersing cleaned-paddy in a water bath at 35°C for 12 h, the water was drained off and the GP was cooled by a freezer (Refrigerant: R22, Model YR-1000SRT) at temperatures of 5, 10 and 15°C for 3, 2 and 1 h, respectively. After cold treatment, the GP was soaked in water at ambient temperature after which the sample was germinated under the aforementioned hypoxic state for 12, 24 and 36 h. At the predetermined germination time, it was sampled to determine GABA content.

#### *Preparation of dried germinated paddy*

GP was dried by a conventional hot air drying at a temperature of 45°C and at a superficial air velocity of 1 m/s to inhibit germination. GP was dried to final moisture content of 16% (db). The sample was kept in cold storage at 4–6°C until further analysis.

#### Textural property evaluation

The textural property of cooked germinated rice in terms of hardness was measured by a texture analyzer (TA.XT Plus, Stable Micro Systems, Ltd. in Godalming, Surrey UK). 12 grains of cooked germinated rice samples were placed under the cylindrical probe with a size of 50mm diameter and then compressed at a pretest speed of 1 mm/s and posttest speed of 10mm/s. The hardness value was defined as a maximum compressive force at 85% strain of the force deformation curve (Srisang et al., 2011).

#### *Analysis of GABA content*

The GABA content of the dried GP prepared by S, S-H, S-ISD-H and S-C-H was determined by using HPLC, following the procedure of Lin and Wang (1980) with a minor modification.

#### *Total phenolic content determination*

Total phenolic content (TPC) of the dried GP prepared by S, S-H, S-ISD-H and S-C-H was analyzed using Folin–Ciocalteu reagent with modified method of Singleton and Rossi (1965).

## RESULTS AND DISCUSSION

#### *Percentages of germination and GABA Contents*

The percentages of germination of paddy prepared by all treatment methods, S, S-H, S-C-H and S-ISD-H, were increased to the maximum value of 95% at 48 h of the germination process as shown in Fig. 2. When the germination was extended from 48 to 60 h, the germination percentage did not change.

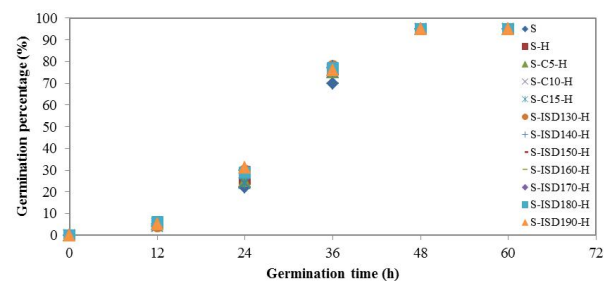


Fig. 2. Percentage of germination of paddy prepared by S, S-H, S-C-H and S-ISD-H method. Values are means  $\pm$  SD (n = 2).

The GABA content of paddy before germination in this study was about  $2.88 \pm 0.21$  mg/100 g DW. During the first 12 h of germination, the GABA contents were significantly increased compared with raw rice as shown in Fig 3. After 12 h, the GABA content was rapidly increased with increasing germination time for all treated samples. The GABA

content in the GP prepared by S-H was  $30.19 \pm 0.31$  mg/100 g DW at 48 germinated, which is 1.2 times higher than that of the GP prepared by S method. Hypoxia treatment could lead to the reduction in cytosolic acidosis (Bown and Shelp, 1997), which in turn stimulates GAD activity provides higher GABA accumulation. Therefore, combination of soaking and hypoxic state (S-H) could increase GABA production. However, the GABA content in the GP prepared by S-H was insignificant different from that in the GP prepared by S-C-H method. This result indicated that GABA accumulation was not influenced by cold stress treatment. This result was dissimilar to that reported by Mazzucotelli et al. (2006) who found that the cold stress condition could increase a significant in total amino acid content and induce the expression of the GABA shunt genes, leading to the higher production of GABA in wheat and barley. It might be stated that rice is a tropical plant which cannot survive under cold acclimation. Thomashow (1998) claimed that plants vary greatly in their ability to survive freezing temperatures. At one extreme are plants from tropical regions that have virtually no capacity to survive even the slightest freeze.

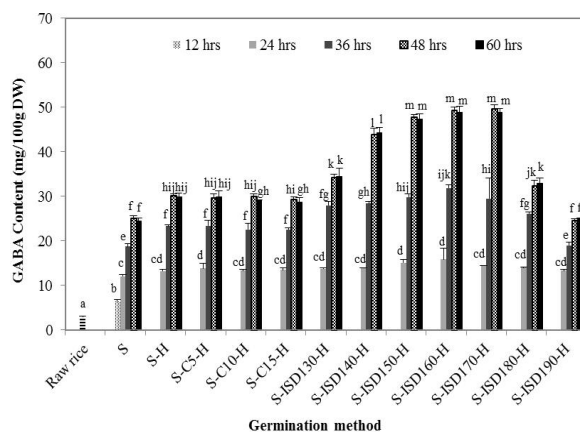


Fig. 3. GABA content in GP prepared by different methods. Data were expressed as the mean  $\pm$  SD (n = 3). Different corresponding letters indicate significant differences amongst different germination times at  $p < 0.05$  by Duncan's test.

