

Changes in Some Physico-Chemical Properties and γ -Aminobutyric Acid Content of *Kimchi* during Fermentation and Storage

Suk-Heung Oh[†], Hye-Jin Kim¹, Yo-Han Kim¹, Jin-Ju Yu¹, Ki-Bum Park², and Jong-In Jeon²

¹Department of Food and Biotechnology, Woosuk University, Jeonju 565-701, Korea

²Bioscience Research Team, WinniaMando Co., Inc., Chungnam 336-843, Korea

Abstract

In this study, we investigated changes in some physico-chemical and biochemical properties of *Kimchi* during fermentation and storage. After fermenting *Kimchi* at 15°C for 23, 36, 40, 44, and 60 hrs during the first week, we stored it at -1°C in a *Kimchi* refrigerator until the end of 8th week. The pH of samples fermented for 36 hr, 40 hr, 44 hr and 60 hr sharply decreased during the first seven days and then slowly decreased. Acidities of samples fermented for 36 hr, 40 hr and 44 hr sharply increased for the first seven days. According to measured changes of lactic acid bacteria number, samples fermented for 60 hr revealed the largest augmentation in the number of lactobacilli for the first seven days. The γ -aminobutyric acid (GABA) content of the sample fermented for 40 hr was the most superior, with an early increase and maintenance of GABA content, which maintained for a maximum 20 mg per 100 g of *Kimchi* sample on the seventh, fourteenth, and twenty eighth days. These results suggest that relatively enhanced levels of GABA in *Kimchi* samples can be produced and maintained by controlling the fermentation and storage processes, as with the 40 hr fermented sample conditions.

Key words: *Kimchi*, fermentation, storage, GABA

INTRODUCTION

Korean traditional fermented foods contain abundant lactobacilli, which give nutritional advantages such as prevention of adult disease, anti-cancer effects and enhancement digestion and health. Among Korean traditional fermented foods, *Kimchi* is known as a functional foods with various ingredients and fermentation products such as dietary fiber, vitamins, carotene, special compounds of red pepper and garlic, lactic acid, lactic acid bacteria, acetylcholine, dextran and acetate, all of which enhance immunity, have antioxidant, antibacterial, and anti-cholesterol functions as well as preventing formation of carcinogens and mutagens (1-3).

In fact, if a person eats about 300 g of *Kimchi* a day, lactic acid bacteria in the colon increases to 100 fold above those of who do not eat *Kimchi*. Thus it has been reported that *Kimchi* works to make the intestine healthy and has anti-bacterial & anti-virus effects while inhibiting and destroying harmful bacteria (4). In addition, bioactive materials such as various organic acids, γ -aminobutyric acid (GABA) and ornithine are formed as a result of active growth of lactic acid bacteria and many effective materials such as indole-3-carbinol, isothiocyanate, allyl sulfide, capsaicin, carotenoids, flavonoids, anthocyanins, polyphenols and vitamin C are derived from

Chinese cabbage, garlic, red pepper powder, ginger and salted fish, which all together make *Kimchi* effective on anti-cancer, anti-oxidation and the prevention of skin aging and arteriosclerosis (3,5,6).

GABA, one of non-protein amino acids which exist widely in nature, can be found in brain, kidney, heart and lungs in animals and in mulberry leaves, green tea, germinated brown rice, beans, mandarin oranges, vegetables and fruits in plants (7-9). It was proven that GABA plays a role in regulating many physiological mechanisms such as suppression of blood pressure, preventing increases in blood cholesterol and triglycerides, improvement of cerebral blood flow, prevention of obesity, improvement of eyesight, and anti-anxiety and anti-convulsant actions (7,10,11). In addition, GABA also plays a role in improving mental concentration and regulating secretion of growth hormones and is effective to improve brain function, which is pharmacologically remarkable (11,12). With these effects of GABA, interest in GABA as a functional food ingredient is increasing recently.

