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Effect of Starter Cultures and Storage Conditions on the Gamma-Aminobutyric Acid and Phytic Acid Contents in a Yoghurt-Like Product Made from Germinated Hyacinth Beans

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Abstract

Hyacinth beans were used for developing a functional yoghurt-like product with a high level of gamma aminobutyric acid (GABA). Germinated hyacinth bean extract was fermented with three different yoghurt cultures, namely (1) Lyofast Y 258 A and (2) Lyofast Y 450 B, containing a combination of *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, and (3) XPL-20 containing *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis*, and *S. thermophilus*, incubated to pH 4.5, and then stored at 4-6°C for 21 days. The variations in GABA and phytic acid levels during cold storage were evaluated. The type of culture and storage time significantly influenced the GABA and phytic acid levels. The mixture of *Lactococcus lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis*, and *Streptococcus thermophilus* produced a yoghurt-like product with the highest level of GABA (2.2 mg/g DM). In addition, the levels of GABA and phytic acid significantly decreased during cold storage. This study provides a foundation for the development of yoghurt-like products from water hyacinth beans with enhanced GABA content.

Keywords

Hyacinth bean yoghurt, gamma-aminobutyric acid, phytic acid, youghurt culture, cold storage

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Introduction

Hyacinth bean (*Lablab purpureus* (L.) Sweet) is a tropical and subtropical legume originating from Africa (Subagio, 2006; Naeem *et al*., 2020), but has also been widely cultivated in Europe, America, the Middle East, and Asia (Mohan & Mellem, 2020). Its seeds are a valuable source of protein, available carbohydrates, dietary fiber, and essential minerals such as phosphorus, calcium, and iron (Akpapunam, 1996; Naeem *et al*., 2020), as well as flavonoids like

kievitone and genistein (Naeem *et al*., 2020). However, hyacinth bean seeds also contain anti-nutritional factors such as phytic acid, trypsin inhibitors, and tannins (Naeem *et al*., 2020).

Among the bioactive compounds of nutritional interest, γ-aminobutyric acid (GABA) is a four-carbon non-protein amino acid with multiple health-promoting properties, including functioning as an inhibitory neurotransmitter, regulating blood pressure and heart rate, and alleviating physical pain and anxiety (Park & Oh, 2007). GABA is synthesized by glutamate decarboxylase (EC 4.1.1.15) through the decarboxylation of L-glutamic acid, highlighting the critical role of both glutamate decarboxylase activity and L-glutamic acid availability in its formation (Zhang *et al*., 2014). According to Akpapunam (1996), hyacinth bean seeds are rich in glutamic acid. During germination or fermentation, endogenous or exogenous proteases hydrolyze proteins into amino acids, including glutamic acid, which serves as the precursor for GABA synthesis.

In past decades, germination has been widely used to enhance GABA and isoflavone levels in adzuki beans and soybeans (Liao *et al*., 2013; Xu & Hu, 2014), and to improve the apparent protein content and protein quality due to dry-matter loss and increased protein digestibility (Ghavidel & Prakash, 2007). Additionally, germination contributes to reducing the undesirable "beany" flavor in soybean-derived products (Wang *et al*., 2015). A recent study by Nguyen Thi Thom *et al*. (2021) demonstrated that the germination of hyacinth beans significantly increases GABA levels while reducing the phytic acid content.

Hyacinth beans are consumed in different forms such as unripe pods and mature green beans (Athmaselvi *et al*., 2020). The dry seeds are also commonly used in tempeh production or cooked with rice as a protein supplement (Subagio, 2006). Recent studies have reported that hyacinth bean seeds contain 20-25% protein (Athmaselvi *et al*., 2020; Mohan & Mellem, 2020), suggesting their potential as a raw material for producing protein-based ingredients (Athmaselvi *et al*., 2020). Despite these nutritional attributes, no previous study has reported the development of GABA-enriched yoghurt-like products from hyacinth beans.

