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STUDY ON APPROPRIATE CONDITIONS TO OBTAIN GERMINATED BROWN RICE WITH HIGH BIOLOGICAL ACTIVITIES

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Abstract. Germinated brown rice strains contain more bioactive substances than germinated regular rice ones, however germination conditions play an important role in the activity and the content of those substances. The proper germination process provides the optimized active ingredients from rice that can be used for the production of nutritious beverages. In this study, we investigated the effects of pH, temperature and incubation time in microaerobic culture condition on the change of bioactive substances in Anh Dao brown rice. The optimal germination conditions with pH 3, temperature of 35 ^{o}C and duration of 36 hours release 109.11 U/g of the α-amylase activity, 17.22 (U/g) of the enzyme glutamate decacboxylase (GAD), 1.38 (U/g) of protease, 231.76 (mg/100 g dried weight) of GABA content and 21.9 (mg GAE/100 g dried weight) of polyphenol from germinated Anh Dao brown rice. In nutrient evaluation, germinated Anh Dao brown rice contains 65.53 % of starch, 2.49 % of lipid, 9.13 % of protein, 2.04 % of reducing sugar, and 1.26 % of ash.

Keywords: germinated brown rice, bioactive substances, α-amylase, protease, glutamate decacboxylase (GAD), gamma aminobutyric acid (GABA).

Classification numbers: 1.2.1., 1.3.2.

1. INTRODUCTION

Many studies have shown that germination improved the nutritional quality of the rice. The germination process of rice grain changed the nutritional component and structure of the grain [1]. During germination of brown rice, the intracellular enzymes work and cleave large molecular substances such as proteins, starches, etc. into small molecular substances. Starch in germinated grains is hydrolyzed by the amylase into simple sugars, which enhances the digestion process. Germination leads to the important nutritious changes such as the higher levels of GABA (Gamma Aminobutyric Acid), other amino acids, unsaturated fats (tocotrienols and Gamma oryzanol) [2].

The components of bioactive substances in germinated brown rice are influenced by several factors such as germination time, temperature, pH, and nutrient contents [3]. The condition of germination determines the quality of the bioactive substances in the germinated rice. There are several studies about the germinated brown rice, however they mainly focus on the nutrient contents but not on the bioactive substances or the hydrolysis products. Therefore, our study aims to determine the factors that affect the enzyme activity and the content of bioactive substances in brown rice germination.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

The selected rice strain for the experiment is Anh Dao glutinous strain purchased at Thai Binh Grain Corporation. Rice straw is removed by rice husk with Satake (Japanese) milling machine. Brown rice obtained after grinding (average size 0.3 - 0.4 mm) was stored in plastic bag at a temperature of 3–5 ^{o}C in the refrigerator for maximum 7 days.

Chemicals used: DNS (dinitrosalicylic acid), HCl, Na_2HPO_4, KH_2PO_4, $NaCH_3COO$, $Na_3C_6H_5O_7$ (sodium citrate), I_2, KI from Merck company, Germany, starch and other chemicals of Chinese companies.

2.2. Methods

2.2.1. Experimental method

Experiment 1: Study the effect of pH on the protease, GAD, Polyphenol and GABA contents in the germination process of Anh Dao brown rice.

50 g of Anh Dao brown rice was soaked in citrate buffer at different pH levels (pH 2, 3, 4, 5, 6) at 30 °C for 6 hours with the ratio of 1:2 (w/v) rice/water. Then the rice was harvested and incubated at 30 °C for 24 hours for germination process. The obtained germinated rice was ground finely and collected for the determination of the protease, GAD, Polyphenol and GABA contents. The contents were determined by the percentage of dry weight.

Experiment 2: Study the effect of temperature on the protease, GAD and Polyphenol and GABA contents in the germination process of Anh Dao brown rice.

The rice was carried out with the selected pH condition in the experiment 1 and then incubated at different temperatures: 25, 30, 35, 37, 40 °C for 24 hours. The obtained germinated rice was ground finely and collected for the determination of the protease, GAD, Polyphenol and GABA contents.

Experiment 3: Study the effect of time on the protease, GAD and Polyphenol and GABA contents in thegermination process of Anh Dao brown rice.

The rice was carried out with the selected pH condition in the experiment 1 and the selected incubation temperature in the experiment 2. Then the rice was incubated at different times: 20, 24, 28, 32, 36, 40, 44 and 48 hours. The obtained germinated rice was ground finely and collected for the determination of the protease, GAD, Polyphenol and GABA contents.

