



저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

A THESIS FOR THE DEGREE OF
MASTER OF SCIENCE IN FOOD AND NUTRITION

Production of γ -Aminobutyric Acid
during Fermentation of *Gastrodia elata*
Bl. by *Lactobacillus brevis* GABA 100

Lactobacillus brevis GABA 100을 이용하여
 γ -aminobutyric acid를 함유한 발효 천마 개발

August, 2013

Department of Food and Nutrition

The Graduate School

Seoul National University

Jeong Ah Kim

Abstract

Production of γ -Aminobutyric Acid during Fermentation of *Gastrodia elata* Bl. by *Lactobacillus brevis* GABA 100

Jeong Ah Kim

Department of Food and Nutrition

The Graduate School

Seoul National University

Gastrodia elata Bl. (GE) is a traditional herbal medicine used for the prevention of cerebrovascular disease and regulation of blood pressure. The γ -aminobutyric acid (GABA) is known to be beneficial for preventing neurological disorders and hypertension. The objective of this study was the development of fermented GE products containing high levels of GABA. Optimal medium conditions for the production of GABA during fermentation were as follows: initial pH 6.5, 3% L-monosodium glutamate, 10% GE powder, and 0.5% yeast extract. The production of GABA was

further enhanced by the co-culture of *Lactobacillus brevis* GABA 100 (*L. brevis* GABA 100) with *Bifidobacterium bifidum* BGN4 (*B. bifidum* BGN4). Several nutritional materials and phenol compounds of the fermented GE were analyzed by HPLC. During fermentation, high amounts of organic acids and GABA were produced while the gastrodin (GAS), the main polyphenol compound of GE, was completely converted to 4-hydroxybenzyl alcohol (HBA). The fermented GE product with GABA was successfully produced by optimizing the fermentation conditions. These findings suggest an opportunity to develop functional GE products with GABA.

Keywords

γ -aminobutyric acid (GABA), *Gastrodia elata* Bl., fermentation, 4-hydroxybenzyl alcohol, *Lactobacillus brevis*, *Bifidobacterium bifidum*

Student Number: 2011-23686

Contents

Abstract	i
Contents	iii
List of Figures	v
List of Tables	vi
List of Abbreviations	vii
INTRODUCTION	1
MATERIALS AND METHODS	4
2.1. Bacterial strains and viable cell counts	4
2.2. Reagents and sample	5
2.3. Scale-up fermentation for the production of GABA	6
2.4. Qualitative analysis of GABA and L-monosodium glutamate by thin layer chromatography (TLC)	7
2.5. Determination of valuable nutrients and phenolic compounds by high-performance liquid chromatography (HPLC)	8
RESULTS AND DISCUSSION	11
3.1. Bacterial cell growth and GABA production during GE fermentation	11
3.2. Effect of co-culture of <i>L. brevis</i> GABA 100 with <i>B. bifidum</i> BGN4 on the production of GABA	14

3.3. Effects of carbon and nitrogen sources on the production of GABA	19
3.4. Scale-up production of the fermented GE	22
3.5. The assessment of glutamate and GABA in the scale-up fermentation of GE	26
3.6. The assessment of gastrodin and 4-hydroxybenzyl alcohol in the scale-up fermentation of GE	29
CONCLUSION	31
REFERENCES	32
KOREAN ABSTRACT	39

List of Figures

Figure 1. Cell numbers of <i>L. brevis</i> GABA 100 in the fermented GE medium	13
Figure 2. Cell numbers of lactic acid bacteria in MRS medium	16
Figure 3. TLC chromatogram of the GABA produced in MRS medium	17
Figure 4. TLC chromatogram of the GABA produced in 4 days fermentation of GE medium	18
Figure 5. TLC chromatogram of the GABA produced in the fermented GE medium (The effect of yeast extract)	21
Figure 6. Comparison of organic acids levels between GE and the scale-up fermented GE	24
Figure 7. Comparison of free amino acids levels between GE and the scale-up fermented GE	25
Figure 8. Conversion of gastrodin to 4-hydroxybenzyl alcohol of the scale-up fermented GE	30

List of Tables

Table 1. Operation conditions of HPLC analysis of phenolic compounds of the fermented GE	10
Table 2. Content of glutamate and GABA in GE and the scale-up fermented GE	28

List of Abbreviations

GE: *Gastrodia elata* Bl.

GABA: γ -Aminobutyric acid

GAD: Glutamate decarboxylase

LAB: Lactic acid bacteria

L. brevis GABA 100: *Lactobacillus brevis* GABA 100

B. bifidum BGN4: *Bifidobacterium bifidum* BGN4

TFA: Trifluoroacetic acid

PLP: Pyridoxal 5'-phosphate

FGE: The fermented GE

GAS: Gastrodin

HBA: 4-Hydroxybenzyl alcohol

INTRODUCTION

Gastrodia elata Bl. (GE), which is a traditional herbal medicine, has been widely cultivated in Korea, Japan, and China (1). GE contains various phenolic compounds such as gastrodin (GAS), 4-hydroxybenzyl alcohol (HBA), 4-hydroxybenzyl aldehyde, vanillyl alcohol, and vanillin (2). These compounds exert preventive effects on neurological disorders like seizures, headaches, and epilepsy (3). GE inhibited the degradation of the γ -aminobutyric acid (GABA) (4) and protected against neuronal damage in the brain (5). Furthermore, recent studies reported on the role of GE in regulating blood pressure (6,7). Among the phenolic constituents, HBA attenuated ischemic injury in the transient focal ischemia model and exerted neuroprotective effects (8,9).

GABA is a ubiquitous non-protein amino acid that is the main inhibitory neurotransmitter in the central nervous system (10). It is produced by glutamate decarboxylase (GAD) that catalyzes the irreversible α -decarboxylation of L-glutamate (11), and was shown to possess a variety of physiological functions like reducing anxiety (12), preventing chronic alcohol-related diseases (13), and controlling cancer cell apoptosis (14). Furthermore, GABA exerted preventive effects against cerebrovascular disease and hypertension (15). The administration of GABA to

spontaneously hypertensive rats decreased blood pressure significantly in several studies (16,17), and this antihypertensive effect was also shown in human subjects (18).

