J Korean Soc Appl Biol Chem (2014) 57(6), 703–708
DOI 10.1007/s13765-014-4227-5

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Characterization of Soybean Fermented by Aflatoxin Non-producing *Aspergillus oryzae* and γ-Aminobutyric Acid Producing *Lactobacillus brevis*

Nam Yeun Kim · Geun Eog Ji

Received: 24 July 2014 / Accepted: 31 August 2014 / Published Online: 31 December 2014

Abstract Fermented soybean products may contain aflatoxins due to the contamination of the aflatoxin producing mold during natural fermentation or as a result of un-prudential use of starter strain. The aim of the present study was to develop and characterize fermented soybean products with enhanced safety and bioactive compound using aflatoxin non-producing *Aspergillus oryzae* FMB S46471 and γ-aminobutyric acid (GABA) producing *Lactobacillus brevis* GABA 100. For fermentation, steam-processed soybeans were inoculated with *A. oryzae* FMB S46471. The scaled-up fermentation product was fermented for 17 days, including 12 days fermentation with *A. oryzae* and 5 days with *L. brevis*, and contained 6.5±0.3 g/kg of GABA and 45.7±0.3 g/kg of total free amino acids. After ripening for 90 days, the fermented soybean contained about 11.6±0.3 g/kg of GABA and 72.7±1.8 g/kg of total free amino acids. Furthermore, aflatoxins were not detected in both mature and immature soybean products. The soybean product that fermented by an aflatoxin non-producer and a powerful GABA producer will contribute to the development of fermented foods with enhanced safety levels and functional benefits of GABA.

Keywords aflatoxin · *Aspergillus oryzae* · fermentation · *Lactobacillus brevis* · γ-aminobutyric acid

N. Y. Kim · G. E. Ji (✉)
Department of Food and Nutrition, Research Institute of Human Ecology, Seoul National University, Seoul 151-742, Republic of Korea

G. E. Ji (✉)
Research Institute, Bifido Inc., Hongcheon, Gangwon 250-804, Republic of Korea
E-mail: geji@snu.ac.kr

Introduction

Asia has a long history of using fermented soybean foods such as Korean *doenjang* and *ganjang* and Japanese *miso* and *shoyu,*. Fermented soybean sauces have been served as an indispensible sauce in their respective cuisines for thousands of years (Kitamoto, 2002; Lee and Lee, 2002; Tamang and Samuel, 2010). One study on the dynamic changes of aflatoxin during the fermentation of soybean sauce revealed that the naturally occurring aflatoxin was produced by toxigenic fungi at the early fermentation stage (Kim et al., 2001; Xie et al., 2014). Park et al. (2001) reported that 6 out of 24 *Aspergillus* strains isolated from the commercial starter product in Korea produced mycotoxins. Kim et al. (2001) also showed that contamination of aflatoxin B_1 was detected in 25 out of 60 *meju* samples (41.6%). Kim et al. (2011) reported that 4 out of 65 *Aspergillus* strains isolated from the soybean starter collected from 2009 to 2010 turned out to be aflatoxin producers. Furthermore, Lee et al. (2012) reported that aflatoxin B_1 was detected by high-performance liquid chromatography (HPLC) in 1 out of 7 soybean sauce samples and 2 out of 56 soybean paste samples, which were collected from various markets (Korea Food and Drug Administration, 2008). To enhance the safety levels of fermented foods, mycotoxin non-producing *Aspergillus* strains were screened and collected. (Kim et al., 2014b). In this study, we adopted *Aspergillus oryzae (A. oryzae*) FMB S46471, a non-producer of both aflatoxins and cyclopiazonic acid (CPA).

During soybean fermentation, glutamic acid is produced by the hydrolytic action of the fermenting microorganisms, which can be used as a substrate for the production of γ-aminobutyric acid (GABA) by GABA-producing lactic acid bacteria (LAB). GABA is a non-protein amino acid (Ueno, 2000). The consumption of GABA-enriched foods can suppress the occurrence of hypersensitivity in humans (Inoue et al., 2003). GABA consumption regulates pain and anxiety and lower lipid levels in sera (Kono

and Himeno, 2000; Miura et al., 2006). Furthermore, the consumption of GABA-rich foods inhibits cancer cell proliferation (Park and Oh, 2007) and improves memory and learning abilities (Miura et al., 2006). The Korean Food and Drug Administration (2009) has approved a daily consumption of 20 mg GABA for the regulation of blood pressure in health functional foods. Recently, Lee et al. (2014) suggested that fermented soybean products containing a high concentration of GABA showed a therapeutic effect on phthalic anhydride-induced atopic dermatitis in IL-4/Luc/CNS-1 Tg mice. In addition, Takeshima et al. (2014) showed that the result of toxicity evaluation supported the safety of GABA consumption and its potential use as a functional food ingredient. A number of microorganisms have been reported to produce GABA (Smith et al., 1992; Kono and Himeno, 2000; Lu et al., 2008; Li and Cao, 2010; Dhakal et al., 2012). Fermentation by a GABA producer has been considered as a promising possibility to increase the nutritional, functional, sensory, and technological properties of food products (Coda et al., 2010).

