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Production of Carrot Pomace Fortified with Mucilage, Fibrinolytic Enzyme and Probiotics by Solid-state Fermentation Using the Mixed Culture of *Bacillus subtilis* and *Leuconostoc mesenteroides*

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Abstract

Bioactive compounds were produced from carrot pomace by solid-state fermentation using *Bacillus subtilis* HA and *Leuconostoc mesenteroides*. The carrot pomace (CP) fermented by *B. subtilis* HA with 3% monosodium glutamate (MSG) showed higher production of various bioactive compounds, with 1.64 Pa·s^n of consistency, 2.31% of mucilage content, 16.95 unit/g of fibrinolytic enzyme activity, 35.3 unit/g of proteolytic activity and 37.5 mg% of tyrosine content. The mucilage production was greatly dependent upon the concentration of MSG added. Most MSG added in CP was converted into mucilage (2.3%) including 0.83% poly-γ-glutamic acid (PGA) with 1,505 kDa of molecular weight. The CP fermented secondly by *Leuc. mesenteroides* showed acidic pH and lower consistency. However, the fibrinolytic and proteolytic activities were increased. The secondly fermented CP showed the viable cell counts with 2.5×10^8 CFU/g of *B. subtilis* HA and 3.7×10^9 CFU/g of *Leuc. mesenteroides*, respectively. The freeze-dried fermented CP showed 2.88 Pa·s^n of consistency, 24% of mucilage content and 104.9 unit/g of fibrinolytic enzyme activity, respectively. Also, the powder of fermented CP indicated viable cell counts of 8.0×10^7 CFU/g of *B. subtilis* and 4.0×10^8 CFU/g of *Leuc. mesenteroides*. Therefore, the fermented CP that was fortified with dietary fibers, fibrinolytic enzyme and probiotics could be utilized as valuable ingredients of functional foods in food or cosmetic industries.

Key words: carrot pomace, mucilage, fibrinolytic enzyme, *Bacillus subtilis*, *Leuconostoc mesenteroides*

INTRODUCTION

Carrots, one of the vegetables classified as *Apiaceae*, contain a number of vitamins and minerals as well as many glucosides, such as sugar and fibrous materials, and also the reddish-yellow carotenoid pigments (1). In particular, the β-carotene found in carrots is valuable for what it can add both visually and nutritionally: it colors foods and is a precursor of vitamin A (2). Carotenoids are known to reduce rates of cancer, coronary heart disease, age-related disease of the eye (3), and they exhibit anti-cancer activity through activation of the immune system (4). Additionally, they maintain the strength of mucosa of digestive organs, which helps prohibit entrance into the body of many types of bacteria (5).

Carrot juice is collected using a juicer or centrifugal force. The yield rate of commercially produced carrot juice is approximately 50%, with the other half of the carrot eliminated as the by-products carrot pomace. In medium sized industries, over 10 tons of carrot pomace is produced every month, and is used as feed for animals and fertilizer. To increase competitiveness of the companies, carrot pomace needs to be used for more valuable applications than animal feed. Carrot pomace contains large amounts of valuable compounds, including dietary fiber (6), which is a functional ingredient in terms of nutrition and health. The water holding capacity of dietary fiber plays an important role in preventing a range of diseases, such as cancer of the large bowel, as well as cardiovascular disease (7). However, carrot pomace also has over 85% moisture content, which causes it to deteriorate easily, making it difficult to use as a food source for humans. Therefore, the fermentation of carrot pomace has been studied as one possible method to enhance its function and value (8).

B. subtilis in traditional fermented soybean foods produces hydrolytic enzymes of protein and carbohydrate (9) and contributes to the production of biologically active substances, such as functional peptides (10) and mucilage, during fermentation (11). In particular, it produces polyglutamic acid (PGA) as macromolecular mucilage by using glutamate as a substrate in both liquid culture and solid state fermentation (12). Lactic acid bacteria, which are a type of probiotic, are used for fermenting foods. These "healthy" bacteria prohibit viability of pathogenic organisms and harmful bacteria by metabo-

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lizing saccharides such as glucose to produce lactic acid (13). In particular, the *Leuconostoc* strain is a homo-fermentative lactic acid bacterium and is mainly isolated from Korean fermented vegetables. It is useful for changing rheological properties of fermented foods by synthesizing biological dextran, a macromolecular polysaccharide, using sugar as a carbon source (14).