Due to the physiological functions of GABA, several researches reported on the ability of lactic acid bacteria (LAB) to produce GABA (19,20). Fermented foods like raspberry juice (21), grape must (22), red seaweed (23), sea tangle (24), soya yogurt (25), and fishery products (26) that contain high levels of GABA have been produced using LAB as a starter. Kim *et al.* (21) reported that *Lactobacillus brevis* GABA 100 (*L. brevis* GABA 100), which was isolated from *Kimchi*, showed a high GABA-producing ability. In particular, GE was considered to be a good candidate for the production of GABA using *L. brevis* GABA 100, since the fermented GE with GABA might exhibit synergistic anti-hypertensive functions due to GE and GABA. In addition, the neuroprotective effects of GABA and 4-hydroxybenzyl alcohol (HBA) which is a bioconverted form of gastrodin (GAS) during fermentation may be enhanced.

The purpose of this study was to develop fermented GE products containing high levels of GABA. To obtain the greatest conversion of glutamate to GABA, the optimum conditions of the GE fermentation were

investigated. Based on these conditions, a scale-up fermentation was processed and several nutritional materials and phenol compounds of the fermented GE were analyzed by HPLC. The findings of this study may provide an opportunity to develop functional GE products containing GABA.

MATERIALS AND METHODS

2.1. Bacterial strains and viable cell counts

L. brevis GABA 100, which was previously isolated from *Kimchi*, was used for fermentation (21). *Bifidobacterium bifidum* BGN4 (*B. bifidum* BGN4) (KCTC 3202), a commercial strain of probiotics, was used for co-culturing (27). These strains were obtained from the Food Microbiology Lab. at the Department of Food and Nutrition in Seoul National University (Seoul, Korea). *L. brevis* GABA 100 and *B. bifidum* BGN4 were cultured in deMan-Rogosa Sharpe (MRS) broth (Difco™, Detroit, MI, USA) with 0.05% (w/v) L-cysteine-hydrochloride anhydrous (Cysteine) (Sigma, USA) at 37°C for 18 h under the anaerobic state prior to inoculation.

The cell viability of *L. brevis* GABA 100 and *B. bifidum* BGN4 was determined by MRS agar plates. The plates were incubated at 37°C for 2-3 days under the anaerobic state. Viable counts of co-culture were determined using BL agar (Difco™) with 5% (v/v) horse blood (MEDEXX, Seongnam, Korea), in which the colony morphologies of *L. brevis* GABA 100 and *B. bifidum* BGN4 were distinguished. The colonies were counted after the anaerobic growth at 37°C for 3 days. The results were expressed as log colony forming unit (CFU)/mL. The values were presented as mean results

of duplicate determinations.

2.2. Reagents and sample

GE powder was obtained from a farm in Kyoungbuk province, South Korea and stored at 4°C prior to the experiment. GABA and L-monosodium glutamate were purchased from Sigma (USA) and Yakuri Pure Chemicals Co., Ltd (Kyoto, Japan), respectively. Gastrodin (GAS), 4-hydroxybenzyl alcohol (HBA) and various carbohydrates were purchased from SAFC (France), Sigma (USA), and Duksan (Korea). The carbohydrates were dissolved in distilled water and filtered by a 0.2-µm membrane (Corning[®], Sterile Syringe Filter, Germany). Yeast extract was supplied by BD Bacto[™] and Gistex[®] LS Powder AGGL (for scale-up). Methanol was of a high-performance liquid chromatography grade (Duksan, Korea). Distilled water was obtained from an aqua MAX[™]-Ultra Water Purification System (Younglin Co. Ltd., Korea). All other reagents used were of analytical grade.

2.3. Scale-up fermentation for the production of GABA

The fermentation medium contained 3% (w/v) L-monosodium glutamate, 10% (w/v) GE powder, and 0.5% (w/v) yeast extract. Fifty liter fermentor (Biotron, Inc., Korea) was used. The initial pH was 6.5 adjusted with 25% ammonium hydroxide (OCI Company Ltd., Korea). Extracting valuable components from GE was performed at 180 rpm at 85°C for 4 h and then the GE medium sterilized at 121°C for 15 min. The activated strains (*L. brevis* GABA 100 and *B. bifidum* BGN4) in MRS broth were inoculated into the GE medium at a 1% (v/v) level. The fermentation was processed at 195-200 rpm at 30°C for 6 days under the anaerobic state. The fermented GE was lyophilized and stored at 4°C before being subjected to analysis.

2.4. Qualitative analysis of GABA and L-monosodium glutamate by thin layer chromatography (TLC)

The fermented GE samples were diluted 1:10 with distilled water and centrifuged at 2,700 g at 4°C for 20 min. The supernatants were collected, and two microliters of each culture supernatant were loaded onto the TLC Kieselguhr F 254 plate (Merck, Germany) for the assessment of the levels of GABA and L-monosodium glutamate. An *n*-butanol: acetic acid: water (4:1:1, v/v/v) solvent mixture was prepared for the mobile phase using an ascending technique. A 2% ninhydrin (in absolute ethanol) as indicator reagent was sprayed on the plate. The chromatogram was viewed after developing in a dry oven at 110°C for 10 min.

2.5. Determination of valuable nutrients and phenolic compounds by high-performance liquid chromatography (HPLC)

The fermented GE samples were diluted 1:10 with distilled water. One ml of 10-fold diluted broth was centrifuged in Micro high-speed centrifuge (Hanil Science industrial Co. Ltd., Korea) at 18,000 g at 4°C for 10 min. The supernatants were filtered through a 0.2- μ m PVDF membrane (PALL Acrodisc[®] Syringe Filters, Ann Arbor, MI, USA) and analyzed *via* HPLC. Determinations of monosaccharides, organic acids, and free amino acids (containing GABA and L-glutamate) were carried at NICEM, Seoul National University (Seoul, Korea).