These days, diverse functional foods and fermentation machines are being developed coupled with increased national interests in health. In this research, we measured the changes in pH, acidity, lactic acid bacteria number

[†]Corresponding author. E-mail: shoh@woosuk.ac.kr
Phone: +82-63-290-1433, Fax: +82-63-290-1429

and GABA content during fermentation and storage of *Kimchi* and will propose some ideas on the basis of these results of how to improve GABA content in *Kimchi*.

MATERIALS AND METHODS

Making, fermenting and storing *Kimchi*

We made *Kimchi* with a standard method using Chinese yellow cabbage purchased at a market place in Asan-city, Korea. After washing the Chinese cabbages, we salted them in 13% salt water solution for 20 hrs and added 3.0 g red pepper powder, 2.0 g garlic, 0.5 g ginger, 2.0 g onions, 1.0 g scallion, 1.5 g salted shrimp, 0.5 g salted anchovies, 1.0 g sugar and 0.5 g Korean leeks to 74.5 g salted cabbages for making Chinese cabbage *Kimchi*. We fermented *Kimchi* under at 15°C for 23, 36, 40, 44 and 60 hrs during first week and consequently stored it in a *Kimchi* refrigerator (WinniaMando CV-A208DW, Korea) under at -1°C up to the end of 8th week. *Kimchi* samples of this research were designated CH1 (23 hr), CH2 (36 hr), CH3 (40 hr), CH4 (44 hr) and CH5 (60 hr) according to fermentation time and *Kimchi* sample without fermentation, which was stored directly at -1°C, was named as CH6 (0 hr).

Measurement of pH, acidity and salinity

The pH of the juice from each *Kimchi* sample was measured with pH Meter (420A, Orion). After preparing 10 mL juice of *Kimchi* sample, acidity (%) was repeatedly measured adding 0.1 N NaOH solution with burette until pH 8.2. Acidity (%)=(the titrated amount of 0.1 N NaOH solution×0.009)/ (the amount of juice of *Kimchi* sample)×100. The salinity of *Kimchi* was measured with digital salinity Meter (ES-421, Atago).

Counting the number of lactic acid bacteria

After collecting 1 mL juice of each *Kimchi* sample, we diluted the juice with sterilized saline solution using 1:10 dilution method. A smear of 0.1 mL of the solution on MRS (Difco) agar plate with micropipette was prepared and cultured at 20°C for 72 hrs. After that, the number of colonies shown on MRS plate was counted and expressed in terms of colony forming unit (CFU/mL).

Measurement of GABA content

We added 50 mL double distilled water to 50 g of each *Kimchi* sample and mashed them in a mixer (HMF-392, Hanil). Then mashed samples were filtered through two-ply gauze, collecting 200 µL filtered juice and 200 mg of the rest of suspended solids, we performed analysis of samples using GABA analysis method described below. *Kimchi* juice (200 µL) was also col-

lected and assayed by the same method.

GABA was analyzed by HPLC (ACCQ·Tag™ Amino Acid Analysis System, Waters) in as previously described (13). To measure GABA content, the same amount of methanol : chloroform : water (12:5:3) solution was added to samples and mixed. The aqueous layer containing GABA was obtained using a centrifugal separator (12,000×g, 15 min, 4°C) and secondary extraction was conducted to obtain the rest of the GABA remaining in the precipitate by adding chloroform : water (3:5) solution. Upper layers obtained by the primary and secondary centrifugal separation were mixed and freeze-dried. After that, the extract was dissolved in small amount of water and filtered through 0.45 µm PVDF filter (Millipore) to be used for analysis. For fluorescence derivation of GABA, AccQ·Fluor Reagent (Waters) was used as reagent, and the separations of those derivations were carried out using 3.9×150 mm AccQ·Tag™ (Nova-Pak™ C18, Waters) column. To elute derivations in column, AccQ·Tag Eluent A and 60% acetonitrile were released into column at a flow rate of 1 mL/min. GABA content was calculated by comparing the analysis data with that of the standard GABA.

