Vietnam Journal of Science and Technology ***57*** *(3B) (2019) 137-143*

doi:10.15625/2525-2518/57/3B/14499

TECHNICAL ASSESSMENT OF GAMMA-AMINO BUTYRIC ACID (GABA) PRODUCTION FROM RICE BRAN

Lai Quoc Dat*, Ngo Thanh An, Le Thi Kim Phung, Nguyen Hoang Dung, Nguyen Quang Long

Faculty of Chemical Engineering, University of Technology, VNU-HCM, 268 Ly Thuong Kiet Street, Ward 14, District 10, Ho Chi Minh City

*Email: *lqdat@hcmute.edu.vn*

Received: 14 October 2019; Accepted for publication: 4 November 2019

Abstract. This research focused on technical assessment of GABA production from rice bran through fermentation by *Lactobacillus brevis*. Influence of operating pressure on separation of GABA by nanofiltration membrane was investigated and 4 bar was suitable for the nanofiltration process. The purification of GABA by nanofiltration with constant feed volume was carried out and purity of GABA reached 4.8 folds, compared to feed, at 5 volumes of added water. At 40 of concentration factor in concentration of GABA solution by nanofiltration with full recycle of retentate, content of GABA reached 49.8 g/L. The production of GABA from defatted rice bran at pilot scale was carried out at 1,000 L/batch (equal to 200 kg of rice bran) of fermentation. Results obtained from pilot production showed that, with 200 kg of defatted rice bran, 7.0 kg of GABA powder was obtained. Results indicated that, it is potential to produce GABA from rice bran through the fermentation by *Lactobacillus brevis*.

Keywords: rice bran, gamma-amino butyric acid, nanofiltration, purification.

Classification numbers: 1.3.1, 1.1.5.

1. INTRODUCTION

Recently, GABA has been widely utilized as bioactive for human health due to their physiological functions including: anti-stress effect [1], relation with unipolar depressive disorder [2], antihypertensive effect [3, 4], and responsibility for muscle tone regulation [5]. GABA was supplied for human from tea [6], germinated brown rice [7] and beans [8]. For pharmaceutical utilization, GABA has been produced by fermentation of glutamic acid by *Lactobacteria* [9].

Rice bran, byproduct of rice processing, has been known as a source of protein, containing huge amount of glutamic acid [10]. In previous work, Lai *et al*. [11] utilized defatted rice bran extract for synthesis of GABA through fermentation by *Lacbacillus brevis*. Feasibility of the recovery of GABA from fermentation broth by ion exchange was reported [12]. It proved that GABA can be produced from rice bran. Nevertheless, solution obtained from elution of ion

exchange process contains NaCl with high content. Thus, it is necessary to purify GABA by an appropriate process.

This research aimed to study the feasibility of GABA production from defatted rice bran. The purification and concentration of GABA solution by nanofiltration was investigated. The production of GABA from defatted rice bran at pilot scale was conducted and yield of GABA was determined.

2. MATERIALS AND METHODS

2.1. Materials

Rice bran was collected from milling of rice, with 10 % of milling degree and 8 % of moisture. Rice bran was defatted by extraction with n-hexane, followed by heating at 80 ^{o}C with hot air to remove the solvent. The fatty content in defatted rice bran was 3 % w/w. *Lactobacillus brevis* VTCC – B – 454, isolated from Vietnamese pickled vegetable, was supplied by Institute of Microbiology and Biotechnology, Vietnam National University, Ha Noi.

GABA and analytical reagents were supplied by Sigma Aldrich (USA) with analytical grade. Termamyl, α- amylase produced by a genetically modified laboratory strain of *Bacillus licheniformis*; and Flavorzyme, a mixture of endoprotease and exopeptidase synthesized by *Aspergillus oryzae*, were supplied by Novozymes (Denmark). All chemicals, reagents and enzymes were stored at 4 ^{o}C.

Membrane utilized in this research was made from polypiperazine amide, spiral module (NF 90, 8040), manufactured by Dow – Filmtech (USA).

