Production of Sesaminol and Antioxidative Activity of Fermented Sesame with *Lactobacillus plantarum* P8, *Lactobacillus acidophilus* ATCC 4356, *Streptococcus thermophilus* S10

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Abstract This study was carried out to select the most competent bacterial cultures that could convert sesaminol glycosides to aglycone by β -glucosidase produced by lactic acid bacteria such as *Lactobacillus acidophilus*, *Lactobacillus plantarum* (LP), and *Streptococcus thermophilus* in sesame fermented at 37°C for 24 h. The pH of fermented sesame was decreased compared to non-fermented controls. The pH of LP was lower than that of the other two during fermentation. Fermented sesame had higher antioxidant activity compared to non-fermented controls during the entire fermentation time. Total phenol content, DPPH free radical scavenging assay, reducing power assay of sesame fermented by LP was the highest compared to the others. In addition, sesame fermented by LP had more bioconversion of sesaminol glycoside to aglycone compared to the others. Therefore, LP was the best bacterial culture of the three strains studied for producing functional fermented sesame for good health.

Keywords: sesame, fermentation, lactic acid bacteria, sesaminol, aglycone

Introduction

Sesame is an important health food in Korea, China, and other Asian countries. Korea has the highest consumption of sesame in the world with approximately 6-7 g/day per capita (1). Sesame seed contains 50% lipid, 20% protein, and 14% carbohydrate. It is one significant oil seed crop in the world. Sesame seed provides nutritious meal and protein as well as highly stable oil. (2). Sesame exhibits many beneficial physiological effects due to its bioactive lignan compounds. Sesame seed contains abundant lignan glycosides such as sesamin, sesamolin, and sesaminol glucosides as significant functional components (1,3,4). Sesamin is a major lignan in the sesame oil. Its beneficial biological effects have been studied extensively. Many studies have reported that sesamin has anti-hypertensive effect (5) and anti-hypercholesterolemic activity through its inhibitory activity on cholesterol absorption and synthesis (6). Sesamolin and sesaminol glucoside have antioxidant activities related to their high stability against oxidation during long period storage of sesame oil (7). The major lignan glycoside exists mainly in sesame seed, hydrophilic antioxidants are sesaminol triglucosides (STG) that have less bioactivity *in vitro* (8-10). Although sesaminol glucosides have no antioxidant activity *in vitro*, they can be converted to phenolic

compounds with antioxidant activity *in vivo* (11,12). Experiments have proved that STG could be metabolized by human and rat intestinal microbiota and form phenolics that possess anti-inflammatory, antioxidant, and estrogenic activities. They can be hydrolyzed by intestinal β -glucosidase to sesaminol, an antioxidant. According to the Jan *et al.* (13), the transformation of STG in gut involved the demethylation of a methoxy group, hydrolysis of glucoside and reductive cleavage of furfuran ring. And then STG incorporated through lymphatic absorption into the cardiovascular system (14-18). Many scientific studies have proved that sesaminol in sesame is mostly in the glycoside form (3). β -Glucosidic bond of glycoside can be hydrolyzed by β -glucosidase produced by microorganism to form aglycone after fermentation. In other words, higher β -glucosidase activity will release more aglycone contents (19). The formed aglycone will lead to more active biological activity. Since the intestinal absorption of aglycone is faster than glycoside, aglycone is more effective in preventing diseases. Aglycones have peroxyl radical scavenging activities by β -glucosidase after being liberated in the gastrointestinal tract (8,20). *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* have been reported to be able to produce β -glucosidase. Therefore, they are typically used for fermentation (21). For example, *S. thermophilus* and *Lactobacillus*

have been used to produce sesame yogurt from sesame milk by Afaneh *et al.* (22). Several studies have demonstrated antioxidant activity of sesame, however, there is no sufficient study on fermented sesame with lactic acid bacteria. Unlike previous studies, the objective of this study was to investigate whether sesaminol triglucoside was converted to sesaminol by β -glucosidase produced by lactic acid bacteria such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Streptococcus thermophilus* in sesame fermented at 37°C for 24 h. This study is carried out to select the most competent cultures among *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Streptococcus thermophilus* that could convert sesaminol glycosides to aglycone in fermented sesame.

Materials and Methods

Materials and reagents Sesame was purchased at a local market in Gwangjin-gu, Seoul, Korea. Sesaminol triglucoside and sesaminol at HPLC standard grade were obtained from Nacalai USA Company (Kyoto, Japan). Mobile phase reagents of HPLC analysis such as methanol was purchased from J. T. Barker Company (Phillipsburg, NJ, USA). All other chemicals used were of analytical grade.