RESULTS AND DISCUSSION

Measurement of pH

According to the results of measured changes of pH in samples CH6 (0 hr) and CH1 (23 hr) (Fig. 1), there was a slow decrease in the pH of samples during the first seven days. In the case of CH2 (36 hr), CH3 (40 hr), CH4 (44 hr) and CH5 (60 hr), pH levels were sharply decreased by fermentation and storage during the first seven days, and then slowly decreased thereafter. It is

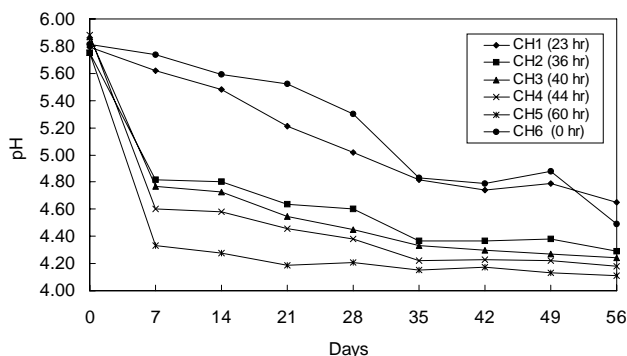


Fig. 1. Changes in the pH of *Kimchi* samples. After making *Kimchi* (0 day samples), the CH1, CH2, CH3, CH4, and CH5 samples were fermented at 15°C for 23, 36, 40, 44 and 60 hrs of first week and subsequently stored at -1°C up to the end of 8th week. The CH6 sample was stored at -1°C without fermentation. The salt concentration of *Kimchi* samples was $2.18 \pm 0.10\%$.

considered that these results were affected by fermentation time. When the longer samples were fermented before storage, more hydrogen ions were produced, which is regarded as a component that makes obvious decreases in pH in the early phase. Generally, *Kimchi* samples showed rapid decreases in pH during early phase of fermentation, continued to decrease, and then showed little change at the end of fermentation (6).

Measurement of acidity

Measured changes in acidity of samples CH6 (0 hr) and CH1 (23 hr) (Fig. 2) revealed that there were slow increases in acidity during the first seven days. Acidities of CH2 (36 hr), CH3 (40 hr) and CH4 (44 hr) showed clear increases in acidity for the first seven days and CH5 (60 hr) revealed obvious increases in acidity for the first seven days as well. It is considered that these results were affected by fermentation time, which was the same as decreased pH, and the longer samples were fermented before storage, the more acid was produced, which is regarded as making obvious increases in acidity. It is considered that the degree of acidity increase in sample CH2 (36 hr) and CH3 (40 hr) was very similar. Generally, *Kimchi* samples showed rapid increases in acidity during early phase of fermentation, continued to increase, and then showed little change at the end of fermentation (6). Changes in acidity of *Kimchi* largely depend on temperature and salt concentrations (6). Recently Bang et al. (14) showed that it took 28 days to reach an acidity of 0.5% at the temperature of 7°C and salt concentration of 2.0%. The CH1 (23 hr) *Kimchi* sample which was fermented at 15°C for 23 hrs during the first week and subsequently stored at -1°C took 28 days to reach the acidity of 0.5% at the salt

concentration measured as $2.18 \pm 0.10\%$. However, the CH2 (36 hrs) *Kimchi* sample which was fermented at 15°C for 36 hrs during first week and subsequently stored at -1°C took 7 days to reach the acidity of 0.6% at the salt concentration of $2.18 \pm 0.10\%$. It is assumed that fermentation proceeded up to a certain point during storage period at -1°C due to the initial temperature of *Kimchi* samples. This is a similar observation to the report of Noh et al. (15), but more or less different from the report of Choi et al. (16) showing that, after reaching the acidity level of 0.43%, the pH and acidity were almost unchanged during the storage period at $-1 \pm 1^\circ\text{C}$.