The polyphenol compounds were analyzed by YL 9100 HPLC system (Younglin Co. Ltd., Korea) using C₁₈ reversed-phase column (Waters Sunfire[™], 4.6 mm ID x 150 mm, 3.5 μ m) at 220 nm UV length. The flow rate was set at 0.3 ml/min in room temperature. Sample aliquots of 20 μ L were injected into the column. Mobile phases were distilled water (A mobile phase) containing 0.1% (v/v) trifluoroacetic acid (TFA) (Sigma, USA) and methanol (B mobile phase). The mobile solutions were passed through a 0.45- μ m membrane filter (Omnipore[™], Dublin, Ireland) by a solid suspension filtering apparatus and degassed by sonication. The gradient

elution was performed as follows: 10% B (0-5 min), 10-40% B (5-30 min), 40-100% B (30-40 min), 100% B (40-60 min), 100-10% (60-61 min), and 10% B (61-95 min). Each sample was assayed at 0, 1, 2, 3, and 6 days during fermentation. Standard solutions of GAS and HBA were prepared in distilled water. The GAS and HBA concentrations were calculated using a commercial standard based on a standard curve. The coefficient of determination (R^2) was greater than 0.99. HPLC assay conditions are shown in table 1. The standards and each sample were determined in triplicate.

Table 1. Operation conditions of HPLC analysis of phenolic compounds of the fermented GE

Parameters	Conditions
Eluent component A	Water (Containing 0.1% (v/v) of TFA)
Eluent component B	Methanol
Composition	Multi-step gradient: 10% B (0-5 min), 10-40% B (5-30 min), 40-100% B (30-40 min), 100% B (40-60 min), 100-10% B (60-61 min), and 10% B (61-95 min)
Column	Waters Sunfire™, 4.6 mm ID x 150 mm, 3.5 μ m
Flow rate	0.3 ml/min
Inj. volume	20 μ l
Detection wavelength	220 nm
Column temperature	Room temperature
Instruments	Younglin YL 9100 HPLC system

RESULTS AND DISCUSSION

3.1. Bacterial cell growth and GABA production during GE fermentation

Glutamate decarboxylase (GAD) is an intracellular enzyme that catalyzes the irreversible α -decarboxylation of L-glutamate into GABA (10,11). LAB produces the organic acids during fermentation, resulting in an acidic environment that inhibits bacterial growth. Various strains of LAB can produce GAD to maintain a neutral cytoplasmic pH in an acidic medium since the α -decarboxylation of L-glutamate consumes a cytosolic proton (28). *L. brevis* GABA 100, which is a facultative anaerobe, has been reported to show a GABA-producing ability (21). To investigate the effect of aeration on the GABA production, *L. brevis* GABA 100 was grown either under aerobic or anaerobic conditions.

As shown in Fig. 1, the initial count of the bacteria was 6.3×10^7 CFU/ml. High levels of bacterial numbers up to 3.5×10^9 CFU/ml were recorded at 24-h fermentation under both states. During anaerobic incubation, the viable cell counts retained 9 logs CFU/ml for 6 days. In contrast, under the aerobic state, viable counts were remarkably decreased after 4 days. The GABA production was also higher during the anaerobic

state than during aerobic (data not shown). Similarly, Aoki *et al.* (29) reported that the anaerobic cultivation of soybeans by *Rhizopus* increased the production of GABA. Consequently, the anaerobic conditions were used for further studies.

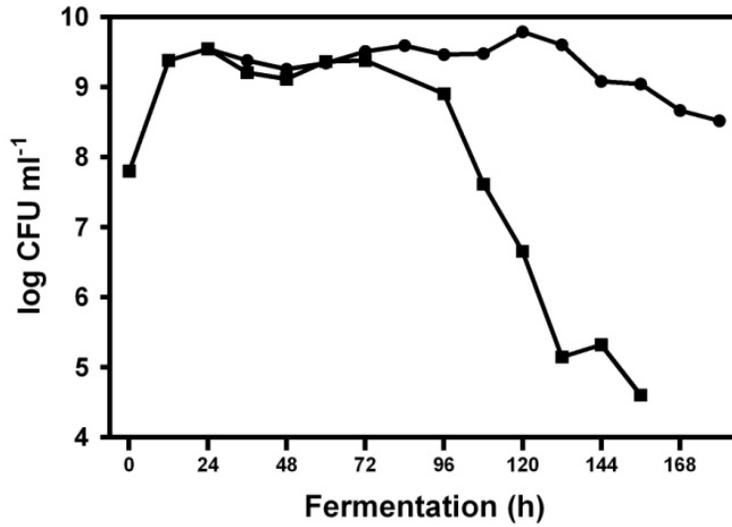


Fig. 1. Cell numbers (log CFU/ml) of *L. brevis* GABA 100 in the fermented GE medium under the anaerobic state (●) and aerobic state (■). Data was expressed as average in duplicate.

3.2. Effect of co-culture of *L. brevis* GABA 100 with *B. bifidum* BGN4 on the production of GABA

The co-culture was designed to enhance the production of GABA. In the first step, the two strains (*L. brevis* GABA 100 and *B. bifidum* BGN4) were simultaneously inoculated in a MRS broth with 3% (w/v) L-monosodium glutamate and 0.05% (w/v) cysteine. Figure 2 and 3 show the cell viability of the two strains and the TLC chromatogram of GABA production during fermentation. The viability of *L. brevis* GABA 100 did not show remarkable changes during co-culture incubation. Interestingly, the production of GABA was considerably enhanced by the co-culture of *L. brevis* GABA 100 with *B. bifidum* BGN4. The enhanced production of GABA by the co-culture was also observed in the fermented GE medium containing 3% (w/v) L-monosodium glutamate (Fig. 4).

The GABA production observed in the co-culture was higher than in the culture inoculated by only *L. brevis* GABA 100. The precise mechanism of the co-culture effect on GABA production was not yet elucidated. We assumed that the presence of *B. bifidum* BGN4 during co-culture decreased the pH values greater than that of *L. brevis* GABA 100 single culture, resulting in the enhanced production of GABA. The pH value of the medium usually increased during the fermentation due to GABA production,

which resulted in exceeding the optimum pH 4-5 of GAD (20). Komatsuzaki *et al.* (30) reported that the maintenance of pH 5 was needed to optimize the production of GABA.