Experiment 4: Evaluating the composition of raw Anh Dao rice after germination

50 g of Anh Dao brown rice was carried out with the selected conditions in the experiment 1, 2 and 3. The obtained germinated rice was ground finely and collected for the determination of the content of nutrients and bioactive substances in rice.

2.2.2. Method for determination of enzyme activity α– amylase by Rukhliadeva – Geriacheva [4]

2.2.3. Determination of protease enzyme activity by modified Anson method [4]

2.2.4. Determination of GAD enzyme activity by method of Zhang *et al*. [5]

2.2.5. Determination of GABA by spectrophotometer (by Karladee and Suriyong [6])

The GABA was extracted from the sample with 80 % ethanol, pH adjusted with 0.2 M borate buffer. GABA then reacts with 6 % phenol reagents and 10 % sodium hypochlorite for color measurement. The reaction solution was measured of optical density by UV/Vis device at 630 nm wavelength. The unit of GABA is mg/100 g dry weight.

2.2.6. Determination of polyphenol by colorimetric method using Folin – Ciocalteu reagent [4]

2.2.7. Other methods

- Determination of ash content was performed according to previous study [4].
- The moisture was analyzed based on dried samples to constant weight [4].
- Determination of the total protein content was performed following Kjeldahl method [4]
- The total lipid content was determined by using Soxhlet method [4].

2.2.8. Data processing methods

Each experiment was carried out 3 times. The results were processed by Microsoft Excel 2010 software with an error less than 0.05.

3. RESULTS AND DISCUSSION

3.1. Effect of pH on the protease, GAD and Polyphenol and GABA contents during the germination of Anh Dao brown rice

The samples were prepared and the experiments were carried out according to Section 2.2.1.

3.1.1. Effect of pH on the protease activity

Figure 1 shows that protease activity is highest at pH 2 with a value of 1.21 U/g, the next value was at pH 3 with 1.05 U/g and the lowest at pH 6 with a value of 0.55 U/g. Protease activity increased by 1.47 - 3.27 times compared to brown rice without germination. This result is similar to the results of Reihaneh Ahmadzadeh Ghavidel, (2011) [7], according to this research group, the protease enzyme activity reaches the optimal activity at pH 2.5 - 5.5 in

acetate buffer environment. However, they also mentioned that the protease activity depended on the quality of rice grain and the size of the grain.

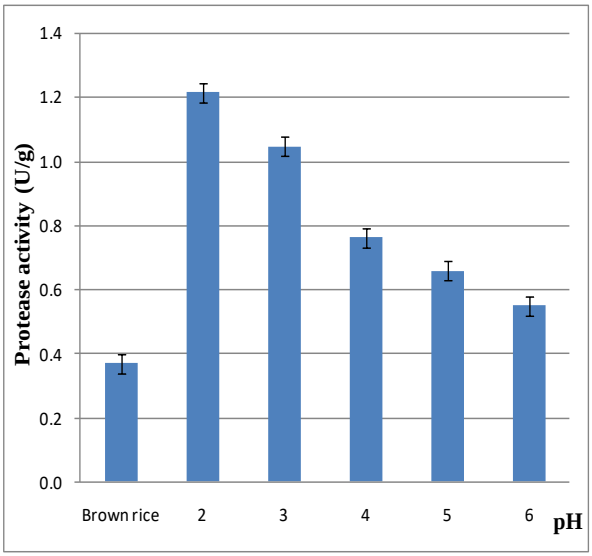

Figure 1. Effect of pH on protease activity.

3.1.2. Effect of pH on GAD activity and GABA content in germinated brown rice

GAD activities in germinated brown rice under different pH conditions are shown in Figure2. It has been shown that pH level had a strong influence on GAD activity of germinated rice. GAD activity of brown rice was 3.32 U/g, after germination the highest GAD activity reached at 10.64 U/g at pH 3, increasing 3.2 times. At pH6, the lowest GAD activity was 4.33 U/g. The research of Le Nguyen Doan Duy and Nguyen Cong Ha (2014) also showed that pH had the effect on GAD activity in rice [8]. GAD is activated during pH treatment, converting glutamic acid into a beneficial function as GABA for the body.

