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ARTICLE

Preparation and functional properties of probiotic and oat-based synbiotic yogurts fermented with lactic acid bacteria

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Abstract The main purpose of the current study was to assess the physicochemical properties of the synbiotic yogurt fermented with oat slurry and probiotic strains and the antioxidative and antibacterial activities of the oat-based synbiotic yogurt. The viable cells of *Lactobacillus brevis* SBP49 and *Lactobacillus acidophilus* SBP55 reached 10^8 CFU/g or more in the probiotic and oat-based synbiotic yogurt, and the resistance to artificial digestive juices and the adherence to intestinal epithelial cells of these lactic acid bacteria were also very high in these yogurts. In addition, oat flour added for the manufacture of the synbiotic yogurt significantly promoted the production of antimicrobial substances by these probiotics, thereby increasing the antibacterial effect of the strains against pathogenic food poisoning bacteria including *Bacillus cereus* American Type Culture Collection (ATCC) 11778, *Escherichia coli* O157 ATCC 43889, *Listeria monocytogenes* Korean Collection for Type Cultures (KCTC) 3569, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* KCTC 2514, and *Staphylococcus aureus* ATCC 6538. Meanwhile, the antioxidative activity of the oat-based synbiotic yogurt was significantly higher than that of the probiotic yogurt and its activity may be due to free radical scavenging ability of phenolic compounds contained in oat slurry.

Keywords Antibacterial activity · Antioxidative activity · Oat slurry · Probiotic yogurt · Synbiotic yogurt

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Introduction

The normal flora that inhabit the human gastrointestinal tract are exceedingly complex and consist of more than 800 different bacterial, which have an enormous influence on the host immune response with important implications for human health [1]. Under normal circumstances, major functions of the indigenous microbiota living in the human digestive system also include the activation of energy metabolic switches and the protection of the host from exogenous pathogens infection [2].

However, the disturbances in the ecological balance of human microflora are associated with the immune-mediated disorders caused by potentially pathogenic bacteria in the gut or the use of antibiotics or other medication [3]. In addition, the intestinal pathogens might secrete harmful toxins that block the epithelial cell function and host's metabolic response and cause the pathological disorders, including multisystem organ failure, colon cancer, and irritable bowel syndrome [4]. Previous studies indicated that the overgrowth of pathogenic bacterial populations and the significant decline of health-promoting bacteria play an important role in innate intestinal inflammation and pathogenesis of gastrointestinal disease [5]. Meanwhile, reactive oxygen species (ROS), which is mainly generated during the oxidative metabolism, can induce various gastrointestinal diseases such as peptic ulcers, cardiovascular disease, and cancers [6].

Fortunately, numerous in vivo and in vitro studies have shown that probiotics, live microbial food supplements with health-promoting attributes have a beneficial effect in the prevention and treatment of various intestinal disorders [7]. The antimicrobial substances produced by the probiotic bacteria may reduce not only the number of viable pathogenic cells but may also affect bacterial metabolism or

toxin production [7]. The major probiotic mechanisms of action probiotic also include the promotion of intestinal homeostasis, the stabilization or the maintenance of gastrointestinal barrier function, and the repression of ROS-induced oxidative stress and procarcinogenic enzymatic activities [8].

Meanwhile, there is currently a great deal of interest in the use of prebiotic as functional substances that encourage the growth and useful activity of healthy bacteria in the colon, reduce the risk of inflammatory bowel disease-associated gut dysbiosis, and promote intestinal barrier integrity and metabolism [9]. Prebiotics are defined as non-digestible food ingredients [e.g., transgalactosylated and fructooligosaccharides (FOS)] that benefit the host by selectively stimulating the growth and/or activity of some intestinal flora in the colon [10]. Prebiotics that are present in significant amounts in several edible fruits, vegetables, and cereals are able to alter the colonic microflora to a healthy composition by inducing beneficial luminal or systemic effects within the host [11]. Among the various prebiotic foods, whole oats are a potential source for β-glucan, a prebiotic polysaccharide resulting in positive effects on human gut health including the blood cholesterol-lowering ability and antioxidative and anticancer activities [12]. Meanwhile, synbiotics that can be simply defined as health-enhancing foods or nutritional supplements combining probiotics and prebiotics in a form of synergism have recently been proposed as a novel preventive and therapeutic agent for a variety of gastrointestinal tract disorders [10]. Synergistic synbiotics containing prebiotics that can stimulate specifically the growth of probiotic provide more additive benefits in gastrointestinal function [13]. Therefore, the main purpose of the current study was to assess the physicochemical properties of the synbiotic yogurt fermented with oat slurry and probiotic strains and the antioxidative and antibacterial activities of the oat-based synbiotic yogurt.