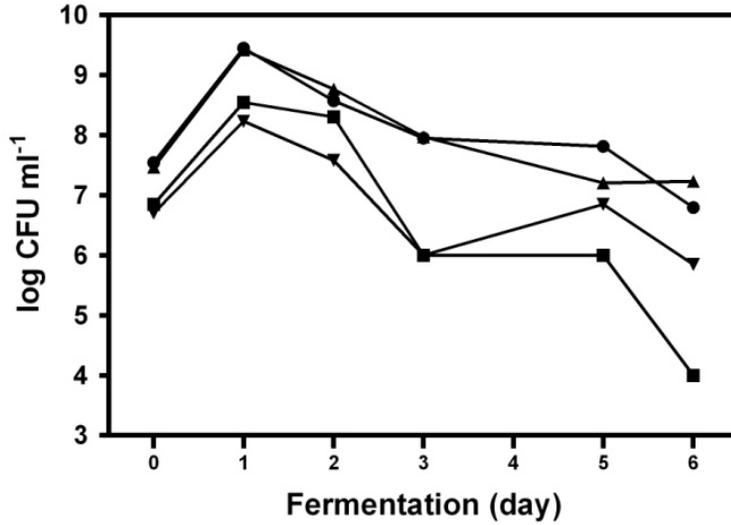


Fig. 2. Cell numbers (log CFU/ml) of lactic acid bacteria in MRS medium with 3% (w/v) L-monosodium glutamate and 0.05% (w/v) cysteine at 37°C. Symbols: (●) *L. brevis* GABA 100 during the single culture; (▲) *L. brevis* GABA 100 co-cultured with *B. bifidum* BGN4; (▼) *B. bifidum* BGN4 co-cultured with *L. brevis* GABA 100; (■) *B. bifidum* BGN4 during the single culture. Data was expressed as average in duplicate.

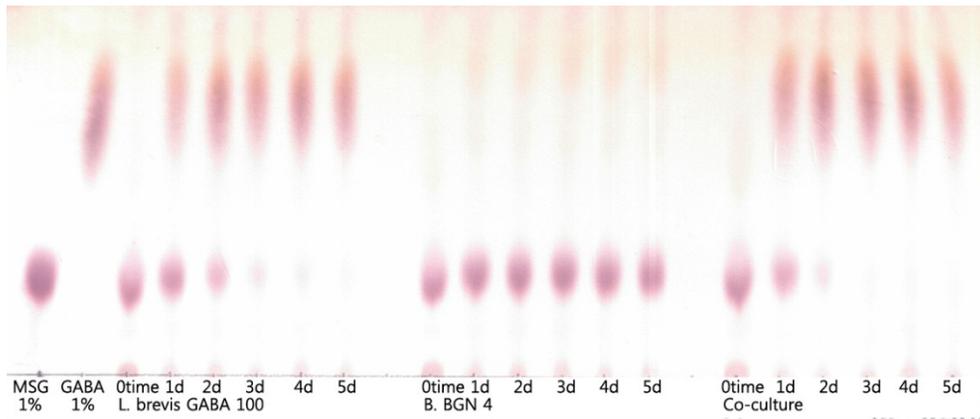


Fig. 3. TLC chromatogram of the GABA produced in MRS medium with 3% (w/v) L-monosodium glutamate and 0.05% (w/v) cysteine at 37°C. The co-culture of *L. brevis* GABA 100 with *B. bifidum* BGN4 shows a higher conversion rate than the single culture of *L. brevis* GABA 100. MRS medium inoculated by only *B. bifidum* BGN4 did not show GABA production. Chromatography using an *n*-butanol: acetic acid: water (4:1:1, v/v/v) solvent mixture was carried out in an ascending technique. The standard concentration of GABA and L-monosodium glutamate was 1%. Sampling volume of 10-fold dilutions of the fermented GE was 2 uL.

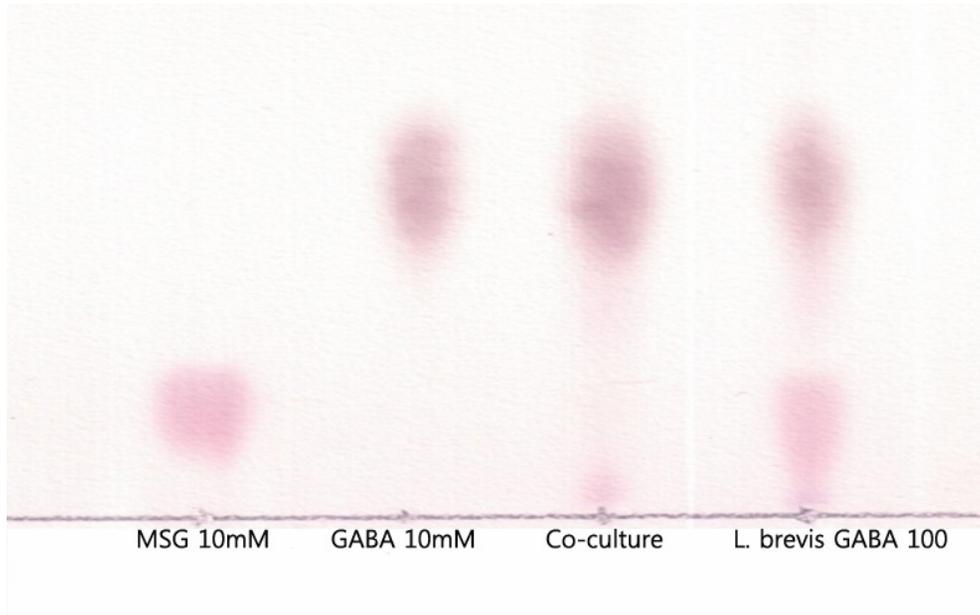


Fig. 4. TLC chromatogram of the GABA produced in 4 days fermentation of GE medium containing 3% (w/v) L-monosodium glutamate at 37°C. The enhanced production of GABA by co-culture was observed in GE medium. The standard concentration of GABA and L-monosodium glutamate was 10 mM. Sampling volume of 10-fold dilutions of the fermented GE was 2 uL.