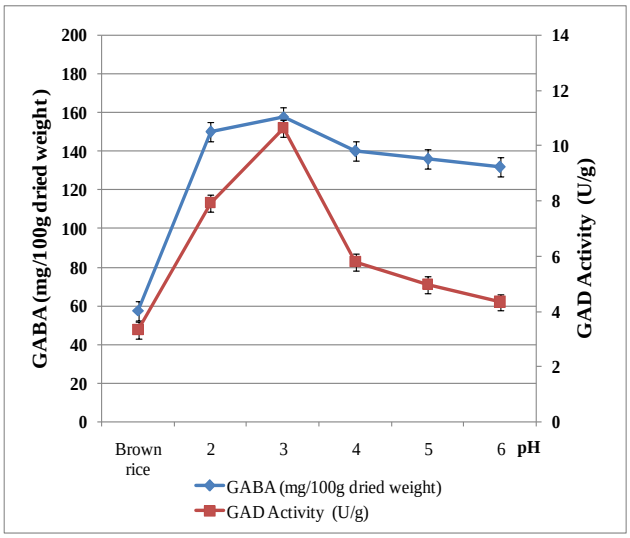

Figure 2. Effect of pH on GAD activity and GABA content.

GABA is synthesized during decarboxylase process accompanied by H^+ ion consumption (Karladeea, D., Suriyong (2012)) [9]. Therefore, the decreased pH enhances GABA production. In addition, the previous studies also showed the pH levels from 2 to 6 were suitable for GAD activity resulting to the high GABA content in brown rice. In addition to the pH values, the type of buffer may also affect the GABA content during the germination [10, 11].

The results of the study in Figure 2 show that GABA content is highest (157.72 mg/100 g dried weight) when brown rice soaked at pH 3, corresponding to 2.75 times higher than that in untreated brown rice. The activity decreases gradually in the pH 4, 5 and lowest at pH 6 with GABA content of 131.74 mg/100 g dried weight. This result correlates to the other previous studies. For example, Charoenthaikij *et al*. [12] showed that the highest GABA content (67 mg/100 g dried weight) in KhaoDawk Mali 105 was obtained with citrate buffer at pH 3 for 48 hours. The study of Banchuen (2010) [10] showed that the highest GABA content at 8.36 mg/100 g dried weight of the rice strain Sangyod Phatthalung and Chiang Phatthalung with citrate buffer at pH 3 for 36 hours. Similarly, the GABA content in the study of 6 rice strains of (Koornneef *et al.*) [13] reached the highest 30.69 mg/100 g rice after 24-hour incubation with citrate buffer at pH 3. Taken all together, our results show the correlation between GAD activity and GABA content as in theory and in other previous studies [14].

3.1.3. Effect of pH on the polyphenol content in germinated brown rice

Figure.3 shows that polyphenol content of untreated brown rice is 10.59 mg GAE/100 g dried weight. After germination process, polyphenol content reaches the highest amount at pH2 (21.23 mg GAE/100 g dried weight), as twice higher compared to that of untreated brown rice. At pH 3 the polyphenol amount is 20.07 mg GAE/100 g dried weight, at pH 6 the lowest polyphenol content is 13.71 mg GAE /100 g dried weight.

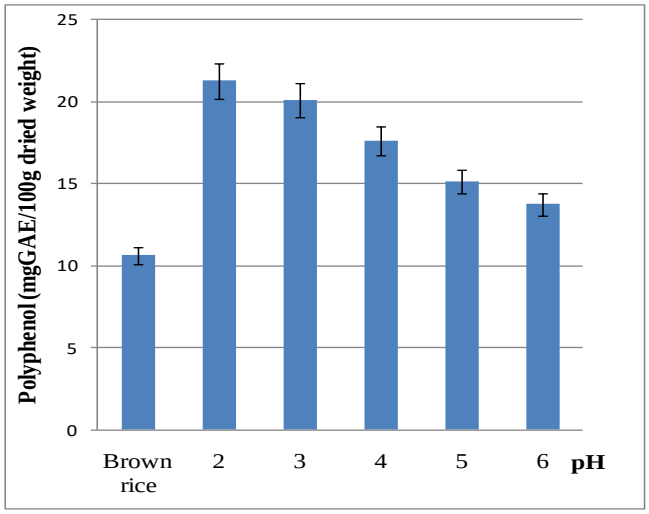

Figure 3. Effect of pH on polyphenol content.

Taken together, this study has found that germinated Anh Dao brown rice contained the highest amount of GAD and GABA contents at pH 3 treatment and the highest protease activity and polyphenol content at pH 2 treatment. Our purpose is to prepare germinated brown rice for the production of nutrient beverages, therefore we prefer the higher value of GAD and GABA contents. We selected pH at 3 for further experiments.

