A THESIS FOR THE DEGREE OF

MASTER OF SCIENCE IN FOOD AND NUTRITION

Development of *Doenjang* Enhanced with GABA and Aglycone Isoflavone by Mixed Fermentation of *Aspergillus oryzae* and *Lactobacillus brevis*

*Aspergillus oryzae*와 *Lactobacillus brevis*의 혼합 배양을 통하여 GABA와 Aglycone Isoflavones 강화 된장의 개발

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Abstract

Development of *Doenjang* Enhanced with GABA and Aglycone isoflavones by mixed fermentation of *Aspergillus oryzae* and *Lactobacillus brevis*

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A new fermentation process for *doenjang* with increased GABA and aglycone isoflavones content was developed by a mixed culture of *Lactobacillus brevis* and *Aspergillus oryzae*as starters. *Lactobacillus brevis* GABA 100 (2.5 mL 10^{10} cfu/mL) and *Aspergillus oryzae* FMB S40250 (5 mL 10^{8} cells/mL) were co-inoculated into the enzyme-treated soybean medium and cultured for 24 h to produce starter for *doenjang* fermentation. Then, the starter was mixed with steamed soybean for *doenjang* fermentation. Content of GABA and isoflavones were determined by TLC and HPLC. The *doenjang* at its seventh fermentation day contained 7162 μg/g GABA, 60 μg/g daidzein and 59 μg/g genistein, which were higher or comparable to traditional *doenjang* fermented more than one year. The results showed that the

contents of GABA and aglycone isoflavones in the *doenjang* were enriched by co-cultivation of *L. brevis* GABA 100 and *A. oryzae* FMB S40250.

Key words: *Doenjang*, Mixed Fermentation, *Aspergillus oryzae*, *Lactobacillus brevis* GABA 100, GABA, Isoflavones.

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List of Abbreviations

A. oryzae: Aspergillus oryzae

DW: Distilled Water

GABA: γ-Aminobutyric Acid

GAD: Glutamic Acid Decarboxylase

L. brevis GABA 100: Lactobacillus brevis GABA 100

MRS: Mann-Rogosa-Sharpe medium

MSG: Monosodium L-Glutamate

PDA: Potato Dextrose Agar

INTRODUCTION

Soybean is an agricultural crop with very high nutritional value. Soybean cultivation has a long history in Asia and fermented soybean foods such as *doubanjiang* in China, *natto* in Japan and *doenjang* in Korea are very popular on the dining table of Asians.

During soybean fermentation, several health promoting compounds such as γ-aminobutyric acid (GABA) and isoflavone aglycones are known to be produced. Glutamic acid is produced via the hydrolyzation of soybean protein and can be transformed into GABA under the catalysis of glutamic acid decarboxylase (GAD) [22]. GABA is a four-carbon non-protein amino acid [3], and acts as a major inhibitory neurotransmitter in not only the central nervouse system, but also the peripheral nervouse system [5]. GABA has various physiological functions in humans such as hypotensive effect [13], and the effect to improve insomnia and depression [17]. It is known that some lactic acid bacteria have the ability to synthesize GAD that produces GABA from glutamate. This decarboxylation reaction is a response to an acid stress occurring below pH 5 in anaerobic conditions [15]. *Lactobacillus brevis* isolated from *kimchi* has been used for the production of GABA [19]. Especially, *L. brevis* GABA 100, which was originally isolated from *kimchi*, produced a large amount of GABA under the presence of

monosodium L-glutamate (MSG) [9].

Another physiological active compound in the fermented soybean foods is soy isoflavones. Soy isoflavones is reported as a phytoestrogen [20, 21] and have potential to prevent and treat chronic diseases such as osteoporosis, coronary heart disease, postmenopausal complications, and sex-hormone related cancers[2, 16, 18].There are twelve chemical forms of isoflavones in soybeans and soy foods [23]. The aglycone forms, genistein, daidzein and glycitein, are produced by the hydrolytic action of β-glucosidase on the glucosidic isoflavones [14]. *Aspergillus oryzae* is a major fungus growing in *meju*, which is a soybean paste prepared by the traditional fermentation method [6]. Previously, *A.oryzae*was shown to possess relatively highβ-glucosidase activity, as compared to other *koji* starters such as *A.awamori* and *Rhizopus azygosporus* [12]. However, some strains of *A. oryzae* are knownto produce mycotoxions such as cyclopiazonic acid or aflatoxin [1]. In this study, we used *A. oryzae* which were previously shown not to produce aflatoxin and cyclopiazonic acid [11].

In the present study, for the enhancement of a health-benefit, co-cultivated *L. brevis* GABA 100 and *A. oryzae* were used to produce fermented *doenjang* leading to the increase in the content of GABA and aglycone isoflavones.

MATERIALS AND METHODS

2.1. Fungal strains and cultures

The strains of *A. oryzae* and *L. brevis* GABA 100 were obtained from the Food Microbiology Laboratory at the Food and Nutrition Department at Seoul National University. *A. oryzae* was grown on potato dextrose agar (PDA) (Becton, Dickinson and Company, MD, USA) at 30℃ under aerobic conditions for 7 days before being inoculated to the soybean medium. *L. brevis* GABA 100 was cultured in *Lactobacillus* MRS broth (Becton, Dickinson and Company, MD, USA) with 0.05% (w/v) L-cysteine-hydrochloride (Sigma-Aldrich Co., LLC., USA) at 37℃ under anaerobic conditions for 18 h before inoculation to the soybean medium.