3.3. Effects of carbon and nitrogen sources on the production of GABA

To further increase the production of GABA in the GE medium containing 3% (w/v) L-monosodium glutamate, 11 different carbohydrates (arabinose, cellobiose, dextrin, fructose, galactose, glucose, lactose, maltose, rhamnose, sucrose, and xylose) were supplemented at a 1% (v/v) level. Each carbohydrate was prepared as a solution sterilized by filtration. However, there was no carbohydrate which can increase the production of GABA higher than the control (no-addition) (data not shown). Interestingly, the addition of xylose and arabinose negated the production of GABA. The suppression of GABA production by xylose and arabinose during co-culture fermentation was also shown in the MRS medium containing L-monosodium glutamate inoculated by only *L. brevis* GABA 100. The reason for these results has not been clarified in the present study.

Next, the effect of yeast extract on GABA production was investigated. The added yeast extract (0.5% (w/v)) increased the production of GABA (Fig. 5). Yeast extract has been generally used as a source of nitrogen and essential vitamins such as pyridoxal 5'-phosphate (PLP). PLP was reported to increase the production of GABA as a coenzyme for GAD reaction (31). However, there exists a contradictory study which reported

that the addition of PLP did not increase GABA production (28). It is not yet clear whether the enhanced production of GABA was due to the presence of PLP in yeast extract or increased viable cell growth by the yeast extract.

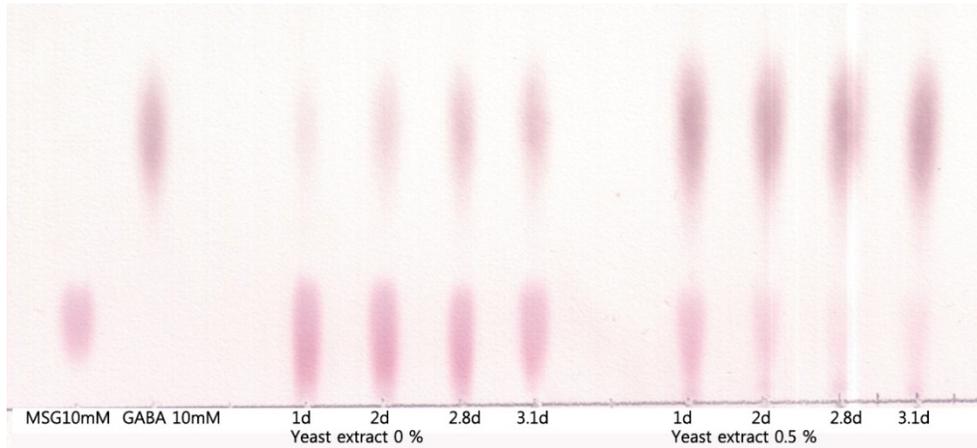


Fig. 5. TLC chromatogram of the GABA produced in the fermented GE medium containing 3% (w/v) L-monosodium glutamate using co-cultured *L. brevis* GABA 100 with *B. bifidum* BGN4 at 37°C. When yeast extract was supplemented to the medium, the production of GABA was considerably increased. The standard concentration of GABA and L-monosodium glutamate was 10 mM. Sampling volume of 10-fold dilutions of the fermented GE was 2 uL.

3.4. Scale-up production of the fermented GE

The optimal medium conditions for the fermentation were as follows: initial pH 6.5, 3% (w/v) L-monosodium glutamate, 10% (w/v) GE powder, and 0.5% (w/v) yeast extract. The activated strains (*L. brevis* GABA 100 and *B. bifidum* BGN4) in MRS broth were inoculated at 1% (v/v) level into the sterilized GE medium. Using a 50-liter fermentor, the GE medium was fermented with shaking at 195-200 rpm at 30°C for 6 days under the anaerobic state. Consequently, the fermented GE (FGE) containing GABA was produced and lyophilized. The FGE product displayed a deeper yellow color with the reduced off-flavor of GE. The viable cell counts of *L. brevis* GABA 100 and *Bifidum* BGN4 increased initially from 3.2×10^7 CFU/ml and 6.0×10^6 CFU/ml to 2.5×10^8 CFU/ml and 8.7×10^6 CFU/ml, respectively (data not shown).

Several nutritional materials of FGE were analyzed by HPLC. Monosaccharides (glucose and fructose) were not retained in the fermented GE (data not shown). During the fermentation, high levels of organic acids, such as citric acid, lactic acid, succinic acid, and acetic acid, were produced (Fig. 6). The total amounts of organic acids progressively increased from 14.6 mM to 72.3 mM. Furthermore, FGE contained various free amino acids. The total free amino acid content was 18.0 mM for the GE medium and 16.3

mM for the FGE (Fig. 7). The levels of amino acids, such as alanine, glycine, histidine, tyrosine, and proline, were increased. Among them, the alanine showed the highest increase after fermentation. Since GABA could decompose to alanine and succinic semialdehyde by amino transferase, FGE may contain a higher content of alanine than other amino acids. Corresponding to this result, alanine was highly accumulated during anaerobic fermentation of soybeans using *Rhizopus* (29). Arginine, asparagine, aspartate, isoleucine, leucine, phenylalanine, serine, and threonine decreased after fermentation.

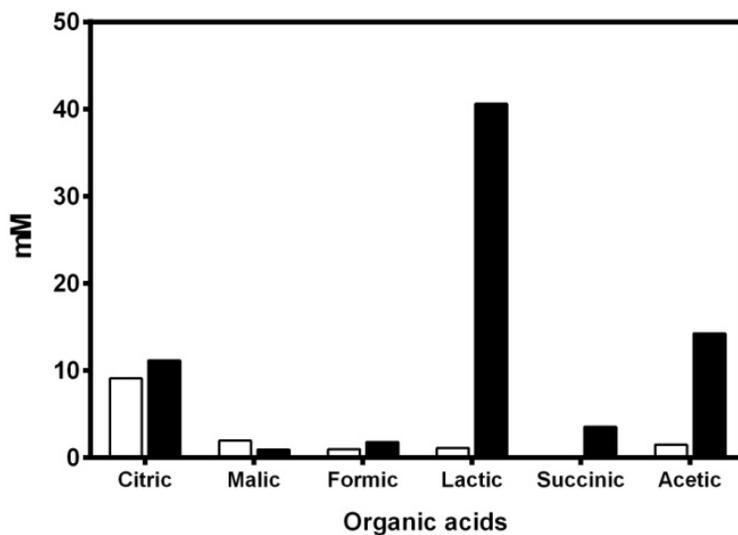


Fig. 6. Comparison of organic acids levels between GE and the scale-up fermented GE using co-cultured *L. brevis* GABA 100 with *B. bifidum* BGN4 at 30°C for 6 days. The open bar (□) and the closed bar (■) indicate the organic acids levels of GE medium and the fermented GE medium, respectively.