Microorganisms *Streptococcus thermophilus*, *Lactobacillus plantarum* P8 (LP), and *Streptococcus thermophilus* S10 (ST) were isolated from the Laboratory of Dairy Biotechnology and Bioengineering, Inner Mongolia Agricultural University. Pure cultures of *Lactobacillus acidophilus* ATCC 4356 (LA) was obtained from Korean Collection for Type Culture (Daejeon, Korea). Three strains were activated in de Mann Rogosa Sharps (MRS) broth (Oxoid Ltd., Basingstoke, England) at 37°C for 24 h aerobically. Each microorganism concentration was adjusted to 10^8 CFU/mL.

Sample preparation Sesame seeds were extracted with n-hexane (1:10, w/v) by stirring at room temperature for 24 h to obtain defatted sesame (23). Sesame was soaked in distilled water at 1:10 (w/v) ratio, boiled at 100°C for 40 min, and ground with a blender (KNJ-991100R; NUC Co., Ltd., Daegu, Korea). To extract sesame juice, the resultant slurry was filtered with cotton cloth. The sesame juice was sterilized at 121°C for 15 min, chilled, and refrigerated at 4°C. The sesame juice was then inoculated (1%, v/v) with LP, ST and LA and incubated at 37°C for 24 h. Samples were collected at intervals of 4 and 6 h and stored at –80°C after freeze-drying.

Determination of total count of lactic acid bacteria, pH, and titratable acidity Cell viability of lactic acid bacteria (LAB) was determined by using a spread plate method with MRS agar (Oxoid Ltd.) after incubation at 37 for 24 h. The number of total viable cells was expressed in log values. The pH of sesame sample was analyzed by using a pH meter (pH 900; Precisa Co, London, UK). Titratable acidity (TA) was measured by titration with 0.1N NaOH. Result was

presented as percent lactic acid (24).

Proximate analysis The proximate analysis of non-fermented and fermented sesame was analyzed by using AOAC method (24). Total solid contents were estimated by subtracting the moisture percent from 100. Crude ash contents were measured by burning at 450. Protein and crude fat contents were measured by Kjeldal method and Soxhlet method, respectively. Carbohydrate content was calculated by deducting the sum of crude ash, crude protein, and crude fat contents from the total solid contents.

HPLC analysis of lignans The amount of lignan was measured by HPLC using procedures described by Zhu *et al.* (25). Dried sample was extracted with 80% methanol (1:20, w/v) using a Sonicator (Branson Ultrasonics Co., Danbury, CT, USA) for 60 min followed by centrifugation at 3,000×g for 20 min. The supernatant was filtered through PVDF syringe filter (0.45 μ m, Pall Corporation, Port Washington, NY, USA). Filtered samples were analyzed with an Agilent 1100 series HPLC system equipped with a Binary pump, Autosampler, multiple wavelength detector, and a ChemStation software. The analyses were carried out on a Higgins 100 C18 column (4.6×250 mm, 5 μ m). The oven temperature was set at 35. The mobile phase of HPLC was methanol/water (60:40, v/v) and the flow rate was 0.6 mL/min. The detector was set at 290 nm. Injection volume was 10 μ L.

Determination of total phenol contents The amount of total phenol contents for antioxidative activity was determined with procedures described by Folin-Ciocalteu (26) with slight modifications. Same as the extraction method in HPLC, sample extraction was processed by the methanol method. Briefly, a 0.5 mL of sample extract was added to 2.5 mL of 0.2 N Folin-Ciocalteu's phenol reagent and mixed. After standing for 5 min at room temperature, 2 mL of 75 g/L sodium carbonate was added to the mixture and incubated at room temperature for 2 h. The samples were measured with a spectrophotometer at 760 nm. The results were expressed as mg of gallic acid equivalents (GAEs) per 10 g using gallic acid as the standard.