2.2. Processing of GABA production from defatted rice bran

Firstly, defatted rice bran was added into water with ratio of 1:5 (w:w) of defatted rice bran:water; then heating to 100 ^{o}C, remaining in 45 min for gelatinization. After gelatinization, it was cooled down to 95 ^{o}C, adjusting pH to 6.5, adding Termamyl with 1 % w/w of content and remaining in 60 min for liquefaction of starch. After that, the mixture was cooled to 50 ^{o}C, adjusting pH to 8.0, adding 2 %w/w Flavozyme and remaining in 120 min. Then, it was filtrated with cloth to obtain defatted rice bran extract which was sterilized at 100 ^{o}C in 10 min prior to fermentation. The fermentation was conducted by *Lactobacillus brevis* at 10^8 cfu/mL of cell density. The fermentation was carried out under the conditions as follows: 30 ^{o}C, pH 5.0 and 48 hours of fermentation time. After that, the broth was heated to 100 ^{o}C in 5 min to inactivate bacteria, followed by cooling to obtain ambient temperature. Then, it was filtrated by ultrafiltration (1,000 Da of molecular weight cut off) at 6 bar. The filtrate was pumped through the cartridge containing cation exchanger (Purelite C100). Then, the elution was carried out with 0.5 M of NaCl solution at pH 5.5. The effluent from elution was taken to nanofiltration for purification, followed by concentration by nanofiltration. The concentrate, then, was frozen at – 20 ^{o}C, followed by freeze drying to obtain the powder.

2.3. Purification and concentration of GABA solution

Effluent from elution of cation exchanger containing GABA and NaCl was purified by nanofiltration process with adding of pure water. Volume of pure water was equal to permeate flux to keep constant volume of feed.

Concentration of GABA solution by nanofiltration: After the purification, GABA solution was concentrated by nanofiltration process operating with full recirculation of retentate.

2.4. Analysis methods

GABA content was determined by spectroscopy method. 0.5 mL of sample was added to a mixture of 0.2 mL of 0.2 M borate buffer (pH 9.0), 1 mL of 6 % w/w phenol and 0.4 mL of 9 % w/w sodium hypochlorite. Then, it was vortexed and put in water bath at 100 ^{o}C in 10 min. After that, it was put in ice bath in 20 min. Then, it was vortexed again until the blue color was obtained. Finally, adding 2 mL of 60 % v/v ethanol and determined the absorption at 645 nm [13]**.**

NaCl content was determined by method proposed by AOAC method [14].

Moisture was analyzed by drying at 105 ^{o}C until obtaining constant weight.

3. RESULTS AND DISCUSSION

3.1. Nanofiltration for purification and concentration of GABA solution

3.1.1. Influence of pressure on separation of GABA

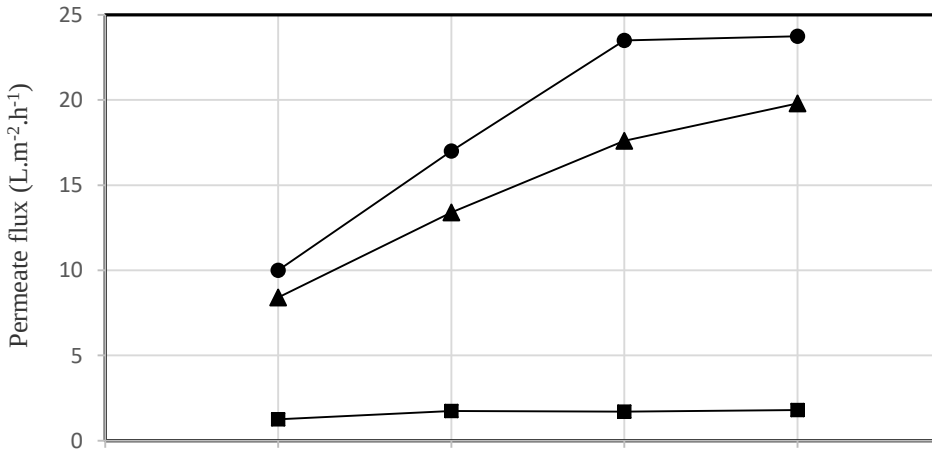

Figure 1. Influence of pressure on nanofiltration of effluent obtained from elution of ion exchange process in GABA production (▲: permeate flux, ■: NaCl rejection, ●: GABA rejection).

Influence of operating pressure on rejection of GABA and NaCl in nanofiltration of effluent obtained from elution of ion exchange process is showed in Fig. 1. Increasing in operating pressure resulted in increase of permeate flux. Relationship between operating pressure and permeate flux was linear. It implies that, the influence of concentration polarization was eliminated by crossflow fluid on membrane surface [15]. This is an important point in nanofiltration: the elimination of concentration polarization leads to significantly reduce fouling, which causes decline of permeate flux in filtration [16].