Counting the number of lactic acid bacteria

According to measured changes in lactic acid bacteria number, as Fig. 3 shows, the CH1 (23 hr) sample exhibited a slow increase in the number of lactic acid bacteria. And in the case of sample CH6 (0 hr), there was a small decrease during the first seven days, and then an increase after the 7th day, and then the counts tended to remain stable through the 35th day. In the case of samples CH2 (36 hr), CH3 (40 hr), CH4 (44 hr) and CH5 (60 hr), there were clear increases in the number of lactic acid bacteria for the first seven days. CH5 (60 hr) showed the largest increase in the number of lactic acid bacteria for the first seven days. All of these samples' lactobacilli counts tended to remain stable after the 7th day, and decrease a little after the 35th day. Generally, *Kimchi* samples showed rapid increases in the number of lactic acid bacteria during early phase of fermentation, continued to increase, and then showed little change at the end of fermentation (6). It has also been regarded that the different degrees of lactic acid bacteria numbers are dependant on fermentation temperature and salt con-

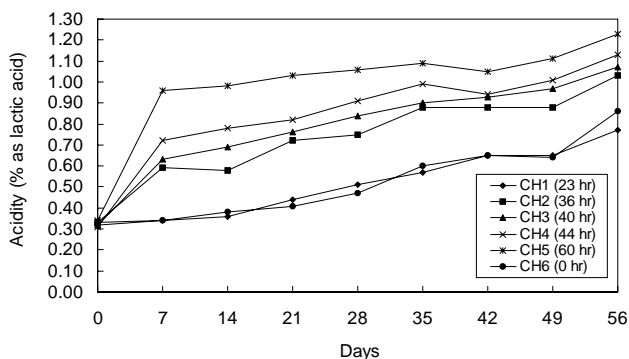


Fig. 2. Changes in the acidity of *Kimchi* samples. After making *Kimchi* (0 day samples), the CH1, CH2, CH3, CH4, and CH5 samples were fermented at 15°C for 23, 36, 40, 44 and 60 hrs during the first week and subsequently stored at -1°C up to the end of 8th week. The CH6 sample was stored at -1°C without fermentation. The salt concentration of *Kimchi* samples was $2.18 \pm 0.10\%$.

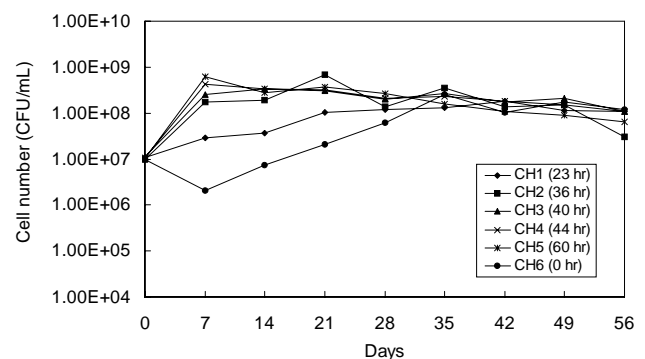


Fig. 3. Changes in the number of lactic acid bacteria of *Kimchi* samples. After making *Kimchi* (0 day samples), the CH1, CH2, CH3, CH4, and CH5 samples were fermented at 15°C for 23, 36, 40, 44 and 60 hrs of first week and subsequently stored at -1°C up to the end of 8th week. The CH6 sample was stored at -1°C without fermentation. The salt concentration of *Kimchi* samples was $2.18 \pm 0.10\%$.

centration (17).

Measurement of GABA content in Kimchi

GABA in *Kimchi* samples [CH1 (23 hr), CH2 (36 hr), CH3 (40 hr), CH4 (44 hr), CH5 (60 hr), CH6 (0 hr)] and *Kimchi* juice of each sample was analyzed using the ACCQ · Tag™ method on a Waters HPLC. As Fig. 4 shows, the analysis of *Kimchi* samples CH1 (23 hr) shows that GABA content was found in the order from highest-to-lowest in solids, pressed *Kimchi* juice and juice. In the case of total content, there the highest amount was present on the 21st day and remained constant at 11~16 mg of GABA per 100 g of sample. As Fig. 5 shows, the analysis result for sample CH2 (36 hr) showed GABA content of highest in order of solids, pressed *Kimchi* juice, and juice. According to total data, there was the highest increase for the first seven days, small decreases after that, and large decreases after 35th day. In the case of total content, there was the highest amount at 7th day reaching up to maximum 20 mg per 100 g of *Kimchi* sample. As Fig. 6 shows, the analysis