Materials and methods

Bacterial strains and growth conditions

Lactobacillus brevis SBP49 and *Lactobacillus acidophilus* SBP55 confirmed as putative probiotic candidates in the previous study [14] were selected as starter for yogurt manufacture in this research. The harmful intestinal bacteria including *Bacillus cereus* American Type Culture Collection (ATCC) 11778, *Escherichia coli* O157 ATCC 43889, *Listeria monocytogenes* Korean Collection for Type Cultures (KCTC) 3569, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* KCTC 2514, and *Staphylococcus aureus* ATCC 6538 were obtained from ATCC and KCTC. These selected pathogens were inoculated into brain heart infusion broth (Difco, Detroit, MI, USA) and cultured under aerobic conditions for 24 h at 37 °C. Stock cultures were maintained at − 20 °C in culture medium containing 20% (v/v) glycerol until further use. To obtain fresh cultures from the freezed stocks, these strains were thawed at room temperature and propagated twice in cultural medium at the optimal temperature (37 °C) before the experiments.

Preparation of probiotic and oat-based synbiotic yogurts

For the production of probiotic yogurt, fresh cow milk with skimmed milk powder fortification (5%, w/v) was homogenized and pasteurized by heating to 85 °C for 20 min. Then it was cooled to 45 °C and inoculated with lactic acid bacteria (LAB, 1.0×10^6 CFU/mL) recovered from culture broth after incubation for 16 h at 37 °C. Oat-based synbiotic yogurt was prepared according to Mahrous et al. [15] with some modifications. Steel-cut oats were purchased at a local grocery store in Korea, washed with tap water and then dried at 50 °C in a dry oven. Fine oat flour containing 7.3 ± 0.2% β-glucan) was obtained by milling the oat grains in a hammer mill (Falling Number-3100 Laboratory Mill, Perten Instruments, Huddinge, Sweden) and fitting with a sieve of 800 μm aperture size. The milled oat flour (20 g) was used as a substrate and blended with distilled water (80 mL) to make the slurry. For manufacturing of oat-based yogurt, the oat slurry at different concentrations (2.5, 5, and 10%, w/v) was added to 10% skim milk obtained from a local food market and pasteurized at 85 °C for 20 min under stirring conditions (100 rpm). Each of the cultures of LAB starter was inoculated into MRS broth and incubated at 37 °C until the early stationary phase of growth. The cells were harvested by centrifugation (7000×*g*, 10 min, 4 °C) and washed twice with PBS (pH 7.0). The milk was cooled to 40–42 °C, and the samples were inoculated with LAB cell pellets (1.0×10^6 CFU/mL) obtained from culture medium. The inoculated milk samples were mixed thoroughly and incubated for 16 h at 37 °C. Yogurt samples were then stored in refrigerator for 10 days.

Stability of probiotic potential in probiotic and oat-based synbiotic yogurts

Fermented yogurt samples were tenfold serially diluted in PBS (pH 7.0) and plated for colony forming units (CFU) determination. LAB colonies were enumerated on MRS agar after aerobic incubation at 37 °C for 48 h. Bacterial numbers were expressed as CFU per gram of sample. The tolerance to simulated gastric and intestinal juices of the