Rejection of NaCl insignificantly changed with increasing in operating pressure: approximately 2 – 3 %. It means that, concentration of NaCl in permeate and retentate size was

approximate with each other. Rejection of GABA increased with increase in operating pressure from 20 to 40 bar. At 50 bar of operating pressure, rejection of GABA was insignificantly different with that at 40 bar: approximately 95 %. In nanofiltration, solutes passed through membrane by three mechanisms: convection, diffusion and electro-migration [17]. Increase of operating pressure leads to increase in convection of water and solutes with molecular size being smaller than pore size of membrane [18]. The bigger the molecular size is, the lower the increase in convective motion is [19]. Compared to water, Na^+ and Cl^-, molecular size of GABA is significantly larger. Consequently, increase in convective motion of GABA was lower than that of the others. On the other words, GABA remained in retentate was more than the others, leading to increase in rejection. Result in Fig. 1 indicated that, GABA can be separated from NaCl and concentrated by nanofiltration due to the significant difference of their rejections.

3.1.2. Purification of GABA

Purification of GABA solution was conducted at 40 bar of operating pressure. The change in NaCl and GABA contents in feed was showed in Fig. 2. The result indicated that, increasing water supply led to increase in purity of GABA in solution. Due to low rejection, NaCl passed through membrane and rejected in permeate. On the other hand, GABA was remained in retentate. Nevertheless, amount of GABA was lost in permeate. That is the reason why content of GABA decreased in nanofiltration, although volume of feed was constant. At 5 volumes of water adding to feed, ratio of GABA concentration to NaCl concentration was 0.77, compared to 0.16 in feed. It means that, purities of GABA increased 4.8 folds by nanofiltration with adding water. The permeate flux during nanofiltration was insignificantly changed. Perhaps, the decline of permeate by fouling in nanofiltration was compensated by reduction of difference in osmotic pressure between both sides of membrane [20].

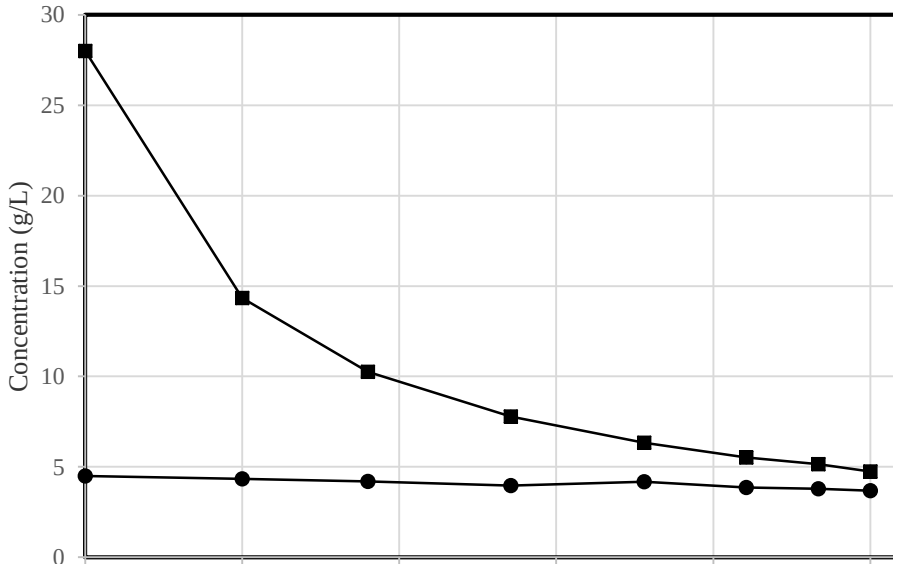

Figure 2. GABA (●) and NaCl (■) concentration against ratio of volume of water to feed volume in purification of GABA solution by nanofiltration.

3.1.3. Concentration of GABA

Concentration of GABA solution was carried out at 40 bar of operating pressure. The

change in NaCl and GABA contents in concentrate (retentate) against concentration factor (ratio of initial and final volumes of feed) was shown in Fig. 3. Due to the low rejection, content of NaCl was insignificantly changed in nanofiltration. It was approximately 4.8 g/L. At 40 of concentration factor, concentration of GABA reached 49.8 g/L, 13.6 folds higher than that in feed. Ratio of GABA concentration to NaCl concentration was 10.3, purity of GABA increased 13.3 folds, compared to that in feed.