Most research on fermentation of carrot reports methods for manufacturing mixed fruit and vegetable juice through lactic acid fermentation of carrot (15,16) or deals with the development of functional foods by the fermentation with *Bifidobacterium* of mixed fruits and vegetables (17). Although many studies have been performed with carrots, there is no report about research on the production of fermented carrot pomace to enhance the storage stability, to improve functional substances or to become a probiotic ingredient through mixed fermentation using *B. subtilis* and lactic acid bacteria. In contrast with the production of various biological active substances from beans fermented by *B. subtilis*, this study conducted two-stage fermentation of carrot pomace by using *B. subtilis* and lactic acid bacteria to produce multi-functional ingredients, including PGA as functional mucilage, fibrinolytic enzymes, peptides, hydrolytic enzymes of protein and higher viable cell counts of *Bacillus* and *Leuconostoc* strains. The evaluation of various bioactive components in fermented carrot pomace will provide the basic data for production of valuable ingredients from carrots and their application in creating functional foods.

MATERIALS AND METHODS

Material

Carrots were harvested in Kyungsangbukdo seonsan in June of 2007. Carrot juice was prepared using a Juice extractor (NJ-9300A, NUC Electric., Daegu, Korea) and carrot pomace (CP) was obtained as by-product. MRS broth was purchased from Difco (Sparks, MD, USA). Fibrin, fibrinogen, and thrombin were obtained from Sigma (St. Louis, MO, USA). The standard dextran (molecular weight 130, 400, 770, 1200 kDa) was purchased from American Polymer Standard Corporation (Mentor, USA). Monosodium glutamate (MSG) was purchased from Wei-chuan Foods Co. (Taipei city, Taiwan). White sugar was obtained from CJ Co. (Incheon, Korea).

Starter culture

Bacillus subtilis HA previously deposited in the Korean Culture Center of Microorganisms (KCCM) as KCCM 10775P was used (18). The solid state fermentation of CP was performed by *B. subtilis* HA. *B. subtilis* HA was inoculated on an MRS agar plate and then incubated at 42°C for 24 hr. For seed culture, 5% suspension of defatted soybean flour was prepared and sterilized at 121°C for 15 min. *B. subtilis* HA strain was inoculated in 50 mL culture broth and cultured with shaking at 180 rpm (SI-900R, JEIO TECH Co., Ltd., Korea) at 42°C for 24 hr (6.5×10^8 CFU/mL). *Leuconostoc mesenteroides* subsp. *dextranicum* (KCTC 3530) was activated on MRS agar plate at 25°C for 24 hr. One inoculum of *Leuc. mesenteroides* was inoculated in carrot juice sterilized at 121°C for 15min. The starter culture was prepared by shaking at 180 rpm at 25°C for 24 hr (9.0×10^9 CFU/mL).

Solid-state fermentation of carrot pomace

The 50 g of carrot pomace (89% moisture content) was fortified with MSG (0～7%) and finally adjusted to the final moisture content. Carrot pomace mixture was sterilized using a sterilizer (MLS-3020, Sanyo Electric Co. Ltd, Japan) set at 121°C for 15 min. The starter of *B. subtilis* HA was inoculated in carrot pomace at a 1% level and then incubated at 42°C for 15 hr. For the second fermentation, the fermented carrot pomace was fortified with 2% sucrose and then inoculated with 4% of *Leuc. mesenteroides* subsp *dextranicum* culture. Lactic acid fermentation was performed at 25°C for 24 hr.

Preparation of mucilage

Fermented CP (5 g) was completely mixed with 45 mL of distilled water and followed by centrifugation ($2490\times g$, 10 min). The recovered supernatant was mixed with two volumes of isopropanol and then the aggregate was obtained by centrifugation ($1106\times g$, 10 min). The crude mucilage aggregate was washed with 95% ethyl alcohol and then was dried under the vacuum using vacuum drying oven (VO-200, Schwabach, Germany) at 50°C.

Consistency of fermented carrot pomace

Fermented CP (5 g) was mixed with 20 mL of distilled water and then passed through a sieve (0.99 mm). The consistency of filtrate was determined using Rheometer System (HAKKE RheoStress 1, Karlsruhe, Germany) with a cone plate device (Plate PP35Ti, 3.5 cm diameter). The filtrate (1 mL) was loaded on the plate with a cone device as the moving head and then the shear rate (1/s) and shear stress (Pa) were measured. The measuring was performed at a temperature of 20°C and the shear rate with the range of 1 to 100 1/s. The consistency index was determined by the power law model (19).