2.2. Preparation of soybean medium

Soybean was purchased from Nonghyup (KOREA), which is a major Korean food manufacturer in Korea. The soybean was milled after washing using a hood blender (FM-9097, Hanil, KOREA). The milled soybean (5 mg) was mixed with 50 mL of distilled water. One gram of monosodium glutamate was added to the mixture according to Radhika Dhakal [4]. The pH of the mixture was adjusted to 5.0±0.2 using 1 M citric acid according to Nam Yeun Kim [10] and autoclaved for 15 min at 121℃ using a steam sterilizer to prepare soybean medium. As for the

enzyme treated medium, the soybean medium was treated by using commercial proteolytic enzymes, Prozyme 2000P and Multifect PR 7L (Bision Co., Ltd., KOREA), before steam-sterilized at 121℃. The amount of enzyme added and the enzyme treatment condition is shown inTable 1. After enzyme treatment, the pH of the soybean mediums was adjusted to 5.0±0.2 using 1 M citric acid and the mediums were steam-sterilized at 121℃ for 15 min.

Table 1. The amount of enzymes added and the enzyme treatment condition

Enzyme	Amount	Time	pH	Temperature
Prozyme 2000P & Multifect PR 7L	Prozyme 2000P 0.30% (w/w) & Multifect PR 7L 0.55% (w/w)	30min	6.75	55℃

2.3. Determination of optimum fermentation conditions and screening of *Aspergillus oryzae* for fermentation

Five mL of *A. oryzae* FMB S46471 (10^7 spores/mL) and 2.5 mL of *L. brevis* GABA 100 (10^9 cfu/mL) were inoculated into soybean medium and incubated in various conditions in order to find optimum fermentation conditions such as inoculation order, anaerobiosis, temperature and fermentation time. Then, optimum *A. oryzae* strain for the fermentation of soybean medium was assessed in the culture as follows; 5 mL of 10^7 spores/mL of various strains of *A. oryzae* without aflatoxin or cyclopiazonic acid were co-cultured with 2.5 mL of *L. brevis* GABA 100, respectively, and cultured in the optimum fermentation condition found in the previous stage.

2.4. Starter preparation

The soybean medium used for starter fermentation contains milled soybean and distilled water in the ratio of 1:2 (w/w). MSG (2% (w/w)) was added to the medium and the pH was adjusted to 6.75 using 1 N NaOH. After enzyme treatment as described above, the pH of the medium was adjusted to 5.0±0.2 using 1 M citric acid and steam-sterilized at 121℃ for 15 min. Five mL of 10^8 spores/mL of the optimum *A. oryzae* were co-inoculated with 2.5 mL of 10^{10} cfu/mL of *L. brevis* GABA 100 in 50 g soybean medium and fermented in a shaking incubator at 30℃

and 150 rpm for 1 day.

2.5. *Doenjang* fermentation

The soybean was washed and soaked for 15 h. Before being steamed at 121℃ for 1 h using a steam sterilizer, every 100 g soybean was packed into crocks with 2 g of MSG. When the sterilized soybean was cooled to room temperature, the soybean was crushed inside the clean bench and the pH of the soybean was adjusted to 5.0±0.2 using 1 M citric acid. The two enzymes, prozyme 2000P and multifect PR 7L, were made into 10% and 50% aqueous solution, respectively, in advance and sterilized by 0.2 μm filter. Ten percent prozyme 2000P aqueous solution (3.06 mL) and 1.12 mL of multifect PR 7 L aqueous solution were added into the crushed soybean. Fifty grams of starter were added into the crocks and mixed evenly. The crocks were put into 30℃ incubator for fermentation. After 3 days of fermentation, 100 mL of 20% sterilized NaCl solution was added and mixed thoroughly. Then the crocks were put back into the 30℃ incubator for another 10 days of fermentation.

2.6. TLC analysis of GABA

Samples were diluted 10 times using distilled water (D.W.) and the 2 μL samples were loaded onto the thin-layer chromatography (TLC) Silica gel 60F254 (Merck,

Germany) for analyzing GABA and glutamic acid. Pure GABA (Sigma-Aldrich Co., LLC., USA) and glutamic acid (Sigma-Aldrich Co., LLC., USA) were used as standards. The mobile phase was prepared by mixing n-butanol, acetic acid, and water in a 4 : 1 : 1 (v/v/v) ratio (Samchun Pure Chemical Co., Ltd., Korea). 2% (w/v) ninhydrin (Sigma-Aldrich Co., LLC., USA) in absolute ethanol (Samchun Pure Chemical Co., Ltd., Korea) was sprayed on the TLC plate after running for 3.5 h. After the indicator reagent dried, the TLC plate was put into a 110℃ oven for 1.5 seconds to develop the spot.

2.7. Extraction of isoflavones from fermented soybean

The method of T. H. Kao and B. H. Chen [8] was modified to extract isoflavone from fermented soybean as follows. The sample (1 g) was mixed with 3 mL of hexane (Sigma-Aldrich Co., LLC., USA) and vortexed for 30 min. The mixture was then centrifuged at 2,900×g and 25℃ for 30 min in a high speed centrifuge (2236R, LaboGene, DENMARK). After the supernatant was discarded, 300 μL of hexane (Sigma-Aldrich Co., LLC., USA) was added and centrifuged at 2,900×g and 25℃ for 30 min. After three repeated times, sediment was dried in a speed vacuum concentration (ScanSpeed 40, SCANVAC, LaboGene, DENMARK) at 1,500 rpm and 27℃ for 10 h. Twenty-eight mL of 70% ethanol was added and the sediment was extracted in 60℃ water bath for 2 h, followed by 1 h of sonication at

60℃. Then the sample was centrifuged at 11,000×g and 25℃ for 15 min. The supernatant was collected and volatilized in the speed vacuum concentration at 1,500 rpm and 27℃ for 14.5 h. Finally, 1 mL of 70% ethanol was added to dissolve the solid.

