Effects of Acid Glutamic and Lighting on GABA Content in Germinated Brown Rice

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ABSTRACT

In this research, to survey some conditions which influence germination process of brown rice 4900. After surveying the condition and the additional nutrient content which influence the germination activity and GABA (Gamma–Aminobutyric Acid) content in germinated brown rice, we have determined the optimal parameters: the rate of water and rice (2:1), germination temperature is 36°C, the content of glutamic acid is added to the 200 ppm soaking process with a soaking time of 24h and a temperature of 35°C using a white light illuminating and combining a continuous moist spray with germination time of 18 h. After germination, the germinated brown rice should be dried at 55°C with a moisture content of less than 14% (w/w). When germinating brown rice under the above conditions, the research results showed that the content of GABA was 12.48 mg/100 g of dry matter, higher than Thailand’s commercially germinated rice product. The results of this study can be considered to improve the process of the germination of brown rice production.

Keywords: GABA (γ-aminobutyric acid), Germination, Brown Rice, Acid Glutamic

Introduction

Rice is one of the world’s five food crops, along with maize (Zea Mays L.), wheat (Triticum sp.), cassava (Manihot exculenta Crantz) and potatoe (Solanum tuberosum L.). Germinated Brown rice provides essential amino acids, glucid and B vitamins which are increasingly concerned. They have the ability to synthesize many essential biochemical compounds, including amino acids and GABA by the germination process. Japan has analyzed researched on germinated rice, soaked brown rice in warm water at 40°C from 8–24 h (Saikura, 1994). Not only the nutrient compounds initially increases in content but they aslo produces many new substances during germination such as GABA, lysin, vitamin E, fiber, niacin, magnesium, vitamin B1 and B6 (Kayahara et al., 2001). Choi et al., 2006, studied and concluded that after 24 h of germination, the content of nutrients will vary compared to pre-germinated brown rice such as:

- The amount of fructose increased by 3.4 times
- The amount of GABA increased 7.97 times

In this study we determined glutamic acid supplementation during soaking and the effect of the direct spraying process on germination of milling brown rice.

Materials and Methods

Brown Rice Samples

- OM4900 rice variety is supplied by Mekong Delta Rice.
- Commercial GA (collected from Lychnis viscaria extracted plant, the trade name is Comcat 150WP - Germany)
- Incubators INB 400 - Firm Memmert – Germany.
Germination process

Rice was milled with rice husks soaked for 6 hours at a ratio of rice:water (2:1) with added glutamic acid and continuously sprayed during germination to reach 95% moisture. Brown rice is sprouted for 18 h in the incubator INB 400 with 3 LEDs. After germination process, germinated brown rice was dried at 55°C until the moisture was below 14% (w/w). The analytical indicators are the content of GABA, reducing sugars and amino acids.

Determination of Moisture Content

The content of moisture in dried soaked and GBR was determined by keeping the samples at 105ºC to a constant weight according to AOAC 925.09 (AOAC, 2000).

Determination of γ-aminobutyric acid (GABA)

γ-Aminobutyric acid (GABA) content was determined by HPLC as described previously (Cáceres et al., 2014b). Briefly, 50 µL aliquot of concentrated water-soluble extract and 10µL allyl-L-glycine solution (Sigma-Aldrich) used as internal standard were derivatized with 30 µL phenyl isothiocyanate (PITC 99%, Sigma-Aldrich) and dissolved in mobile phase A for GABA analysis. An Alliance Separation Module 2695 (Waters, Milford, USA), a photodiode array detector 2996 (Waters) setted at 242 nm wavelenth and an Empower II chromatographic software (Waters) were used as chromatographic system. A volume of 20 µL of sample were injected onto a C18 Alltima 250 x 4.6 mm i.d., 5 µm size (Alltech, Spain) column thermostatted at 30ºC. The chromatogram was developed at a flow rate of 1.0 ml/min by eluting the sample with mobile phase A (0.1 M ammonium acetate, pH 6.5) and mobile phase B (0.1 M ammonium acetate, acetonitrile, methanol, 44/46/10, v/v/v, pH 6.5). Replicates samples were independently analyzed and results were expressed as mg GABA/100 g d.w.

Statistical Analysis

Each germination experiment and subsequent drying process were conducted in triplicate. Two extractions were performed for each replicate and the analytical determinations were carried out in triplicate. Data were expressed as mean ± standard deviation. The data obtained from each experimental condition were subjected to one-way Analysis of Variance (ANOVA) using Duncan test to determine the significant differences at P ≤ 0.05 level using Minitab 16 for Windows. This programme was also used for correlation analysis.

