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Overproduction of GABA in Apple Pomace with *Auricularia auricula-judae* by Lactic Acid Fermentation

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ABSTRACT

Lactic acid fermentation of apple pomace was performed using *Lactobacillus plantarum* EJ2014 to produce gamma-aminobutyric acid (GABA), in the presence of 3% MSG and 0.5% yeast extract. After fermenting for 5 days, apple pomace showed pH and acidity of 6.26 and 0.48%, respectively. Fermentation of apple pomace in the presence of wood ear mushroom (*Auricularia auricula-judae*) resulted in low acidity (0.16%) and a pH of 6.26. Initially, the number of viable cells was ~1.91×10^7 CFU/mL, which increased to $>3.15 \times 10^9$ CFU/mL after one day. Addition of wood ear mushroom resulted in the highest bacterial stability during lactic acid fermentation for 5 days, resulting in higher number of viable cells (3.86×10^8 CFU/mL). In particular, GABA production using apple pomace was considerably low; however, it increased following the addition of 2% wood ear mushroom. After 5 days, glutamic acid concentration decreased from 19,552 μg/mL to 1,532 μg/mL, resulting in the production of 13,442 μg/mL GABA. Thus, lactic acid fermentation of apple pomace containing wood ear mushroom resulted in the formation of multifunctional ingredients, including GABA, dietary fibers, and probiotics.

Key words : Apple pomace, *Auricularia auricula-judae*, GABA, Lactic acid fermentation, Wood ear mushroom

Introduction

Apple pomace obtained from *Malus pumila* var. *dulcissima* is a by-product of the apple juice industry [1,2]. It has considerably high moisture, protein, and fiber content, along with good palatability. In particular, it has been reported to contain polyphenols (1.048 mg%) and flavonoids (458 mg%), which exhibit antiallergic, antioxidant, and other physiological activities. It also prevents obesity, diabetes, cholelithiasis, hypertension, high cholesterol, cancer, and heart diseases [3-5]. Studies have shown that dietary fiber inhibits the absorption of various toxic substances. Apple pomace effectively increases muscle mass, controls body weight, and lowers blood pressure by the action of ursolic acid, which is widely found in the peels of apple [6-9].

Auricularia auricula-judae, known as the wood ear mushroom, belongs to the phylum Basidiomycota that is distributed worldwide. Edible mushrooms, such as the wood ear mushroom, are the major ingredients of Chinese dishes and contain health food ingredients including proteins, potassium, phosphorus, iron, calcium, and dietary fibers. The wood ear mushroom has been used as a functional food for the prevention and treatment of constipation [10]. Recently, the wood ear mushroom has been listed as a healthy functional food ingredient certified by KFDA. In addition, the anticancer and cholesterol-lowering effects of wood ear mushroom extracts have been reported [11]. It has also been found that old antler containing wood ear mushroom fermented by lactic acid bacteria

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possesses anti-inflammatory effects [12]. Recently, it was shown that GABA was produced during lactic acid fermentation by *Flammulina velutipes* [13,14]. However, the production of high levels of GABA using apple pomace and wood ear mushroom has not been reported.

Fermentation is a traditional method used to enhance the shelf life of oriental and western foods. *Lactobacillus plantarum*, *L. brevis*, *B. subtilis*, and other useful microorganisms have been used in the preparation of various fermented foods with enhanced functionality [15]. *L. plantarum*, known to be a strain for vegetable fermentation, produces organic acids, carbon dioxide, and GABA as fermentation by-products. GABA is a non-protein amino acid produced by lactic acid bacteria, such as *L. plantarum*. It is effective in improving the blood flow and increasing oxygen supply to improve memory and relieve depression. Although GABA is also produced in germinated brown rice, mushrooms, and green tea, it is difficult to expect a physiological effect because of the small amount. Recently, it has been reported that GABA, which is recognized as a functional material for health, is produced using various lactic acid bacteria [16]. In addition, it has been reported that as probiotics, lactic acid bacteria play important roles in maintaining proper intestinal function and enhancing immunity. Therefore, lactic acid fermentation could be the best means to provide multi-functional ingredients.

GABA-producing *L. plantarum* EJ2014, along with the wood ear mushroom were used to optimize the production of GABA in apple pomace mixture. To achieve high GABA production, the concentrations of MSG and yeast extract added to apple pomace were optimized. Further, the effect of addition of wood ear mushroom on GABA production was also studied. The physicochemical properties of fermented apple pomace mixture were analyzed to study the basic functional information for utilization as food and health food ingredient [17].