In the present study, we adopted an innovative two-step fermentation process, where steam-processed soybeans were first fermented aerobically with mycotoxin non-producing *A. oryzae* followed by an anaerobic fermentation using GABA-producing *Lactobacillus brevis* (*L. brevis*). This is first study on the development of soybean paste fermented by an aflatoxin non-producer and a powerful GABA producer.

Materials and Methods

Fungal strains and cultures. *A. oryzae* FMB S46471 was selected from the study of Kim et al. (2014b). *L. brevis* GABA 100 previously isolated from *kimchi* was obtained from the Food Microbiology Laboratory at the Department and Food and Nutrition at Seoul National University (Kim et al., 2009). *A. oryzae* FMB S46471 was grown on potato dextrose agar (Becton, Dickinson and Company, USA) at 30°C under aerobic conditions prior to the inoculation of steamed soybeans. *L. brevis* GABA 100 was cultured in Difco *Lactobacilli* MRS broth (Becton, Dickinson and Company, USA) with 0.05% (w/v) L-cysteine-hydrochloride anhydrous (Sigma-Aldrich Co, USA) at 30°C under anaerobic conditions prior to the inoculation of steamed soybeans.

Extraction of aflatoxins from fermented soybeans. Soybean paste (10 g) was collected and homogenized with a cell strainer (BD). Subsequently, 10 mL of aflatoxin extract (water:methanol, 30:70, v/v, 1% NaCl) was added and vortexed for 5 min. The sample was filtered through a Whatman No. 4 filter (Whatman International Ltd., England), and 10 mL of the filtrate was then diluted with 1% Tween 20 solution to 40 mL. The mixture was passed through a Whatman GF/A glass filter paper (Whatman), and 20 mL of the filtrate was loaded onto an AflaTest WB column (Vicam Co., USA) at a flow rate of 1 drop per s for clean up. After washing the column with 10 mL of water at the same flow rate, total aflatoxins were eluted with 3 mL of acetonitrile (Duksan Pure Chemicals Co., Ltd., Korea). The elute was evaporated at 50°C. The dry residue was derivatized by adding 200 μL of trifluoroacetic acid (Sigma-Aldrich Chemical Co.), and the mixture was left to stand in the dark for 15 min and then diluted with 800 μL of acetonitrile:water (20:80, v/v). The derivatized sample was filtered through a 0.45-μm-pore-sized membrane filter, and the filtrate was used for HPLC analysis. The standards of total aflatoxins (G_1, B_1, G_2, and B_2 at 25 μg/mL) were obtained from Trilogy Analytical Laboratory (Washington, MO). The concentrations of analyzed aflatoxin standards were 1, 10, 20, and 50 ppb.

Detection of aflatoxins using HPLC methods. Aflatoxins G_1, B_1, G_2, and B_2 were separated from the injected 20 μL samples using a Polaris C18 column (250 mm long, 4.6 mm inside diameter, 5 μm particle size; Agilent Technologies, USA). The mobile phase was acetonitrile:water (25:75, v/v), which was pumped at a constant flow rate of 1 mL/min. Each aflatoxin was quantified using a fluorescence detector (360 nm excitation, 450 nm emission). The analysis was performed using the Ultimate 3000 HPLC systems (Thermo Fisher Scientific, USA).

Protease assay. Protease activity was measured with a modified procedure based on the Folin-Ciocalteu method (Kunitz, 1974). Samples were collected by dates. Then the sample was filtered through a Whatman No. 1 filter paper (Whatman International Ltd., England), and 1 mL of the filtrate was then added to 5 mL 0.65% (w/v) casein solution (Sigma-Aldrich Co., LLC.), which was prepared by mixing 6.5 mg/mL of 50 mM potassium phosphate buffer (adjusted to pH 8.0 using 1 M HCl) (Sigma Chemical Co.). After incubation in a 37°C water bath (Jeio Tech Co., Ltd., Korea) for 30 min, the reaction was stopped by adding 5 mL of the 110 mM trichloroacetic acid solution (Sigma-Aldrich Co., LLC.). The stop reaction was performed at 37°C for 30 min. Standards were prepared by using 1.1 mM L-tyrosine solution (Sigma-Aldrich Co., LLC.). Two mL of samples and standards, respectively, was mixed with 5 mL of the 500 mM sodium carbonate (Samchun Pure Chemical Co., Ltd., Korea) and 1 mL of the 0.5 M Folin & Ciocalteu reagent solution (Sigma-Aldrich Co., LLC.) and incubated at 37°C for 30 min. The final products were filtered through a 0.45 μm syringe filter (PALL Life Sciences, USA). The optical density was measured at 660 nm using a OPTIZEN 2120UV (Mecasys Co., Ltd., Korea). One unit (U) of protease activity was defined as the amount of enzyme required to release 1 μg tyrosine from casein per minute under assay conditions. Calibration curves were obtained based on seven levels (0.055, 0.111, 0.221, 0.442, 0.553, 1.106, and 2.212 μmoles) of L-tyrosine solution.

