

# GABA (GAMMA-AMINOBUTYRIC ACID) PRODUCTION OF MUNG BEAN (*PHASEOLUS AUREUS*) DURING GERMINATION AND THE COOKING EFFECT

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## Abstract

Gamma-aminobutyric acid (GABA), a four carbon amino acid produced by the decarboxylation of L-glutamic acid, provides many beneficial effects on human health including decreasing blood pressure and controlling stress. GABA is widely distributed in nature and also found in many kinds of germinated grains such as barley and brown rice. Mung bean (*Phaseolus aureus*) was used in this research to study GABA production during germination process. Mung bean was soaked and incubated for 0, 6, 12, 24, 36, and 48 h. The GABA content was determined by HPLC analysis. In addition, dietary fiber content, antioxidant activity, and cooking effect on GABA content of germinated mung bean were also evaluated. The important finding was that mung bean could be a potential source of GABA production. The amount of GABA significantly increased during germination and the highest production of GABA (80.68 mg/100 g dry weight) was found at 24 h of incubation period. The IDF is the main dietary fiber fraction in mung bean and its content increased up to 20.57 g/100g (dry weight) after soaking and incubation for 24 h. The DPPH activity of germinated mung bean slightly decreased and total phenolics content showed no significant change during germination. Additionally, all the cooking processes were found to decrease GABA in germinated mung bean. However, microwave was found to be the least destructive cooking method for GABA content in germinated mung bean.

Keywords: GABA, mung bean, dietary fiber, antioxidant, cooking effect

## Introduction

Gamma-aminobutyric acid (GABA, C<sub>4</sub>H<sub>9</sub>NO<sub>2</sub>) distributed in nature along with eukaryotes is non-protein amino acid. GABA is widely and prokaryotes which is produced by the

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decarboxylation of L-glutamic acid that catalyzed by glutamate decarboxylase enzyme (GAD) (Komatsuzaki *et al.*, 2007). It is well known that GABA functions in animals as a major inhibitory neurotransmitter. GABA provides many beneficial effects for human health such as decreasing blood pressure, controlling stress, diuretic effect, and tranquilizer effect (Matsuyama *et al.*, 2009). GABA production by various microorganisms has also been reported. The study of Komatsuzaki *et al.* (2007) showed the accumulation of GABA in several lactic acid bacteria (LAB), and the potential of GABA producing was depended on LAB strains and cultivation conditions. GABA was found to accumulate in plants such as germinated rough rice and brown rice (Moongnarm and Saetung, 2010), germinated barley (Chung *et al.*, 2009), buckwheat sprouts (Kim *et al.*, 2004), and green tea (Wang *et al.*, 2006). Nevertheless, a legume is one of the potential sources for GABA production. This is because of legumes contain rich amount of protein. Glutamic acid is the substrate for GABA production which one of the most abundant amino acids found in legumes such as soybean (Matsuyama *et al.*, 2009), faba bean (Khalil and Mansour, 1995) and mung bean (Mubarak, 2005).

Mung bean is an excellent source of protein with high vitamins and minerals. Its essential amino acid profile was comparable to that of soybean and kidney bean (Mubarak, 2005). Mung bean is inexpensive source of sprout. Mung bean sprout is the most popular bean sprout in Asian countries, especially China and Thailand. Several Thai traditional dishes have mung bean sprout as essential ingredients such as Pad-Thai and noodle. Randhir and Shetty (2007) reported that mung bean consumption showed a small increase in blood glycemic index in humans making it an attractive option for diabetic patients.

In addition, mung bean is a good source of dietary fiber. Dietary fiber could promote the movement of material through the digestive system. Dietary fibers play an important role in prevention of several diseases such as cardiovascular disorder, cancer, and diabetes (Girish *et al.*, 2012). Each of insoluble (IDF)

and soluble (SDF) dietary fractions has different physiological effect. The IDF relates to both water absorption and intestinal regulation, whereas SDF associates with cholesterol in blood and diminishes its intestinal absorption. The previous report revealed that the total dietary fiber was increased during germination process in mung bean, cowpea, lentil, and chickpea (Ghavidel and Prakash, 2007). Also, the total dietary fiber was increased in germinated lupin seed and soybean, but slightly changed in germinated black bean (Donangelo *et al.*, 1995).

