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Properties of Kimchi Fermented with GABA-Producing Lactic Acid Bacteria as a Starter

Kang Wook Lee[1], Jae Min Shim[1], Zhuang Yao[1], Jeong A Kim[1], and Jeong Hwan Kim[1,2*]

[1]*Division of Applied Life Science (BK21 Plus), Graduate School, Gyeongsang National University, Jinju 52828, Republic of Korea*
[2]*Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea*

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**Corresponding author*
Phone: +82-55-772-1904;
Fax: +82-55-772-1909;
E-mail: jeonghkm@gnu.ac.kr

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Kimchi (a traditional Korean fermented vegetable) was prepared with a starter, *Lactobacillus zymae* GU240 producing γ-aminobutyric acid (GABA), and one precursor of GABA (glutamic acid, glutamic acid monosodium salt (MSG), or kelp extract). *L. zymae* GU240, an isolate from kimchi, can grow at 7% NaCl and low temperature. Five different kimchi samples were fermented for 20 weeks at –1°C. Kimchi with starter alone could not produce GABA. The GABA content was highest in kimchi with co-inoculation of the starter and MSG (1% (w/w)). Kimchi co-inoculated with the starter and kelp extract powder (3% (w/w)) had the second highest GABA content. Addition of glutamic acid powder (1% (w/w)) caused a reduction in the pH level of kimchi and growth inhibition of lactic acid bacteria and yeasts. Kimchi samples with MSG or kelp extract showed improvement of sensory evaluation scores. The results demonstrate the possibility to produce kimchi with improved functionality and taste by using *L. zymae* GU240 as a starter along with a suitable precursor such as MSG or kelp extract.

Keywords: Kimchi, GABA, *Lactobacillus zymae*, MSG

Introduction

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian brain and is known to exert various functionalities such as diuretic, hypotensive, sedative, and antidiabetic effects [1, 2]. GABA is produced from its precursor, L-glutamic acid, by irreversible decarboxylation mediated by glutamate decarboxylase (GAD, E.C.: 4.1.1.15), which is widely conserved among animals, plants, and microorganisms. Lactic acid bacteria (LAB) are the dominant group among GABA-producing microorganisms. Many species of LAB have been isolated from various fermented foods, including kimchi (a traditional Korean fermented vegetable), cheese, yogurt, and other fermented foods [2–4]. GABA-producing LAB have been used as starters for kimchi, yogurt, and cheese fermentation processes in order to produce fermented foods with enhanced GABA contents [5–8].

In recent years, significant portions of the kimchi annually consumed in Korea have been produced commercially using starters [9]. Starters are believed to confer positive changes to kimchi by improving its sensory properties and extending its shelf-life [10]. Kimchi is known to possess functionalities such as anticancer, antioxidative, and anti-obesity effects [11]. If a GABA-producing strain is used as a starter, the functionalities of kimchi will be further potentiated.

Lactobacillus zymae GU240, which was isolated from kimchi, abundantly produces GABA if a precursor of GABA such as L-glutamic acid or monosodium glutamate (MSG) is present in the growth medium [12]. *L. zymae* GU240 grows in the presence of NaCl up to 7% (w/v) and grows at 4°C (data not shown). *L. zymae* GU240 also possesses β-galactosidase activity (data not shown). Thus, the strain seems suitable as a starter for fermented foods, including kimchi and dairy products such as yogurt. In this work, kimchi samples were prepared with *L. zymae* GU240 as a starter. Three different precursors of GABA (MSG, L-glutamate, and kelp extract) were tested for their efficacies for GABA production during kimchi fermentation. The

GABA contents and other properties of the kimchi samples were examined during 20 weeks of fermentation at −1°C. The results confirm the possibility to produce kimchi with improved functionalities and taste at large-scale by use of suitable starters and precursors.

Materials and Methods

Preparation of Kimchi Samples

Baechu (*Brassica rapa* subsp. *pekinensis*) was purchased at a local market in Jinju, Republic of Korea in the Fall of 2016, soaked in brine (10% (w/v)) prepared with solar salt aged for 3 years (TaePong Salt Farm, Korea) for 10 h, and then washed three times with tap water. Washed baechu was stored in plastic baskets for 3 h to drain excess water, then cut into 4 × 4 cm squares, and mixed with ingredients according to the following formula : baechu : red pepper powder : crushed garlic : crushed ginger : jeotgal (salted and fermented seafood) : radish : sugar : scallion = 100 : 3.5 : 1.4 : 0.6 : 2.2 : 13 : 1 : 2 (w/w). *L. zymae* GU240 was grown in MRS broth overnight at 30°C, and cells were recovered by centrifugation. The cells were washed three times with cold sterile water and resuspended in a small volume of sterile water. The cells were added into the sliced baechu (10^7 CFU/g kimchi) together with seasonings and a precursor of GABA. The salt concentration was adjusted to 1.7−1.8% (NaCl content). *L. zymae* GU240 was deposited into the Korean Federation of Culture Collections (Korea) under the accession number KFCC11545P.