When GP was thermally treated, the GABA content was significantly higher that prepared by S-H, S-C-H and S methods, except for those samples heated at 180-190°C. The highest GABA was found at the treatment temperatures, between 150-170°C, which was about 1.8 times higher than those of the GP prepared by S-H, S-C-H and S, respectively. The GABA contents in the GP prepared by S-ISD150-H and S-ISD160-H methods were insignificantly different from that in the GP prepared by S-ISD170-H method whereas the GABA contents in the GP prepared by S-ISD180-H and S-ISD190-H methods became lower. The difference in GABA content of

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 at temperature among 40°C and the corresponding highest GABA content was achieved. As measured from the experiments, the average grain temperatures at the ISD outlet were 34.5, 36, 38, 39.5, 40.5, 42 and 43°C at the heated temperatures of 130, 140, 150, 160, 170, 180 and 190°C, respectively, as presented in Fig 3. Therefore, the GP prepared by ISD at temperatures 150-170°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 50%. These data indicated that it is very important to control precisely the treatment temperature of the ISD.

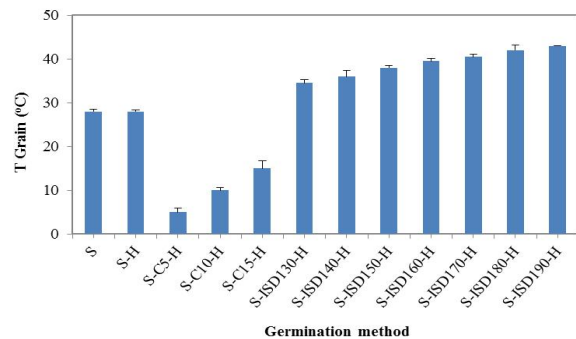


Fig. 4. Average grain temperature at the outlet of ISD (at a germination time of 48 h). Values are means  $\pm$  SD (n = 2)

#### Effect of stress condition on TPC

Figure 5 shows the total phenolic compounds prepared by different germination methods. The TPC content of brown rice before germination was about  $23.69 \pm 0.3$  mg GAE/100 g DW when it was germinated, the TPC content for all GP samples significantly increased with increasing germination time. This might be due to the fact that the activation phenylalanine ammonia lyase (PAL), which is a catalyst in the phenylpropanoid pathway being responsible for the synthesis of a variety of phenolic compounds, is induced during the germination (Keles and Oncel, 2002; Shao and Bao, 2015). In addition to the germination time, the germination method affected the TPC content significantly. The germination by the S method provided the lower TPC content than that the S-H method. This is because under hypoxia condition and reactive oxygen species e.g., superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals, all of which are highly reactive and toxic, were generated (Blokhina et al., 2003). Plant cells develop antioxidant defense mechanisms to protect against these toxic oxygen species (Keles and Oncel, 2002).

The protective mechanisms in response to the hypoxic stress include enzymatic system such as superoxide dismutase, catalase and ascorbate peroxidase and non-enzymatic system including low molecular weight antioxidants such as phenolic compounds, tocopherols, ascorbate and glutathione (Shen et al., 2015). Therefore, the TPC of GP prepared by S-H method was higher than that prepared by S method. When the cold and heat treatments was applied in between soaking and hypoxia treatment, however, the TPC content of GP were not different from those prepared by S-H.

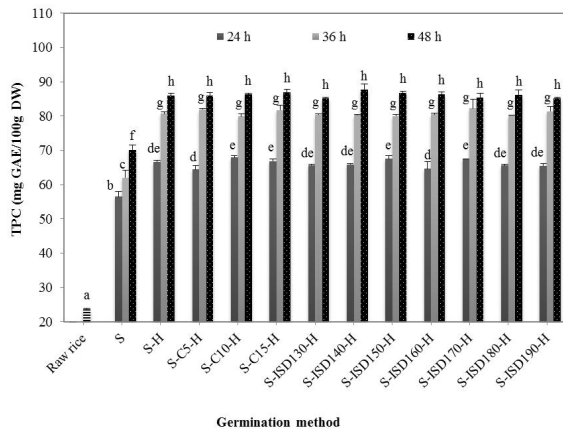


Fig. 5. TPC of GP prepared by S, S-H, S-C-H and S-ISD-H methods. Data are expressed as the mean  $\pm$  SD (n = 3). Different corresponding letters indicate significant differences amongst different germination times at  $p < 0.05$  by Duncan's test.