Plant-based yoghurt-like products have gained increasing attention due to growing consumer demand for dairy alternatives and functional foods. These products are typically produced by fermenting plant protein sources such as soybeans, almonds, coconuts, oats, and various legumes with lactic acid bacteria (LAB) traditionally used in dairy yoghurt production (Mårtensson *et al*., 2002; Bernat *et al*., 2015). Common starter cultures include *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and probiotic strains such as *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, and *Bifidobacterium* species, which contribute to acidification, flavor development, and textural properties (Rivera-Espinoza & Gallardo-Navarro, 2010). Fermentation not only improves the sensory quality and digestibility but also enhances the nutritional and functional attributes of plant substrates by increasing the levels of bioactive compounds such as GABA, phenolics, and peptides, while reducing anti-nutritional factors like phytic acid and trypsin inhibitors (Sawant *et al*., 2025).

Despite the nutritional potential of hyacinth beans, research on their application in fermented plant-based yoghurt-like products remains very limited, and no study to date has explored their use for producing GABA-enriched fermented foods. The present study aimed to develop a functional yoghurt-like product using germinated hyacinth beans in combination with different yoghurt cultures, and to investigate the changes in GABA and phytic acid levels during cold storage.

Materials and Methods

Chemicals and starter cultures

The hyacinth bean seeds were collected from a local supermarket in Hanoi, Vietnam. The GABA standard was purchased from Sigma Aldrich (St. Louis, MO, USA). Phytic acid sodium salt hydrate was purchased from Sigma Aldrich (Buchs, Switzerland). The yoghurt cultures were (1) Lyofast Y 258 A and

(2) Lyofast Y 450 B, containing a combination of *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Sacco, Italy), and (3) XPL-20 containing *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis* and *S. thermophilus* (Chr-Hansen, Denmark).

Preparation of the yoghurt-like product

The hyacinth bean seeds were soaked in tap water at 36°C for 10h. The soaking water was exchanged for fresh water at 2-hour intervals. After being removed from the soaking water, the seeds were drained and then germinated at 30°C for 36 hours in an incubator at a relative air humidity of 65%. The germinated seeds were ground with hot water at a 1:5 ratio (w/v) at 80°C and then filtrated through cheesecloth. The slurry was mixed with sugar, whey powder, whole milk powder, and butter oil (8, 1, 4, and 2% (w/v), respectively), and this mixture was called "hyacinth bean extract". After heating at 90°C for 5 min and cooling to 43°C, the hyacinth bean extract was divided into three equal portions and then inoculated with the yoghurt cultures. A 5% (v/v) inoculum of the yoghurt starter cultures, namely Lyofast Y 258 A, XPL-20, or Lyofast Y 450 B, was added to the hyacinth bean extract to produce yoghurt. The samples were coded as Y-258, Y-XPL, and Y-450, respectively. The inoculated extracts were incubated at 43°C until the pH reached 4.5 and then stored at 4-6°C for 1, 7, 14, and 21 days.

pH and titratable acidity

The pH of the yoghurt-like samples was measured using a pH meter (SevenCompact S220, Mettler Toledo, Switzerland). Titratable acidity was determined according to ISO/TS 11869:2012. In brief, the yoghurt-like samples (10 ± 0.001g) were thoroughly mixed with 10 mL of ultrapure water and then titrated with 0.1 mol L^{-1} NaOH until reaching the pH 8.30 ± 0.01. The titratable acidity was expressed in millimoles NaOH per 100g.

Analysis of gamma-aminobutyric acid

GABA was determined using a colourimetry method according to Zhang *et al.* (2014). In brief, 5mL of ultrapure water was added to the unfermented hyacinth bean extract and yoghurt-like samples (1 ± 0.001g) and gently shaken for 1 hour. After filtering through Whatman filter paper, 0.2mL of 0.2 M borate buffer (pH 9.0), 1mL of 6% phenol, and 0.4mL of 9% sodium hypochlorite were added to 0.5mL of the filtrate. The mixtures were shaken vigorously and then incubated in boiling water for 10min followed by an ice bath for 20min. Finally, 2mL of 60% ethanol was added to the mixtures.

A series of GABA calibration standards using GABA standard solutions (0, 0.04, 0.08, 0.12, 0.16, and 0.2 mg mL^{-1}) were prepared as described for the samples. The absorbances of the sample solutions and calibration standards were measured at 645 nm using a UV-VIS spectrophotometer (UV 1800, Shimadzu, Japan). The level of GABA was expressed as mg GABA g^{-1} dry matter.

