INFLUENCE OF SOAKING AND GERMINATION CONDITIONS ON THE γ-AMINOBUTYRIC ACID (GABA) CONTENT OF 2 RICE VARIETIES (IR 50404 AND JASMINE 85) FROM MEKONG DELTA

Lê Nguyễn Đoan Duy*, Nguyễn Công Hà

College of Agriculture and Applied Biology, Can Tho university

Email: lndduy@ctu.edu.vn*

Received date: 21.10.2013 Accepted date: 22.01.2014

ABSTRACT

Effects of soaking conditions (time, temperature and pH of the soaking solution) and germination conditions (aerobic and anaerobic) on the γ-aminobutyric acid (GABA) content of IR 50404 and Jasmine 85 rice varieties were investigated. The rice was kept in water at room temperature (30± 2°C) and moisture content monitored until it reached the optimum absorption. The moisture content saturation attained after 6 h was 27.1 % and 32.7 % for IR 50404 and Jasmine 85, respectively. The pH values of soaking solution of pH 3 for IR 50404 and pH 2 for Jasmine 85 were optimal for production of GABA (70.37mg/100g for IR 50404 and 78.50 for Jasmine 85). Germination under anaerobic condition (10% CO_2 concentration) yielded the highest GABA content after 30 hours.

Keywords: γ-aminobutyric acid (GABA), germination condition, IR 50404, Jasmine 85, soaking condition.

Ảnh hưởng của điều kiện ngâm và ủ đến hàm lượng γ-aminobutyric acid (GABA) của 2 giống lúa (IR 50404 và Jasmine 85) trồng ở Đồng bằng sông Cửu Long

TÓM TẮT

Nghiên cứu tập trung đánh giá ảnh hưởng của điều kiện ngâm (nhiệt độ, pH của dung dịch ngâm cũng như thời gian ngâm) và điều kiện ủ nẩy mầm (hiếu khí và yếm khí) lên hàm lượng axit γ-aminobutyric (GABA) của gạo mầm sản xuất từ 2 giống lúa IR 50404 và Jasmine 85 ở Đồng bằng sông Cửu Long. Gạo sau khi tách vỏ được ngâm trong nước ở nhiệt độ phòng (30± 2°C) và theo dõi hàm ẩm cho đến khi gạo đạt được độ ẩm bão hòa. Sau 6 giờ ngâm, giống IR 50404 sẽ đạt độ ẩm bão hòa là 27,1%, trong khi giống Jasmine 85 đạt độ ẩm bão hòa là 32,7%. pH thích hợp của dung dịch ngâm là 3 đối với IR 50404 và 2 đối với Jasmine 85 để hàm lượng GABA thu được cao nhất tương ứng là 70,37 mg/100 g và 78,50/100 g. Việc cho gạo sau khi ngâm nẩy mầm ở điều kiện yếm khí (hàm lượng CO_2 là 10%) sẽ cho ra gạo thành phẩm có hàm lượng GABA cao nhất là 88,88 mg/100 g đối với IR 50404 và 104,80 mg/ 100 g đối với Jasmine 85 sau khi ủ 30 giờ.

Từ khóa: γ-aminobutyric acid (GABA), chất lượng gạo, điều kiện ngâm, điều kiện nẩy mầm, IR 50404, gạo mầm, Jasmine 85.

1. INTRODUCTION

Rice production in Vietnam is important for the food supply in the country. The Mekong Delta is considered as the rice bowl of Vietnam and accounts for more than 50% of the national total production. Vietnam is the second largest rice exporter worldwide. In 2012, Vietnam exported nearly 8 million tons of rice (http://www.vietfood.org.vn/en/). However, the export value of Vietnamese rice is far lower than that of Thailand as we export mainly the rice. The requirement of value added products from rice is crucial to increase the income of Vietnamese farmers, especially the farmers in the Mekong Delta.

The nutritional value of brown rice is much higher than normal milled rice as it contains more dietary fiber, vitamin E and vitamin B (Champagne et al., 2004). However, brown rice takes longer time to cook and its taste is worse than milled rice. Germinated brown rice can be considered as a suitable product as it improves texture and increases in flavor. In addition, germinated brown rice contains high quantity of γ aminobutyric acid (GABA), a neurotransmitter in the brain and the spinal cord of mammals. A great deal of research showed that GABA provides beneficial effects for human health, including decreasing blood pressure (Ohmori et al., 1987), controlling stress (Hayakawa et al., 2004) and inhibiting cancer cell proliferation (Oh and Oh, 2004).

IR 50405 is a popular rice cultivar in Mekong delta. It can adapt to alkaline soil and it can resist to various plant diseases. However, the quality of this rice is low t leading to low selling price. Jasmine 85 is the fragrant rice, however, some people do not appreciate the soft texture of this rice. Germination can be a good solution to attract consumers for buying these 2 rice cultivars.