Small-scale cultivation conditions for an evaluation of GABA production by GABA producing LAB. 30 g soybeans (Ogok Farm, Korea) were steamed at 121°C for 15 min using a steam sterilizer (Jeio Tech., Co., Ltd., Korea). After cooling, *A. oryzae* FMB S46471 was inoculated with a suspension of spores of *A. oryzae* FMB S46471 (10^6 cells/g) to the steam-processed soybeans. The inoculated beans were incubated for 7 days in a multi-shaking

incubator (Vision Bio Tech Co., Ltd., Korea) at 30°C under aerobic conditions. Following this, *L. brevis* GABA100 (5% (v/v), 10^8 CFU/mL) was added to the fermented soybeans with *A. oryzae* FMB S46471. The inoculated soybeans with *A. oryzae* FMB S46471 for seven days were additionally inoculated with *L. brevis* GABA100 and further fermented for the following 11 days in the multi-shaking incubator (Vision Bio Tech Co., Ltd., Korea) at 30°C under anaerobic conditions. The soybean fermented by only *A. oryzae* FMB S46471 for 14 days was prepared for use as a control. Samples were collected periodically for analysis.

Optimization of GABA production in scaled-up fermentation. Steamed soybeans (3.5 kg) were prepared by Nong-hyup (Korea), which is a major Korean food manufacturer in Korea, by using a commercialized starter manufacturing process. After cooling, *A. oryzae* FMB S46471 was inoculated with a suspension of spores of *A. oryzae* FMB S46471 (10^6 cells/g) to the steamed soybeans. The inoculated soybeans were incubated for 12 days in a multi-shaking incubator (Vision Bio Tech Co., Ltd., Korea) at 30°C under aerobic conditions. After then, *L. brevis* GABA100 (5% (v/v), 10^8 cells CFU/mL) was added to the fermented soybeans with *A. oryzae* FMB S46471. The initial pH was adjusted to 5.0 with 1 M citric acid (Junsei Chemical Co., Ltd., Japan). The inoculated soybeans with *A. oryzae* KACC 46471 were additionally inoculated with *L. brevis* GABA100 and further fermented for the following 7 days in the multi-shaking incubator (Vision Bio Tech Co., Ltd., Korea) at 30°C under anaerobic conditions. Samples were collected periodically for analysis. Subsequently, the soybeans adjusted to 6% salinity were matured for 90 days. For an evaluation of GABA production, commercially available soybean paste (Cheiljedang Co. Ltd., Korea) was purchased from a local market.

Qualitative analysis of GABA and glutamic acid by thin layer chromatography (TLC). Samples were ground by using a 40 μm sized cell strainer (BD Biosciences, USA). The samples were centrifuged at 3,000×*g* and 4°C for 20 min. The supernatants were collected and two μl of each supernatant was loaded onto the TLC Silica gel 60_{F254} (Merck, Germany) to assess the level of free amino acids including GABA and glutamic acid. GABA (Sigma-Aldrich Co., LLC.) and L-monosodium glutamate (MSG) (Yakuri pure chemicals Co., Ltd., Japan) were used as standards. The mobile phase was prepared as a solvent mixture (*n*-butanol : acetic acid : water (4:1:1, v/v/v) (Samchun pure chemical Co, Ltd., Korea). A 2% (w/v) ninhydrin (Alfa Aesar, UK) in absolute ethanol (Samchun pure chemical Co, Ltd., Korea) used as an indicator reagent was sprayed on the TLC plate after running for 4 h. The spot on the TLC plate was shown after drying.

