Beans germination as a potential tool for GABA-enriched tofu production

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Abstract Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter that can be found in many plants, especially beans. Beans are normally used for producing vegetarian foods, such as bean milks, bean sprouts, and tofu. Thus, the aims of this study were to determine the GABA content in various germinated beans (yellow beans, black beans, green beans, and red beans) as well in tofu products made from different types of germinated beans. The results showed that soaking and germination significantly contributed to an increase in GABA production. The GABA content increased to a maximum value of 0.89, 3.09, 3.93 and 4.78 mg/g in yellow beans, red beans, green beans, and black beans, respectively. Moreover, due to the bean characteristics, green beans, red beans, and black beans were collected at 6 h after germination while yellow beans were collected at 0 h after germination. As a result, only yellow bean sprouts could be used for tofu production since they are composed of a high amount of proteins and a low amount of carbohydrates. The GABA content in tofu was 0.55 mg/g, which was lower than that in soybean milk (0.65 mg/g), likely due to the filtration and pressing processes of tofu production.

Keywords Beans · Germination · GABA · Tofu

Abbreviations

PS	Peeled split bean
NG	Nongerminated bean (whole bean seed)
0 h	Immediately after soaking
GABA	Gamma-aminobutyric acid

Introduction

Gamma-aminobutyric acid (GABA) is a secondary metabolite in plants that is produced by the decarboxylation of glutamic acid via glutamate decarboxylase (Li et al. 2016). Basically, it is considered to be an inhibitory neurotransmitter in the human nervous system, which is involved in relieving anxiety, relieving depression, reducing sleep difficulty and decreasing high blood pressure (Inoue et al. 2003; Chuang et al. 2011; Yamatsu et al. 2015; Ma et al. 2015). GABA is naturally found in many kinds of seeds such as beans, rice, and sesame. Their GABA content typically ranges from 0.03 to 2.00 $\mu\text{mol/g}$ -wet weight (Luo et al. 2018). Among these seeds, beans are not only a main source of GABA but also play an important role in overcoming human malnutrition. Beans, such as yellow beans (*Glycine max*), green beans (*Phaseolus aureus*), black beans (*Phaseolus vulgaris*), and red beans (*Vigna angularis*), have been considered as highly nutritious food ingredients since they are an inexpensive source of protein, carbohydrates, fiber, and phytochemical substances including GABA (Medic et al. 2014; Gohara et al. 2016; Saleh et al. 2018). Beans have been used in many kinds of foods, such as bean milks, tofu, and sweets. Among these foodstuffs, tofu is a popular traditional Asian food product, and it is predominantly used because of its inclusion in the vegetarian, vegan and hypocaloric diets. The increase in tofu consumption is motivated by the high nutritional value

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of tofu because it contains minerals (calcium, iron, and potassium), vitamins (thiamin B1; riboflavin B2; and niacin B3), essential amino acids, and some phytochemical compounds (Fukutake et al. 1996). Moreover, it consists of 6.0–8.4%, protein, 79–89%, water, 3%, ash, and the pH is around 5.2–6.2 (Medic et al. 2014).

Germination is a physiological process of plant seeds, which stimulates endogenous enzymatic activity and changes biochemical reactions. Recent studies discovered that soybean sprouts could be used as an alternative way to increase the nutritional quality of phytochemical contents, especially GABA (Kayembe and Jansen van Rensburg 2013). Hence, GABA could be produced from the decarboxylation reaction of glutamic acid catalyzed by glutamate decarboxylase (GAD) during germination (Wang et al. 2015a). The objective of this research was to determine the concentration of GABA in various germinated beans, including yellow beans (*G. max*), green beans (*P. aureus*), black beans (*P. vulgaris*), and red beans (*V. angularis*). Additionally, tofu products were also investigated utilizing different types of germinated beans.