LAB grown in probiotic and synbiotic yogurts was assayed as described by Maragkoudakis et al. [16]. Simulated gastric juice was prepared fresh daily by suspending NaCl (125 mM), KCl (7 mM), $NaHCO_3$ (45 mM), and pepsin (1:10,000, Sigma, St Louis, MS, USA) in PBS, and adjusting the pH to 2.5 with concentrated HCl. Simulated small intestinal juice was prepared by dissolving NaCl (1.25 M), $NaHCO_3$ (82 mM), $NaHPO_4$ (44 mM), KCl (48 mM), $CaCl_2{\cdot}2H_2O$ (20 mM), $MgCl_2{\cdot}6H_2O$ (5 mM), bile salts (17.5 g/L), and pancreatin (5 g/L) in PBS buffer adjusted to pH 8.0 using 1 N HCl or NaOH. The yogurt samples (1 g) were transferred into a test tube containing simulated gastric juice (9 mL) and then incubated at 37 °C for 2 h. The culture (1 mL) was tenfold serially diluted with PBS (pH 7.0), and the residual viable population was calculated by plate counting on MRS agar after incubation at 37 °C for 48 h under aerobic conditions. After incubation in vitro with simulated gastric juice, the yogurt samples were exposed into an artificial intestinal juice for 3 h. Cell viability was determined by counting the viable cells after serially dilution.

For in vitro adhesion assay, human adenocarcinoma cell line HT-29 cells were used for the assay to determine adherence of the tested LAB in MRS broth and yogurt samples. HT-29 cells were cultured in a culture medium containing Dulbecco's modified Eagle's minimal essential medium (DMEM; HyClone Laboratories Inc., Logan, UT, USA) supplemented with 10% (v/v) heat-inactivated (30 min, 56 °C) fetal bovine serum (FBS, GIBCO, Invitrogen Ltd., Carlsbad, CA, USA), glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 units/mL), and streptomycin (50 mg/mL). HT-29 cells were seeded at a density of 10^5 cells/well 24-well tissue culture plates (Thermo Fisher Scientific, Denmark) containing growth medium. The cultures were maintained in humidified atmosphere with 5% of CO_2 and 95% of air at 37 °C. The cell culture media was changed every other day until confluent monolayer was reached. Prior to the adhesion assay, the monolayers were washed three times with ice-cold PBS (pH 7.0) and the cell suspension was transferred to a six-well tissue culture plates (1×10^4 cells/well) containing antibiotic-free DMEM. The aliquot (0.5 mL) of each yogurt sample was added to each well containing HT-29 cells and medium and then incubated at 37 °C in 5% CO_2 95% air atmosphere for 2 h in the humidified incubator. After incubation for 2 h, non-adherent bacterial cells were eliminated by washing twice with PBS (pH 7.0) and the adherent cells were lysed by incubation at room temperature for 15 min in the presence of 0.25% trypsin–EDTA (Gibco, Invitrogen, USA). The suspension from each well was then serially diluted and plated onto MRS agar to determine adhesion ability. The adhesion ability (%) of the LAB to HT-29 cells was determined by counting the viable bacterial counts before the adhesion assay and viable bacterial counts adhered to the epithelial cell layers.

Physicochemical properties of oat-based synbiotic yogurt

The pH values of yogurts were measured using pH meter (Metrohm 744, the Netherlands) after completion of the calibration. The titratable acidity values of each yogurt samples were determined by dissolving 10 g of the sample in 100 mL of distilled water and titrating with 0.1 N NaOH using a 1% phenolphthalein indicator to produce a faint pink color. Meanwhile, the β-glucan contents of the samples were determined with the enzymatic (K-BGLU) kit (Megazyme International Ireland Ltd., Co. Ireland) following the manufacturer's instructions. Briefly, mixed-linkage β-glucans, [(1–3), (1–4)-β-D-glucans], were subjected to selective degradation of the (1–3) linkage using lichenase [a specific endo-(1–3),(1–4)-β-D-glucan 4-glucanohydroase] β-glucosidase, and glucose oxidase [17]. The extent syneresis of fermented yogurts was determined by the method given by Hassan et al. [18], with slight modification. The yogurts (100 g) were transferred into a funnel equipped with a 100-mesh stainless screen. After 5 h of drainage at 4 °C, the volume of whey separated into the graduated cylinder was used as an index of syneresis. The difference in viscosity between probiotic and synbiotic yogurts was measured as described by Ranadheera et al. [19] with small modifications using a viscometer (Brookfield Engineering Laboratories Inc., USA). Apparent viscosity was measured at constant speed (20 rpm) of spindle (No. 4) rotation at 1-min intervals. Results were typically expressed in Centipoise (cP).