Methods

1. Raw material

Apple pomace was purchased from Sunlight Agricultural Food Co. (Seongju, Korea). *A. auricula-judae* was purchased from natural farming association law (Gyeongsan, Korea). Yeast extract and monosodium L-glutamate (MSG) were purchased from Choheung Co. (Ansan, Korea) and Yakuri Pure Chemicals Co. Ltd. (Kyoto, Japan), respectively. The reagents used in the analysis were reagents of over grade.

2. Moisture and soluble solid content

Moisture content of the apple pomace paste and wood ear mushroom was measured using the atmospheric pressure drying method at 105°C [18]. Water solubility index (WSI) of the mixed fermented product of apple pomace and wood ear mushroom was measured [19].

3. Strains and starter cultures

L. plantarum EJ2014 isolated from rice bran was used as the starter. For this, a single colony of this strain was subcultured on MRS agar. After isolation, *L. plantarum* EJ2014 was inoculated in MRS broth, which was incubated at 30°C for 24 h and subsequently used as the starter culture.

4. Mineral analysis

For determining the mineral content of apple pomace mixture, fermented apple pomace mixture (10 g) was heated at 550~600°C for 12 h. It was solubilized in 10 mL HCl and subjected to filtration. The mineral content was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 700DV; Perkin Elmer, Waltham, MA, USA), and the concentrations were calculated from the standard curve.

5. Lactic acid fermentation of apple pomace with wood ear mushroom

Frozen apple pomace was thawed and diluted two-fold using water. Apple pomace paste was obtained by passing through a sieve (25 mesh). For long-term storage, apple vinegar (13% acetic acid; Ottogi, Eumseong County, Korea) was added to the apple pomace paste. Approximately 5 g wood ear mushroom was soaked in 100 mL distilled water for 1 h. Subsequently, 5 g swollen wood ear mushroom was added to the apple pomace paste. The mixture was homogenized for 10 min using an electric blender (800W HR-1673; PHILPS, Hungary), and heated at 85°C for 30 min. Approximately 3% MSG and 1% yeast extract were added to the mixture of apple pomace and wood ear mushroom. Thereafter, *L. plantarum* EJ2014 (1%) was inoculated.

6. pH, acidity, and viable cell count

The pH was measured using a pH meter (model 420+; Thermo, Washington DC, USA). Titrable acidity was calculated by adding 9 mL distilled water to 1 mL sample, and calculating the consumption amount (%, v/v) of lactic acid by 0.1 N NaOH to pH 8.3. Viable cell count was determined by serial 10 time and then was plated on MRS agar plate. After culturing at 30°C for 24 h, the number of viable cells was expressed as colony forming units per milliliter (CFUs/mL).

7. Soluble solid and reducing sugar content

To determine the soluble solid content, the supernatant obtained after centrifuging for 10 min at 13,200 rpm using a centrifuge (Centrifuge 5415 R; Eppendorf, USA) was determined using an electronic sugar meter (ATAGO, pocket REFRACTOMETER PAL-3, ATAGO, JAPAN). Reducing sugar content was measured using the dinitrosalicylic acid (DNS) method. Briefly, 3 mL DNS reagent was added to 1 mL diluted supernatant, obtained after centrifuging at 13,200 rpm for 10 min, and the solution was developed at 100°C for 5 min. After cooling from the dark place to room temperature for 40 min, the absorbance at 550 nm was measured and calculated. The reducing sugar content was calculated using glucose as the standard.

8. Polyphenol and flavonoid content

Polyphenol content was measured using the Folin–Denis method [20]. The fermented apple pomace mixture was diluted and allowed to react with the Folin reagent. After the reaction, polyphenol content was evaluated by measuring the absorbance at 700 nm. Total polyphenol content was calculated using gallic acid as the standard. Flavonoid content was measured using the method described by Nieva Moreno et al. [21]. Absorbance of the reacted mixture was measured at 415 nm. Total flavonoid content was determined from the standard curves prepared using quercetin.

9. Analysis of GABA and free amino acids

For qualitative analysis of MSG and GABA, silica gel thin-layer chromatography (TLC) plates (10 × 20 cm) and a square developing chamber (30 × 25 × 10 cm) were used. The developing solvent used was a n-butyl alcohol : glacial acetic acid : distilled water mixture (6 : 2 : 2 v/v) saturated at room temperature for at least 4 h. The fermented sample and standard solution (containing 1% MSG and 1% GABA) were spotted (2 μL) on the TLC plate, which was subsequently developed. The developed TLC plate was dried at room temperature. A 0.2% ninhydrin solution, used as a coloring reagent, was sprayed onto the dried TLC plate and color was allowed to develop for about 5 min at 100°C. The MSG and GABA spots of the fermented product were confirmed.