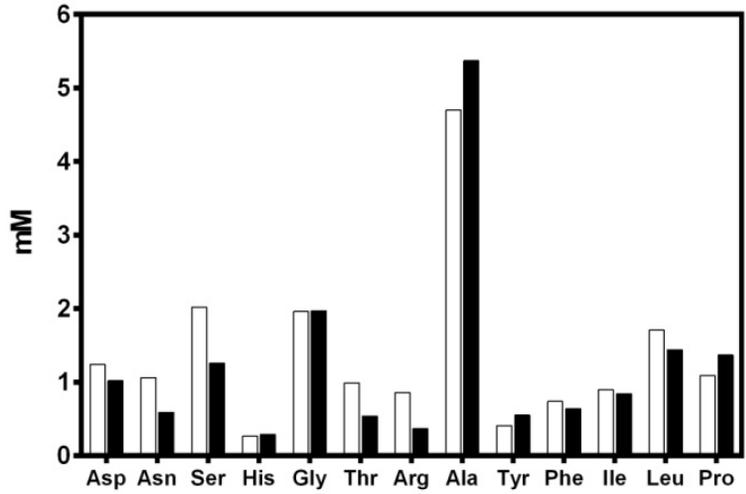


Fig. 7. Comparison of free amino acids levels between GE and the scale-up fermented GE using co-cultured *L. brevis* GABA 100 with *B. bifidum* BGN4 at 30°C for 6 days. The open bar (□) and the closed bar (■) indicate the free amino acids levels of GE medium and the fermented GE medium, respectively.

3.5. The assessment of glutamate and GABA in the scale-up fermentation of GE

L-monosodium glutamate was used as the source of GABA synthesis using *L. brevis* GABA 100. Ratanaburee *et al.* (23) reported that the addition of L-monosodium glutamate to the medium significantly increased the production of GABA. On the other hand, the extra high L-monosodium glutamate content inhibited the viability of *L. brevis* GABA 100. Komatsuzaki *et al.* (30) reported that the addition of L-monosodium glutamate above 500 mM inhibited cell growth. In the present study, the concentration of glutamate was initially decreased from 162.3 mM to 34.1 mM after fermentation (Table 2). Although residual glutamate amount was low, the presence of L-monosodium glutamate might be received by consumers negatively. Thus, further studies focusing on reducing or eliminating the residual glutamate would be useful to increase the attraction of FGE product. The conversion rate of glutamate to GABA was 74.3%

(The conversion rate = $\frac{\text{The produced GABA content}}{\text{Initial glutamate content}} \times 100$).

FGE contained 122.2 mM of GABA (Table 2). The GABA content of FGE was much higher than that of other fermented products like grape must (22) and sea tangle (24), which contained 4.8 mM (from 18.4 mM L-

glutamate) and 2,465 mg/L (from 1% L-monosodium glutamate), respectively. The fermented culture bulk contained 14.5% (w/v) solid content and 1.3% (w/v) GABA. Consequently, the GABA content was 8.7% of the FGE powder. The intake of 20 mg GABA per day was approved for the regulation of blood pressure in health functional foods by the Korean Food & Drug Administration (KFDA). The consumption of only 10 mg of GABA daily for 12 weeks was effective at lowering blood pressure in mildly hypertensive people (18). Furthermore, the intake of 100 mg GABA by 13 subjects showed biological functions for relaxation and alleviation of anxiety on brain waves (12). Therefore, the GABA content of FGE powder was high enough to exert diverse functional effects.

Table 2. Content (mM) of glutamate and GABA in GE and the scale-up fermented GE (FGE) using co-cultured *L. brevis* GABA 100 with *B. bifidum* BGN4 at 30°C for 6 days

Medium	Glutamate	GABA
GE	162.3	1.6
FGE	34.1	122.2

3.6. The assessment of gastrodin and 4-hydroxybenzyl alcohol in the scale-up fermentation of GE

FGE contained various phenolic compounds, such as gastrodin (GAS), 4-hydroxybenzyl alcohol (HBA), 4-hydroxybenzyl aldehyde, vanillyl alcohol, and vanillin. The retention times of GAS and HBA were shown after 15.37 ± 0.15 min and 29.34 ± 0.16 min, respectively. The peaks corresponded to standard reagents.

HPLC assay revealed that GAS was completely converted to HBA during fermentation (Fig. 8). GAS concentration was decreased, and most of the GAS was converted to HBA in 48 h of fermentation. Ultimately, FGE contained 291.1 ± 7.7 μ M HBA. The effect of HBA on learning and memory was more significant than those of GAS (32). Furthermore, HBA was shown to possess neuroprotective effects (8,9) and antioxidant effects (33). HBA administered to mice did not show toxicity at doses up to 200 mg/kg (34). Thus, FGE may provide the synergetic effects of GABA and HBA on the prevention of diverse neurological diseases and be safely used for various neuroprotective effects.

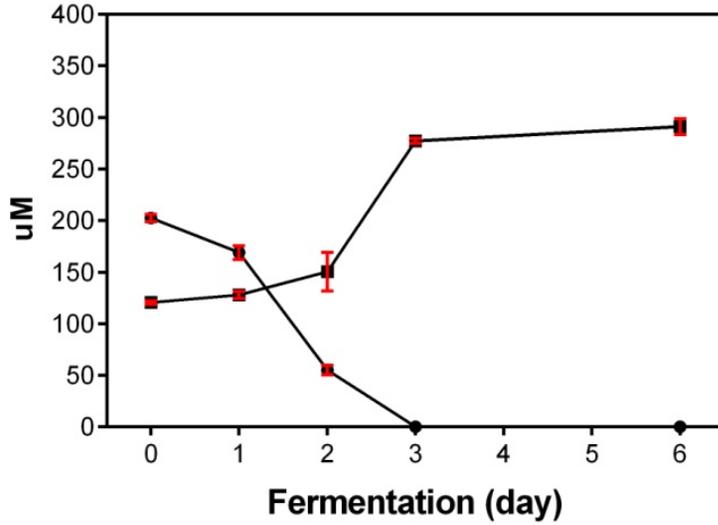


Fig. 8. Conversion of gastrodin (GAS) (●) to 4-hydroxybenzyl alcohol (HBA) (■) of the scale-up fermented GE using co-cultured *L. brevis* GABA 100 with *B. bifidum* BGN4 at 30°C for 6 days. The retention times of GAS and HBA were presented after 15.37 ± 0.15 min and 29.34 ± 0.16 min, respectively. The data was expressed as average of triplicate determinations. Bars of standard deviations are also represented.