2.8. Detection of isoflavones by HPLC

The extracted solution was filtered through a 0.2 μm syringe filter (PALL Life Sciences, USA) before high-performance liquid chromatography (HPLC) analysis. Daidzin, genistin, daidzein and genistein were separated from the 20 μL samples using an Eclipse XDB-C18 column (150 mm long, 3 mm inside diameter, 5 μm particle size; Agilent Technologies, USA). Standard daidzin, genistin, daidzein and genistein were purchased from Nanjing Dilger Medical Technology Co., Ltd, China.

The four kinds of standard were dissolved in 80% methanol. The concentration of analyzed isoflavone standards were 10 μg/mL, 15 μg/mL, 20 μg/mL, 25 μg/mL. The mobile phase and the ratio changes for each are shown in Table 2. The column temperature was 35℃, and the flow rate was 0.5 mL/min. The UV detection wavelength was 254 nm. The system used for HPLC analysis was the 1090 Series-II Model HPLC System (Hewlett Packard, USA).

Table 2.HPLC analysis condition for isoflavones

Time	A: 0.1% Acetic Acid in Water	B: 0.1% Acetic Acid in Acetonitrile
0 min	95%	5%
4 min	95%	5%
48 min	65%	35%
54 min	15%	85%
56 min	0%	100%
66 min	0%	100%
75 min	95%	5%
80 min	95%	5%

2.9. Quantitative analysis of free amino acids by HPLC

One g sample was mixed with 10 mL of 70% ethanol. After 1 h of sonication, the mixture was put on a rocker (NB-104, N-Biotek, INC) and extracted at 20 rpm at room temperature for 24 h. The mixture was then centrifuged at 2,900×g for 1.5 min at 25℃. The supernatant was collected and filtered through a 0.2 μm syringe filter (PALL Life Sciences, USA) before HPLC analysis.

HPLC analysis for free amino acids was performed at the National Instrumentation Center for Environmental Management (NICEM) at Seoul National University. The column used for separation was the VDSpher 100 C18-E (150 mm long, 4.6 mm inside diameter, 3.5 μm particle size; VDS optilab, Germany). Standard amino acids were made by dissolving 17 kinds of amino acid in 0.1 N HCl. The concentration of analyzed amino acid standards were 10 pmol/μL, 100 pmol/μL, 500 pmol/μL, and 1,000 pmol/μL. The mobile phase and the ratio changes are shown in Table 3. The column temperature was 40℃, with the flow rate 1.5 mL/min. The quantitative determination of free amino acids was performed by using a fluorescence detector at 338 nm. The analysis was performed using the Dionex Ultimate 3000 HPLC systems (Thermo Fisher Scientific Inc., USA).

Table 3. HPLC analysis condition for free amino acids

Time	A: 40 mM Sodium Phosphate Dibasic pH 7	B:Water-Acetonitrile-Methanol (10:45:45, v/v/v)
0 min	95%	5%
3 min	95%	5%
24 min	45%	55%
25 min	0%	100%
31 min	0%	100%
34.5 min	95%	5%
35 min	95%	5%

RESULTS AND DISCUSSION

3.1. Determination of the optimum fermentation conditions

The order of inoculated microorganism, presence of oxygen, and temperature during fermentation are shown in Table 4. The samples were obtained every 7 days and the quantity of GABA in the fermentation products were determined by TLC (**Fig. 1)**.

Group 11 in **Fig. 1** shows that, co-inoculation of *A. oryzae* FMB S46471 and *L. brevis* GABA 100 at the beginning of fermentation resulted in the maximal transformation of glutamic acid into GABA under aerobic condition at 30℃ within 7 days. Then, the optimum fermentation time was assessed under aerobic condition at 30℃. As shown in **Fig. 2**, most of the MSG were converted to GABA after 3 days by co-inoculating *A. oryzae* FMB S46471 and *L. brevis* GABA 100 at the beginning of fermentation and fermenting the soybean under an aerobic environment at 30℃.

Table 4.Inoculation order and fermentation conditions of each group

Group	0~7days	7~14days
1	*A. oryzae* aerobic 30℃	*A. oryzae* aerobic 30℃
2	*A. oryzae* aerobic 30℃	*L. brevis* aerobic 37℃
3	*A. oryzae* aerobic 30℃	*L. brevis* anaerobic 37℃
4	*L. brevis* aerobic 37℃	*L. brevis* aerobic 37℃
5	*L. brevis* aerobic 37℃	*A. oryzae* aerobic 30℃
6	*L. brevis* aerobic 37℃	*A. oryzae* anaerobic 30℃
7	*L. brevis* anaerobic 37℃	*L. brevis* anaerobic 37℃
8	*L. brevis* anaerobic 37℃	*A. oryzae* aerobic 30℃
9	*L. brevis* anaerobic 37℃	*A. oryzae* anaerobic 30℃
10	*L. brevis, A.oryzae* anaerobic 37℃	*L. brevis, A. oryzae* aerobic 30℃
11	*L. brevis, A.oryzae* aerobic 30℃	*L. brevis, A. oryzae* anaerobic 37℃
12	Blank aerobic 30℃	Blank anaerobic 37℃

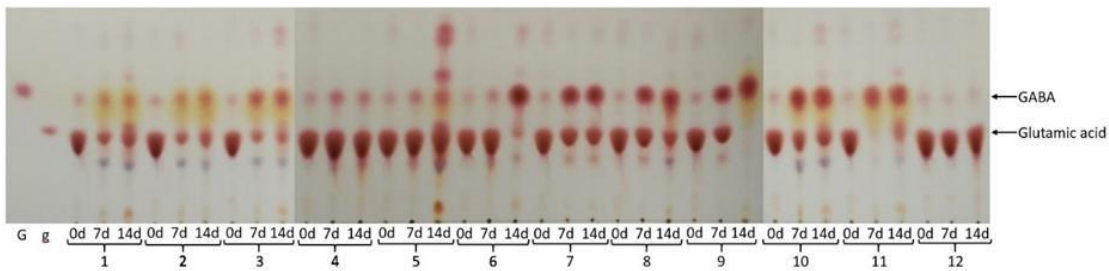


Figure 1. Production of GABA during co-culture of *A. oryzae* FMB S46471 and *L. brevis* GABA 100 under the various fermentation conditions. G: GABA; g: Glutamic acid; 1~12: groups as shown in Table 4.