Free amino acid content, including GABA content, was analyzed using high-performance liquid chromatography (HPLC). The dried sample was derivatized with 20 μL phenylisothiocyanate (PITC) solution (MeOH : H_2O : TEA : PITC = 7 : 1 : 1 : 1) and allowed to react at room temperature for 30 min. After the sample dried completely, it was dissolved in 200 μL centrifuged. The supernatant was filtered using a 0.45-μL syringe filter and analyzed. Amino acid analysis was performed with a C_{18} column (Waters Nova-Pak, 4 μm) using HPLC (Hewlett Packard 1100 series; PaloAlto, CA, USA) and mobile solvents were A (140 mM NaHAc, 0.15% TEA, 6% CH_3CN, 0.015% EDTA) to 100%. Amino acid analysis was performed in the UV range at an absorbance at 254 nm.

10. Statistical analysis

Differences between groups were statistically evaluated using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test or Student's *t*-test using SPSS (Chicago, IL, USA). P values < 0.05 were considered to be statistically significant.

Results and Discussion

1. Water content and water solubility index

Approximately 2.5-fold diluted apple pomace was mixed with swollen wood ear mushroom to obtain 0.5%, 1%, 1.5%, and 2% (w/v) mixtures. The moisture content of each apple pomace mixture before fermentation was > 98%. However, it slightly decreased as the content of wood ear mushroom increased. In order to investigate the water solubility of fermented product, WSI was measured. It was observed to be 1.03% for apple pomace without the wood ear mushroom. As the content of wood ear mushroom in apple pomace paste increased, the WSI value also increased (Table 1).

2. Mineral content

Mineral content of apple pomace with wood ear mushroom was compared to that of 2% wood ear mushroom. As shown in Table 2, the wood ear mushroom consisted of potassium [26.222 mg/% (w/w)] as a major mineral. Compared to this, in

Table 1. Water content and water soluble index of apple pomace mixture with wood ear mushroom

Items	Wood ear mushroom (%)				
	0	0.5	1.0	1.5	2.0
Water content (%)	99.08 ± 0.10^a	98.84 ± 0.06^b	98.67 ± 0.04^c	98.58 ± 0.03^c	98.24 ± 0.01^d
WSI (%)	0.91 ± 0.10^d	1.15 ± 0.06^c	1.33 ± 0.04^b	1.42 ± 0.03^b	1.75 ± 0.01^a

*WSI: water solubility index. Values represent mean ± SD (n = 3). Values with different letters in the same row are significantly different (analyzed using ANOVA and Duncan's multiple range test; $p < 0.05$, a > b > c > d).

Table 2. Analysis of minerals in apple pomace mixture with wood ear mushroom

Mineral	mg/100 g
K	31.541
Na	25.141
P	9.522
Mg	2.584
Ca	3.214
Fe	0.084
Mn	0.025

apple pomace containing 2% wood ear mushroom, potassium content was higher [31.541 mg% (w/w)]. Previous studies have shown that the mineral content of *A. auricular* was ~30 mg/100 g [22]. The sodium content of apple pomace mixture increased from 19.141 mg% (w/w) to 25.141 mg% (w/w), after the addition of 2% wood ear mushroom. The phosphorus content of apple pomace also increased with 9.522 mg% (w/w), after the addition of 2% wood ear mushroom.

Calcium is a component of teeth and bones. It is an inorganic substance that participates in major metabolic processes in the body which control muscle contraction and relaxation [23]. It is also known to be the most deficient nutrients in Korean diet [24]. The content of calcium in wood ear mushroom was 1.884 mg% (w/w). After mixing with wood ear mushroom, the calcium content of apple pomace mixture increased with 3.214 mg% (w/w). Thus, the wood ear mushroom was a good source of various minerals. Seven minerals, including manganese, were present in the wood ear mushroom. The level of these minerals in apple pomace increased after the addition of wood ear mushroom to it. This implies that compared to apple pomace, various minerals present in the apple pomace mixture provided a more favorable environment for bacterial growth (Table 2).