Materials and methods

Bean germination

Yellow, green, red, and black beans were purchased from Khaothong Company, Thailand. The germination condition was revised from Tiansawang et al. (2016). A hundred seeds from each bean were collected randomly and soaked in tap water (1:5, w/v) for 12 h. Then, the soaked bean seeds were placed in a plastic basket lined with napkin paper and covered with wetted napkin paper for germination. The beans were observed at the different germination times of 0 h (immediately after soaking), 6 h, 12 h, 24 h, and 36 h. The germination percentages were calculated as expressed below:

$$\% \text{Germination} = \frac{\text{total number of bean sprouts}}{\text{total of bean seeds}} \times 100.$$

Additionally, each germinated bean was randomly selected for analysis at different germination times. Then, all samples were ground by using a high-speed blender for approximately 10 min and kept at 4 °C for GABA determination.

Bean milk preparation

The different germinated beans at a certain period of germination time were mixed with tap water at a ratio of 1:2 (w/v) and were ground at high speed for 10 min. Then, the

slurry was filtered to obtain bean milk. After that, all of the bean milk was cooked until it reached a temperature of approximately 80 °C for 20 min (Serrazanetti et al., 2013). The bean milk was kept at 4 °C for GABA analysis and tofu preparation.

Tofu preparation

Twenty-four milliliters of a tofu salt coagulant (2.55 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) was added to 1 L of bean milk and homogenized well for 15 min at 80 °C. Then, the curd formation of tofu was pressed to remove the excess water and left for approximately 2 h to obtain tofu (Shih et al. 2002; Serrazanetti et al. 2013). All samples were kept at 4 °C for GABA analysis.

Determination of GABA contents

GABA extraction

The bean sprout, bean milk, and tofu samples were extracted by following the modified method from Bai et al. (2009). One gram of each sample was extracted by mixing it with 4 mL of 4% (v/v) acetic acid. The mixtures were shaken at 150 rpm (room temperature) for 20 min and centrifuged at 12,000 rpm (16 K 230 V, EU; BIO-RAD Laboratories) for 10 min. The supernatant was kept in a deep freezer at − 20 °C for further analysis.

GABA determination by thin layer chromatography (TLC)

GABA content was determined by following the method from Lim et al. (2017). Two microliters of each sample was spotted onto TLC plates (TLC Silica gel 60 F₂₅₄, Merck), which were then put into TLC tanks. A TLC plate was developed with a solvent mixture of n-butanol, acetic acid, and deionized water (4:1:1, v/v/v). After development was complete, the plate was dried and sprayed with 0.5% (w/v) ninhydrin reagent. GABA spots were visualized after drying in a convection oven at 90 °C for 15 min.

GABA determination by high-performance liquid chromatography (HPLC)

GABA content was also determined by high-performance liquid chromatography (Waters, USA), which used a reverse-phase Inertsil ODS-3 column (4.6 × 250 mm, 5 μm; GL science Inc., Tokyo, Japan) and a postcolumn reaction module (Waters, USA). The mobile phase in this separation was carried out by following the modified methods from Ishida et al. (1985) and Villegas et al. (2016). To prepare the mobile phase, 0.5 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 1.25 g Na-P-toluenesulfonate were dissolved in

400 mL of deionized water. The solution was adjusted to pH 3.5 before the addition of 2.5 mL of absolute ethanol. The OPA (orthophthalaldehyde) reagent was modified from Vasani et al. (2000) and generated by dissolving 0.5 g of *N*-acetyl-L-cysteine in 400 mL of 0.2 M borate buffer and mixed well with 2.5 mL of ethanolic OPA (0.05 g of OPA in 2.5 mL of absolute ethanol). The flow rates of the mobile phase and OPA reagent were 0.50 mL/min and 0.20 mL/min, respectively. The extracts were separated at a column temperature of 40 °C and detected by a fluorescence detector with excitation at 340 nm and emission at 450 nm.