Analysis of phytic acid

Phytic acid was determined using a colourimetry method according to Gao *et al.* (2007) with some minor modifications. The unfermented hyacinth bean extract and the yoghurt-like samples (0.5 ± 0.001g) were mixed with 10mL of 2.4% HCl and shaken at 220rpm for 16 hours. After centrifuging at 1000rpm for 20min at 10°C, the supernatants were decanted off and 0.5g NaCl (purity 99.5%) was added to each supernatant. The mixtures were then shaken at 350rpm for 20min, stored at -20°C for 20min, and then centrifuged at 1000rpm for 20min at 10°C. The clear supernatant was removed and diluted with ultrapure water at a 1:24 ratio (v/v). Wade solution (0.03% $FeCl_3.6H_2O$ + 0.3% sulfosalicylic acid) was added to the diluted solution at a 1:3 ratio (v/v) and then the mixture was centrifuged at 1000rpm at 10°C for 10min.

A series of calibration standards using phytic acid sodium salt hydrate standard solution (0; 5; 10; 20; 40; and 80 µg mL^{-1}) were prepared as described for the diluted solution. The absorbances of the sample solutions and the calibration standards were measured at 500nm using a UV-VIS spectrophotometer (UV 1800, Shimadzu, Japan). The phytic acid level was expressed as mg phytic acid g^{-1} dry matter.

Data analysis

All analyses were performed in triplicate and data were presented as mean ± standard deviation. Statistical analyses were performed with Minitab 16. Data were analyzed using a two-way analysis of variance within a general linear model to evaluate the effects of yoghurt culture and storage time and their interaction, according to the following model: $y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$, where μ is the overall mean; α_i is the i^{th} yoghurt culture; β_j is the j^{th} storage day; $(\alpha\beta)_{ij}$ is the yoghurt culture-storage day interaction; and ε_{ij} is the experimental error. Tukey's multiple range test was applied to compare means among treatment combinations with $P < 0.05$.

Results and Discussion

pH and titratable acidity

Whey powder, which contains a substantial amount of lactose in addition to proteins and minerals, was added to the hyacinth bean extract to increase the availability of fermentable substrates for lactic fermentation. After incubating at 43℃ for approximately 8 h, the pH for all the hyacinth bean extracts was 4.5. The titratable acidity of the yoghurt-like products during storage are presented in **Table 1**. The data show that pH and titratable acidity were significantly influenced by the yoghurt culture and storage time ($P < 0.001$). The interaction between the yoghurt culture and storage time was also significant for pH ($P < 0.01$) and titratable acidity ($P < 0.001$).

As shown in **Table 1**, the pH for all the yoghurt-like products significantly decreased during storage at 4-6℃ ($P < 0.001$). The pH values decreased from 4.44-4.47 on day 1 to 4.32-4.39 on day 21. On the other hand, the pH values were significantly different among the yoghurt-like samples made from the three different cultures after 1, 7, 14, and 21 days of cold storage ($P < 0.001$). The pH value for Y-XPL was the highest followed by that for Y-258 and Y-450 (**Table 1**).

Titratable acidity significantly increased during cold storage (**Table 1**). The titratable acidity values varied from 7.42-7.91 milimoles NaOH $100g^{-1}$ on day 1 to 8.14-9.07 milimoles NaOH $100g^{-1}$ on day 21. The results also showed that the titratable acidity values were statistically different among the cultures. At all periods of storage, the titratable acidity value for Y-450 was higher than that for Y-258 and Y-XPL.