3.2. Effect of incubation temperature on the protease, GAD and Polyphenol and GABA contents during the germination of Anh Dao brown rice

The samples were prepared and the experiments were carried out according to Section 2.2.1.

3.2.1. Effect of temperature on the protease activity

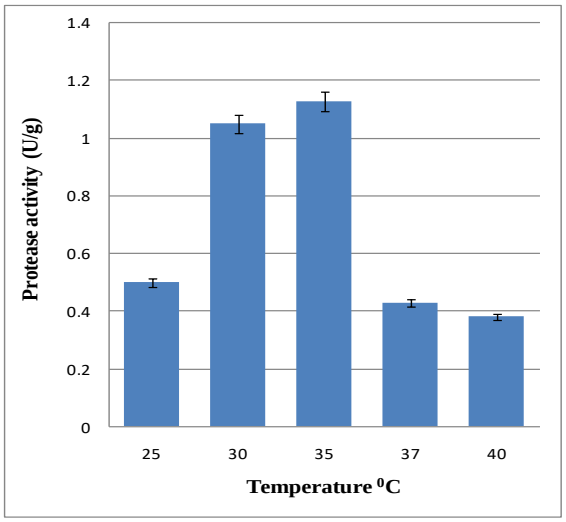

Figure 4. Effect of incubation temperature on protease activity.

The results in Figure 4 show that the germination temperature affects the protease enzyme activity in brown rice. The highest protease activity is 1.13 U/g dried weight at incubation temperature of 35 °C, increasing 3.05 times compared to that of untreated rice (0.37 U/g). There are not many studies on the protease activity in brown rice during the germination, however, the results of Quan Le Ha [15], on the study of Moc Tuyen rice germination also showed that the protease activity increased by 2.5 times after 8 days of germination.

3.2.2. Effect of incubation temperature on GAD activity and GABA content

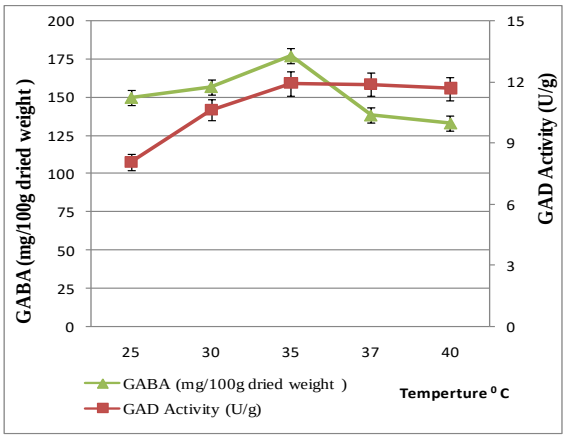

Figure 5. Effect of temperature on GAD enzyme activity and GABA content.

In Figure 5, GAD activity and GABA content increase during the germination process. In details, GAD activity in germinated brown rice is highest as 11.93 U/g at 35 °C that equivalent to 3.59 times higher than that of untreated one (3.32 U/g). When the incubation temperature increases to 40 °C, GAD activity does not change significantly.

The GABA content in germinated brown rice reaches the highest as 177.12 mg/100 g dried weight at 35 °C incubation, corresponding to 3.1 times higher than that of untreated one (57.33 mg/100 g dried weight) and the lowest GABA content is only 133.2 mg /100 g dried weight at 40 °C incubation. The highest obtained GABA is consistent with the highest GAD activity at 35 °C. However, at 40 °C GAD enzyme activity did not change much but the GABA content was significantly low, which may be due to the inhibition of high temperature on the conversion of glutamate to GABA content or activation of glutamate into other substances.

3.2.3. Effect of incubation temperature on the polyphenol content

The polyphenol contents in germinated brown rice under different temperatures are shown in Figure 6. It could be seen that temperature affects polyphenol content in germination process; however, the alteration is only in the range of 1.6 - 1.8 times. Polyphenol content reaches the highest level of 19.05 mg GAE/100 g dried weight when incubated at 35 °C, and then gradually decreased without significance. The incubation temperature range from 25 to 40 °C does not have significant effect on the polyphenol content.