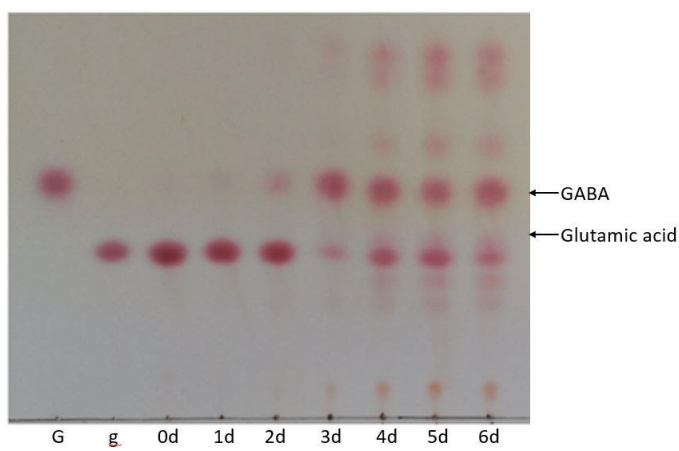


Figure 2. Production of GABA by co-cultivation of *A. oryzae* FMB S46471 and *L. brevis* GABA 100 under aerobic condition at 30℃ at different fermentation times. G: GABA; and g: Glutamic acid.

When the isoflavones were analyzed by HPLC during fermentation, the contents of aglycone isoflavones, daidzein and genistein, reached the peak on the first day of fermentation and fell to less than 5 μg/mL on the third day of fermentation (**Fig. 3**).

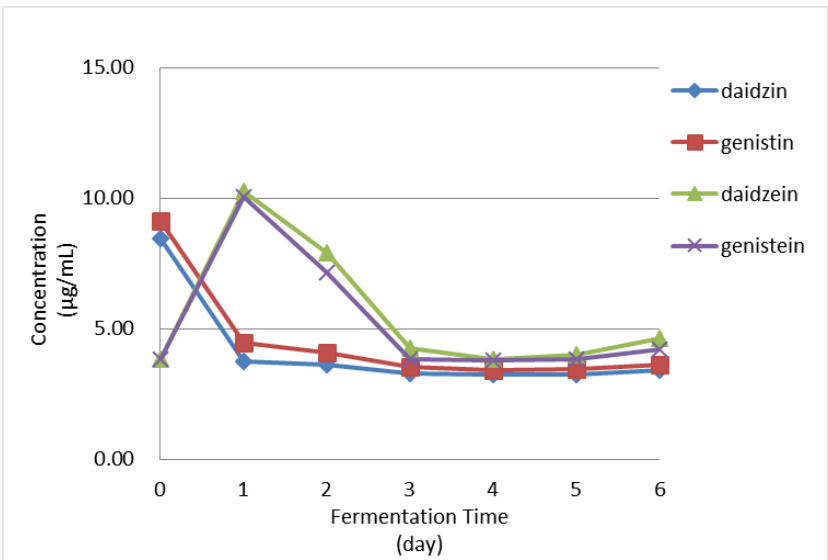


Figure 3. Changes of isoflavones during fermentation of *doenjang* by co-cultivation of *L. brevis* GABA 100 and *A. oryzae* FMB S46471.

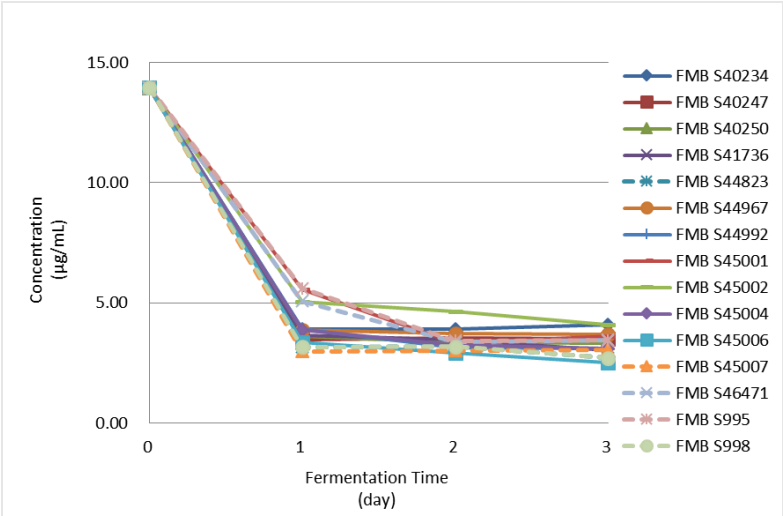
3.2. Screening of *Aspergillus oryzae* for the enhancement of isoflavone aglycones during fermentation

L. brevis GABA 100 was co-inoculated with the 15 strains of *A.oryzae* including *A. oryzae FMB S*40234, *A. oryzae FMB S*40247, *A. oryzae FMB S*40250,*A. oryzae FMB S*41736, and *A. oryzae FMB S*998, which were previously shown not to produce aflatoxin or cyclopiazonic acid [11]. The samples were taken every day and the concentration of the isoflavones and GABA in the products was determined by HPLC and TLC, respectively.