Quantitative analysis of free amino acids by HPLC. Samples were ground by using a 40 μm sized cell strainer (BD Biosciences, USA) and filtered through a 0.2-μm syringe filter (PALL Life Sciences, USA). The quantitative determination of the free amino acids, including glutamate and GABA, was performed at the National Instrumentation Center for Environmental Management (NICEM), Seoul National University (Korea). The samples were diluted with distilled water and filtered through a 0.2-μm syringe filter (PALL Life Sciences, USA). Primary and secondary amino acids were automatically derivatized into fluorescent substances within the auto sampler using *O*-phthalaldehyde (Agilent Technologies) and 9-fluorenyl methyl chloroformate (Agilent Technologies), respectively. After derivatizing amino acids, separation was performed using an Inno C18 column (150 by 4.6 mm i.d., 5 mm; Innopia, Korea). Standard amino acids were purchased from Agilent Technologies. The concentrations of analyzed amino acid standards were 10, 100, 250, and 500 μM. The mobile phases A and B were a mixture of 10 mM Na_2HPO_4 (Sigma-Aldrich Co., LLC.), 10 mM $Na_2B_4O_7$ (Sigma-Aldrich Co., LLC.), and water-acetonitrile-methanol (10:45:45, v/v/v) (Honeywell Burdick & Jackson Inc., USA) and was pumped at a constant flow rate of 1.5 mL/min. The quantitative determination of free amino acids was performed using a fluorescence detector (excitation: 340 nm; emission: 450 nm). The analysis was performed using the Ultimate 3000 HPLC systems (Thermo Fisher Scientific Inc., USA).

Results

Characterization of soybean fermented by an aflatoxin non-producing *A. oryzae*. Aflatoxins were not detected in soybean paste fermented by *A. oryzae* FMB S46471 and *L. brevis* GABA 100 regardless of maturity (data not shown). The optimal protease activity of soybeans fermented by *A. oryzae* FMB S46471 was determined (Fig. S1).

Evaluation of GABA production by GABA-producing LAB. The soybeans fermented with *A. oryzae* FMB S46471 for 7 days under aerobic state were additionally inoculated with *L. brevis* GABA100 and further fermented for the following 11 days under anaerobic state. GABA production gradually increased from the day when *L. brevis* GABA100 was inoculated up to day 7 and plateaued thereafter (data not shown). GABA production is shown in the Fig. 1 with a TLC image. The optimal GABA production after inoculating *L. brevis* GABA100 was shown on day 7 when most of the glutamic acids were converted into GABA, (Fig. 1, lane 6). In contrast, the fermented sample by only *A. oryzae* FMB S46471 produced a considerably low level of GABA (Fig. 1, lane 4). Furthermore, various amino acids were actively produced in the samples fermented with *A. oryzae* FMB S46471 but not in the un-inoculated sample (Fig. 1, lane 3). The quantitative analysis of free amino acids was performed by HPLC analysis. The chromatograms of HPLC for free amino acids including GABA and gultamic acid are shown in Fig. S2. Consistent with the TLC result, the enriched GABA production was shown only in the sample fermented by *L. brevis* GABA100 together with *A. oryzae* FMB S46471 (Fig. S2).

Optimization for the GABA production in scaled-up fermentation. For increasing the scale of fermentation, the soybeans fermented by *A. oryzae* FMB S46471 were additionally inoculated with *L. brevis* GABA100 on day 12 and further

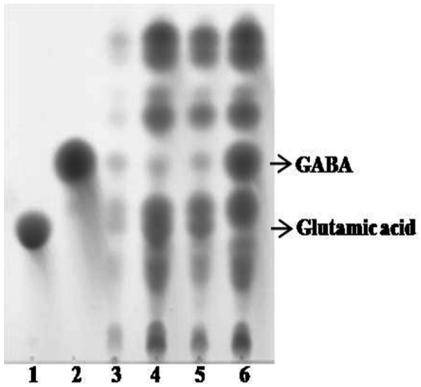

Fig. 1 Evaluation of GABA production by *L. brevis* GABA100. Thin layer chromatogram showing the GABA production from glutamic acid; 1% Glutamic acid (lane 1); 1% GABA (lane 2); the sample fermented without both *A. oryzae* FMB S46471 and *L. brevis* GABA100 for 14 days (lane 3); the sample fermented by only *A. oryzae* FMB S46471 for 14 days (lane 4); the sample fermented by *A. oryzae* FMB S46471 for 7 days were additionally inoculated with *L. brevis* GABA100 without further fermentation (lane 5); the sample fermented by *A. oryzae* FMB S46471 for 7 days were additionally inoculated with *L. brevis* GABA100 and further fermented for the following 7 days (lane 6).