Permeate flux can be divided into 2 sessions (Fig. 3). In early session, permeate flux significantly declined due to the formation of boundary layer on membrane surface that led to fouling [21]. In the later session, permeate flux insignificantly declined. In this stage, the boundary layer became stable and not caused the decline of permeate flux more.

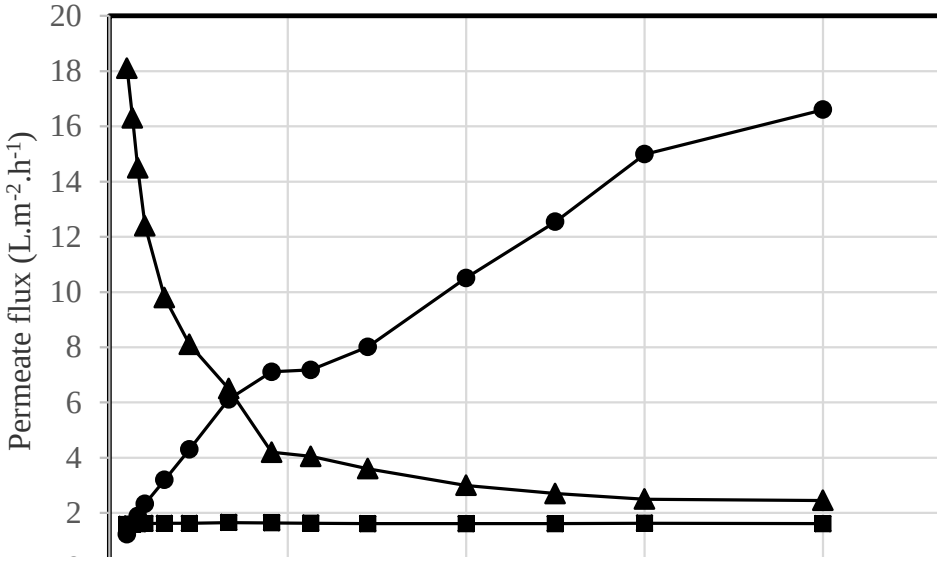

Figure 3. Permeate flux, GABA and NaCl concentrations against concentration factor in concentration of GABA solution by nanofiltration (▲: permeate flux, ■: NaCl rejection, ●: GABA rejection).

3.2. Production of GABA from defatted rice bran by fermentation at pilot scale

Table 1. Material consumption of production of GABA from defatted rice bran by fermentation at pilot scale (1,000 L/batch of fermentation).

Materials	Defatted rice bran	Flavorzyme	Termamyl	NaCl	Peptone	HCl (35%)	NaOH	Water
Unit	Kg	Kg	Kg	Kg	Kg	kg	Kg	L
Amount	200	4	2	14	20	0.2	0.6	7,300

Consumption of materials in production of GABA at pilot scale with 200 kg of defatted rice bran/batch was showed in Table 1. The product, in form of powder, was 7.0 kg, with composition as the follows: GABA: 91 % w/w, NaCl: 4.5 % w/w, moisture: 5.8 % w/w. Content of GABA in product was approximately with commercial products, which was produced by fermentation of glutamic acid. The result indicates that, production of GABA from defatted rice bran is potential.

4. CONCLUSIONS

Increasing of operating pressure led to increase in rejection of GABA in nanofiltration. Purification of GABA solution obtained from elution of ion exchange process by nanofiltration with adding water could improve purity of GABA: reaching 4.8 folds compared to that in feed, with 5 feed volumes of added water. Concentration of GABA solution could increase concentration of GABA: reaching 13.6 folds higher than that in feed. With 200 kg of defatted rice bran, 7.0 kg of GABA powder was obtained. It is potential to produce GABA powder from defatted rice bran by fermentation with *Lactobacillus brevis* and application of nanofiltration for purification and concentration.

Acknowledgements. This research was funded by VNU-HCM under grant number KHCN-TNB.ĐT/14-19/C06.