In this study, effect of various conditions for soaking and germination on the accumulation of GABA in germinated brown rice was investigated.

2. MATERIALS AND METHODS

2.1. Materials

IR 50404 and Jasmin 85 rice cultivars were provided by Cuu Long Rice Research Institute (CLRRI) in Cantho city. The paddies were milled in Satake miller and packed in plastic bags, then kept in a cool room (approximately 8°C) throughout the experiment.

2.2. Hydration characteristics of brown rice

Brown rice was washed with chlorine water (10 ppm) to rinse out contaminants and then thoroughly rinsed in distilled water. Rice sample was soaked in distilled water at room temperature (30± 2°C) for 10 hours. At various time intervals, the rice samples were analyzed for moisture content by drying in the convection oven at 130°C until constant weight.

2.3. Effect of pH of soaking solution on GABA content accumulation

Brown rice was steeped in soaking solutions at various buffer solutions: buffer pH 2.0 of 0.1 M glycine-HCl; buffer pH 3.0 and 5.0 of 0.1 M citrate and buffer pH 7 of 0.1 M phosphate and also distilled water with pH 6.7. The ratio of grain to solution was 1:2 (w/v). The soaking time was 6 hours at room temperature (30± 2°C). After 6 hours, the rice were taken and then wrapped in a cloth to maintain the moisture and left in the incubator (30± 2°C) during 24 hours. The germinated brown rice was dried to <13% of moisture content and analyzed for GABA content using the method of Banchuen et al. (2010). The pH of soaking solution that gave the highest GABA content was chosen for further study.

2.4. Effect of the germination conditions of brown rice on GABA content

The brown rice was soaked in the solution with pH chosen above. The rice were then wrapped in a cloth and left in either normal incubator or anaerobic incubator with 10% CO_2 (30± 2°C). The sample of germinated rice was taken every 12, 24, 36 and 48 hours. The germinated rice was dried to <13% of moisture content and analyzed for GABA content using the method of Banchuen et al. (2010).

2.5. Determination of GABA content

GABA content was determined with the method of Banchuen *et al* (2010). One-half gram (0.5 g) of ground germinated brown rice sample was weighted in plastic tubes and 1.8 mL of deionized water was added and the slurries were shaken for 1.5 hours at room temperature. Then 200 mL of 3% (by volume) sulfosalicylic acid was added and the mixture were centrifuged at 4500xg during 10 min. 50 mL of supernatant were mixed with 50 mL of

100 mM $NaHCO_3$ and 50 mL of 4 mM 4-dimethylaminoazobenzene -4- sulfonyl chloride acetonitrile solution. The mixture was heated at 70°C for 10 min to effect derivatization. After that, 250 mL of absolute ethanol and 250 mL of 25 mM phosphate buffer (pH 6.8) were added. The sample was then filtered and 5 mL of the filtrate were injected into Shimadzu HPLC (Japan), with C18 column (3.5 µm 4.6x150mm). The HPLC was equipped with an UV-Vis photodiode array detector set at 465 nm wavelength. The mobile phase was 25 mM acetate buffer and acetonitrile (65:35) operated at the flow rate of 0.5 mL/min at 55°C. Pure GABA (Merck, Germany) was used as standard for calibration.

2.6. Statistical analysis

All experiments were carried out using 3 freshly prepared germinated rice samples, 100 g for each samples and three replicates of each sample were analyzed. The results were statistically analyzed using one way analysis of variance by Duncan multiple range test with mean square error at 5% probability calculated with the Statgraphic Centurion version 15 software.

3. RESULTS AND DISCUSSION

3.1. Hydration characteristics of brown rice

The hydration of 2 rice varieties is shown in Fig. 1. At the early stage of soaking, the water uptake rapidly, increasing from 13% to 24% due to the difference in moisture content inside and outside of the rice. In addition, during this stage, there was a quick absorption into the embryo of the kernel (Bello et al., 2004). From 2 hours to 6 hours, the moisture content increased slowly and it nearly reached the saturation point after 6 hours for both rice varieties. The moisture content at this moment was 29% and 32 % for IR 50404 and Jasmine 85, respectively.

Similar result was found by Bello et al. (2004) that moisture content of rice increased rapidly during the first 2 hours, then slowed down until it reached the saturation after 5 hours. Banchuen et al. (2010) also noted that after 5 – 7 hours soaking, the moisture content of 3 Thai rice varieties (Niaw Dam Peuak Dam, Sangyod Phatthalung và Chiang Phatthalung) also arrived to the saturation.