DPPH free radical scavenging assay This experiment was to identify antioxidative activity. The 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich Co., St. Louis, MO, USA) radical has an odd electron that has a deep violet color. When the radical accepts a hydrogen radical or an electron, its absorbance value is changed. In this study, the DPPH radical scavenging activities of the extract of fermented sesame were measured with the method of Blois (27) with slight modifications. The sample was extracted by the methanol method and analyzed by HPLC A 0.5 mL aliquot of sample extract and a 2.5 mL of 75 μ M DPPH solution were reacted in the dark at room temperature for 30 min. The absorbance value was analyzed with a spectrophotometer (Otizen 2120UV; Mecasys Co., Ltd., Daejeon, Korea) at 517 nm. Free radical scavenging activity was determined

using the following: DPPH free radical scavenging effect (%) = $(1 - \text{absorbance of sample}) / \text{absorbance control} \times 100$.

Reducing power assay The reducing power of sample was determined according to the method of Oyaizu (28). Briefly, a 250 μL of sample extract was mixed with a 0.2 M sodium phosphate buffer (250 μL , pH 6.6) and a 250 μL of potassium ferricyanide (1%, w/v, Sigma-Aldrich). After the mixture was reacted at 50°C for 30 min, 10% trichloroacetic acid (250 μL , Sigma-Aldrich) was added to the mixture. The mixture was then centrifuged at 1,500 $\times g$ for 10 min. A 500 μL of distilled water and a 100 μL of 0.1% ferric chloride (Junsei Chemical Co., Ltd., Tokyo, Japan) were mixed with 500 μL of the supernatant. The absorbance was measured at 700 nm using a UV-visible spectrophotometer.

Statistical analysis Statistical analyses were performed using SPSS statistics 18.0 (SPSS Inc., Chicago, IL, USA). All data were analyzed by one-way analysis of variance (ANOVA) and expressed as mean $\pm$ standard deviation (SD). A Turkey test was used to determine whether there is a significant difference between samples. Significant difference was considered when the p value was less than 0.05.

Results and Discussion

Acid production and microorganism growth during fermentation

Results of pH and titratable acidity of fermented and non-fermented sesame are shown in Fig. 1. The pH of fermented sesame was significantly ($p < 0.05$) decreased during fermentation at 37°C for 24 h due to the production of lactic acid by three bacteria. The initial pH ranged was 6.44 to 6.52. It was decreased to 4.69 and 5.34 after 24 h fermentation. The pH of sesame fermented with LP was the lowest compared to that fermented with the other two strains of bacteria at

Fig. 2. Changes in cell viability of LAB in fermented sesame during different fermentation time. Con, control, non-fermented sesame; LP, fermented sesame with *Lactobacillus plantarum* P8; ST, fermented sesame with *Streptococcus thermophilus* S10; LA, fermented sesame with *Lactobacillus acidophilus* ATCC4356.

the end of fermentation. The initial titratable acidity (TA) was 0.26-0.28. It was increased to 0.61-0.83, among which TA of the sesame fermented with LP was the highest after the fermentation. As the time of fermentation was increased, TA of fermented sesame was significantly ($p < 0.05$) increased. It is possible to identify the quality of fermented sesame through the production of acid. Wu *et al.* (29) have mentioned that the pH value of yogurt fermented by lactic acid bacteria such as *Streptococcus* and *Lactobacillus* have reached 4.0 to 4.7. Although ST and LA exceeded the usual pH range of yogurt, fermented sesame with LP showed an appropriate pH during fermentation. Results of lactic acid bacteria count of fermented sesame are shown in Fig. 2. The count of LAB was significantly

Fig. 1. Changes in acid production in fermented sesame during different fermentation time. Con, control, non-fermented sesame; LP, fermented sesame with *Lactobacillus plantarum* P8; ST, fermented sesame with *Streptococcus thermophilus* S10; LA, fermented sesame with *Lactobacillus acidophilus* ATCC4356.

($p < 0.05$) increased during the fermentation at 37°C for 24 h. The initial LAB count ranged from 7.1 to 7.3 log CFU/mL. It reached up to 8.1 to 8.6 log CFU/mL during the fermentation. The minimum accepted microflora counts ranged at 10^6 – 10^9 CFU/mL in yogurt and thus sesame is a proper growth medium for LAB. Afaneh *et al.* (22) have reported similar LAB results.

Proximate analysis Proximate analyses for moisture, crude fat, crude protein, ash, and carbohydrate of fermented and non-fermented sesame are summarized in Table 1. There was no significant difference in crude fat or carbohydrate of sesame fermented with three strains of bacteria as compared with non-fermented sesame. However, crude protein contents were significantly ($p < 0.05$) increased in sesame fermented with LP compared to the control or sesames fermented with the other two strains. On the contrary, ash contents decreased significantly ($p < 0.05$) in sesame fermented with LP compared to control or that with the other two strains of LAB. No significant difference was found in sesame fermented with ST or LA. Lee *et al.* (30) showed similar results that fermentation with *Lactobacillus plantarum* decreased in ash and increase in crude protein after fermentation at 37°C for 24 h.