Fibrinolytic enzyme activity of fermented carrot pomace

The fibrinolytic enzyme activity was determined by fibrin plate method (20). To prepare fibrin plate, 10 mL of 0.5% fibrinogen dissolved in 0.067 M sodium phosphate buffer (pH 7.4) was evenly spread on petri-dish (dia. 9 cm) and then 0.1 mL of thrombin (100 unit/mL) dissolved in the same buffer was added and quickly mixed. The mixed solution was solidified by standing for 30 min at room temperature. The water extract (20 μL) of fermented CP was spotted on the fibrin plate, followed by incubation at 37°C for 2 hr. The diameter of the clear zone was measured and its activity was determined by comparing the activity of a standard plasmin enzyme.

Proteolytic activity and tyrosine content of fermented CP

Proteolytic activity was determined by the modified method of Anson (21). The fermented CP (5 g) was thoroughly mixed with 20 mL of 20 mM phosphate buffer (pH 7.0) by shaking at room temperature and then centrifuged at 2490×*g* for 15 min. The obtained supernatant (0.35 mL) was mixed with 0.35 mL of casein solution (0.6%) and then incubated at 37°C for 10 min and followed by addition of 0.7 mL of 0.44 M TCA solution to stop the reaction. After incubating at 37°C for 30 min the formed aggregate was removed by centrifugation (2490×*g*, 10 min). The supernatant was reacted with Folin reagent and then the absorbance was determined at 660 nm. The control was performed by adding 0.44 M TCA solution in the crude enzyme extract before enzyme reaction. One unit of enzyme activity was defined as the generation of 1 μg tyrosine for 1 min at the same reaction condition.

To determine the crude peptide content, the amount of tyrosine in the water extract from fermented CP was measured by a reaction with Folin phenol reagent (22). The blue color of reaction mixture was determined using a spectrophotometer (UVIKON Kontron Co, Milano, Italy) at 660 nm.

Bioconversion rate of glutamate

The glutamate content remaining in fermented CP was determined by TLC method (23). After the water extract (50 mL) of fermented CP (5 g) was treated with two volume of isopropanol and followed by centrifugation, the supernatant was concentrated using vacuum evaporator (BUCHI, Flawil, Swizerland) at 50°C. The concentrate (10 mL) was filtered with 0.45 μm syringe filter (Minisart RC 15, Sartorius, Flankfurt, Germany) and then 2 μL was spotted on the TLC plate. The moving solvent was used by the first solvent with 1-butanol, acetate, water (5:4:3) and the second solvent with ethanol, water (63:37). The TLC plate was dried at room temperature and developed by spreading reagent (0.2% ninhydrin in acetone) and followed by drying at 105°C for 5～10 min. The standard MSG solution (1, 5, 10 mg/mL) was used as control.

Analysis of γ-polyglutamic acid

The dried mucilage was dissolved in 0.1 M Na_2SO_4 and 0.05 M NaN_3 (adjusted to pH 4 with glacial acetic acid) to prepare a 1% solution and then followed by centrifugation. The supernatant was filtered with a 0.45 μm syringe filter and then its molecular weight was analyzed using gel permeation chromatography (GPC) (24). The GPC column was Shodex SB805HQ (Kawasaki, Japan), and moving phase and flow rate were 0.1 M Na_2SO_4 and 0.05 M NaN_3 (adjusting to pH 4 with glacial acetic acid) and 1.0 mL/min, respectively. The eluate was determined with RI detector (Knauer Co., Berlin, Germany). The standard dextrans were used for determining the retention time according to molecular weight. The purified PGA was used for determining the PGA content in crude mucilage (25).

Physicochemical and viable cell counts

The pH of fermented CP was determined from 10% (w/w) water extract using a pH meter (Model 420A$^+$, Thermo Orion, Batavia, USA). The titratable acidity was measured by determining the 0.1 N NaOH content necessary for adjusting to pH 8.3. The acidity of fermented CP was calculated based on lactic acid. The viable cell counts were determined by plating 20 μL of serial diluted CP water extract on MRS agar plates. The viable cell counts (CFU/g) was determined after incubating at 42°C and 25°C for 24 hr.