3. pH and acidity

The pH and acidity of fermented apple pomace mixture were measured. As shown in Fig. 1, apple pomace paste before fermentation showed an acidic pH (< 5). The addition of wood ear mushroom at concentrations of 0, 0.5, 1, 1.5, and 2%, slightly increased the pH to 4.78, 4.88, 4.9, 4.96, and 5.02, respectively. The mixture of apple pomace with wood ear mushroom showed an acidic pH because apple pomace was previously mixed with vinegar to prevent the growth of fungi. The pH of apple pomace mixture decreased 1-day post-lactic acid fermentation, and the pH was observed to be 4.02, 4.14, 4.16, 4.15, and 4.25, respectively. However, the pH of fermented apple pomace mixture increased slightly after fermentation for 3 days.

On the fifth day of lactic acid fermentation, the pH of apple pomace mixture increased depending on the concentration of wood ear mushroom added. In the case of apple pomace without wood ear mushroom, the pH was 5.12. The pH of apple pomace with 2% wood ear mushroom increased to 6.71. Generally, lactic acid fermentation decreases pH because of lactic acid production. However, lactic acid fermentation of apple pomace mixture containing MSG showed contradistinctive results, and indicated an increase in the pH. It has been reported that protons are consumed by glutamate decarboxylase (GAD) in microorganisms that survive in acidic environments. It was also reported that pH increased with an increase in GABA production [25,26]. This implied that MSG, a precursor, was converted into GABA due to the increase in pH during lactic acid fermentation.

The acidity of apple pomace mixture before fermentation was moderately acidic (~0.61~0.72%). On the first day of fermentation, the acidity of each apple pomace mixture increased to 0.81%, 0.78%, 0.71%, 0.71% and 0.67%, respectively. After 3 days of fermentation, the acidity was observed to decrease (Fig. 2).

After 5 days of fermentation, the acidity markedly decreased, and was observed to be 0.49%, 0.32%, 0.27%, 0.19%, and 0.15%, respectively. In particular, apple pomace with 2% wood ear mushroom showed the lowest acidity. Increase in pH and decrease in acidity are related to the lack of protons in the culture broth. Therefore, it was assumed that *L. plantarum* pro-

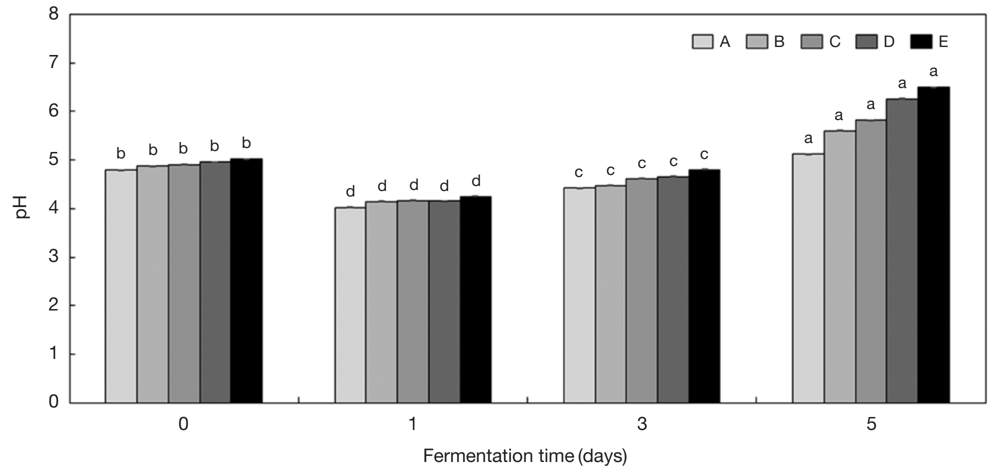

Fig. 1. Changes in the pH of fermented apple pomace with wood ear mushroom. Content of wood ear mushroom: A. 0%, B. 0.5%, C. 1%, D. 1.5%, and E. 2%. Values represent the mean ± SD (n = 3). Values with different letters for the same concentration are significantly different (analyzed using ANOVA and Duncan's multiple range test; $p < 0.05$, a > b > c > d).