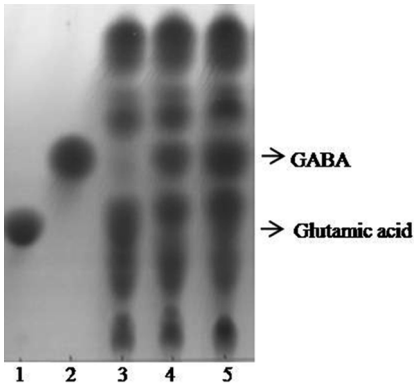

Fig. 2 GABA production in scaled-up fermentation. Thin layer chromatogram for GABA production in scaled-up fermentation showing a relative production compared with small-scale cultivation conditions; 1% Glutamic acid (lane 1); 1% GABA (lane 2); the sample fermented by only *A. oryzae* FMB S46471 for 14 days in cultivation conditions (lane 3); the sample fermented by *A. oryzae* FMB S46471 for 7 days were additionally inoculated with *L. brevis* GABA100 and further fermented for the following 7 days under cultivation conditions (lane 4); the sample fermented by *A. oryzae* FMB S46471 for 12 days were additionally inoculated with *L. brevis* GABA100 and further fermented for the following 5 days in scaled-up fermentation (lane 5).

fermented for additional 7 days (Fig. S3). GABA production was gradually increased from the day when *L. brevis* GABA100 was inoculated up to day 5 and plateaued thereafter (Fig. S3). Fig. 3 shows a relative comparison of the contents of free amino acids from fermented soybeans produced under small-scale cultivation conditions and scaled-up fermentation for GABA production. Dynamic changes were shown only in GABA and glutamic acid depending on the inoculation of *L. brevis* GABA 100 (Fig. 3). Furthermore, high GABA production was evident in scaled-up fermentation (Figs. 2 and 3). The immature soybean samples contained 6.5±0.3 g/kg of GABA and 45.7±0.3 g/kg of total free amino acids. After ripening for 90 days, the fermented soybean contained about 11.6±0.3 g/kg of GABA and 72.7±1.8 g/kg of total free amino acids. Meanwhile, commercially available soybean paste contained 0.2±0 g/kg of GABA and 24.0±0.2 g/kg of total free amino acids. Fig. S4 includes the coefficiency of determination value of standards for the validation of HPLC analysis. Table S1 includes the information for the evaluation of sanitary indicative bacteria, LAB counts and pH in tested soybean pastes.

Discussion

Due to the heat stability of aflatoxins, even quite severe processes such as freezing or cooking cannot eliminate them (Marin et al., 2013). Given the metabolism of aflatoxins in the liver, the consumption of aflatoxins for a long time may be highly dangerous even at a very low concentration (Xie et al., 2014). To ensure the safety of fermented foods, a mycotoxin non-producing *A. oryzae* that was screened and selected in a previous study (Kim et al., 2014b) was applied to the fermentation of soybean in the present study. As expected, we confirmed that aflatoxins were not detected in the fermented soybean products. Protease activity is an important criterion for estimating the quality of fermented soybean products. The *Aspergillus* strain with high protease activity was preferred, because active production of glutamic acid to be used as a GABA precursor was desired. The fermented soybean product by *A. oryzae* FMB S46471 showed better protease activity than the commercially available fermented soybean product based on Folin-Ciocalteu's analysis (data not shown).

With reference to previous studies (Kim et al., 2009; Kim et al., 2014a) the optimal pH 5.0 and anaerobic environment for GABA production was adopted in the present study. There have been many attempts to synthesize GABA in foods due to the beneficial functions of GABA (Dhakal et al., 2012; Wichamanee and Teerarat, 2012; Zhang et al., 2006). GABA enriched foods fermented with LAB in various foods such as yogurt, cheese (Nomura et al., 1998; Park and Oh, 2006; Park and Oh, 2007), *kimchi* (Lu et al., 2008; Seok et al., 2008), sourdough (Rizzello et al., 2008), *paocai* (Li et al., 2008), soy milk (Tsai et al., 2006), and sea tangle (Lee et al., 2010), have been developed. The content of GABA in cheeses produced by *Lactobacillus paracasei* PF6, PF8, and PF13, *Lactobacillus plantarum* PF14, *Lactobacillus* sp. strain PF7, and *Enterococcus durans* PF15 was 391 mg/kg (Siragusa et al., 2007). In a Japanese distilled alcoholic beverage, free 10.50 mM glutamic acid was converted to 10.18 mM GABA by *L. brevis* IFO-12005 (Yokoyama et al., 2002). GABA enriched grape must beverage was produced by fermentation using *L. plantarum* DSM19463 showing 498 mg/L GABA production (Di et al., 2010). While most of the previous studies added MSG to increase