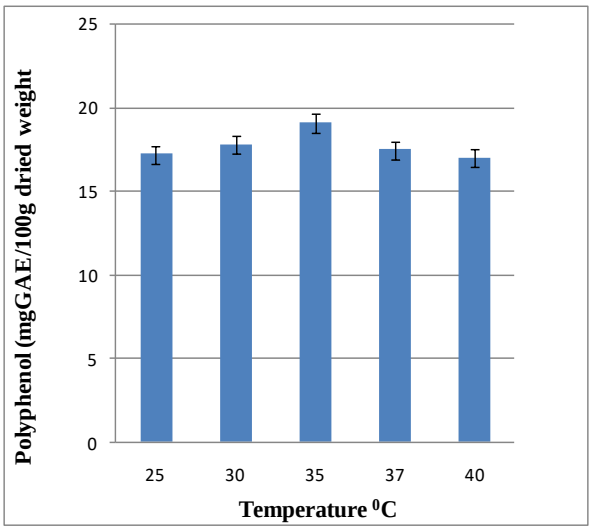

Figure 6. Effect of temperature on Polyphenol content.

Taken together, our result has been shown that the germination of brown rice at 35 °C leads to the highest Protease, GAD and GABA, Polyphenol contents. Therefore, the temperature of 35 °C was selected for further experiments.

3.3. Effect of incubation time on the protease, GAD and Polyphenol and GABA contents during the germination of Anh Dao brown rice

The samples were prepared and the experiments were carried out according to Section 2.2.1.

3.3.1. Effect of incubation time on the protease activity

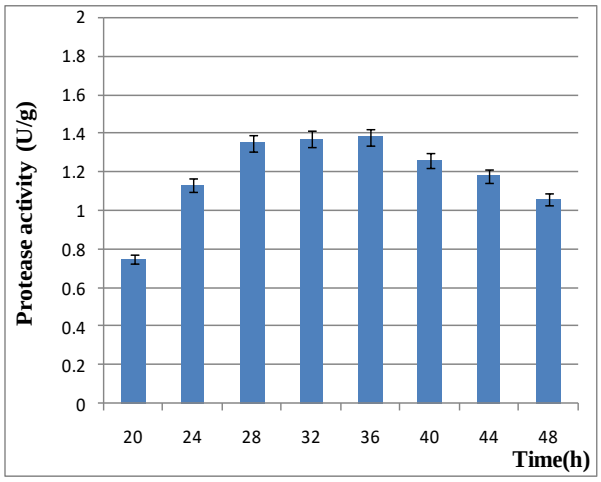

Figure 7. Effect of time on protease activity.

Protease activity increases during germination (Figure7). After 36 hours, the highest protease activity is obtained at 1.38 U/g, and with further increased time, protease activity reduces gradually.

3.3.2. Effect of incubation time on GAD activity and GABA.

In the brown rice grain, GAD activity is very low because of the low moisture in the grain. GAD activity changes during the germination process because of the water supplement that activates the GAD enzyme to synthesize GABA from glutamic acid (Dong-Hwa Cho, Seung-Taik Lim [3]).

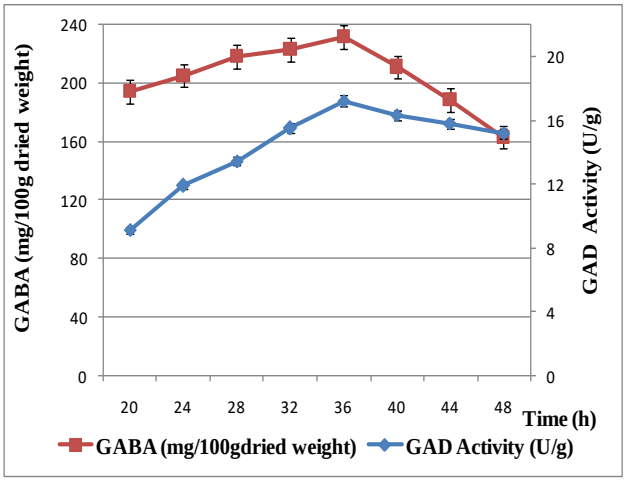

Figure 8. Effect of time on GAD enzyme activity and GABA content.

Research results in Figure 8 show that incubation time has a great influence on GAD activity. GAD activity increased highly during germination time with the highest activity as

17.22 U/g after 36 hours of incubation, nearly 5 times higher than that in untreated rice. However, when the incubation time increases to 48 hours, GAD activity decreases slightly. In addition, GABA content also increases during incubation time from 20 hours to 36 hours and reaches the highest content after 36 hours of incubation (231.76 mg/100 g dried weight). With increasing further incubation time to 48 hours, GABA content decreases into 143.21 mg/100 g dried weight. The GABA content correlates to GAD activity during the germination time.