Result and Discussions

Surveying of Initial Rice Seed Material

Percentage of Rice Recovered After Milling

After the process of separating rice husk with Kett HP-200 equipment, the percentage of rice is presented in Table 1:

| Rice | Husk | Broken rice has sprout | Broken rice has not sprout |
|------|------|------------------------|---------------------------|
| 62.71±0.47 | 21.46±0.33 | 8.21±0.31 | 7.59±0.11 |

The results showed that the percentage of rice has used the Kett HP-200 which was approximately 63% (w/w), compared to the actual gain of about 67% (w/w) (Floukes, 1998)

Chemical composition of Brown Rice

| Chemical composition of Brown rice | (Content) |
|----------------------------------|-----------|
| Moisture | 10.2 ± 0.2 % (w/w) |
| Amino acid total | 45.95 ± 2.35 (mg/100g) |
| GABA | 2.19 ± 0.26 (mg/100g) |
| reducing sugar | 0.278 ± 0.023 % (w/w) |
| Ash | 1.68 ± 0.05 % (w/w) |

The Rate of Germination Removed Husk

Table 3 shows the germination ratio of white brown rice (69% (w/w)) after being separated by rice husk separator Kett HP - 200. Thus, the amount of embryos loss is about 20% (w/w) compared to raw rice.

The effect of acid glutamic on GABA content germinated Brown Rice

The results of table show that the germination was 24 h. The highest GABA content at sample which added 200 ppm acid glutamic to the immersion solution compared with other sample, including 8,48 mg/ 100g raw material. The GABA content did not added acid glutamic 1,59 times and higher than raw material is 8,37 times. This can be explained: during hygroscopic process, the amount of acid glutamic in the supplement will be diffused into sprout; acid Glutamic is the substrate source for GABA biosynthesis. When substrate is increased, the amount of GABA will be generated. This is can be explained with Roohinejad (2009): in rice samples with a high glutamic acid content, a higher amount of GABA is obtained compare with other sample.

When added acid glutamic content at different concentrate show that the effect of different acid glutamic content on created GABA content.
At the highest obtained GABA content is that the effect of acid glutamic on GABA content is clearly. Not much research investigates to survey the effect of acid glutamic on GABA content. Due to a high glutamic acid content, will inhibited with GAD enzyme, reduced GABA concentration during germination process. With OM 4900 rice, when glutamic acid concentration is higher (200 mg/kg), the influence of glutamic acid on GABA concentration in germination process is decreased. The variation of reducing sugar in germination process when it is soaked by added glutamic acid solution is shown below in Table 5. Results show that the reducing sugar content increases during 24 h of germination. Before 24 h germination, there was no difference among the samples. The effect of glutamic acid on reducing sugar content is not clear.

**Table 4. The effect of glutamic acid concentration on GABA content germinated Brown Rice**

| Germination time (hour) | Acid glutamic concentration (ppm) |
|------------------------|----------------------------------|
|                        | 0                      | 100   | 200   | 300   | 400   |
| 0                      | 2.44 ± 0.14            | 2.88 ± 0.41 | 3.04 ± 0.16 | 2.96 ± 0.19 | 2.81 ± 0.31 |
| 6                      | 3.13 ± 0.30            | 3.51 ± 0.42 | 3.86 ± 0.16 | 3.73 ± 0.23 | 3.65 ± 0.19 |
| 12                     | 3.42 ± 0.18            | 4.24 ± 0.54 | 4.54 ± 0.19 | 4.37 ± 0.43 | 3.96 ± 0.37 |
| 18                     | 4.16 ± 0.14            | 5.24 ± 0.44 | 6.35 ± 0.38 | 6.10 ± 0.43 | 5.46 ± 0.18 |
| 24                     | 5.24 ± 0.42            | 7.14 ± 0.16 | 8.48 ± 0.24 | 7.75 ± 0.49 | 6.72 ± 0.45 |
| 36                     | 3.61 ± 0.52            | 6.10 ± 0.34 | 7.01 ± 0.55 | 6.33 ± 0.35 | 6.04 ± 0.35 |
| 42                     | 2.83 ± 0.27            | 5.40 ± 0.24 | 5.67 ± 0.40 | 5.96 ± 0.25 | 5.89 ± 0.56 |

**Table 5. The effect on glutamic acid concentration on reducing sugar content (%w/w) during germination process**

| Germination time (hour) | Glutamic acid content (mg/kg) |
|------------------------|------------------------------|
|                        | 0                      | 100     | 200     | 300     | 400     |
| 0                      | 0.174 ± 0.005           | 0.164 ± 0.002 | 0.188 ± 0.004 | 0.169 ± 0.004 | 0.163 ± 0.001 |
| 6                      | 0.217 ± 0.006           | 0.233 ± 0.012 | 0.201 ± 0.005 | 0.271 ± 0.018 | 0.340 ± 0.029 |
| 12                     | 0.260 ± 0.009           | 0.311 ± 0.027 | 0.463 ± 0.035 | 0.531 ± 0.025 | 0.402 ± 0.036 |
| 24                     | 1.547 ± 0.068           | 1.446 ± 0.092 | 2.234 ± 0.128 | 2.007 ± 0.162 | 2.091 ± 0.101 |
| 30                     | 1.795 ± 0.134           | 1.705 ± 0.071 | 2.837 ± 0.125 | 2.836 ± 0.045 | 2.938 ± 0.049 |
| 36                     | 2.177 ± 0.016           | 2.233 ± 0.027 | 3.659 ± 0.084 | 3.720 ± 0.059 | 4.138 ± 0.205 |
| 42                     | 2.728 ± 0.043           | 3.029 ± 0.060 | 4.845 ± 0.022 | 5.541 ± 0.053 | 4.514 ± 0.044 |