result of sample CH3 (40 hr) shows GABA content also in the order of solids, pressed *Kimchi* juice, and juice. According to total data, there was the highest increase for the first seven days, maintenance of GABA concentration for some time, and then a large decrease after the 35th day. In the case of total content, there was the highest amount on the 14th day reaching a maximum of 20 mg per 100 g of *Kimchi* sample according to the analysis result of 7th, 14th and 28th day. As Fig. 7 shows, the analysis sample CH4 (44 hr) shows GABA content from highest to lowest in solids, pressed *Kimchi* juice, and juice. According to total data, there were increases with a tendency to continue to increase until the 28th day and then the GABA concentration remained stable until the 35th day after which it decreased a little. In the case of total content, there was the highest amount at 28th and 35th day and it reached up to 14~15 mg maximum per 100 g of *Kimchi* sample. As Fig. 8 shows, the GABA content of CH5 (60 hr) in order of highest to lowest was found in solids, pressed *Kimchi* juice and

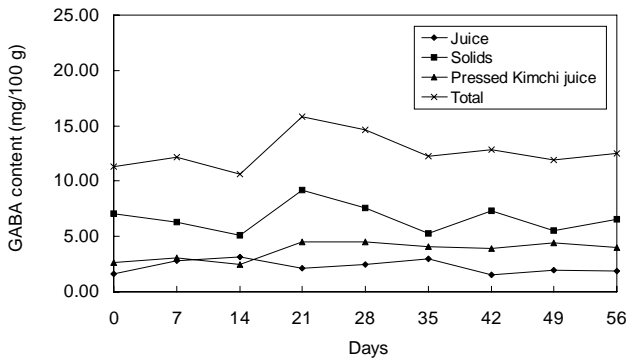


Fig. 4. Changes in the levels of GABA of CH1 (23 hr) *Kimchi* sample. After making *Kimchi* (0 day sample), the sample was fermented at 15°C for 23 hrs of first week and subsequently stored at -1°C up to the end of 8th week.

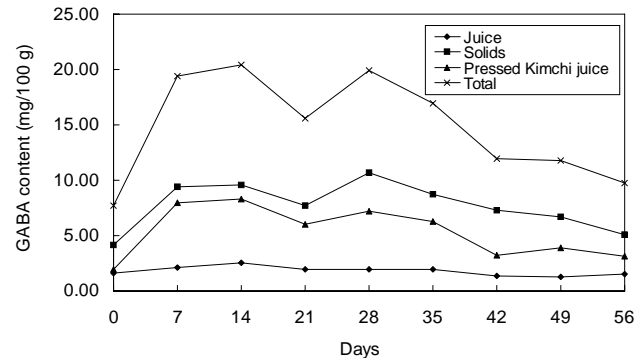


Fig. 6. Changes in the levels of GABA of CH3 (40 hr) *Kimchi* sample. After making *Kimchi* (0 day sample), the sample was fermented at 15°C for 40 hrs during the first week and subsequently stored at -1°C up to the end of 8th week.

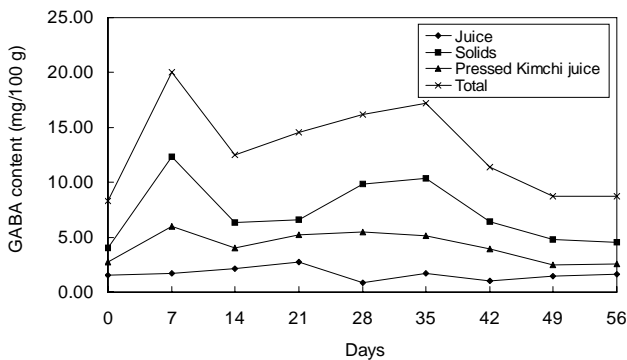


Fig. 5. Changes in the levels of GABA of CH2 (36 hr) *Kimchi* sample. After making *Kimchi* (0 day sample), the sample was fermented at 15°C for 36 hrs of first week and subsequently stored at -1°C up to the end of 8th week.