Statistical analysis

Analytical determination was performed 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 and discussion

The germination of various bean seeds

Normally, seeds consist of a seed coat that protects them during development and growth. Inside the seed, there is an embryo (the baby plant) and cotyledons. When the seed starts to grow, one part of the embryo becomes the plant, while the other part becomes the root of the plant. As the seed develops, the plants always grow upward whereas the roots always grow downward. Therefore, in this study, characteristics of each germinated bean were observed and counted regarding the embryo appearance from the seed coat. From this observation, green beans grew the fastest among the four types of beans since they were observed as being seedlings at 0 h. Their germination rapidly reached 100%. However, the appearances of the green beans at this condition were not different from those at 6 h and 12 h. Germination was markedly distinct at 24 h and 36 h because at these incubation times, the beans developed shoots and roots (Fig. 1a). Moreover, the yellow beans were the second most rapidly growing beans. Their germination increased dramatically from 0% to 79%, 82%, 87%, 91%, and 95% of nongerminated beans (NG) at 0 h, 6 h, 12 h, 24 h, and 36 h, respectively (Figs. 1b, 2). Red beans and black beans grew very slowly since the germination rate of these two beans were developed only after 24 h and 36 h, as indicated in Figs. 1c, d and 2.

The average seed sizes of green beans, yellow beans, red beans, and black beans had dimensions of approximately 4×6 mm, 5.5×7.5 mm, 6.3×9.1 mm, and

11.45×16.45 mm, respectively (Lumpkin et al. 1993; Soares et al. 2013; Wani et al. 2014). As reported by De Ron et al. (2016), seed germination is the process that commences with the uptake of water by dry seeds. The seeds grew well when their moisture content reached the optimum value of approximately 40–50%. Thus, these data have revealed that green beans were the smallest in size, so their water absorption might reach the optimum moisture content faster than the other beans. In agreement with Souza and Fagundes (2014), they reported that small seeds were more water permeable and germinate faster than large seeds since smaller seeds had thinner coats and higher relative surface areas. The sooner the radicle protrudes through the seed coat, the faster the germination. As a result, the germination rate of green beans was higher than that of the other beans, as shown in Fig. 2.

GABA content in germinated beans

Generally, yellow beans are predominantly used rather than other beans since it can produce many kinds of foodstuffs. Thus, the bean industry has provided two kinds of yellow beans: peeled split beans and whole beans (nongerminated beans). The GABA content in peeled split beans, the nongerminated beans, and the germinated yellow beans were compared in this study as shown in Fig. 3. According to the color spot on the TLC plate, GABA had already accumulated in nongerminated yellow beans since the GABA content in each bean sprout was close to 50 mg/L of the GABA standard concentration. However, the GABA content in black beans, red beans, and green beans probably increased from time to time. At 6 h after germination, bean sprouts likely provided more GABA than at the nongerminated time and at 0 h after germination. The GABA contents at 6 h were approximately 50–100 mg/L. However, TLC was just a simple method for predicting the GABA content. It is not precise or accurate enough to determine the amount of GABA in beans. Thus, it is ideal if the GABA content in each sample was confirmed with HPLC.

The results from HPLC demonstrated that the accumulation of GABA in all germinated beans at 0 h was significantly higher than in the nongerminated beans, as identified in Table 1. It was reported that the GABA content in nongerminated yellow beans was 0.19 mg/g, which was 1.7-fold higher than the peeled split bean (0.11 mg/g), but it was approximately four times lower than at 0 h of germination (0.84 mg/g). At 12 h, the GABA content in yellow beans reached a maximum at 0.89 mg/g, which was not significantly higher than the GABA content at 0 h. Thus, yellow beans were collected at 0 h for further experiments. This current study showed that the nongerminated yellow bean gave more advantages than the peeled

Fig. 1 The appearances of various bean sprouts through different germination times at 0 h, 6 h, 12 h, 24 h, and 36 h. **a** Green bean, **b** yellow bean, **c** red bean, **d** black bean