Furthermore, phenolic antioxidant was one of the health beneficial components found in legumes (Lee and Lee, 2005). It was found to reduce oxidative damage that generally recognized as a cause of cell degeneration and related to cancer, inflammatory, and aging. Randhir and Shetty (2007) reported that general mung bean had low content of total phenolics. However its content could be improved by solid-state bioconversion.

Additionally, it is recommended to inactivate heat-labile antinutritional factors in raw mung bean before consumption. However, cooking processes can cause considerable losses of nutrients (Mubarak, 2005). As mentioned above, mung bean possesses several nutritional properties and has been used popularly. However, there is no report on GABA production during germination and the effect of cooking process on GABA loss in germinated mung bean has been presented. Therefore, the aim of this study was to investigate GABA production during germination of mung bean and cooking effect on its loss. Also, dietary fiber content, physicochemical composition and antioxidant activity of germinated mung bean were determined.

## Materials and Methods

### Germination of Mung Bean and GABA Content Analysis

Mung bean (*Phaseolus aureus*) was soaked in distilled water (1:5, w/v) for 6 h, drained, and incubated at room temperature for 0, 6, 12,

24, 36, and 48 h. The germination process was regulated to maintain near 100% relative humidity by sprayed water. The germinated mung bean was dried in hot air oven at 50°C for 8 h and used for further determination of GABA content, dietary fiber, physicochemical composition and antioxidant activity.

The analysis of GABA content was carried out by the modified method of Srisang *et al.* (2011). GABA was extracted with 3% sulfosalicylic acid (0.5 g/200 ml). It was then analyzed by dimethylaminoazobenzene derivatization and High Performance Liquid Chromatography using Supelcosil-LC-DABS column and detected under visible light 465 nm.

#### **Dietary Fiber Content, Physicochemical Compositions and Antioxidant Activity**

The contents of total (TDF), soluble (SDF) and insoluble dietary fiber (IDF) were determined by the enzymatic-gravimetric method modified from American Association of Cereal Chemists (AACC) methods 32-05 and 32-21 (AACC, 2001) using Total Dietary Fiber Assay Kit (Megazyme). The contents of protein, fat, ash, and carbohydrate were determined using the standard methods of Association of Official Analytical Chemists (AOAC, 1995).

The free-radical 2, 2-Diphenyl 1-picrylhydrazyl (DPPH) scavenging capacity of each extract was evaluated according to the procedure of Butsat and Siriamornpun (2010). Briefly, 0.1 ml of the sample extract was added to 1.9 ml freshly prepared 0.1 mM DPPH solution, then mixture was kept in dark room at room temperature for 30 min. The absorbance was measured at 517 nm. The total phenolics content was determined by the Folin-Ciocalteu method (Randhir and Shetty, 2007). Briefly, 1 ml of the sample extract was mixed with 1 ml of 95% ethanol and 5 ml of distilled water. To each sample Folin – Ciocalteu reagent was added to a final concentration of 50% (v/v) and mixed. After 5 min, 1 ml of 5% Na<sub>2</sub>CO<sub>3</sub> was added and the reaction mixture was allowed to stand for 1 h. The absorbance was measured at 725 nm. The result was expressed as mg of gallic acid per gram of dry weight sample.

#### **The Effect of Cooking Process on GABA Content**

The germinated mung bean was soaked in distilled water (1:5, w/v) for 3 h at room temperature to soften the grains and then treated with various cooking processes: boiling in distilled water 98-100°C for 20 min; steaming at 95-100°C in steaming pot for 40 min; and microwave cooking with 2450 MHz, 800 W (Sharp; model IEC 60705) for 10 min. Also non soaked germinated mung bean was roasted in open pan (90-95°C) for 10 min. Cooked bean was analyzed for their GABA content.

#### **Statistical Analysis**

All analyses were performed in triplicate. Statistical analyses were carried out with Duncan's multiple test ( $p < 0.05$ ) using statistical software SPSS V. 17 (SPSS Institute Inc., Cary, NC).

## **Results and Discussion**

#### **GABA Content, Total Phenolics and Antioxidant Activity**

In nongerminated mung bean, the concentration of GABA was 13.25 mg/100g dry matter. After germination, the amount of GABA significantly increased and up to 80.68 mg/100g dry matter at 24 h of incubation period. However there was no further increase after 24 h germination (Figure 1(a)). This indicates that the storage protein in mung bean was probably decomposed at least partially and supplied to the growing part of the seedlings and within this process glutamate decarboxylase enzyme was activated which converted glutamic acid to GABA. This result agrees with a previous report found in germinated brown rice that GABA was significantly increased during soaking and germination (Komatsuzaki *et al.*, 2007). It is difficult to compare the actual GABA content found in grains with difference in plant varieties and germination conditions. However, our study supports the fact that soaking and germination process were efficient processes to increase the GABA content in mung bean. In general, mung bean has higher

content of protein than that of brown rice. This contributes to higher GABA production in germinated mung bean than in germinated brown rice.