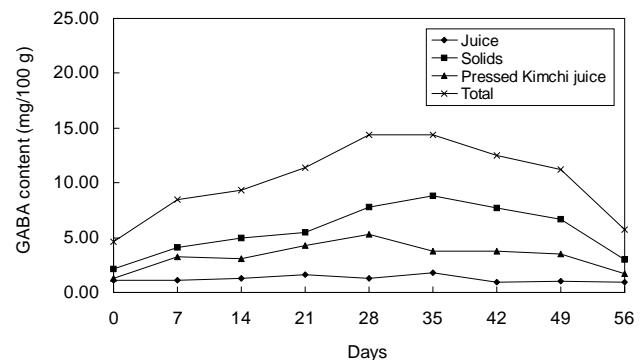


Fig. 7. Changes in the levels of GABA of CH4 (44 hr) *Kimchi* sample. After making *Kimchi* (0 day sample), the sample was fermented at 15°C for 44 hrs during the first week and subsequently stored at -1°C up to the end of 8th week.

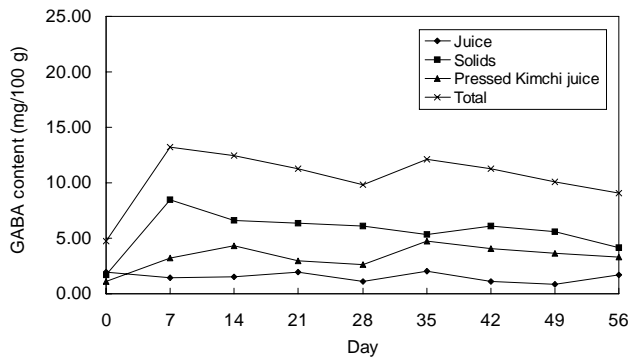


Fig. 8. Changes in the levels of GABA of CH5 (60 hr) *Kimchi* sample. After making *Kimchi* (0 day sample), the sample was fermented at 15°C for 60 hrs during the first week and subsequently stored at -1°C up to the end of 8th week.

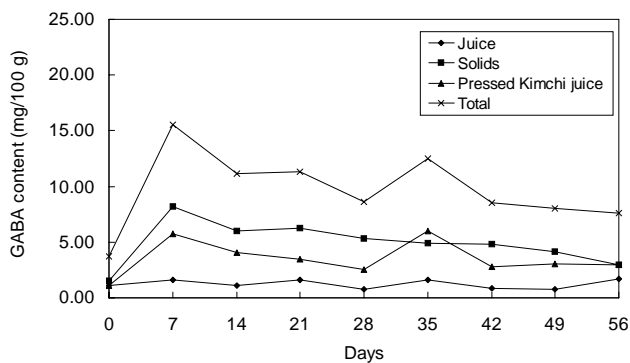


Fig. 9. Changes in the levels of GABA of CH6 (0 hr) *Kimchi* sample. After making *Kimchi* (0 day sample), the sample was stored directly at -1°C up to the end of 8th week.

juice. According to total data, there was the highest increase at 7th day and after that there was a tendency to continue to decrease, but a big decrease was not observed. In the case of total content, the highest amount was on the 7th day and it reached up to 13~14 mg maximum per 100 g of *Kimchi* sample. As Fig. 9 shows, the analysis result of sample CH6 (0 hr) shows GABA content highest in solids, followed by pressed *Kimchi* juice and juice. In the case of total content, the highest amount was observed on the 7th day and it reached up to 15~16 mg maximum per 100 g of *Kimchi* sample. The Fig. 10 shows the comparison of changes in the levels of total GABA among *Kimchi* samples. Sample CH3 (40 hr) was the most superior in early increase and maintenance of content, which reached up to 20 mg maximum per 100 g of *Kimchi* sample at 7th, 14th and 28th day. Also, in the case of sample CH2 (36 hr), there was a similarity between sample CH3 (40 hr) in early increase of GABA concentration but sample CH2 (36 hr) showed a tendency to be lower than sample CH3 (40 hr). In the case of sample CH4 (44 hr), it had a tendency