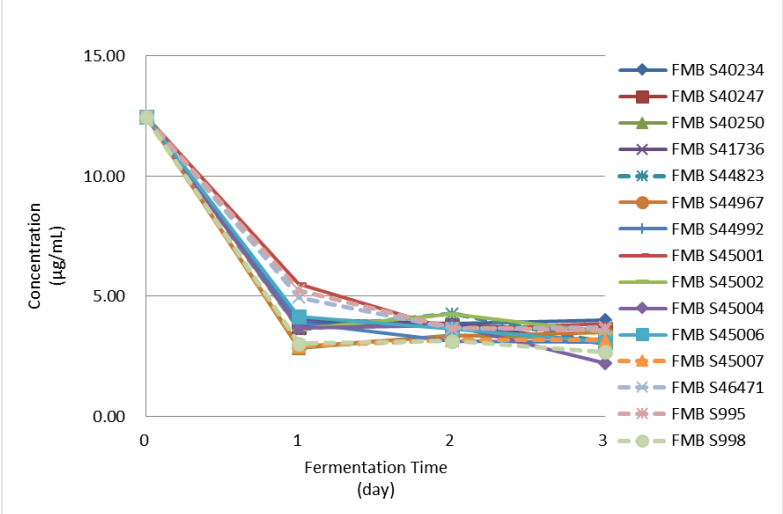
As shown in **Fig. 4**, groups inoculated with FMB S40234, FMB S40250, FMB S44823, FMB S44992, FMB S45002, FMB S45006, and FMB S998 contained relatively more daidzein and genistein than the other groups.

When assessed for the GABA (**Fig. 5**), *A. oryzae* FMB S44992 showed excellent productivity followed by FMB S44823, FMB S998, FMB S40234 and FMB S40250. Considering the contents of both GABA and the aglycone isoflavones, *A. oryzae* FMB S40250 and *A. oryzae* FMB S998 were chosen as starters for the *doenjang* fermentation.

A.


15.00
10.00
5.00
0.00
Concentration (µg/mL)
0
1
2
3
Fermentation Time (day)
FMB S40234
FMB S40247
FMB S40250
FMB S41736
FMB S44823
FMB S44967
FMB S44992
FMB S45001
FMB S45002
FMB S45004
FMB S45006
FMB S45007
FMB S46471
FMB S995
FMB S998

B.


15.00
10.00
5.00
0.00
Concentration (µg/mL)
0
1
2
3
Fermentation Time (day)
FMB S40234
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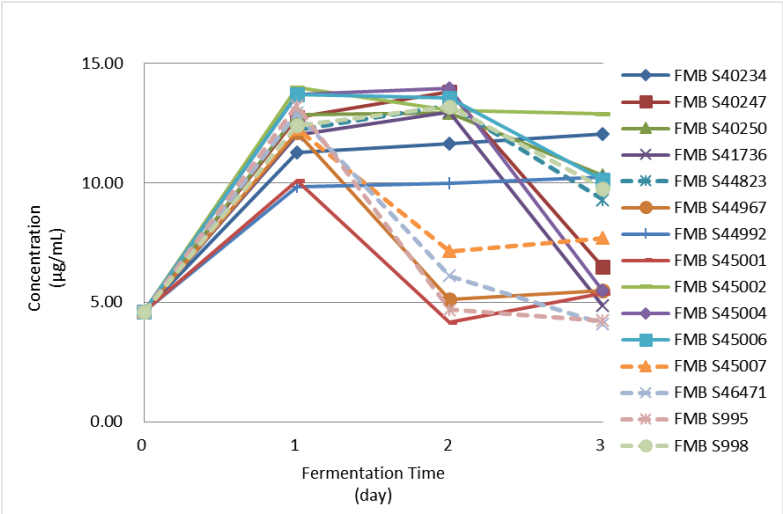
FMB S40250
FMB S41736
FMB S44823
FMB S44967
FMB S44992
FMB S45001
FMB S45002
FMB S45004
FMB S45006
FMB S45007
FMB S46471
FMB S995
FMB S998

C.



D.

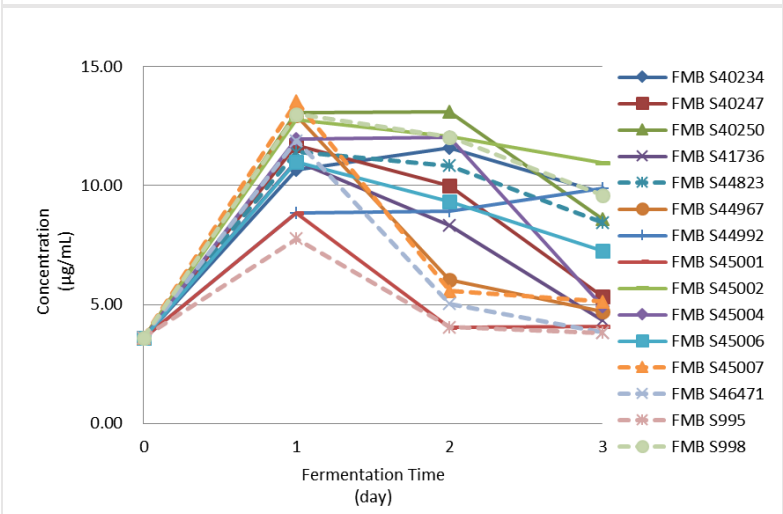


Figure 4. Changes of isoflavones during fermentation of *doenjang* by co-cultivation of *L. brevis* GABA 100 and various *A. oryzae* strains. (A) changes of daidzin during fermentation; (B) changes of genistin during fermentation; (C) changes of daidzein during fermentation; (D) changes of genistein during fermentation.