In summary, incubation time has an influence on GABA, and GAD enzyme activity in germinated brown rice. After 36 hours of incubation, germinated brown rice has the highest GABA content of 231.76 mg/100 g dried weight and the highest GAD activity of 17.22 U/g.

3.3.3. Effect of incubation time on the polyphenol content

The results in Figure 9 show that incubation time affects polyphenol content during the germination process. The polyphenol content reaches the highest value of 21.9 mg GAE/100 g dried weight at 36 hours, afterward decreases gradually with the extension of incubation time.

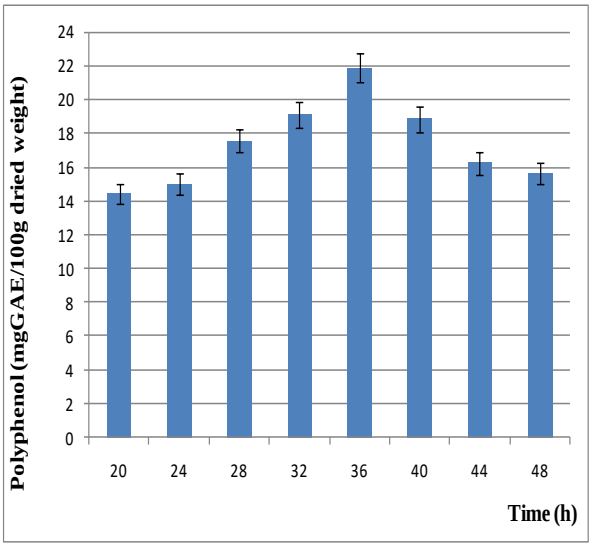

Figure 9. Effect of time on Polyphenol content.

Taken together, 36 hours incubation time is selected for germination process.

3.4. Evaluation of the composition of Anh Dao brown rice before and after germination

From Table 1, the amount of starch in germinated brown rice is lower than that of untreated brown rice (from 86.11 % to 65.53 %, equivalent to 23.9 % reduction). Lipid content decreases during the germination process, however, the reduced amount was not significantly (from 2.72 % to 2.49 %, equivalent to 8.5 % reduction). This result is consistent with other studies of different grains such as soybean [16], corn [17] and spinach [18].

The reduction of nutrients such as starch and lipid is due to their conversion into energy for germination process. Beside the reduction of starch and lipid, germination process provides higher levels of protein, reduced sugar, α-amylase, GAD, GABA and polyphenol contents in brown rice.

Table 1. Evaluation of nutritional composition in brown rice and germinated brown rice.

Nutrients	Untreated Brown rice	Geminated Brown rice
Starch (% total dry weight)	86.11 ± 0.46	65.53 ± 0.53
Lipid (% total dry weight)	2.72 ± 0.15	2.49 ± 0.35
Protein (% total dry weight)	8.57 ± 0.21	9.13 ± 0.23
Reduced sugar (% total dry weight)	0.34 ± 0.02	2.04 ± 0.15
Ash (% total dry weight)	1.53 ± 0.07	1.26 ± 0.27
α–amylase (U/g)	34.91 ± 0.43	109.11 ± 0.7
Protease (U/g)	0.37 ± 0.03	1.28 ± 0.05
GAD (U/g)	3.32 ± 0.26	17.22 ± 0.57
GABA (mg/100g dry weight)	57.33 ± 0.56	231.76 ± 0.7
Polyphenol (mg GAE/100g dry weight)	10.59 ± 0.6	21.9 ± 0.8

4. CONCLUSION

This study investigated the effect of conditions of germination process on bioactive substances in Anh Dao brown rice. The results indicate that the right conditions for obtaining the highest bioactive substances of Anh Dao brown rice are pH 3, temperature at 35 ^{o}C and time of 36 hours. At that condition, geminated Anh Dao brown rice has GABA content of 231.76 (mg/100 g), α-amylase enzyme activity of 109.11 (U/g), GAD activity of 17.22 (U/g), and protease of 1.28 (U/g), polyphenol of 21,9 (mg GAE/100 g). After germination, nutrient contents of Anh Dao brown rice contain 65.53 % of starch, 2.49 % of lipid, 9.13 % of protein, 2.04 % of reducing sugar, and 1.26 % of ash. Based on this study, germinated Anh Dao brown rice is promising to be a suitable material for production of nutritious drinks.

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