Determination of antibacterial substances contents in probiotic and oat-based synbiotic yogurts

To determine the organic acid contents, the yogurt samples were prepared according to the method of De Liano et al. [20] with minor modification. Each sample (5 mL) was mixed with 45 mM H_2SO_4 (20 mL) and homogenized by vortexing for 1 min. The samples were allowed to stand for 10 min in an ice bath and centrifuged at $5500\times g$ for 30 min at 4 °C. The supernatant was injected into a high-performance liquid chromatography system (Hitachi, Tokyo, Japan) after filtration through 0.22-μm filters (Millipore, USA) to remove any small particles. Organic acids (acetic acid and lactic acid) in yogurt samples were analyzed on an Aminex HPX-87N cation-exchange resin column (300 × 7.8 mm) equipped a cation H^+ Micro-Guard cartridge (Bio-Rad Laboratories, Hercules, USA). The column was kept at 65 °C and the elution was carried out with 13 mM H_2SO_4 mobile phase at a flow rate of

0.8 mL/min. The organic acid identification was performed by UV–Vis detection at 220 nm.

The enzymatic method mentioned by Gilliland [21] was used to determine the amount of hydrogen peroxide in probiotic and oat-based synbiotic yogurts. Owing to the high viscosity of yogurt, the content of hydrogen peroxide was measured after dilution and filtration of sample. Yogurt (10 g) was adjusted to pH 4.5 with 0.1 N HCl, and 20 mL of 0.1 M acetate buffer (20 mL) was added. The final volume was brought up with sterile water to 20 mL, and the filtrate (5 mL) was collected using a Whatman filter (Fisher Scientific, USA), and then transferred to a sterile glass tube containing 1% o-dianisidine (100 µL) and horseradish peroxidase (0.01 mg/mL, 1 mL) (Fisher Scientific). Sample blank was prepared by replacing horseradish peroxidase with distilled water. After 10 min of incubation at room temperature, the reaction was terminated by the addition of 4 N HCl (200 µL) and the hydrogen peroxide content (µg/mL) was calculated from the standard curve by measuring the absorbance at 400 nm.

In vitro growth control of selected pathogens by probiotic and oat-based synbiotic yogurts

After incubation under the optimal conditions mentioned above, the cultures of the harmful intestinal bacteria were harvested by centrifuging at 7000×*g* for 10 min. The cell pellets were washed twice with sterile PBS (pH 7.0) and gently resuspended in the same buffer to obtain the final density of approximately 1.0×10^6 CFU/mL. The cell suspension (10%, v/w) of each pathogen was added to the probiotic and synbiotic yogurts (10 g). The samples were taken at 5 and 10 days in order to evaluate the concentration of each bacterium during storage at 20 °C in probiotic and oat-based synbiotic yogurts. The viable cell numbers of the pathogen in yogurt samples were quantified by pour plate technique onto mannitol–egg yolk–polymyxin agar (for *B. cereus* ATCC 11778), sorbitol MacConkey agar (for *E. coli* O157 ATCC 43889), Oxford agar (for *L. monocytogenes* KCTC 3569), xylose lysine deoxycholate agar (for *S. enteritidis* ATCC 13076 and *S. typhimurium* KCTC 2514), mannitol salt with egg yolk agar (for *S. aureus* ATCC 6538). All the dishes were incubated at 37 °C for 48 h, and the colonies grown on the plate were counted and the reduction (%) in the initial number of the pathogenic bacteria in the yogurt samples was calculated.