CONCLUSION

To our knowledge, this is the first study which reported that GABA-producing LAB was applied as a starter culture for the fermentation of GE. The fermented GE product containing GABA was successfully produced by optimizing fermentation conditions. The fermented GE contained high levels of bioactive substances such as GABA and HBA. Newly developed FGE products may be utilized as an effective ingredient to regulate blood-pressure and exert neuroprotective functions.

REFERENCES

1. Taguchi H, Yosioka I, Yamasaki K, Kim IH. Studies on the constituents of *Gastrodia elata* Blume. Chem. Pharm. Bull. 29: 55-62 (1981)
2. Ojemann LM, Nelson WL, Shin DS, Rowe AO, Buchanan RA. Tian ma, an ancient Chinese herb, offers new options for the treatment of epilepsy and other conditions. Epilepsy Behav. 8: 376-383 (2006)
3. Tsai CF, Huang CL, Lin YL, Lee YC, Yang YC, Huang NK. The neuroprotective effects of an extract of *Gastrodia elata*. J. Ethnopharmacol. 138: 119-125 (2011)
4. Huh K, Yi SJ, Shin US, Park JM. Effect of the ether fraction of *Gastrodia elata* methanol extract on the pentylenetetrazole-induced seizures. J. Appl. Pharmacol. 3: 199-204 (1995)
5. Kim HJ, Moon KD, Oh SY, Kim SP, Lee SR. Ether fraction of methanol extracts of *Gastrodia elata*, a traditional medicinal herb, protects against kainic acid-induced neuronal damage in the mouse hippocampus. Neurosci. Lett. 314: 65-68 (2001)

6. Feng L, Manavalan A, Mishra M, Sze SK, Hu JM, Heese K. Tianma modulates blood vessel tonicity. *Open Biochem. J.* 6: 56-65 (2012)
7. Lee OH, Kim KI, Han CK, Kim YC, Hong HD. Effects of acidic polysaccharides from *Gastrodia* rhizome on systolic blood pressure and serum lipid concentrations in spontaneously hypertensive rats fed a high-fat diet. *Int. J. Mol. Sci.* 13: 698-709 (2012)
8. Yu SS, Zhao J, Zheng WP, Zhao Y. Neuroprotective effect of 4-hydroxybenzyl alcohol against transient focal cerebral ischemia via anti-apoptosis in rats. *Brain Res.* 1308: 167-175 (2010)
9. Yu SJ, Kim JR, Lee CK, Han JE, Lee JH, Kim HS, Hong JH, Kang SG. *Gastrodia elata* Blume and an active component, *p*-hydroxybenzyl alcohol reduce focal ischemic brain injury through antioxidant related gene expressions. *Bio. Pharm. Bull.* 28: 1016-1020 (2005)
10. Shelp BJ, Bown AW, McLean MD. Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci.* 4: 446-452 (1999)
11. Ueno H. Enzymatic and structural aspects on glutamate decarboxylase. *J.*

Mol. Catal. B-Enzym. 10: 67-79 (2000)

12. Abdou AM, Higashiguchi S, Horie K, Kim MJ, Hatta H, Yokogoshi H. Relaxation and immunity enhancement effects of γ -aminobutyric acid (GABA) administration in humans. *BioFactors*. 26: 201-208 (2006)

13. Oh SH, Soh JR, Cha YS. Germinated brown rice extract shows a nutraceutical effect in the recovery of chronic alcohol-related symptoms. *J. Med. Food*. 6: 115-121 (2003)

14. Oh CH, Oh SH. Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *J. Med. Food*. 7: 19-23 (2004)

15. Billingsley M, Suria A, Gilman R, Shokes L, Shahvari M. Evidence for GABA involvement in the peripheral control of blood pressure and vascular resistance. *Brain Res. Bull*. 5: 329-333 (1980)

16. Yoshimura M, Toyoshi T, Sano A, Izumi T, Fujii T, Konishi C, Inai S, Matsukura C, Fukuda N, Ezura H, Obata A. Antihypertensive effect of a γ -aminobutyric acid rich tomato cultivar 'DG03-9' in spontaneously

hypertensive rats. *J. Agr. Food Chem.* 58: 615-619 (2010)

17. Hayakawa K, Kimura M, Kamata K. Mechanism underlying γ -aminobutyric acid-induced antihypertensive effect in spontaneously hypertensive rats. *Eur. J. Pharmacol.* 438: 107-113 (2002)

18. Inoue K, Shirai T, Ochiai H, Kasao M, Hayakawa K, Kimura M, Sansawa H. Blood-pressure-lowering effect of a novel fermented milk containing γ -aminobutyric acid (GABA) in mild hypertensives. *Eur. J. Clin. Nutr.* 57: 490-495 (2003)

19. Siragusa S, De Angelis M, Di Cagno R, Rizzello CG, Coda R, Gobbetti M. Synthesis of γ -aminobutyric acid by lactic acid bacteria isolated from a variety of italian cheeses. *Appl. Environ. Microb.* 73: 7283-7290 (2007)

20. Li H, Cao Y. Lactic acid bacterial cell factories for gamma-aminobutyric acid. *Amino acids.* 39: 1107-1116 (2010)

21. Kim JY, Lee MY, Ji GE, Lee YS, Hwang KT. Production of γ -aminobutyric acid in black raspberry juice during fermentation by *Lactobacillus brevis* GABA 100. *Int. J. Food Microbiol.* 130: 12-16 (2009)