Changes in lignan contents HPLC analysis results of lignan such as

sesaminol triglucoside and sesaminol contents of fermented sesame or non-fermented sesame are summarized in Table 2. While the concentration of sesaminol triglucoside was significantly ($p < 0.05$) decreased, the concentration of sesaminol was significantly ($p < 0.05$) increased. No significant difference was found in sesame fermented with LP or ST after 18 h of fermentation or after 12 h of fermentation with LA. This indicated that more aglycone was produced in sesame fermented with LP compared to that of the other two strains of bacteria. Zhu *et al.* (25) have reported that STG should be considered as suitable substrate for intestinal microbiota. Sesaminol aglycone was also produced during the *in vitro* fermentation of STG. The maximal production of sesaminol was observed at 12 h of fermentation. These results suggested that the bioconversion by lactic acid bacteria fermentation could be through hydrolysis of sesaminol glucoside. Hence, fermented sesame that produces sesaminol might have the potential as functional food ingredients. *L. plantarum* could be used as a starter culture for fermented sesame to produce aglycones.

Total phenol contents and antioxidant activities Sesame is good source of carbohydrate, fat, protein, and lignan glycoside. However, it is difficult to extract and isolate sesaminol glycosides from sesame due to the existence of carbohydrate, fat, and protein. To extract sesaminol triglucoside from sesame effectively, ethanol or methanol

Table 1. Proximate component of fermented and non-fermented sesame (unit: g/100 g)

Group ¹⁾	Total solid	Crude fat	Crude protein	Ash	Carbohydrate
Con	0.23±0.00 ^{AB2)}	71.93±2.52 ^A	9.95±0.15 ^{AB}	2.73±0.23 ^B	15.17±2.76 ^A
LP	0.17±0.05 ^B	72.50±3.70 ^A	10.63±0.38 ^A	3.59±0.21 ^A	13.12±3.41 ^A
ST	0.31±0.06 ^A	75.15±2.12 ^A	7.37±0.55 ^C	2.56±0.20 ^B	14.61±2.26 ^A
LA	0.15±0.05 ^B	69.12±2.73 ^A	9.11±0.14 ^B	2.48±0.51 ^B	19.15±2.33 ^A

¹⁾Con, control; non-fermented sesame; LP, fermented sesame with *Lactobacillus plantarum* P8; ST, fermented sesame with *Streptococcus thermophilus* S10; LA, fermented sesame with *Lactobacillus acidophilus* ATCC4356

²⁾Values are mean±SD ($n=3$); Values in the same column with different superscripts are significantly ($p < 0.05$) different.

Table 2. Changes in lignan contents of sesame with and without fermentation with LAB (unit: mg/10 g)

Lignan	Time (h)	Strains			
		Con ¹⁾	LP	ST	LA
Sesaminol triglucosides	0	104.9±0.22 ^{ABns2)}	104.8±0.28 ^A	104.5±0.16 ^A	104.5±0.30 ^A
	4	105.1±0.02 ^{ABa}	88.3±0.10 ^{Bc}	92.9±0.09 ^{Bb}	88.3±0.71 ^{Bc}
	8	104.6±0.41 ^{Ba}	68.7±0.06 ^{Cc}	74.5±0.08 ^{Cb}	74.2±0.10 ^{Cb}
	12	104.9±0.45 ^{ABa}	58.6±0.07 ^{Dd}	64.1±0.14 ^{Dc}	65.6±0.15 ^{Db}
	18	105.3±0.23 ^{ABa}	51.8±0.12 ^{Ed}	57.4±0.19 ^{Ec}	65.6±0.11 ^{Db}
	24	105.6±0.50 ^{Aa}	51.7±0.14 ^{Ed}	57.8±0.45 ^{Ec}	65.6±0.12 ^{Db}
Sesaminol	0	ND ^{NSns}	ND ^E	ND ^E	ND ^D
	4	ND ^d	19.5±0.04 ^{Da}	17.3±0.04 ^{Db}	15.2±0.05 ^{Cc}
	8	ND ^d	25.1±0.07 ^{Ca}	22.9±0.07 ^{Cb}	22.0±0.03 ^{Bc}
	12	ND ^d	29.1±0.14 ^{Bb}	28.9±0.05 ^{Bc}	30.5±0.16 ^{Aa}
	18	ND ^d	35.1±0.04 ^{Aa}	32.9±0.20 ^{Ab}	30.6±0.52 ^{Ac}
	24	ND ^d	34.1±0.05 ^{Aa}	32.6±0.14 ^{Ab}	30.6±0.09 ^{Ac}