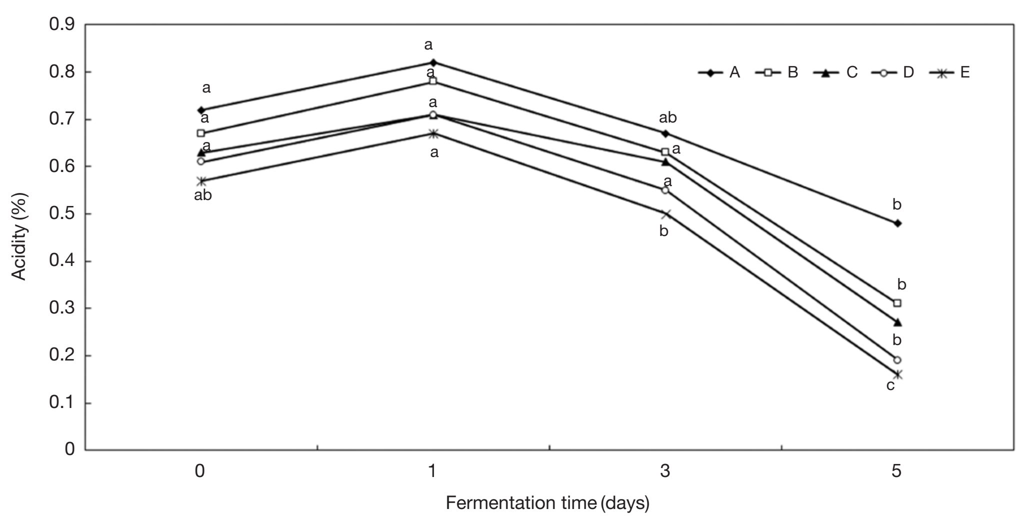

Fig. 2. Changes in the acidity of fermented apple pomace with wood ear mushroom. Content of wood ear mushroom: A. 0%, B. 0.5%, C. 1%, D. 1.5%, and E. 2%. Values represent the mean ± SD (n = 3). Values with different letters for the same concentration are significantly different (analyzed using ANOVA and Duncan's multiple range test; $p < 0.05$, a > b > c).

duced lactic acid during initial fermentation; however, the pH and acidity of apple pomace mixture decreased following longer periods of fermentation. Thus, during lactic acid fermentation of apple pomace, addition of the wood ear mushroom decreased the acidity of the mixture.

4. Viable cell count

The viable cell count in apple pomace mixture was determined, as shown in Table 3. Initial (i.e., before fermentation) viable cell count in the apple pomace mixture containing wood ear mushroom was 1.92×10^7 CFU/mL. The number of viable cells in each apple pomace mixture was 3.15×10^9 CFU/mL, 3.16×10^9 CFU/mL, 3.11×10^9 CFU/mL, 3.72×10^9 CFU/mL, and 3.85×10^9 CFU/mL, respectively, after fermenting for one day. Following longer fermentation time, a decrease in the viable cell counts (evident from the growth curve) of lactic acid bacteria was observed, due to the accumulation of fermented products and lack of nutrients [27]. However, the addition of wood ear mushroom had a positive effect on the viability of *L. plantarum* during lactic acid fermentation. After fermentation for 3 days, the viable cell count in apple pomace without wood ear mushroom tended to decrease (6.79×10^8 CFU/mL). On the other hand, apple pom-

Table 3. Changes in viable cell counts in the fermented product of apple pomace and wood ear mushroom

Fermentation time (days)	Viable cell counts (CFU/mL × 10^6)				
	A	B	C	D	E
0	19.1 ± 8.2^{c}	19.5 ± 8.1^{c}	19.5 ± 8.1^{c}	19.6 ± 7.6^{b}	25.6 ± 7.2^{b}
1	$3{,}152 \pm 121^{a}$	$3{,}160 \pm 133^{a}$	$3{,}110 \pm 123^{a}$	$3{,}715 \pm 136^{a}$	$3{,}855 \pm 121^{a}$
3	679 ± 52^{b}	$1{,}709 \pm 105^{ab}$	$1{,}710 \pm 121^{ab}$	$1{,}822 \pm 158^{ab}$	$1{,}912 \pm 162^{ab}$
5	135 ± 72^{bc}	358 ± 51^{b}	386 ± 48^{b}	655 ± 81^{ab}	692 ± 71^{ab}

*Content of wood ear mushroom: A. 0%, B. 0.5%, C. 1%, D. 1.5%, and E. 2%. Values represent the mean ± SD (n = 3). Values with different letters for the same concentration are significantly different (analyzed using ANOVA and Duncan's multiple range test; $p < 0.05$, a > b > c).