Statistic analysis

The statistic analysis was performed by SPSS (Standard version 17.0, Chicago, IL, USA). One-way ANOVA was carried out and was verified with Tukey test. The statistical significance was confirmed at the above $p<0.05$.

RESULTS AND DISCUSSION

Consistency and mucilage content of fermented carrot pomace

The fermented carrot pomace (CP) was obtained by adding 1～7% (v/w) MSG to optimize production of PGA from solid state fermentation with CP. The consistency of the fermented CP recorded the highest level, 1.64 Pa·s^n when 3% MSG was added, and it reduced to 1.29 Pa·s^n when 5% MSG was added in Table 1. Oh

Table 1. Comparison of consistency index and mucilage content in carrot pomace fermented by *B. subtilis* HA and mixed culture in the different concentration of MSG

MSG (%)	Consistency index (Pa·s^n)		Mucilage content (%)	
	1st	2nd	1st	2nd
0	0.04±0.00	0.05±0.01	0.20±0.06	0±0.00
1	0.05±0.03**	0.06±0.01	0.57±0.07	1.15±0.01
3	1.64±0.01***	1.03±0.02***	2.31±0.01***	2.96±0.00***
5	1.29±0.03***	0.73±0.01***	2.61±0.05***	2.80±0.00***
7	1.19±0.01***	0.42±0.02***	2.72±0.09***	2.80±0.01***

1st: fermentation by *B. subtilis* HA, 2nd: mixed fermentation by *B. subtilis* HA and *Leuc. mesenteroides*. Mean±SD (n=3); Compared to control as determined by Tukey's studentized range (HSD) test ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

et al. (21) reported that in *B. subtilis* KU-A and *B. subtilis* GT-D fermentation of soybean curd residue according to the concentration of glutamate, the consistency increased with more content of glutamate and it recorded 3.7 and 1.1 Pa·s^n in the addition of 3% and 5% MSG, respectively. The CP fermented by *B. subtilis* HA also showed a similar pattern with those of soybean curd residue as the concentration of glutamate affected the consistency. The mucilage content became significantly higher with the addition of more MSG and the highest level, 2.72%, occurred when 7% MSG was added. Woo et al. (26) found that the mucilage contents after fermentation by *B. subtilis* (KCCM 3014) and *B. subtilis* (KCCM 11315) were 3.40% and 3.23%, respectively. Based on these results, the solid state fermentation of CP with a little protein content appears to provide slightly lower mucilage content as compared to those of soybean fermented by *Bacillus* sp.

Consistency and mucilage content in mixed fermented CP

After the second fermentation using lactic acid bacteria, both the mucilage content and the consistency of fermented CP were the highest when 3% MSG was added. The consistency tended to decrease from 1.64 Pa·s^n in the first fermentation of *B. subtilis* to 1.03 Pa·s^n in the second fermentation of lactic acid bacteria in Table 1. This is thought to occur because the pH of fermented CP becomes lower during the second lactic acid fermentation due to the amount of lactic acid produced. Lee et al. (27) revealed that, for PGA accounting for most of mucilage made by *B. subtilis*, the viscosity declined with reduced ionization of carboxyl residue under acidic conditions. The mucilage content tended to increase from 2.96% in the first fermentation to 3% MSG in the second one. *Leuconostoc* strain can produce dextran, a macromolecular polysaccharide with sucrose as a carbon source, by dextransucrase, an extracellular enzyme (28). Therefore, the production of dextran in the second fermentation was thought to lead to the increase of the mucilage content.

Fibrinolytic enzyme activity in mixed fermented CP

As shown in Fig. 1, the activity of a fibrinolytic enzyme known to be an alkaline protease produced in *B. subtilis* fermentation was at its highest reading of 16.95 unit/g with the addition of 3% MSG when fermentation proceeded for 15 hr. The activity reduced to 9.37 unit/g with the addition of 5% MSG. Ok and Cho (29) measured the activity of fibrinolytic enzyme in *Bacillus* sp. strain isolated from soybean paste, and reported that the activity was 2.4 unit/g. In other words, the activity of fibrinolytic enzyme in the CP fermented by *B. subtilis* HA was eight times than that in soybean paste. The reported activity of fibrinolytic enzyme in fermented foods was known to be attributed to *Bacillus* strain (30), so *B. subtilis* HA in CP was considered to also lead to production of fibrinolytic enzyme as protease. Like the first fermentation of *B. subtilis*, the second fermentation with