Five different kimchi samples were prepared: kimchi without a starter and precursor (control, kimchi A), kimchi inoculated with a starter (kimchi B), kimchi inoculated with a starter and L-glutamic acid (Sigma G1251, 1% (w/w)) (kimchi C), kimchi inoculated with a starter and L-glutamic acid monosodium salt (MSG; Sigma G1626, USA, 1% (w/w)) (kimchi D), and kimchi inoculated with a starter and kelp extract (3% (w/w)) (kimchi E).

Each precursor (glutamic acid, MSG, and kelp extract) was added as a powder. Dried kelp (*Laminaria japonica*) (1.3 kg) was purchased at a local market and cut into small pieces that were then ground into powder using a blender. The powder was put into boiling water (15 L), and boiling water extraction was carried out for 2 h. The hot water extract (4.3 L) was concentrated using a vacuum rotatory evaporator, and the concentrate (430 ml) was freeze-dried into a powder for use. Immediately after preparation, the kimchi samples were put into plastic containers and stored at −1°C using Dimchae, a kimchi refrigerator (Winia, Korea), for 20 weeks, and samples were examined every 2 weeks.

Total Viable Bacteria, LAB, and Yeast Counts of Kimchi Samples

Kimchi samples were mixed with 0.1% peptone water and homogenized by using a stomacher 80 (Seward, UK). The homogenate was serially diluted with 0.1% peptone water. Diluents were spread onto agar plates for viable cell counting on plate count agar (PCA; Becton, Dickinson and Company, USA) for total viable bacteria, de Man, Rogosa, and Sharpe (MRS; Becton, Dickinson and Company, USA) agar for LAB; and yeast extract peptone dextrose agar (YPD agar; Becton, Dickinson and Company, USA) with chloramphenicol (50 μg/ml) for yeasts.

pH, Titratable Acidity (TA), and Salinity

Kimchi samples (100 g) were macerated using a laboratory blender (Waring Laboratory Science, USA). After centrifugation for 15 min at 14,000 ×*g*, the supernatant was filtered through sterile gauze, and the filtrate was used as a sample for pH, TA, and salinity measurements.

The pH level was measured using a pH meter. The TA was calculated using the following equation after the filtrate was titrated with 0.1 N NaOH to pH 8.3.

$$TA\ (\%) = \text{amount of 0.1 N NaOH (ml)} \times 0.009 \times 10.$$

The salinity was measured with a salimeter (PAL-SALT; Atago Co., Ltd., Japan).

Measurements of GABA Contents of Kimchi Samples

The GABase method was used to measure the GABA contents of the kimchi samples [12]. Kimchi samples (50 g) were freeze-dried, and the resulting powder was extracted with methanol. The methanol extract was used as the substrate for GABase assay. The assay mixture (1 ml) consisted of 50 μl of sample, 700 μl of 0.5 M potassium pyrophosphate buffer (pH 8.6), 150 μl of 4 mM $NADP^+$, 50 μl of GABase (1 unit/ml; Sigma, USA), and 50 μl of 20 mM α-ketoglutarate. The initial absorbance was read at 340 nm before adding α-ketoglutarate, and the final absorbance was read after 60 min at 25°C. The difference in OD_{340} values was used to calculate the GABA content of the sample. One unit of GAD activity (U) was defined as the amount of enzyme producing 1 μmol of GABA per minute.

Reducing Sugar Contents of Kimchi Samples

A colorimetric method using dinitrosalicylic acid (DNS) solution was used to determine the reducing sugar contents of the kimchi samples [13]. A 0.5 ml aliquot of filtrate, obtained as described above for pH measurement, was mixed with 1.5 ml of DNS reagent and boiled for 5 min. The absorbance at 550 nm was measured using a spectrophotometer (UV-1601; Shimadzu, Japan), and the reducing sugar content was calculated from a glucose standard curve.

Sensory Evaluation of Kimchi Samples

Sensory evaluations of kimchi samples were done twice, with the first at 10 weeks and the second at 20 weeks of fermentation. The panel size was 14 (average age 26.5 years, men:women = 4:3) for the 1st test and 20 (average age 24.9 years, men:women = 2:3) for the 2nd test. A 5-point scale for color, flavor, taste, texture, and overall acceptability was used.

Table 1. Total viable bacteria, LAB, and yeasts in kimchi samples during fermentation.