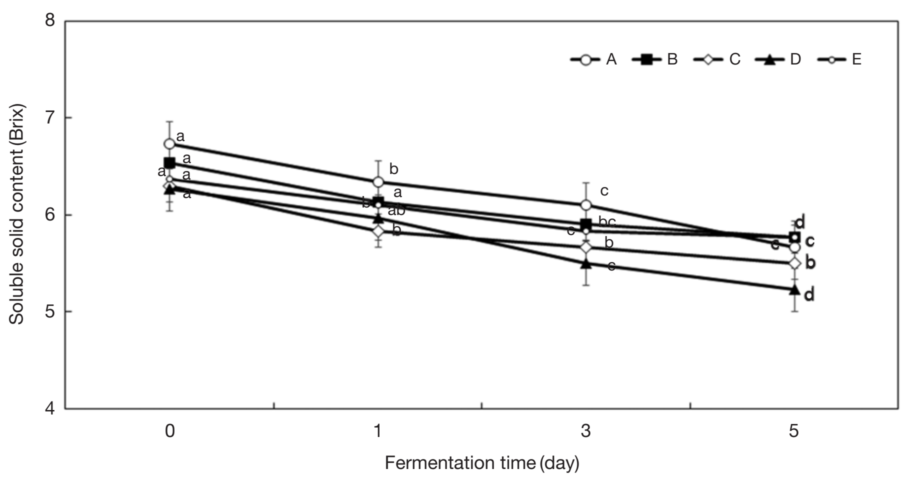

Fig. 3. Changes in the soluble solid content of fermented apple pomace with wood ear mushroom. Content of wood ear mushroom: A. 0%, B. 0.5%, C. 1%, D. 1.5%, and E. 2%. Values represent the mean ± SD (n = 3). Values with different letters for the same concentration are significantly different (analyzed using ANOVA and Duncan's multiple range test; $p < 0.05$, a > b > c > d).

ace containing wood ear mushroom maintained high viable cell counts (10^9 CFU/mL). This is closely associated with the decrease in acidity by the homo-fermentative strain, *L. plantarum*, during fermentation. Also, the presence of glutamic acid results in the maintenance of high viable cell counts [28]. Furthermore, the fermentation of apple pomace without wood ear mushroom for 5 days markedly decreased the viable cell count (1.35×10^8 CFU/mL). On the other hand, other apple pomace mixtures containing wood ear mushroom showed high viable cell counts ($> 3.86 \times 10^8$ CFU/mL). While producing GABA by fermentation using lactic acid bacteria, fermented product was improved in flavor and physical properties and GABA production when thymus mushroom was added [29].

5. Soluble solid and reducing sugar content

L. plantarum is a homo-type strain that produces only lactic acid using sugar during fermentation. It is the presentative means to increases the acidity of the fermented product. During fermentation, soluble solid content and reducing sugar content were measured, as shown in Fig. 3. The soluble solid content of apple pomace mixture before fermentation was about 6.5 Brix. As fermentation progressed, the soluble solid content decreased to 5.5 Brix. This implies that the apple pomace mixture consisted of water-soluble solids with small amounts of fermenting sugar.

Reducing sugar content markedly decreased during lactic acid fermentation, as shown in Fig. 4. Reducing sugar content of the apple pomace mixture was about 4.5%. After fermentation for 1 day, apple pomace without wood ear mushroom showed a reducing sugar content of 2%. Apple pomace with 2% wood ear mushroom showed reducing sugar content of 1.67%. The amount of reducing sugar decreased, as the amount of wood ear mushroom increased. After fermentation for 5 days, the reducing sugar content of each apple pomace mixture was about 1%. We confirmed that 3% fermenting sugar was consumed during lactic acid fermentation.