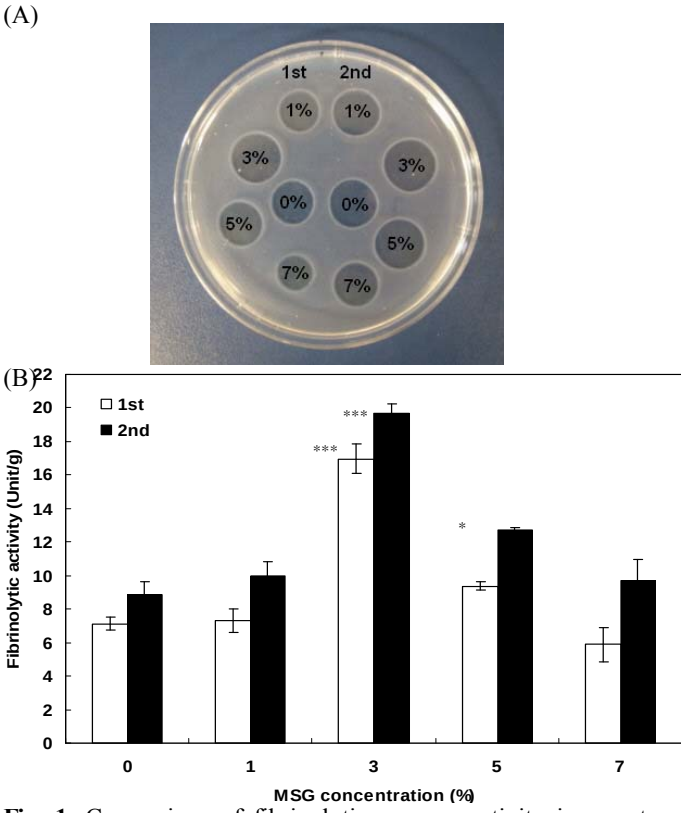

Fig. 1. Comparison of fibrinolytic enzyme activity in carrot pomace fermented by *B. subtilis* HA and mixed culture in the different concentration of MSG. 1st: fermentation by *B. subtilis* HA, 2nd: mixed fermentation by *B. subtilis* HA and *Leuc. mesenteroides*. A: digested fibrin plate, B: fibrinolytic activity. Mean±SD (n=3); Compared to control as determined by Tukey's studentized range (HSD) test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

lactic acid bacteria showed the highest activity of fibrinolytic enzymes (19.71 unit/g) when 3% MSG was added, and the enzyme activity of the second fermentation was higher than that of the first one overall (Fig. 1). These results suggested that *B. subtilis* in the fermented CP still participated in the production of fibrinolytic enzymes as the fermentation of lactic acid bacteria proceeded. Therefore, when fermentation of *B. subtilis* was conducted for CP, fermented CP with high activity of fibrinolytic enzymes could be obtained. Further more, the second fermentation of CP by lactic acid bacterium resulted in enhancing the activity of fibrinolytic enzyme.

Tyrosine content and proteolytic activity in fermented CP

The tyrosine content of ferment CP was measured as a means of indirectly determining the production of hydrolytic protein products. The addition of 3% MSG recorded the highest level, 37.5 mg%, as presented in Table 2. This tendency was similar with that of the consistency and the activity of fibrinolytic enzymes, which were the highest in the addition of 3% MSG. According to Ryu et al. (31) the tyrosine contents in soybean curd residue and in its fermented materials were 192 mg% and 536 mg%, respectively. In comparison with the tyrosine content of fermented soybean with a rich protein, that of CP was low. Therefore, the content of peptides hydrolyzed from proteins in fermented CP, was also considered to be low. In addition, the tyrosine content of fermented CP was not largely different according to the concentration of MSG. This result showed that MSG had little effect on the production of tyrosine. The increase of the tyrosine content in the second fermentation of lactic acid bacteria was considered to follow an additional production of tyrosine by lactic acid bacteria. When proteolytic activity was measured, the first fermentation with 3% MSG resulted in a value of 35.3 unit/g. However, in the second fermentation of lactic acid bacteria, the activity tended to be even higher, with a peak of 43.9 unit/g with the addition of 3% MSG. Both the tyrosine content and the proteolytic activity in fermented CP after the second fermentation were higher than those of the first fermentation.

