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**The alternative source of high GABA content derived  
from germinated black bean**

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**ABSTRACT**

Gamma-aminobutyric acid (GABA) is a kind of biological component which is also considered as one of the inhibitory neurotransmitter in the nervous system. GABA source is generally found in bean seeds, but its production was very difficult to have the biological effect due to the existing of low GABA concentration. To enrich the GABA content in the beans, seed germination process was conducted. Therefore, this study was aimed to investigate the effect of germination times for GABA production in black bean seeds. For this reason, the black bean seeds were germinated and observed at different germination times of 0 h (after soaking immediately), 6 h, 12 h, 24 h, and 36 h, respectively. The result revealed that the highest of GABA content in germinated black beans were found at 4.78 mg/g (24 h after germination) which was 7.83 times higher than the nongerminated black beans (0.61 mg/g). However, the concentration of GABA was decreased probably 2.53 times after 36 h (1.89 mg/g) from the maximum content, likely due to the plant growth metabolism. In overall, this current study confirmed that the germination process could be used as an alternative way to provide high GABA content for the future bean products, especially black bean milk and sprouts.

**Keywords:** Black bean, GABA, Germination

## INTRODUCTION

Black bean (*Phaseolus vulgaris*) is known as the turtle bean which is considered as the world's second most popular bean after soybean seed. Its characteristic is sweet in taste, soft texture, and medium size (Ganesan & Xu, 2017). Black bean enriched with many excellent nutrition, such as 71.4 % carbohydrate, 23.1 % protein, 4.3 % ash, and 1.2 % fat (Amaral et al., 2016). It is also believed as the source of several vitamins, such as thiamin, riboflavin, niacin, vitamin B6, and folic acid and essential minerals including calcium, iron, and potassium (Celmeli et al., 2018). Black bean was normally used as the raw material for manufacturing snack food, sweet, beverage, and cosmetic products. Recent studies have discovered that germination process could be used as the common method for increasing the secondary metabolite of the plant (Kayembe & Jansen van Rensburg, 2013). This technique is involved with the incorporation of some substances in the seed which starts with the water absorption by a quiescent dry seed, and terminate with the elongation of the embryonic axis (Wang et al., 2015). As the result, it would be stimulated the birth of a new life of plant, and meanwhile it could increase the gamma aminobutyric acid (GABA) content in the seed due to the activation of many endogenous enzymes.

GABA is a four carbon non-protein amino acid which is widely known as the inhibitory neurotransmitter. It is the brain's natural calming agent that inhibits the over stimulation of the nerves activities in the central nervous system (Dhakal, Bajpai, & Baek, 2012). Thus, it can help to defend against sleep difficulty, anxiety, and high blood pressure (Kim et al., 2006; Randomized & Trial, 2018; Pouliot-Mathieu et al., 2013). GABA content in the seed can be produced from the decarboxylation reaction of glutamic acid catalyzed by glutamate decarboxylase during germination process. Therefore, the objective of this study was to investigate the GABA content in black beans during germination process.

## MATERIAL AND METHODS

### Bean germination

Black bean (*Phaseolus vulgaris*) was purchased from Khaothong Company, Thailand. The germination condition was conducted by following the description in Vann (2019). The black bean sprout was randomly collected

at 0 h (after soaking immediately), 6 h, 12 h, 24 h, and 36 h. Then, all the samples were ground by using a blender and kept at 4 °C for GABA extraction.

#### **GABA extraction**

Each germinated black bean was extracted by following the method from Vann (2019). The black bean samples were subjected to extract the GABA content with 4 % (v/v) acetic acid in the ratio of 1:4 (w/v). Then, the samples were shaken and centrifuged at 1200 rpm for 10 min. The obtained supernatant was reserved at -20 °C for GABA determination.

#### **GABA determination by high performer liquid chromatography**

The GABA content was carried out according to the method as described in Vann (2019). The reverse-phase Inertsil ODS-3 column (4.6 × 250 mm, 5 µm; GL science Inc., Tokyo, Japan) and a postcolumn reaction module (Waters, USA) were used in this study. The OPA reagent and the mobile phase reagent were generated at 0.20 mL/min and 0.50 mL/min, respectively. The GABA content in the sample was separated with the temperature at 40 °C and monitored by a fluorescence detector with excitation at 340 nm and emission at 450 nm.

#### **Statistical analysis**

All of the measured values are conducted in triplicate. The statistical analysis was carried out by one-way analysis of variance (ANOVA), and the data collections were evaluated with multiple Tukey's HSD tests ( $p < 0.05$ ) by using SPSS Statistic 23.0.

## **RESULTS**

#### **The observation of black bean during germination**

In this study, bean characteristic was observed according to the embryo occurrence from the seed coat of black bean seeds. After germination, the appearance of black beans from 0 h till 12 h have remained the same, but they were then seedling at 24 h and 36 h. The growth of the seed is normally relevance with the bean seed size and the moisture absorption of beans. The common size of this black bean is about 11.45×16.45 mm (Wani, Sogi, Wani, & Gill, 2014) which probably result in making the black bean seed need long time to germinate.