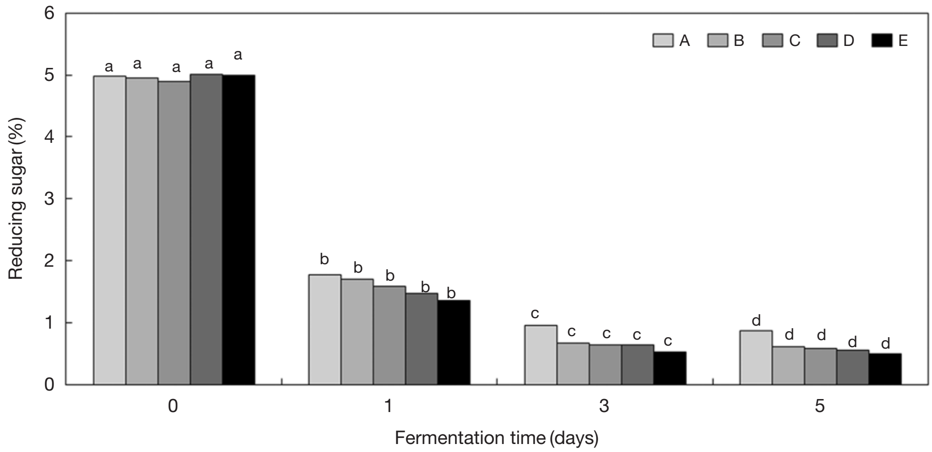

Fig. 4. Changes in the reducing sugar content of fermented apple pomace with wood ear mushroom. Content of wood ear mushroom: A. 0%, B. 0.5%, C 1%, D. 1.5%, and E. 2%. Values represent the mean ± SD (n = 3). Values with different letters for the same concentration are significantly different (analyzed using ANOVA and Duncan's multiple range test; $p < 0.05$, a > b > c > d).

Table 4. Content of polyphenols and flavonoids in fermented apple pomace with wood ear mushroom

Compounds	A	B	C	D	E
Polyphenol	177.56 ± 0.25^{e}	185.52 ± 2.51^{d}	189.10 ± 1.54^{c}	196.19 ± 2.25^{b}	203.52 ± 1.77^{a}
Flavonoid	24.33 ± 1.14^{d}	30.80 ± 0.74^{c}	32.10 ± 1.41^{c}	36.24 ± 0.44^{b}	40.55 ± 1.23^{a}

*Unit: μg/mL. Content of wood ear mushroom: A. 0%, B. 0.5%, C. 1%, D. 1.5%, and E. 2%. Values represent the mean ± SD (n = 3). Values with different letters for the same compound are significantly different (analyzed using ANOVA and Duncan's multiple range test; $p < 0.05$, a > b > c > d > e).

6. Polyphenol and flavonoid content

Phenolic compounds are widely distributed in nature, especially in plants. These compounds are secondary metabolites, and indicators of antioxidant and antimicrobial activities. Among all phenolics tested, guaiacol and resorcinol strongly inhibit nitration [30]. It has shown that the polyphenol content of apple pomace is ~4.46~7.24 g/kg [31,32].

The content of polyphenols and flavonoids in apple pomace mixture containing wood ear mushroom was measured, as shown in Table 4. Apple pomace showed a high polyphenol content (177.56 ± 2.58 μg/mL). The addition of wood ear mushroom increased the polyphenol content further. The addition of 0.5% and 2% wood ear mushroom increased the polyphenol content to 185.52 ± 2.21 μg/mL and 203.52 ± 1.58 μg/mL, respectively. Therefore, polyphenol content could be increased by the addition of wood ear mushroom. It was reported that polyphenols are present at a concentration of 30 μg/mL in the wood ear mushroom [33,34]. The flavonoids of apple pomace paste were also measured to be 24.33 μg/mL. As the amount of wood ear mushroom increased, the flavonoid content also increased (Table 4).

7. GABA analysis

In order to determine the content of GABA in apple pomace containing different amounts of wood ear mushroom and fermented using *L. plantarum* EJ2014, the fermented apple pomace mixture was qualitatively analyzed by TLC. As shown in Fig. 5, GABA production was observed after fermentation for 3 days, and GABA content was proportional to the amount of wood ear mushroom added. Apple pomace mixture fermented without wood ear mushroom showed considerably lesser production of GABA after fermentation for 5 days, when compared with apple pomace fermented with wood ear mushroom.

Yeast extract and MSG were added to the culture broth of apple pomace mixture containing wood ear mushroom for lactic acid fermentation for 5 days using *L. plantarum*. GABA content was quantitatively analyzed using HPLC. As shown in Table 5, the apple pomace mixture containing 2% wood ear mushroom showed the highest GABA production. After fermentation for 5 days, the initial MSG concentration of 19,552 μg/mL was reduced to 1,532 μg/mL, resulting in the production of GABA (13,442 μg/mL).

For overproduction of GABA in the presence of MSG, lactic acid bacteria isolated from kimchi were investigated [35].