Phenolic compounds are known to exhibit free-radical scavenging (antioxidant) activity, which is determined by their reactivity as hydrogen or electron donors (Fernandez-Orozco, Frias, Zielinski, Piskula, Kozłowska, and Vidal-Valverde, 2008). The seed coat or husk of legumes possesses large quantities of phenolic compounds (Girish *et al.*, 2012). Also, The DPPH radical is considered to be a model of a stable lipophilic radical. Antioxidants react with DPPH radical, reducing the number of DPPH molecules equal to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH (Xu *et al.*, 2005). In germinated mung bean, the DPPH activity slightly decreased whereas total phenolics content showed no significant change during germination (Figure 1(b)). The results suggested that total phenolics cannot absolutely predict the activity of DPPH scavenging in germinated sample. In a previous research, Randhir and Shetty (2007) explained that the antioxidant attribute of mung bean extract may depend on the qualitative characteristics of phenolic profile and not just on the total amount of phenolics. For further study, it is beneficial to evaluate the antioxidant activity by two or more com-

plementary methods, such as measuring the  $\beta$ -carotene bleaching method (Randhir and Shetty, 2007).

### Dietary Fiber Content and Physicochemical Compositions

Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. It promotes beneficial physiological effects including laxation, blood cholesterol attenuation, blood glucose attenuation (AACC, 2001). Dietary fiber can be classified as either insoluble dietary fiber (IDF) or soluble dietary fiber (SDF). The insoluble (IDF), soluble (SDF) and total dietary fiber (TDF) contents in non germinated mung bean were 14.64, 0.66 and 15.30 g/100 g dry weight basis, respectively, indicating that the IDF is the main dietary fiber fraction in mung bean (Table 1). The IDF content increased up to 20.57 g/100 g dry weight basis after soaking and incubation for 24 h. Besides, SDF and TDF amounts also increased during germination. The results agree with the report of Ghavidel and Prakash (2007) that the total dietary fiber fractions of germinated mung bean were increased. This increasing could have been due to protein-fiber complexes formed after possible chemical modification induced by the soaking and germination (Bressani, 1993; Alajaji and El-Adawy, 2006), not the weight loss of geminated bean. The results also revealed

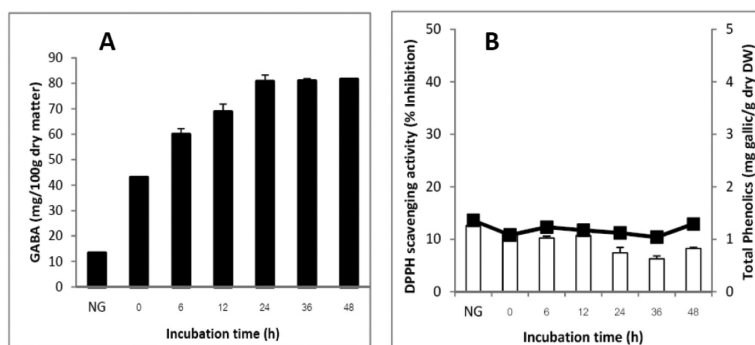


Figure 1. (A) GABA content and (B) DPPH scavenging activity (bar □) and total phenolics (line —) of germinated mung bean for different incubation periods. NG means non germinated mung bean. Data reported are the mean  $\pm$  SD of triplicate determinations

that IDF was found to be higher than the SDF. Thus, the incorporation of germinated mung bean fibrous in function food could be useful particularly in the development of foods with improved digestibility.