#### Textural Properties

Table 1. Hardness of cooked germinated rice produced by different germination methods.

| Sample       | Hardness (N)                     |                                  |                                 |
|--------------|----------------------------------|----------------------------------|---------------------------------|
|              | 24h                              | 36h                              | 48h                             |
| Raw Material |                                  | 159.68 $\pm$ 3.05 <sup>g</sup>   |                                 |
| S            | 148.67 $\pm$ 3.55 <sup>def</sup> | 125.43 $\pm$ 5.11 <sup>ab</sup>  | 120.40 $\pm$ 4.72 <sup>a</sup>  |
| S-H          | 152.70 $\pm$ 3.15 <sup>efg</sup> | 141.05 $\pm$ 4.90 <sup>cd</sup>  | 127.73 $\pm$ 4.91 <sup>b</sup>  |
| S-C5-H       | 154.47 $\pm$ 3.21 <sup>efg</sup> | 144.71 $\pm$ 5.48 <sup>def</sup> | 134.02 $\pm$ 6.70 <sup>b</sup>  |
| S-C10-H      | 155.10 $\pm$ 2.79 <sup>efg</sup> | 146.74 $\pm$ 4.41 <sup>def</sup> | 136.37 $\pm$ 5.33 <sup>b</sup>  |
| S-C15-H      | 156.11 $\pm$ 3.49 <sup>efg</sup> | 145.69 $\pm$ 3.81 <sup>de</sup>  | 135.97 $\pm$ 3.71 <sup>b</sup>  |
| S-ISD130-H   | 155.66 $\pm$ 6.23 <sup>efg</sup> | 148.66 $\pm$ 7.02 <sup>def</sup> | 130.22 $\pm$ 7.63 <sup>bc</sup> |
| S-ISD140-H   | 155.60 $\pm$ 3.01 <sup>efg</sup> | 147.97 $\pm$ 4.16 <sup>def</sup> | 133.43 $\pm$ 6.22 <sup>b</sup>  |
| S-ISD150-H   | 155.39 $\pm$ 5.82 <sup>efg</sup> | 145.97 $\pm$ 4.95 <sup>de</sup>  | 130.08 $\pm$ 5.42 <sup>bc</sup> |
| S-ISD160-H   | 154.54 $\pm$ 5.59 <sup>efg</sup> | 145.39 $\pm$ 2.88 <sup>def</sup> | 131.95 $\pm$ 5.48 <sup>bc</sup> |
| S-ISD170-H   | 154.20 $\pm$ 5.13 <sup>fg</sup>  | 148.33 $\pm$ 6.70 <sup>def</sup> | 135.27 $\pm$ 3.65 <sup>bc</sup> |
| S-ISD180-H   | 156.35 $\pm$ 6.27 <sup>efg</sup> | 147.41 $\pm$ 7.07 <sup>def</sup> | 133.71 $\pm$ 7.62 <sup>bc</sup> |
| S-ISD190-H   | 155.90 $\pm$ 6.61 <sup>efg</sup> | 146.24 $\pm$ 5.31 <sup>def</sup> | 134.33 $\pm$ 3.23 <sup>b</sup>  |

a,b,c,d,e,f,g Means with different superscripts in the same column are significantly different ( $p < 0.05$ ).

The results for hardness of cooked germinated rice produced by different germination methods are given in Table 1. It was decreased with increasing germination time for all treated samples. At 48 h of germination gave the lowest hardness of cooked germinated rice. This is due to the decomposition of high-molecular-weight polymers; that is, starch,

proteins, and nonstarch polysaccharides (Megat-Rusydi et al., 2011). In addition to the germination time, the germination method affected the hardness significantly. The lowest hardness was found in the cooked rice prepared by S method, which was 120.40  $\pm$  4.72 N at 48 h of germination. The hardness in the cooked rice prepared by S-H, S-C-H and S-ISD-H methods was higher than those prepared by S method. Moreover, the cooked rice prepared by S-H, S-C-H and S-ISD-H methods had insignificant difference of hardness. The lower hardness of cooked germinated rice by S method is due to the sample prepared by soaking method was immersed in water throughout the germination period whereas the fact that sample prepared by other treatment was immersed only 12 h. Thus might cause the starch solubilized into water, causing the weaker strength of kernel.

#### CONCLUSIONS

Germination time and germination method had significant effect on the contents of GABA, TPC and the textural property. The highest content of GABA and TPC were found 48 h of germination. The hardness of cooked rice was lowest when germination proceeded up to 48 h. Among the germination methods, the heat shock treatment, at 170°C, resulted in the highest GABA content (about 49.70 mg/100 g DW); a 17-fold increase compared to the un-germinated paddy, except for those samples heated at 180-190°C. However, the GABA content in the heated GP, between 150-160°C, was insignificantly different from that in the GP at 170°C. The GABA accumulation was not influenced by cold stress treatment. Among the stresses tested, heat and cold treatment was not influenced on TPC in GP, whereas hypoxic state treatment could improve the phenolic content. In this study, the hardness of the cooked rice had not affect by environmental stress. These findings can be used for the production of rice that can be uses as a healthy functional food. From this study, it recommended that the heat treatment method was suitable for enhancing GABA in rice during germination.

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