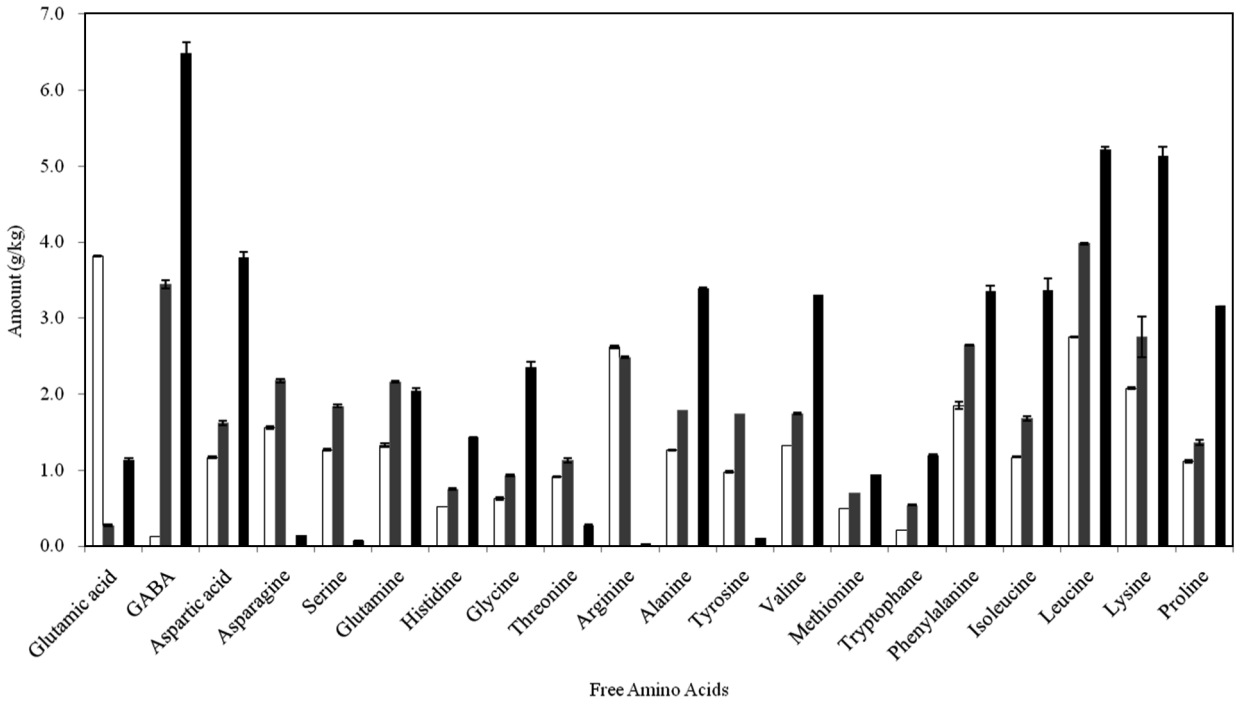

Fig. 3 Dynamic changes in the levels of GABA and glutamic acid. Relative levels of free amino acids in cultivation conditions and scaled-up fermentation *Results were carried out in duplicate. The indicated value was presented as the mean of two determinations with standard error. □, the sample fermented by only *A. oryzae* FMB S46471 for 14 days in cultivation conditions; ■, the sample fermented by *A. oryzae* FMB S46471 for 7 days were additionally inoculated with *L. brevis* GABA100 and further fermented for the following 7 days under cultivation conditions; ■, the sample fermented by FMB S46471 for 12 days were additionally inoculated with *L. brevis* GABA100 and further fermented for the following 5 days in scaled-up fermentation.

GABA production (Lu et al., 2008; Dhakal et al., 2012), the present study did not use MSG but still produced higher level of GABA than the levels reported above. In addition, Jo et al. (2011) reported the production of GABA in traditional fermented soybean paste depending on ripening periods. The contents of GABA were 1,938.7 mg/kg in the soybean paste ripened for 10 years, which suggests that the production of GABA proceeded very slowly. On the other hand, the scaled-up fermentation developed in the present study took 17 days for the optimized GABA production (about 62.9 mM). Furthermore, the matured soybean paste showed much higher levels of GABA compared to the commercially available soybean paste, whereas the GABA ratio to free amino acids were 14.2, 15.9, and 0.69% in a pre-matured, matured, and commercial soybean paste, respectively.

The viable counts of LAB in matured soybean paste maintained about 6.3 log CFU/mL (Table S1). Enriched lactic acids by LAB will contribute to the inhibition of the contamination of spoilage bacteria even at low salinity levels during manufacturing of fermented soybean foods (data not shown).

The fermentation process by *A. oryzae* FMB S46471 was accomplished under a processing line for commercialized starter production. From a feasible perspective, the fermentation process for the GABA rich starter product will be easily applicable to the existing process line by adding simple modifications such as building anaerobic conditions. As a result, it will improve the quality of fermentation product without heavy cost burden for setting up new process lines.