#### **GABA content in germinated black bean**

According to table 1, the GABA accumulation in germinated black beans was significantly increase from time to time. The concentration of GABA was 0.61 mg/g, 1.47 mg/g, 1.78 mg/g, 1.43 mg/g, 4.78 mg/g, and 1.89 mg/g of nongerminated bean, 0 h, 6 h, 12 h, 24 h, and 36 h, respectively. The GABA content in black bean at 0 h was approximately 2 times higher than the nongerminated bean. Hereafter, the concentration of GABA was fluctuated until 12 h of germination. Then, its content has reached a maximum value of 4.78 mg/g, which was 7.83 fold higher than nongerminated black bean. However, the accumulation of GABA was decrease after 36 h of germination. Even though its concentration in this duration was almost 2.53 times lower than the maximum content, the accumulation of GABA within this germination process still leading the GABA level around 3 times higher than the nongerminated bean.

**Table 1.** The GABA content changes in the black beans during germination

Germination times	GABA (mg/g)
<b>Nongermination</b>	0.61 ± 0.03 <sup>a</sup>
<b>0 h</b>	1.47 ± 0.01 <sup>b</sup>
<b>6 h</b>	1.78 ± 0.09 <sup>c</sup>
<b>12 h</b>	1.43 ± 0.01 <sup>b</sup>
<b>24 h</b>	4.78 ± 0.01 <sup>d</sup>
<b>36 h</b>	1.89 ± 0.11 <sup>c</sup>

Note: values in a column with different superscripts are significantly different (P<0.05) according to Tukey's multiple range test.

## DISCUSSION

The result in table 1 indicated that the storage protein in the bean was partially decomposed and supplied to the growing part of seedling. Prolonging with the germination process, glutamate decarboxylase enzyme was also activated which automatically converted glutamic acid to GABA. However, the GABA content in bean sprout was markedly declined (2.53 folds) from the maximum content after 36 h of germination. This decreasing might be involve with the plant growth metabolism since the accumulation of GABA in the plant food was occurred only the early stage of germination (Matsuyama et

al., 2009). As a result, the plant would use GABA as a nitrogen source for supporting the future metabolism of the plant in order to grow bigger and taller (Bouché & Fromm, 2004).

## CONCLUSION

In overall, the germination process could increase the GABA content in the early stage of germinated black bean. The highest of GABA content was found at 24 h with the concentration at 4.78 mg/g. It was clearly demonstrated that germination process could stimulate the activity of glutamate decarboxylase and then produced more GABA. This study has suggested that it might be more useful to health improvement if the further research can apply this study to produce more black bean food products through each germination time of germinated black bean.

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## REFERENCES

- Amaral, J., Berrios, J. D. J., Pinto, V. Z., Antunes, M. D., Vanier, N. L., & Zavareze, R. 2016. Antioxidant activity of black bean (*Phaseolus vulgaris* L.) protein hydrolysates. *Food Science and Technology (Campinas)*, 36: 23–27.
- Bouché, N., & Fromm, H. 2004. GABA in plants: Just a metabolite? *Trends in Plant Science*, 9(3): 110–115.
- Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., & Eker, T. 2018. The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. *Agronomy*, 8(9): 166.
- Dhokal, R., Bajpai, V. K., & Baek, K. H. 2012. Production of GABA ( $\gamma$ -aminobutyric acid) by microorganisms: A review. *Brazilian Journal of Microbiology*, 43(4): 1230–1241.

- Ganesan, K., & Xu, B. (2017). Polyphenol-rich dry common beans (*Phaseolus vulgaris* L.) and their health benefits. *International Journal of Molecular Sciences*, 18(11): 2331.
- Kayembe, N. C., & Jansen van Rensburg, C. 2013. Germination as a processing technique for soybeans in small-scale farming. *South African Journal of Animal Sciences*, 43(2): 167–173.
- Kim, M., Abdou, A. M., Higashiguchi, S., Hatta, H., Horie, K., & Yokogoshi, H. 2006. Relaxation and immunity enhancement effects of  $\gamma$ -Aminobutyric acid (GABA) administration in humans. *BioFactors*, 26(3): 201–208.
- Matsuyama, A., Yoshimura, K., Shimizu, C., Murano, Y., Takeuchi, H., & Ishimoto, M. 2009. Characterization of glutamate decarboxylase mediating  $\gamma$ -amino butyric acid increase in the early germination stage of soybean (*Glycine max* [L.] Merr). *Journal of Bioscience and Bioengineering*, 107(5): 538–543.
- Pouliot-Mathieu, K., Gardner-Fortier, C., Lemieux, S., St-Gelais, D., Champagne, C. P., & Vuillemard, J. C. 2013. Effect of cheese containing gamma-aminobutyric acid-producing lactic acid bacteria on blood pressure in men. *Pharmanutrition*, 1: 141–148.
- Randomized, A., & Trial, D. 2018. Safety and efficacy of gamma-aminobutyric acid from fermented rice germ in patients with insomnia symptoms. *Journal of Clinical Neurology*, 14(3): 291–295.
- Vann Kimroen. 2019. Process improvement of GABA tofu production using *Lactobacillus plantarum* SKKL1. Master of Science Thesis in Biotechnology, Faculty of Technology, Khon Kaen University.
- Wang, F., Wang, H., Wang, D., Fang, F., Lai, J., Wu, T., & Tsao, R. 2015. Isoflavone,  $\gamma$ -aminobutyric acid contents and antioxidant activities are significantly increased during germination of three Chinese soybean cultivars. *Journal of Functional Foods*, 14: 596–604.
- Wani, I. A., Sogi, D. S., Wani, A. A., & Gill, B. S. 2014. Physical and cooking characteristics of some Indian kidney bean (*Phaseolus vulgaris* L.) cultivars. *Journal of the Saudi Society of Agricultural Sciences*, 16(1): 7–15.