The levels of gamma-aminobutyric acid

The levels of GABA in the unfermented hyacinth bean extracts and yoghurt-like products are presented in **Table 2**. Yoghurt culture and storage time strongly affected the level of GABA in the yoghurt-like samples ($P < 0.001$). The

Table 1. pH and titratable acidity of the yoghurt-like products

Yoghurt culture	pH				Titratable acidity (millimoles NaOH $100g^{-1}$)			
	1 d	7 d	14 d	21 d	1 d	7 d	14 d	21 d
Lyofast Y 258 A	4.47^{a} ± 0.01	4.45^{ab} ± 0.01	4.37^{d} ± 0.01	4.34^{de} ± 0.02	7.64^{fg} ± 0.08	7.75^{ef} ± 0.00	8.36^{bc} ± 0.06	8.36^{bc} ± 0.01
XPL-20	4.47^{a} ± 0.01	4.46^{a} ± 0.01	4.40^{c} ± 0.01	4.39^{c} ± 0.01	7.42^{g} ± 0.17	7.58^{fg} ± 0.11	7.66^{f} ± 0.06	8.14^{cd} ± 0.06
Lyofast Y 450 B	4.44^{ab} ± 0.01	4.42^{bc} ± 0.01	4.35^{de} ± 0.01	4.32^{e} ± 0.01	7.91^{de} ± 0.08	8.05^{d} ± 0.02	8.47^{b} ± 0.05	9.07^{a} ± 0.10

Note: Data are expressed as mean value ± standard deviation (n = 3).

LYOFAST Y 258 A contains Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus

XPL-20 contains Lactococcus lactis ssp. cremoris, Lactococcus lactis ssp. lactis, and Streptococcus thermophilus

LYOFAST Y 450 B contains Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus

Different letters indicate significant differences among treatment combinations based on two-way ANOVA and Tukey's test (P <0.05).

Table 2. GABA levels in the yoghurt-like products

Yoghurt culture	GABA level (mg g^{-1} DM)				
	UFE	1 d	7 d	14 d	21 d
Lyofast Y 258 A	3.18^{a} ± 0.15	1.90^{c} ± 0.00	1.88^{c} ± 0.03	1.71^{cd} ± 0.00	1.59^{def} ± 0.04
XPL-20	3.18^{a} ± 0.15	2.20^{b} ± 0.01	2.15^{b} ± 0.01	2.12^{b} ± 0.02	1.64^{de} ± 0.09
Lyofast Y 450 B	3.17^{a} ± 0.14	1.68^{cde} ± 0.01	1.65^{de} ± 0.05	1.47^{ef} ± 0.06	1.39^{f} ± 0.02

Note: Data are expressed as mean value ± standard deviation (n = 3).

LYOFAST Y 258 A contains Streptococcus thermophilus and Lactobacillus delbrueckii ssp. Bulgaricus.

XPL-20 contains Lactococcus lactis ssp. cremoris, Lactococcus lactis ssp. lactis, and Streptococcus thermophilus.

LYOFAST Y 450 B contains Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus

Different letters indicate significant differences among treatment combinations based on two-way ANOVA and Tukey's test (P <0.05).

interaction between the yoghurt culture and storage time was also significant (P <0.001). The data showed that the level of GABA rapidly decreased after 1 day of storage and significantly decreased as the storage time extended. The levels of GABA in the yoghurt-like products were much lower than those in the unfermented hyacinth bean extracts (**Table 2**).

These results are opposite to those previous reported by Liao *et al.* (2013), who showed that the level of GABA increased during the fermentation and storage of fermented adzuki bean extract. These findings suggest that the GABA-producing/degrading ability of lactic acid bacteria (LAB) during fermentation and storage possibly depend on the species. To the best of our knowledge, there are very little data reported in the literature for GABA content in hyacinth beans or fermented hyacinth bean extract. Previous studies have mainly focused on the variation in GABA in yoghurt-like products made from other legumes (Park & Oh, 2007; Liao *et al.*, 2013; Nguyen Duc Doan & Do Thi Ha, 2020) or animal milk (Abd El-Fattah *et al.*, 2018). Although the level of GABA decreased during storage, the data obtained in the present study showed that the levels of GABA in the yoghurt-like products made from germinated hyacinth beans were much higher than that previously reported in soymilk yoghurt (Park & Oh, 2007; Nguyen Duc Doan & Do Thi Ha, 2020) and cow's milk yoghurt (Abd El-Fattah *et al.*, 2018).