The water absorption of these 2 rice varieties was also different. The moisture content of Jasmine 85 after 6 hours soaking was 32.7%,s much higher than IR 50404 (27.1%).

According to Banchuen et al. (2010), the amylose and amylopectin content of rice influence the water absorption. The higher amylopectin content, the higher water absorption. Our results agreed with this study, as the amylopectin content of Jasmine 85 (80%) was higher than that of IR 50404 (74%).

3.2. Effect of pH of soaking solution on GABA content

The GABA content of germinated brown rice in various soaking solutions is shown in Table 1. The highest value of GABA of IR 50404 (70,37mg/100g) was found in samples soaked in citrate buffer at pH 3. The highest value of GABA (78,50 mg/100g) of Jasmine 85 was determined in sample soaked in glycine-HCl buffer at pH 2. The results showed that the high GABA content could be obtained in soaking solution at low pH. These results were similar to the study of Charoenthaikij et al.(2009) who found the highest GABA content of Khao Dawk Mali 105 rice cultivar (67 mg/100 g) after soaking in citrate buffer at pH 3 during 48 hours. Similarly, GABA content of Kor-Khor 6 rice cultivar reached the highest at 30.69 mg/100 g after soaking 24 hours in citrate buffer at pH 3. However, there were different findings from other authors. Watchraparpaiboon et al. (2007) found that brown rice soaked in buffer solution at pH 6 gave the highest GABA content. It was shown that GABA content in germinated brown rice increased when the rice was soaked in acidic solution. The synthesis of GABA is rapidly stimulated by stress conditions including hypoxia. The advantage of this process would be the concomitant H^+ consumption, which ameliorates the cytosolic acidification associated with hypoxia or other stresses (Crawford et al., 1994).

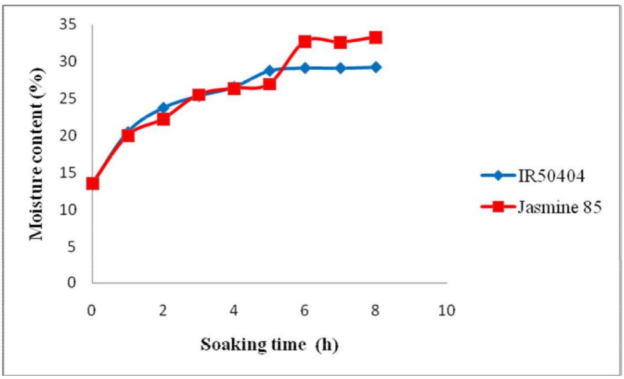

Fig. 1. Hydration of 2 rice varieties (IR 50404 and Jasmine 85) during soaking at room temperature (30± 2°C)

Table 1. GABA content in 2 varieties of germinated brown rice (IR 50404 and Jasmin 85) in different soaking solutions

pH of soaking solution	GABA content (mg/100 g)	
	IR 50404	Jasmine 85
2	67,14^{b}	78,50^{a}
3	70,37^{a}	75,19^{b}
5	47,31^{d}	50,15^{c}
7	64,23^{c}	31,50^{d}
Distilled water (pH 6.6)	36,82^{e}	28,22^{e}

Note: The same letters under the same column indicate no significant difference (p>0.05)

3.3. Effect of the germination conditions of brown rice on GABA content

The GABA contents of brown rice germinated in various conditions are presented in Table 2.

The germination under aerobic condition gave the lower GABA content than under anaerobic condition. After 12h germination in anaerobic condition, the GABA content of Jasmine 85 was 47.14 mg/100 g which was much higher than that of aerobic condition (16.12 mg/100g). The similar result was obtained for IR50404 (Table 2). In addition, the germination time also influenced the GABA content. The highest GABA content of Jasmine 85 was observed after 30 hours under anaerobic germination (104.80 mg/100g) while that of IR 50404 was 88.88 mg/100 g after 30 hours of germination as well. The GABA content of brown rice were 4.5 and 7.0 mg/100g for Jasmine 85 and IR 50404, respectively, so the GABA content increased 12.69 times to 23.88 times compared to the initial content.