22. Di Cagno R, Mazzacane F, Rizzello CG, De Angelis M, Giuliani G, Meloni M, De Servi B, Gobbetti M. Synthesis of γ -aminobutyric acid (GABA) by *Lactobacillus plantarum* DSM19463: functional grape must beverage and dermatological applications. *Appl. Microbiol. Biot.* 86: 731-741 (2010)
23. Ratanaburee A, Kantachote D, Charernjiratrakul W, Penjamras P, Chaiyasut C. Enhancement of γ -aminobutyric acid in a fermented red seaweed beverage by starter culture *Lactobacillus plantarum* DW12. *Electron. J. Biotechnol.* DOI: 10.2225/vol14-issue3-fulltext-2 (2011)
24. Lee BJ, Kim JS, Kang YM, Lim JH, Kim YM, Lee MS, Jeong MH, Ahn CB, Je JY. Antioxidant activity and γ -aminobutyric acid (GABA) content in sea tangle fermented by *Lactobacillus brevis* BJ20 isolated from traditional fermented foods. *Food Chem.* 122: 271-276 (2010)
25. Park KB, Oh SH. Production of yogurt with enhanced levels of gamma-aminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract. *Bioresource Technol.* 98: 1675-1679 (2007)

26. Thwe SM, Kobayashi T, Luan T, Shirai T, Onodera M, Hamada-Sato N, Imada C. Isolation, characterization, and utilization of γ -aminobutyric acid (GABA)-producing lactic acid bacteria from Myanmar fishery products fermented with boiled rice. *Fisheries Sci.* 77: 279-288 (2011)
27. Choi YJ, Kim CJ, Park SY, Ko YT, Jeong HK, Ji GE. Growth and β -glucosidase activity of *Bifidobacterium*. *J. Microbiol. Biotechnol.* 6: 255-259 (1996)
28. Li H, Qiu T, Huang G, Cao Y. Production of gamma-aminobutyric acid by *Lactobacillus brevis* NCL912 using fed-batch fermentation. *Microb. Cell Fact.* 9: 85 (2010)
29. Aoki H, Uda I, Tagami K, Furuya Y, Endo Y, Fujimoto K. The production of a new *Tempeh*-like fermented soybean containing a high level of γ -aminobutyric acid by anaerobic incubation with *Rhizopus*. *Biosci. Biotech. Bioch.* 67: 1018-1023 (2003)
30. Komatsuzaki N, Shima J, Kawamoto S, Momose H, Kimura T. Production of γ -aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiol.* 22: 497-504

(2005)

31. Yang SY, Lü FX, Lu ZX, Bie XM, Jiao Y, Sun LJ, Yu B. Production of γ -aminobutyric acid by *Streptococcus salivarius* subsp. *thermophilus* Y2 under submerged fermentation. *Amino Acids*. 34: 473-478 (2008)

32. Hsieh MT, Wu CR, Chen CF. Gastrodin and *p*-hydroxybenzyl alcohol facilitate memory consolidation and retrieval, but not acquisition, on the passive avoidance task in rats. *J. Ethnopharmacol*. 56: 45-54 (1997)

33. Jung TY, Suh SI, Lee H, Kim IS, Kim HJ, Yoo HS, Lee SR. Protective effects of several components of *Gastrodia elata* on lipid peroxidation in gerbil brain homogenates. *Phytother. Res*. 21: 960-964 (2007)

34. Descamps E, Petrault-Laprais M, Maurois P, Pages N, Bac P, Bordet R, Vamecq J. Experimental stroke protection induced by 4-hydroxybenzyl alcohol is cancelled by bacitracin. *Neurosci. Res*. 64: 137-142 (2009)

KOREAN ABSTRACT

천마는 아시아의 전통적인 천연약초로써 식품으로 활용 가능하다. 가스트로딘, 하이드록시벤질알콜, 바닐린 등의 페놀 화합물을 함유하여, 발작, 두통, 간질과 같은 신경 질환에 예방 효과가 있다. 또한, 혈압조절 효과가 보고된바 있다. 페놀 화합물 중 하이드록시벤질알콜은 뇌혈관 손상을 약화시켰고, 신경보호기능이 있었다.

감마-아미노부티르산 (GABA)은 중추신경계의 억제적 신경전달 물질로써, 다양한 생리기능 활성화와 항 고혈압 효과가 있다고 알려져 있다. GABA의 생리활성으로 인해, 글루탐산을 소스로 하여 GABA를 생합성 할 수 있는 유산균 연구가 많이 진행되었고, 이를 이용한 GABA 함유 발효 식품들이 보고되었다.

Lactobacillus brevis GABA 100 (*L. brevis* GABA 100)은 GABA를 생합성 할 수 있는 유산균이다. 천마를 *L. brevis* GABA 100으로 발효시킴으로써, 혈압조절 및 신경보호 기능성이 향상될 것을 기대하였다. 이 연구의 목적은 유산균을 이용하여 높은 수준의 GABA를 함유하는 발효 천마를 개발하는데 있었다.

발효하는 동안 GABA 생산을 위한 최적 배지 조건은 다음과 같다: 초기 pH 6.5, 글루탐산나트륨 3%, 천마 고형분 10%, 효모추출물 0.5%. GABA의 생산은 *L. brevis* GABA 100을 *Bifidobacterium bifidum* BGN4와 공동 배양했을 때 향상되었다.

50 L 발효조를 이용하여 천마를 scale-up 발효 후, 몇 가지 영양 물질 (유리당, 유기산, 유리아미노산)과 페놀 화합물을 HPLC 분석하였다. 높은 수준의 유기산과 GABA가 생성되었음을 확인 할 수 있었다. 또한, 천마의 주요 폴리페놀 화합물인 가스트로딘은 그 아글리콘 형태인 하이드록시벤질알콜로 완전히 전환되었다.

결론적으로, GABA를 함유한 발효 천마가 배양 조건의 최적화에 의해서 성공적으로 생산되었고, 이는 혈압 조절 및 신경질환예방 기능성 식품으로 활용 될 수 있을 것이다.

주요어: 감마-아미노부티르산, 천마, 발효, 하이드록시벤질알콜, 락토바실러스 브레비스, 비피도박테리움 비피덤

학번: 2011-23686