Samples	Counts (CFU/g)	Fermentation time (Weeks)										
		0	2	4	6	8	10	12	14	16	18	20
A	Total viable cell	9.61×10^{4}	2.68×10^{5}	1.61×10^{7}	1.53×10^{8}	5.13×10^{9}	4.68×10^{8}	1.68×10^{8}	4.87×10^{7}	1.83×10^{7}	7.84×10^{6}	3.26×10^{6}
	LAB	9.42×10^{4}	2.64×10^{5}	1.59×10^{7}	1.48×10^{8}	5.01×10^{9}	4.63×10^{8}	1.65×10^{8}	4.41×10^{7}	1.79×10^{7}	7.80×10^{6}	3.21×10^{6}
	Yeast	-	-	-	-	-	-	-	5.30×10^{3}	3.48×10^{4}	5.14×10^{4}	8.19×10^{4}
B	Total viable cell	1.23×10^{7}	3.29×10^{7}	4.19×10^{7}	4.53×10^{8}	4.64×10^{9}	1.89×10^{9}	3.37×10^{8}	1.52×10^{7}	4.86×10^{6}	4.19×10^{6}	3.38×10^{6}
	LAB	1.14×10^{7}	3.36×10^{7}	4.21×10^{7}	4.41×10^{8}	4.67×10^{9}	1.19×10^{9}	3.34×10^{8}	1.47×10^{7}	4.83×10^{6}	1.15×10^{4}	3.32×10^{6}
	Yeast	-	-	-	-	-	-	-	2.31×10^{2}	8.12×10^{3}	3.61×10^{5}	3.85×10^{4}
C	Total viable cell	1.23×10^{7}	9.49×10^{6}	2.34×10^{6}	1.65×10^{6}	1.44×10^{6}	6.42×10^{5}	6.11×10^{5}	4.28×10^{5}	4.64×10^{5}	3.63×10^{5}	5.66×10^{5}
	LAB	1.26×10^{7}	9.33×10^{6}	2.30×10^{6}	1.66×10^{6}	1.41×10^{6}	6.19×10^{5}	6.02×10^{5}	4.11×10^{5}	4.56×10^{5}	4.27×10^{3}	5.63×10^{5}
	Yeast	-	-	-	-	-	-	-	-	2.03×10^{3}	4.27×10^{3}	6.15×10^{3}
D	Total viable cell	1.70×10^{7}	1.51×10^{7}	4.48×10^{7}	4.85×10^{8}	8.44×10^{8}	6.65×10^{8}	5.61×10^{8}	2.15×10^{9}	1.41×10^{8}	6.81×10^{7}	3.93×10^{7}
	LAB	1.72×10^{7}	1.43×10^{7}	4.35×10^{7}	4.82×10^{8}	8.40×10^{8}	6.51×10^{8}	5.25×10^{8}	2.08×10^{9}	1.28×10^{8}	6.73×10^{7}	3.95×10^{7}
	Yeast	-	-	-	-	-	-	-	-	7.18×10^{2}	1.23×10^{3}	3.18×10^{3}
E	Total viable cell	1.12×10^{7}	3.34×10^{7}	6.61×10^{7}	1.79×10^{8}	4.25×10^{8}	5.64×10^{8}	5.26×10^{8}	1.59×10^{9}	1.24×10^{8}	6.03×10^{7}	4.14×10^{7}
	LAB	1.04×10^{7}	3.33×10^{7}	6.53×10^{7}	1.75×10^{8}	4.17×10^{8}	5.60×10^{8}	5.27×10^{8}	1.53×10^{9}	1.21×10^{8}	6.01×10^{7}	4.10×10^{7}
	Yeast	-	-	-	-	-	-	-	-	6.13×10^{2}	1.18×10^{3}	2.37×10^{3}

Statistical Analysis

All measurements were repeated three times, and the results were expressed as the mean ± standard deviation. Sensory data were analyzed by one-way ANOVA, and Duncan's multiple range test was done using the SPSS ver. 18 (SPSS Inc., USA) package (with statistical significance at $p < 0.05$).

Results and Discussion

Total Viable Bacteria, LAB, and Yeasts Counts of Kimchi Samples

Immediately after preparation, the number of LAB was 9.42×10^{4} CFU/g kimchi for kimchi A (control), and the number increased continuously to 5.01×10^{9} CFU/g kimchi at 8 weeks of storage at −1°C (Table 1). Thereafter, the number decreased gradually to 3.21×10^{6} CFU/g kimchi at 20 weeks. The initial LAB count of kimchi B (starter only) was 1.14×10^{7} CFU/g kimchi, which was 121-fold higher than that of kimchi A. The highest LAB count (4.67×10^{9} CFU/g kimchi) was observed at 8 weeks, after which the number decreased gradually to 3.32×10^{6} CFU/g kimchi at 20 weeks. Although kimchi B showed 121-fold higher LAB counts than kimchi A immediately after preparation, the differences in LAB counts decreased as fermentation proceeded, and the counts were identical at 8 and 20 weeks. LAB counts of kimchi samples inoculated with a starter and GABA precursor were variable and dependent upon the precursor. The LAB count of kimchi C (starter and glutamic acid powder) was 1.26×10^{7} CFU/g kimchi immediately after preparation, and the counts decreased continuously to 1.41×10^{6} and 5.63×10^{5} CFU/g kimchi at 8 and 20 weeks, respectively. Kimchi C showed the lowest LAB count during the entire fermentation period except the first 2 weeks, which was caused by growth inhibition of LAB by glutamic acid. The LAB count of kimchi D was 1.72×10^{7} CFU/g kimchi immediately after preparation. The LAB count then decreased to 1.43×10^{7} CFU/g kimchi at 2 weeks but increased after 2 weeks, reaching a maximum of 2.08×10^{9} CFU/g kimchi at 14 weeks. Thereafter, the LAB count decreased again to 3.95×10^{7} CFU/g kimchi at 20 weeks. The LAB counts of kimchi D were 12.3, 11.9, and 70 times higher than those of kimchi A, B, and C, respectively, at 20 weeks. The LAB counts of kimchi E were 1.53×10^{9} and 4.10×10^{7} CFU/g kimchi at 14 and 20 weeks, respectively. The LAB counts of kimchi E were 12.8, 12.3, and 72.8 times higher than those of kimchi A, B, and C, respectively, at 20 weeks. Kimchi C had lower LAB counts than kimchi B (17% of kimchi B) at 20 weeks. At 20 weeks, kimchi B (starter only) showed the same LAB count as kimchi A (control). However, kimchi D and E showed significantly higher (12-fold) LAB counts than kimchi A. The results indicate that addition of a GABA precursor stimulated the growth of LAB by providing additional nutrients. It is possible that the increased count of *L. zymae* GU240 in kimchi D and E was due to the generation of extra energy by GABA production. It has been reported that GABA production is associated with not only increased acid resistance of LAB against acidic environments but also extra ATP production [14, 15]. Considering this, *L. zymae* GU240 is a good starter for kimchi and other fermented foods if inoculated along with MSG or kelp extract.