¹⁾Con, control; non-fermented sesame; LP, fermented sesame with *Lactobacillus plantarum* P8; ST, fermented sesame with *Streptococcus thermophilus* S10; LA, fermented sesame with *Lactobacillus acidophilus* ATCC4356

²⁾Values are mean±SD ($n=3$); ND, not detected; Values with different superscripts capital letters are significantly different during fermentation time ($p < 0.05$). Values with different superscripts small letters are significantly different among strains ($p < 0.05$); NS, not significant

Fig. 3. Change in total phenol contents in fermented sesame during different fermentation time. Con, control, non-fermented sesame; LP, fermented sesame with *Lactobacillus plantarum* P8; ST, fermented sesame with *Streptococcus thermophilus* S10; LA, fermented sesame with *Lactobacillus acidophilus* ATCC4356.

has been used to remove proteins, sugars, sesaminol glycosides, and sesaminol. Suja *et al.* (31) have reported that 80% methanol is the most effective solvent to extract lignans from sesame. The total phenol contents appeared as GAE of fermented sesame with LAB are shown in Fig. 3. It is known that the antioxidant activity of plant corresponds to the content of their phenolic compounds (32). Total phenol contents of fermented sesame were higher than those of non-fermented sesame. Total phenol contents of fermented sesame were increased significantly ($p < 0.05$) during the fermentation time. The total phenolic compounds had the highest amount in sesame fermented with LP (14.79 ± 0.01 mg/10 g GAE) at 24 h. Nakai *et al.* (33) have reported that sesaminol glucosides could be changed to

phenolic compounds that possess antioxidant activity after oral administration. As DPPH free radical scavenging assay has been widely used to determine the antioxidant activity, DPPH was used to verify the proton radical scavenging action of fermented and non-fermented sesame in this study. DPPH radical scavenging activities of sesame fermented with LAB are shown in Fig. 4. The DPPH free radical scavenging activity of fermented sesame was significantly ($p < 0.05$) higher than that of non-fermented sesame. DPPH free radical scavenging activities of fermented sesame were increased significantly ($p < 0.05$) during 24 h fermentation. The DPPH radical scavenging assay revealed the highest activity (80.56 ± 0.52) in sesame fermented with LP at 24 h. Miyake *et al.* (11) have reported that the medium that contains sesaminol triglucoside exhibits high DPPH radical scavenging activity by culturing with *Aspergillus*.

Reducing power is closely related to antioxidant activity. In this method, depending on the reducing power of the compound, the yellow color of the test solution will change to green and blue. Reducing activity presents the capacity of giving electrons to active oxygen species. Compound containing Fe^{3+} and free radicals can yield hydrogen to stabilize the free radical, thus producing Fe^{2+} (32,34). The changes of reducing power in fermented sesame during the 24 h of fermentation are shown in Fig 4. The reducing power of fermented sesame was significantly ($p < 0.05$) increased during the 24 h fermentation at 37°C . In addition, reducing power of fermented sesame was significantly ($p < 0.05$) higher than that of non-fermented sesame. Reducing power of fermented sesame LP (2.16 ± 0.01) was the highest at 24 h of fermentation. Sesame fermented with LP contained the highest amounts of total phenolic compounds (14.79 ± 0.01), that showed the highest reducing power among all samples. Additionally, the effects of anticancer, antithrombotic and ACE inhibitory activity should be investigated for fermented sesame. To summarize, STG was converted into aglycone (sesaminol) due to

Fig. 4. Changes in antioxidant activities of fermented sesame during different fermentation time. Con, control, non-fermented sesame; LP, fermented sesame with *Lactobacillus plantarum* P8; ST, fermented sesame with *Streptococcus thermophilus* S10; LA, fermented sesame with *Lactobacillus acidophilus* ATCC4356.

hydrolysis by microorganism during fermentation. Sesame fermented with LP had the highest content of aglycone and the highest antioxidant activity. Our results suggest that *Lactobacillus plantarum* P8 can be used as the most efficient strain to produce functional fermented sesame for the health benefit.

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