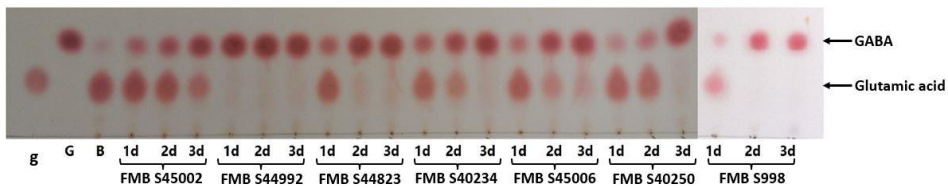


Figure 5. Production of GABA in the fermented products using *L. brevis* GABA 100 and various *A. oryzae* strains. g: Glutamic acid; G: GABA; and B: blank.

3.3. Enzyme treatment for soybean medium before fermentation

The quantities of daidzein and genistein in the fermented products tended to decrease from the second day of fermentation, while the GABA contents tended to gradually increase until the third day of fermentation. In order to speed up GABA production, proteolytic enzyme treatment of the soybean medium was carried out as described above before inoculation of the fermenting microorganisms. When the samples were analyzed at 6 h, 12 h, 18 h, 24 h and 48 h, the GABA production reached the peak level within the first day of fermentation in both groups (**Fig. 6**) and the production of two aglycone isoflavones was faster than the control without enzyme treatment. Furthermore, the contents of the isoflavones were higher than the control (**Fig. 7**).

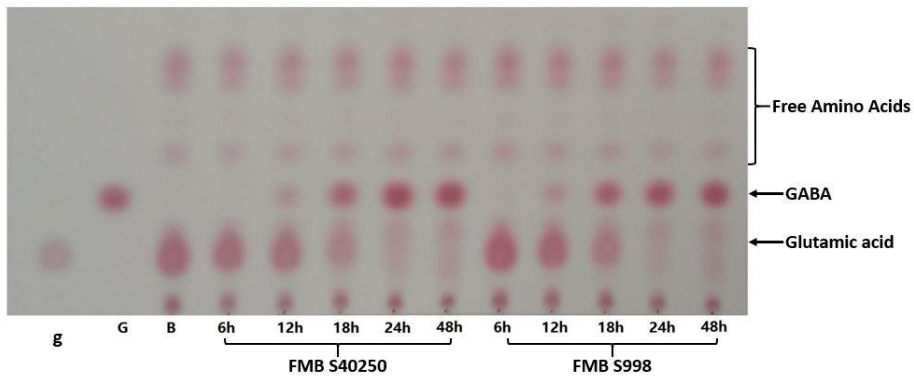
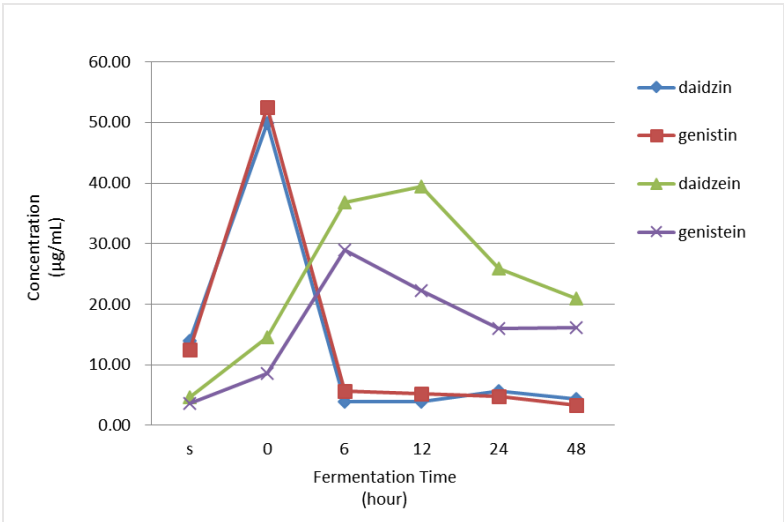


Figure 6. Production of GABA in the products fermented with *L. brevis* GABA 100 and *A. oryzae* FMB S40250 and FMB S998, respectively. g: Glutamic acid; G: GABA; and B: blank.

A.



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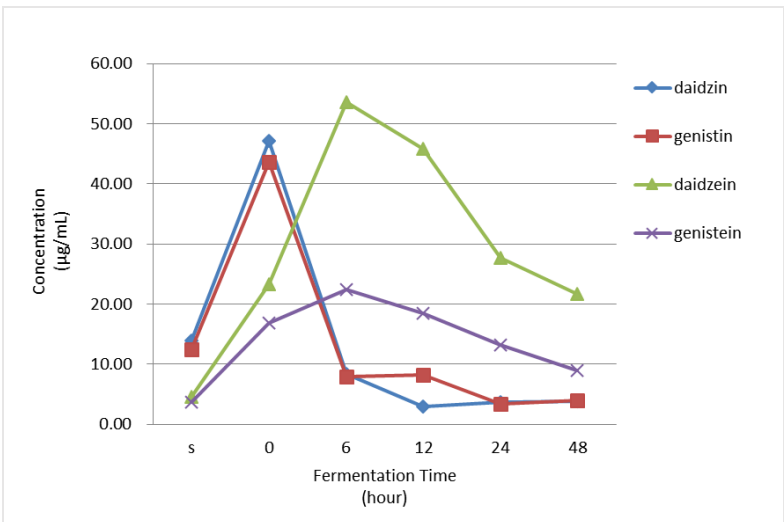

Figure 7. Concentration of the isoflavones in the fermentation products. s: soybean without enzyme treatment. (A) concentration of the isoflavones in the *A.oryzae* FMB S40250 fermentation group; and (B) concentration of the isoflavones in the *A.oryzae* FMB S998 fermentation group.