PCA plates were used to count the total viable bacteria in the kimchi samples, and the numbers were quite similar with those of the LAB counts on MRS plates (Table 1). The results indicate that LAB were the most abundant microorganisms in the kimchi samples. Immediately after preparation, the total viable count of kimchi A (control) was 9.61×10^4 CFU/g kimchi, which was 102% of the total LAB count. The same ratios between total bacterial counts and LAB counts were maintained throughout 20 weeks.

Yeasts were detected from kimchi A and B at 14 weeks as well as from other kimchi samples at 16 weeks. At 14 weeks, the yeast count of kimchi A was 5.30×10^3 CFU/g kimchi whereas that of kimchi B was 2.31×10^2 CFU/g kimchi. Kimchi A and B showed higher yeast counts than other kimchi samples. At 20 weeks, kimchi A showed the highest yeast count (8.19×10^4 CFU/g kimchi) followed by kimchi B (3.85×10^4 CFU/g kimchi). The yeast counts of kimchi C and D were 6.15×10^3 and 3.18×10^3 CFU/g kimchi, respectively. Kimchi E showed the lowest yeast count (2.37×10^3 CFU/g kimchi), which was 2.9% of kimchi A. It is noteworthy that lower yeast counts were observed in kimchi D and E where the LAB counts were higher. One exception is kimchi C, where both counts were low. Glutamic acid powder seemed to inhibit the growth of not only LAB but also yeasts. Yeasts appear during later stages of kimchi fermentation and are considered to be undesirable for kimchi fermentation since they generate off-flavors and deteriorate the texture of kimchi by secreting pectinases [10, 16]. The growth of *L. zymae* GU240 seemed to discourage the growth of yeasts, thereby delaying the appearance of yeasts by 2 weeks. The LAB counts of kimchi D and E were much higher than those of kimchi A and B at 14 weeks. The yeast counts of kimchi D and E remained at low levels during 16–20 weeks.

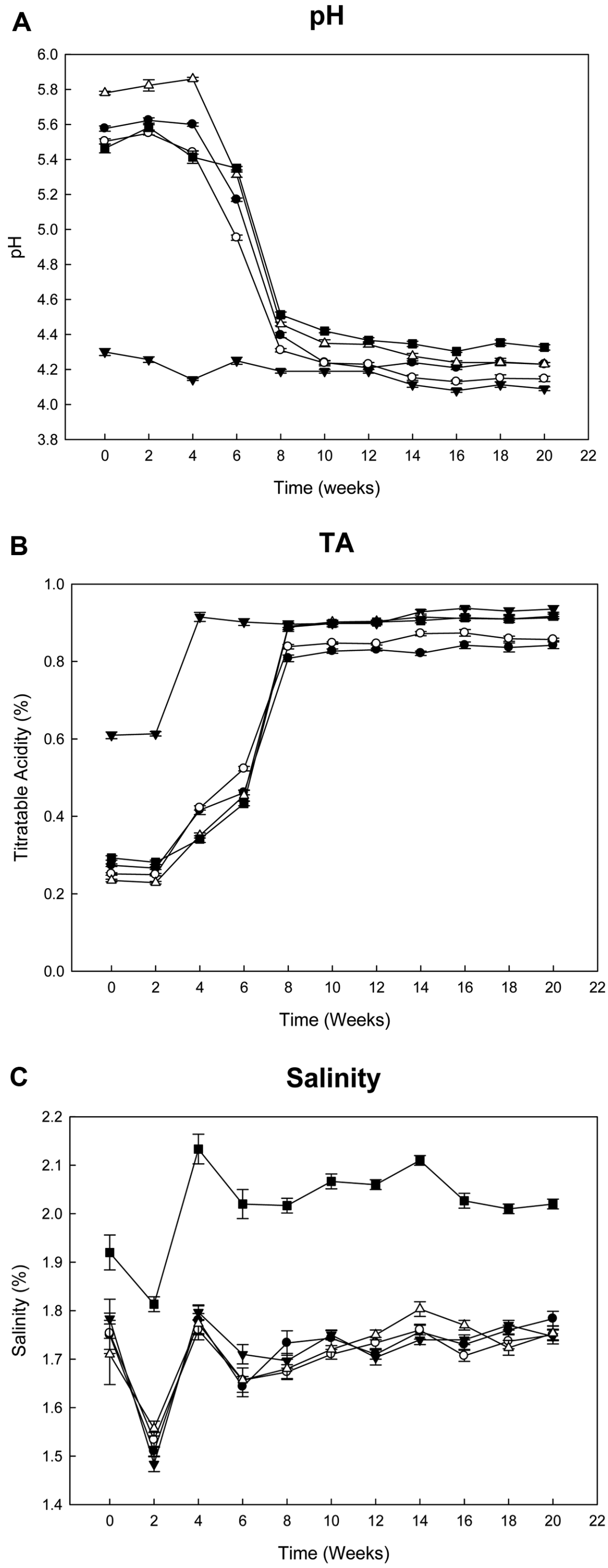

Fig. 1. Changes in pH, titratable acidity (TA), and salinities of kimchi samples during fermentation.