REFERENCES

1. Vaiva G., Thomas P., Ducrocq F., Fontaine M., Boss V., Devosc P., Rascle C., Cottencin O., Brunet A., Laffargue P. and Goudeman M. - Low posttrauma GABA plasma levels as a predictive factor in the development of acute posttraumatic stress disorder, Biological Psychiatry **55** (3) (2004) 250–254.
2. Bjork M. J., Moeller F. G., Kramer L. G., Kram M., Suris A., Rush A. J. and Petty F. - Plasma GABA levels correlate with aggressiveness in relatives of patients with unipolar depressive disorder, Psychiatry Research **101** (2) (2001) 131–136.
3. Hayakawa K., Kimura M. and Kamata K. - Mechanism underlying γ-aminobutyric acid-induced antihypertensive effect in spontaneously hypertensive rats, European Journal of Pharmacology **438** (1–2) (2002) 107–113.
4. Ichimura T., Yamanaka A., Ichiba T., Toyokawa T., Kamada Y., Tamamura T. and Maruyama S. - Antihypertensive Effect of an Extract of Passiflora edulis Rind in Spontaneously Hypertensive Rats, Bioscience, Biotechnology, and Biochemistry **70** (3) (2006) 718–721.
5. Watanabe M., Maemura K., Kanbara K., Tamayama T. and Hayasaki H. - GABA and GABA receptors in the central nervous system and other organs, International Review of Cytolog **213** (2002) 1–47.
6. Tsushida T. and Murai T. - Conversion of Glutamic Acid to γ-Aminobutyric Acid in Tea Leaves under Anaerobic Conditions, Agricultural and Biological Chemistry **51** (11) (1987) 2865–2871.
7. Saikusa T., Horino T. and Mori Y. - Accumulation of γ-Aminobutyric Acid (Gaba) in the Rice Germ during Water Soaking, Bioscience, Biotechnology, and Biochemistry **58** (12) (1994) 2291–2292.
8. Aoki H., Uda I., Tagami K., Furuya Y., Endo Y. and Fujimoto K. - The Production of a New Tempeh-like Fermented Soybean Containing a High Level of γ-Aminobutyric Acid by Anaerobic Incubation with Rhizopus, Bioscience, Biotechnology, and Biochemistry **67** (2003) 1018–1023.
9. Moo-Chang K., Seo M. J., Cheigh C. I., Pyun Y. R., Cho S. C. and Park H. - Enhanced production of γ-aminobutyric acid using rice bran extracts by Lactobacillus sakei B2-16, Journal of Microbiology and Biotechnology **20** (4) (2010) 763–766.

10. Luh S. B. - Rice: Production and Utilization. Second Edition. AVI Publishing Corp, 1991, pp. 925.
11. Lai Q. D., Tran T. K. N. - Nguyen T. X. N. - Gamma-amino butyric acid (GABA) synthesis of Lactobacillus in fermentation of defatted rice bran extract, AIP Conference Proceedings **1878** (2017) 020045
12. Vi N. T., Le T. K. P. and Lai Q. D. - Purification of Gamma-Amino Butyric Acid (GABA) from Fermentation of Defatted Rice Bran Extract by Using Ion Exchange Resin, AIP Conference Proceedings **1878** (2017) 020007.
13. Zhang Q., Xiang J., Lizhen Z., Zhu X., Van der Werf W. and Duan L. - Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice, Journal of Functional Foods **10** (2014) 283–291.
14. AOAC Official Methods of Analysis, 15th Ed. (1990) Methods 32.034 - 32.039.
15. Vincze I. and Vatai G. - Application of nanofiltration for coffee extract concentration, Desalination **162** (2004) 287–294.
16. Van der Horst H. C., Timmer J. M. K., Robbertsen T. and Leenders J. - Use of nanofiltration for concentration and demineralization in the dairy industry: Model for mass transport, Journal of Membrane Science **104** (1995) 205–218.
17. Szymczyk A., Labbez C., Fievet P., Vidonne A., Foissy A. and Pagetti J. - Contribution of convection, diffusion and migration to electrolyte transport through nanofiltration membranes, Advances in Colloid and Interface Science **103** (2003) 77–94.
18. Hussain A. A., Abashar M. E. E. and Al-Mutaz I. S. - Influence of ion size on the prediction of nanofiltration membrane systems, Desalination **214** (2007) 150–166.
19. Pontalier P., Ismail A. and Ghoul M. - Mechanism for the selective rejection of solutes in nanofiltration membranes, Separation and Purification Technology **12** (1997) 175–181.
20. Wijmans J. G., Nakao S., and Smolders C. A. - Flux limitation in ultrafiltration: Osmotic pressure model and gel layer model, Journal of Membrane Science **20** (1984) 115–124.
21. Warczok J., Ferrando M., López F. And Guell C. - Concentration of apple and pear juices by nanofiltration at low pressure, Journal of Food Engineering **63** (2004) 63–70**.**