The similar result was found by Chung et al. (2009) that the GABA content of barley increased 4 times under anaerobic germination condition in comparison with un-germinated barley. Banchuen et al. (2010) also reported that the GABA content under anaerobic germination was highest after 36 hours for Sangyod Phatthalung and Chiang Phatthalung varieties,

Table 2. GABA content in 2 varieties of germinated brown rice (IR 50404 and Jasmin 85) in various germination conditions

Rice variey	Germination method	Germination time (hours)				
		12	18	24	30	36
IR 50404	Aerobic	$35,07^{Be}$	$57,53^{Bc}$	$69,36^{Bb}$	$75,09^{Ba}$	$54,01^{Bd}$
	Anaerobic	$49,69^{Ae}$	$59,59^{Ad}$	$77,68^{Ab}$	$88,88^{Aa}$	$70,60^{Ac}$
Jasmine 85	Aerobic	$16,12^{Bd}$	$55,24^{Bc}$	$78,97^{Bb}$	$84,14^{Ba}$	$54,42^{Bc}$
	Anaerobic	$47,14^{Ae}$	$62,94^{Ad}$	$87,42^{Ab}$	$104,80^{Aa}$	$81,96^{Ac}$

Note: $^{A-B}$ Means with the same letter in the same column within each rice variety indicates no significant difference (p>0.05)
$^{a-e}$ Means with the same letter in the same row within each rice variety indicates no significant difference (p>0.05)

and after 48 hours for NiawDam Peuak Dam variety, and increased 9.43 to 16.74 times in comparison with brown rice.

The high content of GABA during anaerobic germination may be due to the synthesis of GABA through glutamate decarboxylase in reduced oxygen supply that occurred by the effect of decreasing cytoplasmic acid in cell (Caroll et al., 1994). The result also indicated that the glutamic acid was synthesized by the glutamate synthase (GOGAT) and glutamine synthetase (GS) cycle. The G/GOGAT cycle plays an important role in anaerobic accumulation of GABA (Aurisano et al., 1995).

4. CONCLUSION

The research results indicated that the soaking conditions significantly affected the accumulation of GABA during germination. The water uptake happened during soaking and could readily reach the optimum content for germination. The acidic pH of the soaking solution also played an important role in GABA content of the germinated brown rice of IR 50404 and Jasmine 85. Anaerobic treatment of germinated brown rice also increased the GABA content. The appropriate soaking time was 6 hours for both IR 50404 and Jasmine 85. The suitable pH of soaking solution was pH 3 for IR 50404 and pH 2 for Jasmine 85 that gave the GABA content of 70,37mg/100g for IR 50404 and 78.50 for Jasmine 85. The germination under anaerobic conditions yielded highest GABA contents which were 88.88 mg/100 g and 104.80 mg/100g for IR50404 and Jasmine 85, respectively, after 30 hours. The germinated brown rice could become a value-added products to diversify the products from rice and increase the income of farmers in the Mekong Delta.

ACKNOWLEDGEMENT

The study was supported by the research fund of Cantho University.

REFERENCES

Aurisano N., Bertani, A. and Reggianni, R. (1995). Anaerobic accumulation of 4-aminobutyrate in rice seedlings: causes and significance. Phytochemistry 38: 1147-1150.

Banchuen J., Paiboon T., Buncha O., Phaisan W. and Piyarat S. (2010). Increasing the bio-active compounds contents by optimizing the germination conditions of Southern Thai Brown rice. Songklanakarin Journal of Science and Technology 32 (3). 219-230.

Bello M., Tolaba, M. P. and Suarez, C. (2004). Factors affecting water uptake of rice grain during soaking. Lebensm Wiss Technol. 37: 811-816.

Carroll A.D., Fox G.G., Laurie S., Phillips R., Ratcliffe R.G. and Stewart G.R. (1994). Ammonium assimilation and the role of g-aminobutyric acid in pH homeostasis in carrot cell suspensions. Plant Physiology 106: 513-520.

Champagne E.T., Wood D.F., Juliano B.O. and Bechtel D.B. (2004). The rice grain and its gross composition. In Rice, Champagne E.T., editor. American of Cereal Chemistry, St Paul, USA, pp. 77-107.

Chung H. J., Ang S.H., Cho H.Y. and Lim S.T. (2009). Effect of steeping and anaerobic treatment of GABA (Gama aminobutyric acid) content in

germinated waxy hull-less barley. Lebensm Wiss Technol. 42: 1712-1716.

Crawford L.A., Bown, A.W., Breitkreuz, K.E., and Guinel, F.C. (1994). The synthesis of γ-aminobutyric acid in response to treatments reducing cytosolic pH. Plant Physiology 104: 865-871

Oh C. H. and Oh S. H. (2004). Effect of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. Journal of Medicinal Food, 7(1): 19-23.

Ohmori M., Yano T., Okamoto J., Tsuchida T., Murai T., and Higuchi M. (1987). Effect of anaerobically treated tea (Gabaron tea) on blood pressure of spontaneously hypertensive rats. Nippon Nogei Kagaku Kaishi 61: 1449-1451.

Watchraparpaiboon W., Laohakunjit N., Kerdchoechuen O. and Photchanachai S. (2007). Effects of pH, temperature and soaking time on qualities of germinated brown rice. Agricultural Science Journal 38: 169-172.