pH, TA, and Salinities of Kimchi Samples

The pH values of kimchi samples immediately after preparation were 5.46–5.78, except kimchi C where the pH was quite low at 4.30 (Fig. 1A). Addition of glutamic acid powder caused a reduction of pH. Glutamic acid powder was not dissolved readily upon addition, whereas it dissolved slowly during fermentation. During fermentation, *L. zymae* GU240, a heterolactic fermenter, and other LAB produced lactic acid and acetic acid, resulting in pH reductions in the kimchi samples. Addition of glutamic acid further reduced the pH in kimchi C. All kimchi samples showed a reduction of pH during fermentation, and a significant decrease occurred between 6 and 8 weeks. The pH of kimchi C was 4.09 ± 0.01 at 20 weeks, which was the lowest among the kimchi samples. Other kimchi samples showed slightly higher pH values: kimchi B, 4.15; kimchi A and D, 4.23; and kimchi E, 4.33.

The TA values of the kimchi samples changed with the pH values but in the reverse direction (Fig. 1 B). Kimchi C showed the highest TA (0.61 ± 0.01) immediately after preparation, and the other kimchi samples showed TA values of 0.23–0.29. The TA values increased with fermentation time, and significant increases occurred between 6 and 8 weeks, similar to the changes in pH. This observation can be attributed to the significantly increased LAB counts between 6 and 8 weeks. Kimchi C showed the highest TA value of 0.94 ± 0.01 at 20 weeks, whereas the other kimchi samples showed TA values of 0.84–0.94. Kimchi A showed the lowest TA (0.84 ± 0.01). Kimchi is in its best state for consumption when its TA value reaches 0.6–0.8 during fermentation [11, 16]. Therefore, all kimchi samples were well fermented and ready for consumption after 8 weeks, and the states were maintained until 20 weeks.

The salinities of the kimchi samples ranged from 1.71 (kimchi D) to 1.92 (kimchi E) immediately after preparation. The salinities of the kimchi samples except kimchi E were not altered significantly during fermentation (Fig. 1C). Kimchi E showed a higher salinity than the other kimchi samples. The salinity of kimchi E was highest at 4 weeks (2.13 ± 0.03), after which it decreased gradually to 2.02 ± 0.01 at 20 weeks. Unlike MSG and L-glutamic acid, kelp extract contains salt originating from fresh kelp. It was previously reported that 8 g of NaCl is present per 100 g of dried kelp [17]. Therefore, the higher salinity of kimchi E can be attributed to the kelp extract.

GABA Contents of Kimchi Samples

The GABA content of kimchi A was 6.7 ± 1.1 mg GABA/100 g kimchi immediately after preparation and increased during fermentation, reaching a maximum value of 62.0 ± 1.5 mg GABA/100 g kimchi at 8 weeks. The GABA content then decreased gradually to 47.9 ± 2.0 mg GABA/100 g kimchi at 20 weeks. The GABA contents of kimchi B were similar to those of kimchi A, reaching maximum values of 69.3 ± 0.9 mg GABA/100 g kimchi at 10 weeks and 47.8 ± 0.7 mg GABA/100 g kimchi at 20 weeks (Table 2). The results indicate that *L. zymae* GU240 alone could not produce GABA unless a suitable precursor was present. GABA was produced by kimchi samples where a precursor was provided along with *L. zymae* GU240, and the concentration increased during fermentation. Kimchi D showed a higher GABA content than the other samples, and the highest GABA content was observed at 8 weeks (120.3 ± 2.8 mg GABA/100 g kimchi). After 8 weeks, the GABA content decreased slowly to 95.6 ± 1.4 mg GABA/100 g kimchi at 20 weeks. For kimchi E, its highest GABA content (82.2 ± 2.2 mg GABA/100 g kimchi) was observed at 10 weeks, after which the GABA content decreased to 55.1 ± 3.7 mg GABA/100 g kimchi at 20 weeks. For kimchi C, its highest GABA content was 76.6 ± 2.4 mg GABA/100 g kimchi at 10 weeks and decreased to 62.5 ± 1.4 mg GABA/100 g kimchi at 20 weeks. The GABA contents of kimchi D were 194% and 174% higher than those of kimchi A and kimchi B, respectively, when the highest contents of

Table 2. GABA contents of kimchi samples during fermentation.