Table 2. Comparison of tyrosine content and proteolytic activity in carrot pomace fermented by *B. subtilis* HA and mixed culture in the different concentration of MSG

MSG (%)	Tyrosine content (mg%)		Proteolytic activity (unit/g)	
	1st	2nd 2nd	1st	2nd
0	31.0±0.6	51.3±1.1	24.9±0.5	41.4±0.7
1	32.5±0.7	52.2±1.1	25.6±0.4***	42.8±0.6***
3	37.5±0.7***	56.7±1.0**	35.3±0.5***	43.9±0.6***
5	36.7±0.7**	55.7±1.1*	31.3±0.5***	42.7±0.6***
7	35.8±0.7**	55.5±1.0*	36.1±0.4***	37.5±0.7***

1st: fermentation by *B. subtilis* HA, 2nd: mixed fermentation by *B. subtilis* HA and *Leuc. mesenteroides*. Mean±SD (n=3); Compared to control as determined by Tukey's studentized range (HSD) test ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

Physicochemical properties and viable cell counts in mixed fermented CP

As shown in Table 3, the pH of fermented CP was reduced from pH 6.8 in the first *B. subtilis* fermentation to pH 4.8 in the second fermentation of lactic acid bacteria while acidity had a tendency to increase. The acidity was the highest by recording 0.63% in the addition of 3% MSG and was decreased in the addition of 5% MSG. The lower pH of the fermented CP following production of organic acids from lactic acid bacteria was thought to maintain quality of the fermented CP and to improve storage stability by inhibiting the growth of *B. subtilis*. The storage stability of fermented soybean product was reported to be enhanced by organic acids such as lactic acid, acetic acid and oxalic acid (32). Viable cell count of the fermented CP added with 3% MSG was the highest by recording more than 5.4×10^{8} CFU/g of *B. subtilis* HA and 6.6×10^{9} CFU/g of *Leuc. mesenteroides*, respectively. Considering the high viable cell counts of the CP fermented by *B. subtilis* and *Leuc. mesenteroides*, it was expected to be used as a probiotic

Table 3. Comparison of pH, acidity and viable cell counts of carrot pomace fermented by mixed culture in the different concentration of MSG

MSG (%)	pH		Acidity (%)	Viable cell counts	
	1st	2nd		*B. subtilis* ($\times10^{8}$ CFU/g)	*Leuc. mesenteroides* ($\times10^{9}$ CFU/g)
0	6.8	4.4	0.52	1.3	1.0
1	6.9	4.4	0.57	2.1	1.2
3	6.9	4.9	0.63	5.4	6.6
5	6.7	4.9	0.60	1.8	5.4
7	6.8	4.9	0.60	1.7	4.4

Data were presented as mean±SD (n=3).
1st: fermentation by *B. subtilis* HA, 2nd: mixed fermentation by *B. subtilis* HA and *Leuc. mesenteroides*.

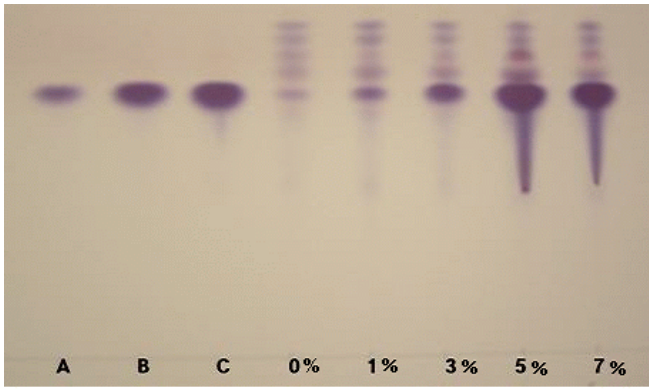

Fig. 2. TLC patterns of residual glutamate in the carrot pomace fermented by *B. subtilis* HA in the different concentration of MSG. Lane A, B, and C: MSG marker (A, 1 mg/mL; B, 5 mg/mL; C, 10 mg/mL); Sample (2 μL) was spotted on the TLC plate.

for functional foods.

Bioconversion of glutamate and PGA analysis in fermented CP

The *B. subtilis* HA used in the fermentation of CP is a glutamate-dependent strain and the production of mucilage was improved when glutamate was added to the culture medium. To determine the degree of conversion into macromolecular PGA according to MSG concentration, the glutamate content existing in the fermented CP was measured with TLC. As shown in Fig. 2, over 90% of the glutamate was transformed in the fermented CP with 3% MSG, but the existing amount of glutamate was increased with the addition of over 5% MSG. Therefore, when *B. subtilis* HA fermented CP with the addition of 3% MSG, most of the added glutamate was utilized so that CP was considered to be suitable for producing fermented materials with a high mucilage content.