3.4. *Doenjang* fermentation

After determining the optimal starter strains and fermentation conditions, two groups of *doenjang* were made using both *L. brevis* GABA 100 and *A. oryzae* FMB S40250 or FMB S998. During the *doenjang* fermentation, samples were takenand analyzed on the third, seventh, and tenth day of fermentation. As shown in **Fig. 8** and **Fig. 9** the contents of aglycone isoflavones and GABA on the seventh day *doenjang* were higher than those of the tenth day *doenjang*. Furthermore, the *doenjang* fermented by *A. oryzae* FMB S40250and *L. brevis* GABA 100 proved to contain more GABA and aglycone isoflavones on the seventh day of fermentation than the *doenjang* fermented by *A. oryzae* FMB S998 and *L. brevis* GABA 100.

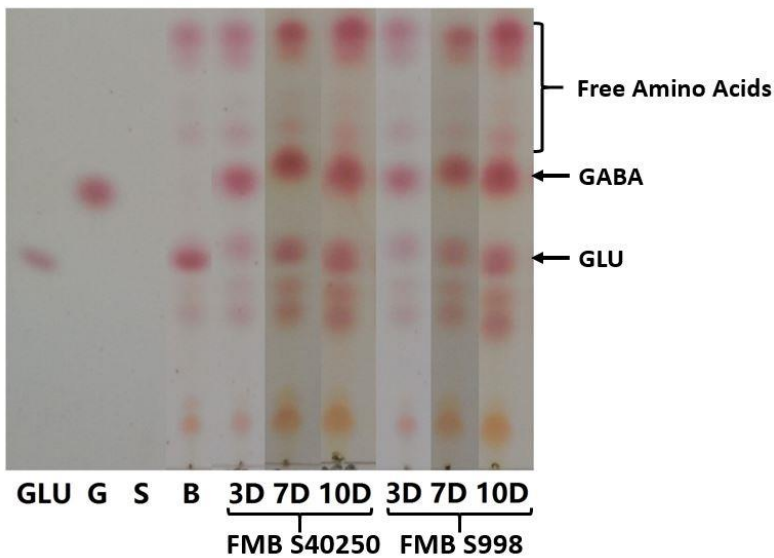

Figure 8. Determination of the GABA in the *doenjang* fermented by co-cultivation of *L. brevis* GABA 100 and *A. oryzae* FMB S40250 and *A. oryzae* FMB S998. g: Glutamic acid; G: GABA; S: soybean without enzyme treatment; and B: enzyme treated soybean.

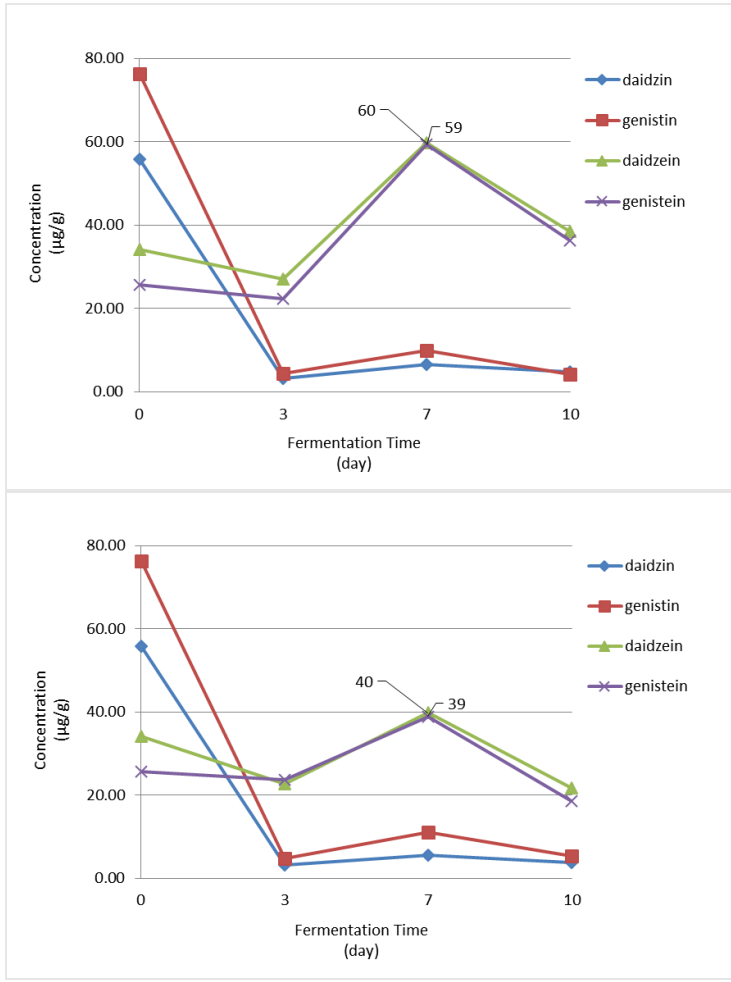

Figure 9. Changes of the isoflavones concentration in the *doenjang* fermented by co-cultivation of *L. brevis* GABA 100 and *A. oryzae* FMB S40250 and *A. oryzae* FMB S998. (A) changes of the isoflavones concentration in the *doenjang* fermented by *L. brevis* GABA 100 and *A. oryzae* FMB S40250; and (B) changes of the isoflavones concentration in the *doenjang* fermented by *L. brevis* GABA 100 and *A. oryzae* FMB S998.