Fig. 2 The percentages of germination from various bean seeds at nongermination time (NG), 0 h, 6 h, 12 h, 24 h, and 36 h

split bean in terms of the GABA content and its cost. In Thailand market, the nongerminated yellow bean was sold at 0.67 USD (21 Bahts) per 500 g, while the price of peeled split bean was 0.70 USD (22 Bahts) per 500 g. It is indicated that the cost of nongerminated yellow bean was approximately 4.55% cheaper than that of the peeled split bean, and it could be used as the main source to get more GABA and bean sprouts through the germination process. On the other hand, the peeled split bean was not able to germinate since the seed was already broken and split in half, which is considered as the dead seed.

The accumulation of GABA in green beans and red beans dramatically increased over the germination time. The concentration of GABA in these two beans reached a maximum at 3.93 mg/g and 3.09 mg/g, respectively, after 36 h, whereas the GABA content in black beans increased slightly until 6 h and then dramatically increased to reach a maximum at 4.78 mg/g 24 h after germination. However, black bean sprouts, red bean sprouts, and green bean sprouts at 6 h were collected for making tofu in subsequent experiments since this incubation period provided more GABA than at 0 h and was more convenient for producing bean milk. The sample collection also depended on the odor of each bean sprout since the prolonged germination affected the flavor with the extension of the germination period.

Interestingly, the results clarified by TLC and HPLC indicated that soaking and germination contributed to an increase in GABA in all bean sprouts. The increasing GABA content in each germinated bean might be relevant to the extensive breakdown of the seed-storage compounds and synthesis of structural proteins and other cell compounds during germination to respond to the requirement of plant growth (Khandelwal et al. 2010). During germination, GABA can increase due to the glutamic acid content in the bean sprouts. Glutamic acid could be synthesized by two different reaction pathways. First, it

Fig. 3 GABA contents in different types of germinated beans at different germination periods. PS and NG denote peeled split bean and nongerminated bean, respectively

could be from carbohydrates bypassing pyruvate, glycolysis, and the Krebs cycle. Then, glutamic acid would be produced by the amination reaction of α -ketoglutarate to glutamic acid by glutamate dehydrogenase (Forde and Lea 2007). The second pathway involves glutamic acid being produced by an aminotransferase reaction with the amino nitrogen (donated by a number of different amino acids) to glutamic acid (Bowsher et al. 2007). Moreover, germination also cause an important change in the biochemical

reactions in the plant metabolism, and these changes also stimulate many enzymes, especially glutamate decarboxylase, continuously converting glutamic acid into GABA (Wang et al. 2015a). According to the plant growth metabolism, the GABA or glutamic acid content in the plant would decrease after its content reached a maximum because the rapid accumulation of GABA and glutamic acid happened only in the early stages of seed germination (Matsuyama et al. 2009). This process is likely because

Table 1 The concentration of GABA (mg/g, dry weight) in various germinated beans

Incubation time	Yellow bean	Green bean	Red bean	Black bean
PS	0.11 ± 0.01 ^a	–	–	–
NG	0.19 ± 0.01 ^b	0.26 ± 0.02 ^a	0.33 ± 0.03 ^a	0.61 ± 0.03 ^a
0 h	0.84 ± 0.03 ^c	1.20 ± 0.16 ^b	0.92 ± 0.09 ^b	1.47 ± 0.01 ^b
6 h	0.77 ± 0.02 ^{cd}	1.87 ± 0.03 ^c	1.47 ± 0.02 ^c	1.78 ± 0.09 ^c
12 h	0.89 ± 0.01 ^c	2.48 ± 0.17 ^d	2.30 ± 0.07 ^d	1.43 ± 0.01 ^b
24 h	0.61 ± 0.02 ^e	2.73 ± 0.01 ^d	2.75 ± 0.04 ^e	4.78 ± 0.01 ^d
36 h	0.71 ± 0.02 ^{cd}	3.93 ± 0.20 ^e	3.09 ± 0.04 ^f	1.89 ± 0.11 ^c

PS peeled split bean, NG nongerminated bean

Different letter superscript within the same column indicated a statistically significant difference ($p < 0.05$)

bean sprouts need to use the nitrogen source to form the cotyledon and expand to synthesize chlorophyll in the developing leaves (Yaronskaya et al. 2006).