To investigate effect of the addition of glutamate on the production of macromolecular PGA through *B. subtilis* fermentation of CP, the mucilage content and molecular weight of PGA in CP fermented under an optimal condition with 3% MSG were analyzed with GPC. The mucilage content and PGA content were 2.3% and 8.34 g/kg, respectively as presented in Table 4, and the molecular weight of PGA was measured to be 1,505 kDa. Therefore, the mucilage of the fermented CP was considered to be composed of PGA as well as other macromolecules. Oh et al. (33) reported that mucilage made through solid state fermentation of *B. subtilis* was composed of PGA and fructan. According to Xu et al. (34) the production of PGA was different according to moisture content of media, carbon source, nitrogen source and fermentation period. While 7～18 g/kg PGA was reported to be produced in the solid state fermentation under an optimal condition, PGA content with 10～50 g/L was produced after liquid fermentation of *B. subtilis* (35). Compared to the result of a study of Kunioka and Furusawa (36) revealing that the molecular weight of PGA was 2,000 kDa, PGA molecular weight of fermented CP was relatively smaller. Therefore, these data suggest that the macromolecular PGA produced by *B. subtilis* is different in its molecular weight, purity and the amount of products according to the composition of media, types of culture and *Bacillus* sp.

Table 4. γ-PGA molecular weight of the carrot pomace fermented by *B. subtilis* HA

	Retention time (min)	Mucilage content (%)	PGA content (g/kg)	Molecular weight (kDa)
F-CP[1)]	6.07	2.3	8.34	1,505

[1)]F-CP: carrot pomace fermented by *B. subtilis* HA with 3% MSG.

Physicochemical and biological properties of fermented CP

Consistency, mucilage content and activity of fibrinolytic enzymes in freeze dried CP after or before mixed fermentation using *B. subtilis* and lactic acid bacteria are shown in Table 5. In the non-fermented CP powders there was no mucilage, fibrinolytic enzyme activity and viable cells, and the viscosity was very low. However, the consistency of CP powders after solid state fermentation using two strains was 2.88 $Pa \cdot s^n$ and the mucilage content was very high, with a value indicating 24%. Fibrinolytic enzyme was also higher after fermentation, with an activity of 104.9 unit/g. This activity was

Table 5. Comparison of consistency index, mucilage content, fibrinolytic enzyme activity and viable cell counts in freeze dried powder of carrot pomace fermented by mixed culture

	Consistency index ($Pa \cdot s^n$)	Mucilage content (%)	Fibrinolytic activity (unit/g)	Viable cell counts	
				B. subtilis ($\times 10^7$ CFU/g)	*Leuc. mesenteroides* ($\times 10^8$ CFU/g)
CP	0.05±0.00	ND[1)]	ND	ND	ND
F-CP[2)]	2.88±0.03	24±0.01	104.9	8.0	4.0

[1)]ND: not detected.
[2)]F-CP: carrot pomace fermented by the mixed culture using *B. subtilis* HA and *Leuc. mesenteroides* with 3% MSG.

about five times higher than that of the initial fermented CP, and about three times higher than that of the freeze dried powders of fermented soybeans (37). Currently, the activity of fibrinolytic enzymes in soybean grit fermented by *B. subtilis* HA is reported to be 30 unit/g (38). These results show that the activity of fibrinolytic enzymes are considerably different according to the raw materials for *B. subtilis* fermentation, and that carrot pomace is more suitable for the production of fibrinolytic enzymes than soybeans. In addition, the viable cell counts of *B. subtilis* and *Leuc. mesenteroides* in the freeze dried powders of fermented CP were 8×10^7 CFU/g and 4×10^8 CFU/g, respectively, so that the fermented CP is considered to be useful as a probiotic source.

In conclusion, the fermented CP, with plenty of dietary fibers, pigments and bioactive compounds, could produce functional food and even possibly cosmetic products through mixed fermentation using *B. subtilis* and lactic acid bacteria. In particular, these new products have high mucilage content as dietary fibers, very high activity of fibrinolytic enzymes, and probiotics including lactic acid bacteria and *B. subtilis*.

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