According to Park & Oh (2007) and Liu *et al.* (2015), *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* did not significantly influence the level of GABA, meanwhile other LAB such as *L. brevis*, *Lc. lactis*, and *L. rhamnosus* could enhance the level of GABA. As shown in **Table 2**, the level of GABA was significantly different among the yoghurt-like products made from different cultures at all periods of storage. Its level in Y-XPL was higher than that in Y-258 and Y-450. Therefore, the degradation of GABA might depend on the strains used for fermentation. The decline in GABA during storage was likely related to its conversion to succinic acid through the GABA-shunt pathway, in which GABA is first converted into succinic semialdehyde and subsequently oxidized to succinic acid (Bouche & Fromm, 2004).

The levels of phytic acid

Phytic acid is an anti-nutrient present in plant-derived foods such as cereals and legumes (Tang *et al.*, 2010). The levels of phytic acid in the unfermented hyacinth bean extracts and yoghurt-like products are shown in **Table 3**. The phytic acid levels during storage were significantly influenced by the yoghurt cultures (P <0.05) and storage time (P <0.001). The interaction between yoghurt culture and storage time was not significant (P >0.05). The levels of

Table 3. Phytic acid levels in the yoghurt-like products

Yoghurt culture	Phytic acid level (mg/g DM)				
	UFE	1 d	7 d	14 d	21 d
Lyofast Y 258 A	21.43^{a} ± 0.09	11.73^{bc} ± 0.06	11.71^{bc} ± 0.09	11.45^{bc} ± 0.06	11.40^{c} ± 0.08
XPL-20	21.42^{a} ± 0.19	11.82^{b} ± 0.12	11.81^{b} ± 0.09	11.58^{bc} ± 0.10	11.47^{bc} ± 0.19
Lyofast Y 450 B	21.42^{a} ± 0.17	11.67^{bc} ± 0.09	11.61^{bc} ± 0.24	11.39^{c} ± 0.04	11.36^{c} ± 0.08

Note: Data are expressed as mean value ± standard deviation (n = 3).

LYOFAST Y 258 A contains Streptococcus thermophilus and Lactobacillus delbrueckii ssp. Bulgaricus.

XPL-20 contains Lactococcus lactis ssp. cremoris, Lactococcus lactis ssp. lactis, and Streptococcus thermophilus.

LYOFAST Y 450 B contains Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus

Different letters indicate significant differences among treatment combinations based on two-way ANOVA and Tukey's test (P <0.05).

phytic acid in the yoghurt-like products were much lower than that in unfermented hyacinth bean extract (**Table 3**), probably because of its degradation by the enzyme phytase released from the yoghurt cultures (Bilgiçli & Ýbanoðlu, 2007; Tang *et al.*, 2010).

Table 3 also shows that during cold storage, the levels of phytic acid significantly decreased from 11.67-11.82 mg g^{-1} dry matter on day 1 to 11.36-11.47 mg g^{-1} dry matter on day 21. This finding is similar to that in soymilk yoghurt reported by Nguyen Duc Doan & Do Thi Ha (2020). To the best of our knowledge, this is the first data to report on phytic acid in yoghurt-like products made from hyacinth beans.

Table 3 shows that the level of phytic acid varied significantly among the cultures. Y-XPL contained the highest phytic acid level followed by Y-450 and Y-280. In recent years, several studies have suggested that *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*, and strains of *L. acidophilus, L. casei*, and *L. plantarum* could degrade phytic acid during the fermentation of soymilk (Tang *et al.*, 2010; Nguyen Duc Doan & Do Thi Ha, 2020). Our findings indicate that fermentation of hyacinth beans with yoghurt cultures might potentially improve mineral bioavailability.

Conclusions

This study demonstrated that hyacinth bean is a promising substrate for the development of a functional yoghurt-like product, as it allows for the enhancement of beneficial bioactive compounds while reducing antinutritional factors, thus offering potential health-promoting properties. The type of yoghurt culture and period of storage significantly influenced the levels of GABA and phytic acid. We have demonstrated for the first time that *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis*, and *S. thermophilus* (XPL-20) are suitable starter cultures for germinated hyacinth bean extract, as they promoted higher GABA formation compared with the other cultures tested. Future research should investigate the sensory properties of the yoghurt-like product to assess consumer acceptability and optimize product formulation.

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