The concentration of GABA in the *doenjang* fermented by *A. oryzae* FMB S40250 on the seventh day of fermentation was determined to be 7162 μg/g, which is much higher than that of soybean (**Fig. 10**).

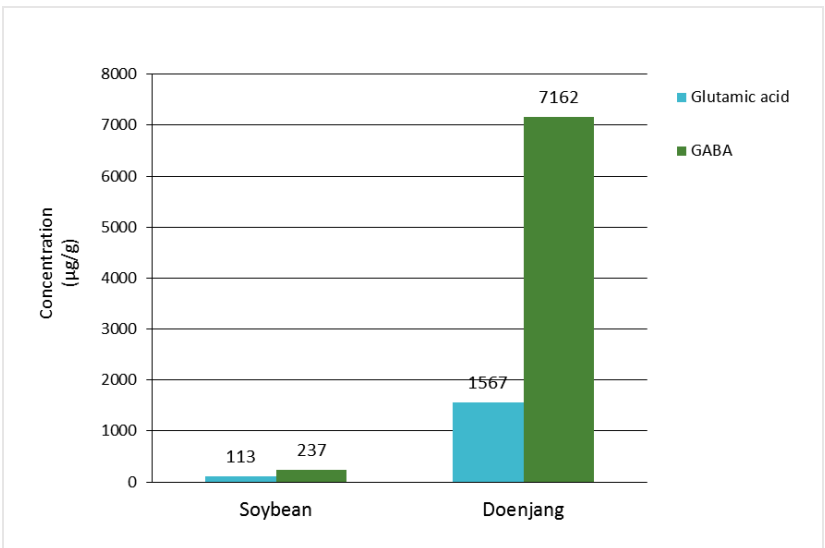

Figure 10. Quantitative determination of GABA in the *doenjang* on the seventh day of fermentation.

The seventh day *doenjang* fermented by *A. oryzae* FMB S40250 and *L. brevis* GABA 100 contained 60 μg/g of daidzein and 59 μg/g of genistein.

According to Seong-Jin Jo [7], traditional Korean *doenjang* fermented for 1 year contained 44 mg/kg of GABA, while a 10 year old *doenjang* contained 1,939 mg/kg of GABA. The content of daidzein in the 1 and 10 year old *doenjang* was 55 mg/kg and 101mg/kg, respectively. As for genistein, 72 mg/kg and 95 mg/kg were contained in the 1 and 10 year old *doenjang*, respectively.

Therefore, there is much more GABA in the *doenjang* fermented by co-culture of *A. oryzae* FMB S40250 and *L. brevis* GABA 100 than in the 10 year old traditional Korean *doenjang*. However in our research, the quantity of GABA can be different according to the quantity of MSG added to the medium. The daidzein contained in the *doenjang* fermented by *A. oryzae* FMB S40250 and *L. brevis* GABA 100 is more than that contained in the 1 year traditional Korean *doenjang*, while the genistein in the *doenjang* fermented by *A. oryzae* FMB S40250 and *L. brevis* GABA 100 is little less than that contained in the 1 year traditional Korean *doenjang*.

CONCLUSION

In this study, we used the co-cultivation method of *A. oryzae* FMB S46471 and *L. brevis* GABA 100 to find the optimum *doenjang* fermentation condition for the efficient production of GABA and aglycone isoflavones during fermentation. The best fermentation condition for GABA production was determined to be co-inoculation and aerobic fermentation for three days. Then, we tried to find the optimum *A. oryzae* strain for both GABA and aglycone isoflavones production. With the additional enzyme treatment of soybean before inoculation, *A. oryzae* FMB S40250 and *A. oryzae* FMB S998 were chosen to produce more GABA and aglycone isoflavones during *doenjang* fermentation. In the *doenjang* fermented by *A. oryzae* FMB S40250 and *L. brevis* GABA 100, the quantities of GABA, daidzein and genistein were 7162 μg/g, 60 μg/g and 59 μg/g, respectively. In conclusion, the content of GABA and aglycone isoflavones in the *doenjang* was enriched by the co-cultivation of *A. oryzae* FMB S40250 and *L. brevis* GABA 100 during *doenjang* fermentation.

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국문초록

본 연구에서 *Lactobacillus brevis* GABA 100 과 *Aspergillus oryzae* FMB S40250 의 공동 배양에 의하여 된장의 γ-aminobutyric acid (GABA)와 비배당체 isoflavones 의 함량을 향상 시키는 것을 목표로 하였다. *L. brevis* GABA 100 (2.5mL 10^{10} cfu/mL)과 *A. oryzae* FMB S40250 (5 mL 10^{8} cells/mL)를 효소 처리한 콩 배지에 접종하여 발효의 최적 조건에서 하루 발효를 통하여 된장 발효 때 쓰는 스타터를 만들었다. 50g 스타터를 100g 삶은 콩과 잘 섞은 다음에 된장 발효 단계로 들어갔다. 발효 7 일째 된장 내에 GABA 가 7162 μg/g, daidzein 이 60 μg/g, genistein 이 59 μg/g 이 각각 검출되었다. 이와 같이 *L. brevis* GABA 100 과 *A. oryzae* FMB S40250 의 공동 배양을 통하여 된장 내의 기능성 성분인 GABA 과 비배당체 isoflavones 의 함량을 향상시킬 수 있다.

Key words: *Doenjang*, Mixed Fermentation, *Aspergillus oryzae*, *Lactobacillus brevis* GABA 100, GABA, Isoflavones.

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