**Table 6. The effect of glutamic acid content on amino acid content (mg/kg) during germination process**

| Germination time (hour) | Glutamic acid content (ppm) |
|------------------------|------------------------------|
|                        | 0                      | 100     | 200     | 300     | 400     |
| 0                      | 39.00 ±1.88             | 32.28±1.53 | 34.26±1.05 | 37.74±1.19 | 31.94±1.53 |
| 6                      | 37.99 ±1.32             | 32.23±1.45 | 38.88±5.57 | 34.25±5.52 | 30.92±3.79 |
| 12                     | 79.68±1.96              | 63.78±11.12 | 80.96±1.61 | 90.39±2.01 | 79.06±2.51 |
| 24                     | 195.51±10.23            | 208.77±8.77 | 226.74±17.11 | 203.35±12.95 | 187.91±9.91 |
| 30                     | 152.23±14.58            | 165.41±13.25 | 204.11±12.89 | 180.34±14.97 | 184.30±9.76 |
| 36                     | 125.49±14.61            | 152.65±13.50 | 141.73±12.25 | 150.98±14.29 | 156.34±12.58 |
Results show that the highest amino acid content was obtained after 24 h of germination process in all experimental samples. The maximum amount of amino acid necessary for germination is stored in rice.

At 200 ppm glutamic acid concentrate, the highest amino acid content were obtained, accounting for 226.74 mg/100 g on dry matter, followed by at 100 ppm and 300 ppm respectively glutamic acid were obtained glutamic acid content, including 208.77 and 203.35 mg/100 g on dry matter respectively; even lower than at compared sample at 195.51 mg/100 g on dry matter basis.

During germination process, in addition to synthetic GABA, Brown Rice was also synthesized with different amino acids at different levels (Moongngarm and Saetung, 2010).

### The Effect of Light on GABA Content During Germination Process

First sample: Using the immersion condition such as: soaked in the appropriate glutamic acid during 24 h without light, Brown Rice was germinated.

Second sample: Using the immersion condition such as: the sample was same but germination was done in the presence of light.

#### Table 7. The effect of light on GABA content (mg/kg)

| Time (hour) | Frist sample | Second sample |
|------------|--------------|---------------|
| 0          | 3.08±0.1 a   | 3.18±0.11 a   |
| 6          | 3.85±0.17 a  | 4.86±0.28 ab  |
| 12         | 4.71±0.21 b  | 6.23±0.37 bc  |
| 18         | 6.95±0.24 d  | 12.48±0.87 a  |
| 24         | 12.45±0.53 e | 12.30±0.1 a   |
| 36         | 11.38±0.42 f | 9.74±0.78 d   |
| 42         | 8.42±0.35 g  | 7.80±0.64 cd  |
| 48         | 6.08±0.24 c  | 7.41±0.35 cd  |

The results from Table 7 and table 9 show that light have effect on the germination process of OM4900 Rice. The highest GABA content was obtained at 18 h with 12.48 mg/100 g on dry matter earlier than first sample which was obtained at 24 h with 12.45 mg/100 g on dry matter GABA content on raw material.

There were currently no report mentioned that the effect of light on GABA content in germination process.

When Rice germinated under the condition white light which impacted on GAD enzyme, was increased GABA content in endosperm (Yoshimuara and Tagawa, 2010); and then obtained the highest GABA content; decreased GABA content after 24 h germination process.

The amount of amino acids in the first stage before 12 h in Sample 2 is higher than that in Sample 1. The rice under the effect of light was stimulated to synthesize more amino acids than in germination stage. Followed by the observation, after 24 h germination under light results in primary sprouts in Brown rice.

Then, amino acid content were used for and decreaed. In germination sample under light condition aslo light increased, due to stimulated amino acid in the growth. And then after 42 h germination, Brown rice sample synthesized eariler, it was used for reducing sugar to create energy more than reducing sugar stimulated in the second sample.

### Conclusion

Glutamic acid content added in soaking process was 200 ppm with 24 h soaking time and 35°C soaking temperature. When germinated Brown Rice in the condition above, this paper show that GABA content is 12.48 mg/100 g on dry matter higher than Tunggula Thai Lan’s commercial germination of GABA Rice is 6.0035 mg/100 g on dry matter.

This paper investigates how the germinated GABA rice processing contributes to enhance the value of rice product, and meets the diverse needs of the current market.
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