Tofu production from various bean seeds

According to the tofu manufacturing, the coagulation was observed after adding magnesium chloride into bean milk due to the electrostatic interactions between the proteins and the cations (Li Tay et al. 2006). The cross-link between the negatively charged proteins and metal ions (Mg^{2+}) leads to aggregation of large molecules, which are separated from the liquid part of bean milk. As shown in Fig. 4a, higher protein content was observed in yellow bean than those of green bean, red bean and black bean since the yellow bean was composed of large amount of proteins (40%) and contain fewer carbohydrates (35%) than the other beans (Medic et al. 2014). After pressing, the tofu has been formed with a familiar smell and a firm texture.

As shown in Fig. 4b, c, the tofu made by green bean and red bean was formed after filtering and removing excess water. However, the texture and the smell of tofu from these germinated beans were not appreciated because these tofu products did not hold their shapes well and showed strong bean-sprout smell, which was not recognized to be tofu.

Compared to other beans, the black bean could not be used to make tofu since the proteins in the black bean did not precipitate after adding the salt. The black bean milk solution became more viscous as shown in Fig. 4d, which might be relevant to the large amount of starch and less protein content in the black bean (Amaral et al. 2016). Thus, during bean milk heating, the starch molecules (amylose and amylopectin molecules) in the bean were passed through the processes of gelatinization and retrogradation. The starch molecules were combined with water with specific ratio, the individual starch granules absorb the liquid and swell. Then, amylose and amylopectin were released from the starch granules and

dissolved in water by heating. The solution showed the high viscosity and changed into the strong gelling state (retrogradation) due to the intermolecular association between amylopectin and amylose by hydrogen bonding (Eliasson 2010; Wang et al. 2015b). The result of this study showed that green beans, red beans, and black beans were not suitable for producing tofu because they contained more carbohydrates (55.4%, 65.7%, 71.4%; respectively) and fewer proteins (21.8%, 6.7%, 23.1%, respectively) than those of yellow beans (Schirmer et al. 2015; Amaral et al. 2016).

The HPLC results indicated that the concentration of GABA in tofu was 0.55 mg/g, which was lower than that in soybean milk (0.65 mg/g). This finding might be attributed to the high water solubility of GABA, which GABA might be lost during tofu preparation processes, for example, the filtration and mechanical pressing. Therefore, this study is the first report presenting the possibility to produce GABA tofu from various beans.

Conclusion

In conclusion, the germination process could be used as an alternative way to increase GABA concentration in bean sprouts, including green beans, red beans, black beans, and yellow beans. This result indicated that the storage proteins in beans changed to supply nutrients to the growing parts of seedling formation, and within this process, glutamate decarboxylase was activated, which automatically converted glutamic acid to GABA. The result also justified that among four different kinds of beans—green beans, yellow beans, red beans, and black beans—only yellow beans were able to produce tofu in the traditional way because they contain a lower amount of carbohydrates and a high amount of proteins. On the basis of the bean characteristics, this study suggested that germinated yellow beans at 0 h could be used as a substitute for peeled split beans and whole beans to produce GABA-enriched soybean milk and tofu products. Meanwhile, the GABA content in yellow

Fig. 4 The characteristic of each bean during making conventional tofu. **a–d** Indicate yellow bean, green bean, red bean, and black bean, respectively. 1, 2, 3, and 4 represent the appearance of each bean after milk boiling, salt adding, filtering, and pressing, respectively

bean sprouts was four- and eightfold higher than that in the whole bean and the peeled split bean, respectively. As a result, the products from germinated yellow beans at 0 h might be found to be effective in improving health.

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