Samples	GABA contents (mg/100 g kimchi) at each week										
	0	2	4	6	8	10	12	14	16	18	20
A	6.70 ± 1.1	12.6 ± 1.2	31.3 ± 2.5	55.5 ± 2.7	62.0 ± 1.5	61.8 ± 0.6	53.4 ± 0.9	48.6 ± 1.6	46.6 ± 1.3	48.5 ± 3.1	47.9 ± 2.0
B	8.3 ± 0.6	13.3 ± 1.4	59.1 ± 2.8	59.5 ± 0.8	58.9 ± 1.9	69.3 ± 0.9	59.3 ± 1.8	52.3 ± 1.4	49.0 ± 1.3	46.8 ± 1.5	47.8 ± 0.7
C	2.3 ± 0.8	14.0 ± 2.4	65.2 ± 1.3	64.6 ± 3.0	73.1 ± 2.0	76.6 ± 2.4	69.9 ± 2.4	68.8 ± 2.1	67.8 ± 2.6	63.5 ± 1.3	62.5 ± 1.4
D	11.8 ± 0.7 (1.93%[a])	18.4 ± 0.5 (3.01%)	115.3± 6.4 (18.92%)	111.9± 0.9 (18.36%)	120.3± 2.8 (19.75%)	118.0 ± 1.7 (19.36%)	112.2 ± 2.9 (18.41%)	107.5 ± 1.7 (17.63%)	105.9 ± 1.4 (17.38%)	96.5 ± 1.4 (15.84%)	95.6 ± 1.4 (15.69%)
E	8.4 ± 0.5	12.7 ± 0.3	66.1 ± 2.9	81.2 ± 3.5	76.3 ± 5.1	82.2 ± 2.2	72.7 ± 4.1	63.6 ± 1.9	64.8 ± 1.4	56.6 ± 1.4	55.1 ± 3.7

[a]Conversion ratio, GABA produced (mmole) divided by MSG (mmole) × 100.

kimchi samples were compared. The GABA contents of kimchi E were 133% and 119% higher than those of kimchi A and B, respectively. MSG (1% (w/w)) was the most effective precursor for GABA production, and kelp extract powder (3% (w/w)) was the least effective. Glutamic acid powder (1% (w/w)) was the least effective precursor for GABA production owing to growth inhibition of *L. zymae* GU240 by glutamic acid. For kimchi D, the conversion yields of GABA from MSG were 19.75% at 8 weeks and 15.69% at 20 weeks.

Considering the GABA contents and TA values, kimchi D and E were the best for consumption after 8 or 10 weeks in a kimchi refrigerator. Seok *et al*. [5] prepared baechu kimchi by inoculating *Lactobacillus* sp. OPK 2-59, a starter (0.2% (w/w)), or MSG (0.1% (w/w)). They also prepared kimchi by co-inoculating a starter and MSG. The kimchi samples were incubated at 15°C for 24 h and then stored at 0−1°C for 24 days. Kimchi co-inoculated with a starter and MSG contained 18 mg GABA/100 g fresh kimchi, whereas kimchi inoculated with either a starter or MSG alone contained 6 mg GABA/100 g fresh kimchi. Kimchi co-inoculated with a starter and MSG showed better scores than the other kimchi samples in the sensory evaluations.

It is difficult to directly compare our results with those of Seok *et al*. [5] since they inoculated freeze-dried cells as a starter (0.2% (w/w)) and fermentation was carried out for 24 days, which is significantly shorter than this work. In our experiment, freshly grown cells resuspended in water were inoculated (10^7 CFU/g kimchi), and 10 times more MSG (1% (w/w)) was used. Fermentation was carried out for a long period (20 weeks) at −1°C without preincubation at 15°C. The analytical methods used for GABA quantification were also different. In this work, the GABase method was used, whereas the HPLC method was used by Seok *et al.* [5]. These differences make it difficult to directly compare the efficacies of GABA production. However, there were some common observations. Addition of a starter and precursor together caused an increase in the GABA contents of the kimchi samples during fermentation. Growth of LAB was accelerated by addition of MSG, and more LAB counts were observed for kimchi D and E. Additionally, we observed production of kimchi with enhanced GABA content, and the higher GABA content was successfully maintained for a long period of at least 6−8 weeks in this work.

Cho *et al.* [6] prepared sour kimchi by inoculating *Lactobacillus buchneri* isolated from Mukeunjee kimchi and carrying out fermentation for 3 days at 30°C. The GABA content of the resulting sour kimchi (61.65 mg/100 g kimchi) was 8 times higher than that of control sour kimchi where no starter was added. These results are interesting since no GABA precursor was used, and fermentation was carried out for just 3 days. Therefore, kimchi with different GABA contents and properties can be produced depending upon the starter organisms and fermentation conditions. Further studies are necessary to compare the efficacies of starters under the same kimchi fermentation conditions and using the same analytical methods.

Reducing Sugar Contents of Kimchi Samples

Immediately after preparation, the reducing sugar content of kimchi A was 33.2 ± 1.8 mg/100 g kimchi. The reducing sugar content increased until 6 weeks, reaching a maximum value of 36.7 ± 1.48 mg/100 g kimchi, after which it decreased gradually to 30.9 ± 3.18 mg/100 g kimchi at 20 weeks. Kimchi B showed a similar pattern as kimchi A. Immediately after preparation, the reducing sugar contents of kimchi C, D, and E were higher than those of kimchi A and B (Table 3), and this was due to the addition of a precursor. The reducing sugar contents decreased gradually during fermentation. The final contents for kimchi C and D were lower than those for kimchi A and B. However, kimchi E had the same reducing sugar content as kimchi A and B. Increased LAB counts seemed to cause elevation of the reducing sugar contents from the raw materials of kimchi during the early stage of kimchi fermentation. Then, LAB utilized reducing sugars for growth, causing reduction of the reducing sugar contents in the middle and later stages

Table 3. Reducing sugar contents of kimchi samples during fermentation.