The soybean starter with rich GABA contents was successfully achieved in scaled-up fermentation. This is first study in which an aflatoxin non-producing *A. oryzae* and GABA producing *L. brevis* were used for the fermentation of soybean product. Thus the product, which has no aflatoxins but has high GABA contents, was successfully developed with scaled-up fermentation. Newly developed soybean paste will contribute to the development of fermented foods with enhanced safety levels and functional benefits of GABA.

Acknowledgments This work was supported by the Technology Development Program (No. 113018-3), Ministry of Agriculture, Food and Rural Affairs and by the R&D Convergence Center Support Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

References

Coda R, Rizzello CG, and Gobbetti M (2010) Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of a functional bread enriched of gamma aminobutyric acid (GABA). *Int J Food Microbiol* **137**, 236–45.

Dhakal R, Bajpai VK, and Baek KH (2012) Production of gaba (γ-

Aminobutyric acid) by microorganisms: a review. *Braz J Microbiol* **43**, 1230–41.
Di Cagno R, Mazzacane F, Rizzello CG, De Angelis M, Giuliani G, Meloni M, De Servi B, and Gobbetti M (2010) Synthesis of gamma-aminobutyric acid (GABA) by *Lactobacillus plantarum* DSM19463: functional grape must beverage and dermatological applications. *Appl microbiol biotechnol* **86**, 731–41.
Inoue K, Shirai T, Ochiai H, Kasao M, Hayakawa K, Kimura M, and Sansawa H (2003) Blood-pressure-lowering effect of a novel fermented milk containing gamma-aminobutyric acid (GABA) in mild hypertensives. *Eur J Clin Nutr* **57**, 490–5.
Jo SJ, Hong CO, Yang SY, Choi KK, Kim HK, Yang H, and Lee KW (2011) Changes in Contents of γ-Aminobutyric Acid (GABA) and Isoflavones in Traditional Korean *Doenjang* by Ripening Periods. *J Korean Soc Food Sci Nutr* **40**, 557–64.
Kim DM, Chung SH, and Chun HS (2011) Multiplex PCR assay for the detection of aflatoxigenic and non-aflatoxigenic fungi in *meju*, a Korean fermented soybean food starter. *Food Microbiol* **28**, 1402–8.
Kim EK, Shon DH, Yoo JY, Ryu D, Lee C, and Kim YB (2001) Natural occurrence of aflatoxins in Korean *meju*. *Food Addit Contam* **18**, 151–6.
Kim JA, Park MS, Kang SA, and Ji GE (2014a) Production of γ-aminobutyric acid during fermentation of *Gastrodia elata* Bl. by co-culture of *Lactobacillus brevis* GABA 100 with *Bifidobacterium bifidum* BGN4. *Food Sci Biotechnol* **23**, 459–66.
Kim JY, Lee MY, Ji GE, Lee YS, and Hwang KT (2009) Production of gamma-aminobutyric acid in black raspberry juice during fermentation by *Lactobacillus brevis* GABA100. *Int J Food Microbiol* **130**, 12–6.
Kim NY, Lee JH, Lee IH, and Ji GE (2014b) An evaluation of aflatoxin and cyclopiazonic acid production in *Aspergillus oryzae*. *J Food Prot* **77**, 1010–6.
Kitamoto K (2002) Molecular biology of the Koji molds. *Adv Appl Microbiol* **51**, 129–53.
Kono I and Himeno K (2000) Changes in gamma-aminobutyric acid content during *beni-koji* making. *Biosci Biotechnol Biochem* **64**, 617–9.
Korea Food and Drug Administration (2008) Korea Foods Industry Association. Food Code, Korea.
Korea Food and Drug Administration (2009) Functional ingredients for human health. Korea.
Kunitz M (1974) Crystalline soybean trypsin inhibitor. *J Gen Physiol* **30**, 291–351.
Lee BJ, Kim JS, Kang YM, Lim JH, Kim YM, Lee MS, Jeong MH, Ahn CB, and Je JY (2010) Antioxidant activity and ã-aminobutyric acid (GABA) content in sea tangle fermented by *Lactobacillus brevis* BJ20 isolated from traditional fermented foods. *Food Chem* **122**, 271–6.
Lee CH and Lee SS (2002) Cereal fermentation by fungi. *Appl Mycol Biotechnol* **2**, 151–70.
Lee GI, Lee HM, and Lee CH (2012) Food safety issues in industrialization of traditional Korean foods. *Food Control* **24**, 1–5.