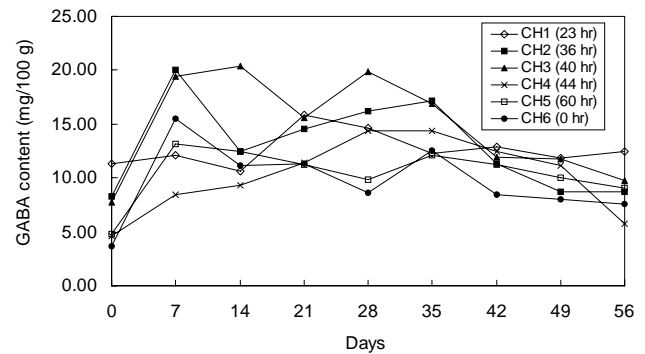


Fig. 10. Comparison of changes in the levels of total GABA among *Kimchi* samples.

to increase slowly until the 35th day.

This research monitored changes of GABA content in *Kimchi* during fermentation and storage and is expected to find ways to make and use *Kimchi* containing natural GABA. According to the results of this research, fermentation and storage of *Kimchi* after it is made are important for obtaining fermented *Kimchi* containing 20 mg of GABA per 100 g of *Kimchi*. This is a good amount considering the GABA content of Chinese cabbage, the main ingredient of *Kimchi*, which contains 0.1 ~ 0.2 mg per 100 g of cabbage (18). Also, its level is considered as excellent considering GABA content of tomato which contains about 2~3 mg per 100 g of tomato and brown rice which contains about 4~8 mg per 100 g of brown rice (18,19). Supposing that Koreans consume an average 300~400 g of *Kimchi* daily, it will be possible to obtain 60~80 mg of GABA when eating *Kimchi* according to this research. WHO recommends taking 6 mg of GABA a day. This level is considered adequate, but efficacy for most functional effects is higher, at about 50~100 mg (20). However, because predicted daily requirements are higher when taking GABA through foods, developing recipes, such as selecting sub-ingredients and using kelp, are required to enhance GABA content in *Kimchi*. Because GABA enhanced *Kimchi* is expected to have superior functional properties to other general *Kimchi*, if a preclinical study and clinical trial on expected efficacy are performed, those will be able to assist in the development of some differentiated *Kimchi* and *Kimchi* fermenting and storing programs.

In Japan, GABA has been already approved as a food additive and a functional food, which offers a wide range of connected business. However, in Korea, using GABA has a narrow business application, and may be the subject of exaggerated advertisement. In Japan, GABA is widely applied in food materials, health foods, functional foods, drugs, beverages, milk, cookies, mayonnaise, porridge, rice cake, fermented milk, brown rice, tofu, natto

(fermented soybeans) and soy milk (10). For examples, as spotlighted products in Japan functional foods market, 'Mental Balance Chocolate GABA' (can package containing 430 mg of GABA, pouch package containing 140 mg of GABA), 'Mental Balance Coffee-GABA COFFEE' (each bottle contains 28 mg of GABA), 'GABA Cool Water' (each bottle contains 50 mg of GABA), 'Jumi GABA bread' (each bread contains 10 mg of GABA) and 'GABA Soy Milk' (contains 10~15 mg of GABA per 100 g of product) are in the market. In Korea, GABA has not been approved as a food additive or a functional food. There are Korean products containing natural GABA such as beverages, biscuits, chocolate, germinated brown rice, fruits and green tea but it is difficult to get information about GABA content marked or reported officially. Also, there is great variation in reported GABA content information, depending on analysis methods and sample collecting method. If GABA is approved as a food additive by Korea Food & Drug Administration in Korea, like Japan, it will be helpful for public health and the range of Korean GABA market will be wider as a result of references to experts' opinions.

ACKNOWLEDGMENTS

This research monitored physico-chemical changes, microbiological changes and GABA content changes in *Kimchi* during fermentation and storage in *Kimchi* refrigerator. WinniaMando Co., Inc. joined as a part of the academic-industrial cooperation program.

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