Samples	Reducing sugar (mg/100 g kimchi) at each week										
	0	2	4	6	8	10	12	14	16	18	20
A	33.2 ± 1.8	34.9 ± 2.6	36.2 ± 3.1	36.7 ± 1.4	34.8 ± 3.1	34.2 ± 2.4	32.4 ± 1.3	31.8 ± 3.3	30.6 ± 2.9	31.5 ± 1.7	30.9 ± 3.1
B	33.4 ± 4.8	34.4 ± 1.7	34.8 ± 2.2	36.2 ± 2.1	39.2 ± 2.9	34.2 ± 1.9	34.0 ± 0.5	34.2 ± 2.5	34.1 ± 1.8	33.6 ± 3.2	32.8 ± 1.4
C	43.1 ± 3.7	40.3 ± 1.6	38.9 ± 3.5	34.2 ± 4.1	27.0 ± 2.4	28.1 ± 2.5	28.0 ± 0.7	24.2 ± 2.1	25.6 ± 5.1	24.4 ± 2.5	23.5 ± 2.1
D	61.2 ± 4.0	45.4 ± 4.5	47.2 ± 2.9	37.1 ± 2.4	23.4 ± 1.8	23.2 ± 3.4	24.2 ± 2.1	21.8 ± 3.4	22.4 ± 1.8	23.1 ± 1.3	21.5 ± 2.8
E	62.0 ± 2.1	62.1 ± 3.4	64.7 ± 3.4	56.3 ± 2.3	42.4 ± 1.9	35.2 ± 1.4	32.4 ± 0.4	31.4 ± 0.9	31.1 ± 4.1	30.3 ± 2.4	31.7 ± 1.9

Table 4. Sensory evaluation results of the kimchi samples.
(**A**) 10 weeks

Samples	Items				
	Color	Flavor	Texture	Taste	Overall acceptability
A	3.64 ± 0.74[a]	3.43 ± 0.65[a]	3.14 ± 0.95[a]	2.50 ± 1.02[a]	3.00 ± 0.78[a]
B	3.14 ± 1.03[a]	3.29 ± 1.07[a]	3.29 ± 1.27[a]	3.14 ± 1.10[ab]	3.14 ± 1.23[ab]
C	3.29 ± 0.91[a]	3.07 ± 1.00[a]	3.79 ± 0.89[a]	3.36 ± 1.22[bc]	3.57 ± 1.02[abc]
D	3.57 ± 0.85[a]	3.57 ± 0.76[a]	3.93 ± 0.73[a]	4.0 ± 0.96[c]	4.14 ± 0.77[c]
E	3.36 ± 0.93[a]	3.36 ± 0.84[a]	3.86 ± 0.77[a]	3.71 ± 0.83[bc]	3.79 ± 0.70[bc]

Different letters indicate significant difference between the values in the same tested items ($p < 0.05$, by least significant difference test).

(**B**) 20 weeks

Samples	Items				
	Color	Flavor	Texture	Taste	Overall acceptability
A	4.00 ± 0.92[a]	2.95 ± 0.76[a]	2.95 ± 1.00[a]	2.50 ± 0.89[a]	2.65 ± 1.04[a]
B	3.60 ± **0.99[a]**	3.35 ± 0.67[a]	3.25 ± 1.07[ab]	2.95 ± 0.94[ab]	3.10 ± 0.85[ab]
C	3.35 ± 0.93[a]	3.60 ± 1.35[a]	3.65 ± 0.99[ab]	3.45 ± 1.32[bc]	3.60 ± 1.23[b]
D	3.65 ± 1.04[a]	3.45 ± 0.94[a]	3.80 ± 1.01[b]	3.75 ± 1.02[c]	3.80 ± 1.06[b]
E	3.50 ± 1.15[a]	3.35 ± 0.81[a]	3.60 ± 1.19[ab]	3.80 ± 1.06[c]	3.80 ± 1.11[b]

Different letters indicate significant difference between the values in the same tested items ($p < 0.05$, by least significant difference test).

of kimchi fermentation. Seok *et al.* [5] reported similar results for reducing sugar changes during kimchi fermentation. Kimchi co-inoculated with a starter and MSG showed lower reducing sugars than kimchi prepared with a starter or MSG alone [5]. Their results also indicated the possibility that higher LAB counts in kimchi co-inoculated with a starter and MSG might be the reason for the lower reducing sugar contents.

Sensory Evaluations

There were no differences in color or flavor among the kimchi samples at 10 and 20 weeks. The results indicate that addition of a starter and precursor did not significantly affect the color or flavor of the kimchi samples. Kimchi samples prepared with a starter received better scores for texture than control kimchi, although the differences were not statistically significant. Kimchi D and E, however, were significantly better than the other kimchi samples in terms of taste. MSG is known as a flavor enhancer and thus contributed to the improved taste of kimchi D [18]. Kelp extract obtained by hot water extraction is known to contain free amino acids such as glutamic acid and aspartic acid in addition to nucleic acids, which are known to improve taste [19, 20]. For these reasons, addition of MSG or kelp extract resulted in higher overall acceptability scores for kimchi D and E.