Lee YJ, Kim JE, Kwak MH, Go J, Kim DS, Son HJ et al. (2014) Quantitative evaluation of the therapeutic effect of fermented soybean products containing a high concentration of GABA on phthalic anhydride-induced atopic dermatitis in IL-4/Luc/CNS-1 Tg mice. *Int J Mol Med* **33**, 1185–94.
Li H and Cao Y (2010) Review Lactic acid bacterial cell factories for gamma-aminobutyric acid. *Amino Acids* **39**, 1107–16.
Li H, Gao D, Cao Y, and Xu H (2008) A high γ-aminobutyric acid-producing *Lactobacillus brevis* isolated from Chinese traditional *paocai*. *Ann Microbiol* **58**, 649–53.
Lu X, Xie C, and Gu Z (2008) Isolation of γ-aminobutyric acid-producing bacteria and optimization of fermentative medium. *Biochem Eng J* **41**, 48–52.
Marin S, Ramos AJ, Cano-Sancho G, and Sanchis V (2013) Mycotoxins: occurrence, toxicology, and exposure assessment. *Food Chem Toxicol* **60**, 218–37.
Miura D, Ito Y, Mizukuchi A, Kise M, Aoto H, and Yagasaki K (2006) Hypocholesterolemic action of pre-germinated brown rice in hepatoma-bearing rats. *Life Sci* **79**, 259–64.
Nomura M, Kimoto H, Someya Y, Furukawa S, and Suzuki I (1998) Production of gamma-aminobutyric acid by cheese starters during cheese ripening. *J Dairy Sci* **81**, 1486–91.
Park JH, Kang SJ, Oh SS, and Chung DH (2001) The screening of aflatoxin producing fungi from commercial *meju* and soybean paste in western Gyeongnam by immunoassay. *J Food Hyg Safety* **16**, 274–9.
Park KB and Oh SH (2006) Isolation and characterization of *Lactobacillus buchneri* strains with high gamma-aminobutyric acid producing capacity from naturally aged cheese. *Food Sci Biotechnol* **15**, 86–90.
Park KB and Oh SH (2007) Production of yogurt with enhanced levels of gamma-aminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract. *Bioresour Technol* **98**, 1675–9.
Rizzello CG, Cassone A, Di Cagno R, and Gobbetti M (2008) Synthesis of angiotensin I-converting enzyme (ACE)-inhibitory peptides and gamma-aminobutyric acid (GABA) during sourdough fermentation by selected lactic acid bacteria. *J Agric Food Chem* **56**, 6936–43.
Seok JH, Park KB, Kim YH, Bae MO, Lee MK, and Oh SH (2008) Production and characterization of *kimchi* with enhanced levels of gamma-aminobutyric acid. *Food Sci Technol* **17**, 940–6.
Siragusa S, De Angelis M, Di Cagno R, Rizzello CG, Coda R, and Gobbetti M (2007) Synthesis of gamma-aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses. *Appl Environ Microbiol* **73**, 7283–90.
Smith DK, Kassam T, Singh B, and Elliott JF (1992) Escherichia coli has two homologous glutamate decarboxylase genes that map to distinct loci. *J Bacteriol* **174**, 5820–6.
Takeshima K, Yamatsu A, Yamashita Y, Watabe K, Horie N, Masuda K, and Kim M (2014) Subchronic toxicity evaluation of ã-aminobutyric acid (GABA) in rats. Food Chem. *Toxicol* **68**, 128–34.
Tamang JP and Samuel D (2010) Dietary cultures and antiquity of fermented foods and beverages. In *Fermented foods and beverages of the world*, Tamang JP and Kailasapathy K (eds.), pp. 1–40, CRC press Inc., USA.
Tsai JS, Lin YS, Pan BS, and Chen TJ (2006) Antihypertensive peptides and gamma-aminobutyric acid from prozyme 6 facilitated lactic acid bacteria fermentation of soymilk. *Process Biochem* **41**, 1282–8.
Ueno H (2000) Enzymatic and structural aspects on glutamate decarboxylase. *J Mol Catal B-Enzym* **10**, 67–79.
Wichamanee Y and Teerarat I (2012) Production of germinated Red Jasmine brown rice and its physicochemical properties. *Int Food R J* **19**, 1649–54.
Xie F, Lai W, Saini J, Shan S, Cui X, and Liu D (2014) Rapid pretreatment and detection of trace aflatoxin B_1 in traditional soybean sauce. *Food Chem* **150**, 99–105.
Yokoyama S, Hiramatsu J, and Hayakawa K (2002) Production of gamma-aminobutyric acid from alcohol distillery lees by *Lactobacillus brevis* IFO-12005. *J Biosci Bioeng* **93**, 95–7.
Zhang H, Yao HY, and Chen F (2006) Accumulation of gamma aminobutyric acid in rice germ using protease. *Biosci Biotechnol Biochem* **70**, 1160–5.