The protein content of non germinated mung bean was 24.57 g/100 g dry weight basis. After germination, the protein content increased up to 29.40 g/100 g dry weight basis at 48 h, while fat content was slightly changed. An increase of crude protein during germination in mung bean could be attributed to net synthesis of enzymes which might have resulted in the production of some amino acids during protein synthesis. However there was decreasing amount of ash and carbohydrates during germination. The former was probably due to leaching of solid matter during soaking process and resulted in mineral loss. The latter could be possibly explained by the consequence of its use as an energy source to start germination and increasing  $\alpha$ -amylase activity during germination. These results are in agreement with the decreasing ash and carbohydrates content during germination in mung bean (Mubarak, 2005)

### The Effect of Cooking Processes on GABA Content

The criterion of well cooked germinated mung bean was they became soften and complete starch gelatinization by laboratory

sensory evaluation and texture analysis (data not shown). However, different cooking process provided various textural properties of germinated mung bean. Therefore, the desirable characteristics of cooked mung bean should also be considered along with GABA content. The result showed that in germinated mung bean, all the cooking processes caused loss of GABA. Contents of GABA were remained some after boiling, steaming, microwave cooking and open pan roasting (6.34, 17.78, 21.84, and 18.34 mg/100 g dry matter, respectively). This suggests that boiling process caused great loss of GABA. These decreasing might be attributed to their diffusion into cooking water (Alajaji and El-Adawy, 2006). However microwave cooking process was the most efficient to prevent loss of GABA in germinated mung bean.

### Conclusions

Germination process caused significant increasing of GABA and dietary fiber production in mung bean. While, there was reduction in ash and carbohydrate content. The antioxidant activity and phenolics were found to be slightly changed. It is suggested that germinated mung bean could be a potential source of GABA and dietary fibers. Additionally, all the cooking processes found to decrease GABA in germinated bean. However, microwave cooking is recommended as suitable

**Table 1. Effect of incubation periods on the insoluble, soluble, and total dietary fiber and physicochemical composition of germinated mung bean (g/100 g dry weight basis)**

Incubation periods (h)	IDF	SDF	TDF	Protein	Fat	Ash	Carbohydrate
NG	14.64 <sup>d</sup> ±0.72	0.66 <sup>d</sup> ±0.05	15.30 <sup>d</sup> ±0.76	24.57 <sup>c</sup> ±0.10	1.70 <sup>a</sup> ±0.23	3.63 <sup>a</sup> ±0.07	69.92 <sup>a</sup> ±0.48
0	16.40 <sup>c</sup> ±0.93	1.42 <sup>c</sup> ±0.06	17.83 <sup>c</sup> ±0.94	24.31 <sup>c</sup> ±0.45	1.67 <sup>a</sup> ±0.32	3.21 <sup>b</sup> ±0.09	70.67 <sup>a</sup> ±0.45
6	18.74 <sup>b</sup> ±0.99	1.62 <sup>a,b,c</sup> ±0.17	20.36 <sup>b</sup> ±1.16	25.22 <sup>c</sup> ±0.16	1.66 <sup>a</sup> ±0.33	3.22 <sup>b</sup> ±0.04	69.75 <sup>a,b</sup> ±0.53
12	19.90 <sup>a,b</sup> ±0.90	1.50 <sup>b,c</sup> ±0.07	21.40 <sup>a,b</sup> ±0.96	26.31 <sup>b</sup> ±0.75	1.63 <sup>a</sup> ±0.26	3.19 <sup>b</sup> ±0.04	68.73 <sup>b,c</sup> ±0.54
24	20.57 <sup>a</sup> ±0.77	1.55 <sup>b,c</sup> ±0.05	22.12 <sup>a</sup> ±0.82	26.89 <sup>b</sup> ±1.14	1.63 <sup>a</sup> ±0.18	3.21 <sup>b</sup> ±0.05	68.12 <sup>c</sup> ±1.15
36	19.65 <sup>a,b</sup> ±0.68	1.66 <sup>a,b</sup> ±0.09	21.31 <sup>a,b</sup> ±0.60	26.42 <sup>b</sup> ±0.23	1.65 <sup>a</sup> ±0.23	3.19 <sup>b</sup> ±0.01	68.60 <sup>c</sup> ±0.10
48	18.96 <sup>b</sup> ±0.56	1.79 <sup>a</sup> ±0.18	20.75 <sup>a,b</sup> ±0.58	29.40 <sup>a</sup> ±0.50	1.66 <sup>a</sup> ±0.29	3.20 <sup>b</sup> ±0.01	65.59 <sup>d</sup> ±3.45

NG means non germinated mung bean. Data expressed as mean  $\pm$  SD of three independent experiments.

<sup>a-d</sup> Means in the same column with different letters are significantly different ( $p < 0.05$ ).

process help to retain the highest content of GABA in germinated mung bean.

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