Our results agree with those of Seok *et al.* [5] who reported that kimchi co-inoculated with a starter and MSG received the highest sensory evaluation scores.

L. zymae GU240 grows well at low temperature and up to 7% NaCl, and the strain possesses strong β-galactosidase activity. Counts of *L. zymae* GU240 increased in kimchi samples where MSG or kelp extract was included. On the basis of these results, it can be concluded that *L. zymae* GU240 is a promising starter for kimchi and other fermented foods such as yogurt. Use of *L. zymae* GU240 as a starter together with MSG was found to be effective in enhancing the functionality and taste of kimchi. Kimchi fermented for 8–10 weeks at -1°C was the best for consumption in terms of GABA content and TA value. Further studies on the optimum concentration of MSG, preparation method for kelp extract, and amount of kelp extract are necessary. Studies are also required to compare starter organisms under the same kimchi production and fermentation conditions. The expected results will be useful to establish methods to produce kimchi with enhanced levels of GABA and improved sensory properties.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Komatsuzaki N, Shima J, Kawamoto S, Momose H, Kimura T. 2005. Production of γ-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiol*. **22:** 497-504.
2. Siragusa S, Angelis MD, Cagno RD, Rizzello CG, Coda R, Gobbetti M. 2007. Synthesis of γ-aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses. *Appl. Environ. Microbiol.* **73:** 7283-7290.
3. Cho YR, Chang JY, Chang HC. 2007. Production of γ-aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from kimchi and its neuroprotective effect on neuron cells. *J. Microbiol. Biotechnol.* **17:** 104-109.
4. Shan Y, Man CX, Han X, Li L, Guo Y, Deng Y, *et al*. 2015. Evaluation of improved γ-aminobutyric acid production in yogurt using *Lactobacillus plantarum* NDC75017. *J. Dairy Sci.* **98:** 2138-2149.
5. Seok JH, Park KB, Kim YH, Bae MO, Lee MK, Oh SH. 2008. Production and characterization of kimchi with enhanced levels of γ-aminobutyric acid. *Food Sci. Biotechnol*. **17:** 940-946.
6. Cho SY, Park MJ, Kim KM, Ryu JH, Park HJ. 2011. Production of high γ-aminobutyric acid (GABA) sour kimchi using lactic acid bacteria isolated from mukeunjee kimchi. *Food Sci. Biotechnol*. **20:** 403-408.
7. Park KB, 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:** 1675-1679.
8. Pouliot-Mathieu K, Gardner-Fortier G, Lemieux S, St.-Gelais D, Champagne CP, Vuillemard JC. 2013. Effect of cheese containing gamma-aminobutyric acid-producing lactic acid bacteria on blood pressure in men. *PharmaNutrition* **1:** 141-148.
9. Jung JY, Lee SH, Jeon CO. 2014. Kimchi microflora: history, current status, and perspectives for industrial kimchi production. *Appl. Microbiol. Biotechnol*. **98:** 2385-2393.
10. Lee ME, Jang JY, Lee JH, Park HW, Choi HJ, Kim TW. 2015. Starter cultures for kimchi fermentation. *J. Microbiol. Biotechnol*. **25:** 559-568.
11. Park KY, Jeong JK, Lee YE, Daily III JW. 2014. Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. *J. Med. Food* **17:** 6-20.
12. Park JY, Jeong SJ, Kim JH. 2014. Characterization of a glutamate decarboxylase (GAD) gene from *Lactobacillus zymae*. *Biotechnol. Lett.* **36:** 1791-1799.
13. Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. **31:** 426-428.
14. Higuchi T, Hayashi H, Abe K. 1997. Exchange of glutamate and γ-aminobutyrate in a *Lactobacillus* strain. *J. Bacteriol*. **179:** 3362-3364.
15. Somkuti GA, Renye Jr. JA, Steinberg DH. 2012. Molecular analysis of the glutamate decarboxylase locus in *Streptococcus thermophiles* ST110. *J. Ind. Microbiol. Biotechnol.* **39:** 957-953.
16. Chang JY, Chang HC. 2010. Improvements in the quality and shelf life of kimchi by fermentation with the induced bacteriocin-producing strain, *Leuconostoc citreum* GJ7 as a starter. *J. Food Sci.* **75:** M103-M110.
17. National Institute of Agricultural Sciences. 2011. *Food Composition Table*, 8th Ed. National Institute of Agricultural Sciences, Rural Development Administration, Wanju-gun, Jeollabuk-do, Republic of Korea.
18. Bellisle F. 1998. Effects of monosodium glutamate on human food palatability. *Ann. NY Acad. Sci*. **855:** 438-441.
19. Bae TJ, Kang DS. 2000. Processing of powdered seasoning material from sea tangle. *Korean J. Food Nutr.* **13:** 521-528.
20. Kim HJ, Yang EJ. 2015. Optimization of hot water extraction conditions of Wando sea tangle (*Laminaria japonica*) for development of natural salt enhancer. *J. Korean Soc. Food Sci. Nutr.* **44:** 767-774.