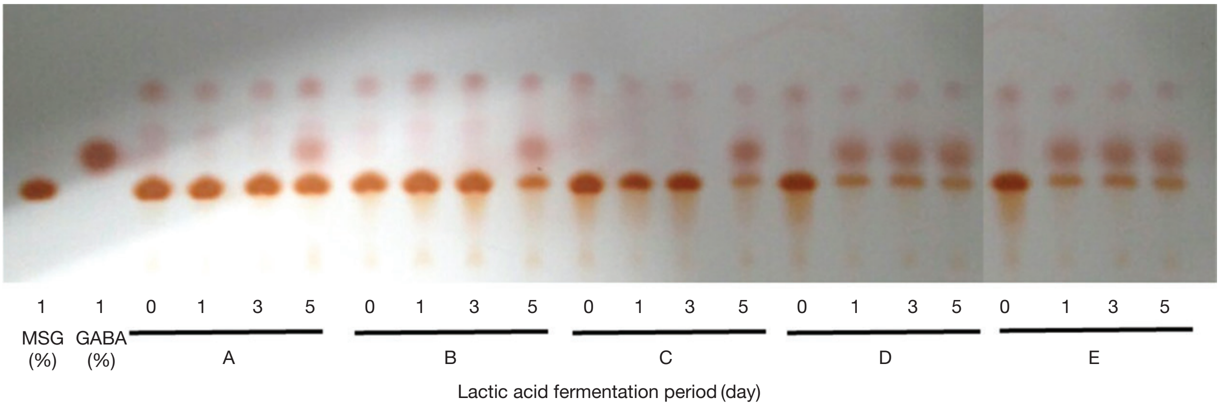

Fig. 5. Comparison of GABA production in fermented apple pomace with wood ear mushroom. Arabic numbers (0, 1, 3, and 5) represent days after fermentation. Content of wood ear mushroom: A. 0%, B. 0.5%, C. 1%, D. 1.5%, and E. 2%.

Table 5. Analysis of GABA and glutamic acid in fermented apple pomace with wood ear mushroom

Fermentation	Glutamic acid (μg/mL)	GABA (μg/mL)
Before	19,552 ± 10.02	N.D.
After	1,532 ± 5.51***	13,442 ± 8.12

*N.D.: not detected. Values represent the mean ± SD (n = 3). Significant differences were observed before and after fermentation using Student's *t*-test at ***$p < 0.001$.

During lactic acid fermentation, as the MSG concentration increased, the amount of GABA produced also increased [36]. Reports on the production of GABA by lactic acid fermentation with apple pomace mixture containing wood ear mushroom are not available. During GABA production using GAD enzymes present in mushrooms, the MSG added was converted to GABA in a topical powder solution [37]. Therefore, high GABA production in apple pomace mixture is considered to be desirable because of the multi-functional properties of GABA. In GABA-producing microorganisms, glutamic acid is decarboxylated by glutamate decarboxylase inside the cells. The biosynthesized GABA is reported to be present outside the cells. Therefore, for efficient microbial production of GABA, it is essential to supply glutamic acid as a precursor. However, in order to produce high concentrations of GABA, bioconversion conditions need to be optimized. Therefore, the concentration of nutrient components, including fermentable sugars and MSG, was considered to be an important factor during the conversion of MSG to GABA in various plants and herbal materials [38-40]. In the apple pomace mixture containing wood ear mushroom, about 4.5% fermentable sugar was present. In addition, it was reported that glutamic acid present in *A. auricula-judae* was about 385.43 mg/mL. Addition of high amounts of the wood ear mushroom resulted in high glutamic acid content in the apple pomace mixture. It was also reported that *Auricularia auricula-judae* used in the present study had more glutamic acid than *Auricularia auricula-judae* and hairy *Auricularia auricula-judae* [41]. The apple pomace mixture containing 2% wood ear mushroom fermented by *L. plantarum* EJ2014 showed higher production of GABA than that observed in other studies [42,43].

Conclusion

Lactic acid fermentation was performed using *L. plantarum* EJ2014 to produce GABA in apple pomace containing wood ear mushroom. The pH and acidity of apple pomace without wood ear mushroom was 5.12 and 0.48%, respectively, after fermenting for 5 days. Fermentation of apple pomace after the addition of 2% wood ear mushroom decreased the acidity (0.16%), while the pH was 6.26. The initial number of viable cells was about 1.91×10^7 CFU/mL, which increased to 10^9 CFU/mL after fermenting for one day. On the other hand, apple pomace mixture containing 2% wood ear mushroom provided the highest stability for lactic acid bacteria, with viable cell counts of 3.86×10^8 CFU/mL. Bioconversion of MSG in apple pomace was efficiently performed following the addition of wood ear mushroom. Apple pomace containing 2% wood ear mushroom fermented by *L. plantarum* EJ2014 was fortified with GABA (13,442 μg/mL). Thus, lactic acid fermentation of apple pomace containing wood ear mushroom resulted in the production of multi-functional ingredients, including GABA, dietary fibers, polyphenols, and probiotics.

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