Total phenolic content (TPC) in probiotic and oat-based synbiotic yogurts

The pH of the samples was adjusted to 4.6 using 1 M HCl to remove the non-hydrolyzed casein in the probiotic and oat-based synbiotic yogurts. The suspension was centrifuged (10,000×*g* for 20 min at 5 °C), and the supernatant was filtered with a 0.45-µm sterile filter. TPC assay was performed according to Shetty et al. [22], with minor modification. In brief, the yogurt extract (1 mL) was dispensed into a test tube fitted with a Teflon-lined screw cap, followed by the addition of 95% ethanol (9 mL) and distilled water (9 mL). After addition of 1 N Folin–Ciocalteu reagent (1 mL) to each tube, the solution was vigorously mixed using a vortex and then left at room temperature for 3 min. 1 N Na_2CO_3 (300 µL) was added to the reaction mixture and allowed to stand at room temperature for 90 min. The absorbance was measured at 765 nm using UV/Vis spectrophotometer (UV-1601, Shimadzu Co., Kyoto, Japan). Gallic acid (5–60 µg/mL) dissolved in ethanol was used as a standard phenolic compound for the quantitative determination. TPC was calculated from the standard curve of gallic acid (5–60 µg/mL) used as standard phenolic compound for the quantitative curve and expressed as µg of gallic acid equivalent (GAE) per 1 mL of yogurt extract (µg GAE/mL).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging abilities of probiotic and oat-based synbiotic yogurts were measured according to the method of Shimada et al. [23], with some modifications. In brief, 50 µM DPPH radical solution (Sigma-Aldrich, St. Louis, MO, USA) prepared in 95% ethanol was added to an equal volume of the yogurt samples. The reaction mixtures were shaken vigorously and kept in the dark at room temperature for 30 min. After centrifugation (8000×*g*, 10 min), the DPPH radical scavenging activity was measured at 517 nm with a microplate reader (Bio-Teck Inc., Winooski, VT, USA). Scavenging effect of DPPH radicals was calculated according to the following equation:

$$\text{Inhibition } (\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)\right]/\left(A_{\text{control}}\right) \times 100,$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Ascorbic acid and butylated hydroxylanisol (BHA) at a concentration of 0.1 mg/mL were used as positive controls.

2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

The ABTS radical scavenging activities of probiotic and oat-based synbiotic yogurts were done using the method of Re et al. [24] with slight modifications. ABTS radical cations ($ABTS^+$) were produced by reacting ABTS (7 mM) in distilled water with potassium persulfate (2.45 mM). The mixture was shaken and left to stand at

room temperature in the dark for 12 h before use, and the resulting solution was diluted with 94% ethanol to obtain the appropriate absorbance (0.17 ± 0.03), which was measured at 734 nm. The yogurt sample (50 μL) was mixed with the $ABTS^{+}$ solution (950 μL), and then, the mixture was kept in the dark at room temperature for 10 min. The absorbance at 734 nm was recorded with a microplate reader, and the inhibition (%) was calculated using the following equation:

$$\text{Inhibition } (\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \times 100,$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Statistical analysis

All experiments were carried out in triplicate, and results for each analysis were expressed as mean ± standard deviations. All statistical analysis was performed using the SPSS for Windows (version 12.0, Chicago, IL, USA). The difference between the treatment and the control groups was analyzed using Student's t test. The p values < 0.05 were regarded as statistical significance.

Results and discussion

Stability of probiotic potential in probiotic and oat-based synbiotic yogurts

The effect of oat slurry on probiotic potential of *L. brevis* SBP49 and *L. acidophilus* SBP55 in probiotic and synbiotic yogurts is shown in Table 1. The viability of *L. brevis* SBP49 ($9.5 \pm 2.1 \times 10^8$ CFU/g) in probiotic yogurt was slightly higher than that of *L. acidophilus* SBP55 ($2.2 \pm 0.6 \times 10^9$ CFU/g). When oat slurry (2.5%) was added to probiotic yogurt fermented with *L. brevis* SBP49, the viable cell counts of probiotic strain were significantly increased compared to the control. When 5% or more of oat slurry was added, the number of viable cells of *L. acidophilus* SBP55 in probiotic yogurt was significantly increased. The viable counts of these LAB in probiotic and synbiotic yogurts were decreased by about 1–1.5 log cycles after incubation for 2 h in artificial gastric juice. On the other hand, the number of remaining cells after incubation in artificial gastric juice was maintained throughout incubation for 3 h in the artificial bile solution. The number of bacterial cells adhered to the intestinal epithelial cells after incubation in the artificial bile solution achieved higher than 10^6 CFU/g. As a result of this study, *L. brevis* SBP49 and *L. acidophilus* SBP55 strains used for the production of probiotic and oat-based synbiotic yogurts showed strong resistance to artificial digestive juices and high adherence to intestinal epithelial cells. Meanwhile, the resistance to artificial digestive juices and the adhesion to intestinal epithelial cells of these LAB strains were not significantly different between probiotic and oat-based synbiotic yogurts, so oat slurry did not have a direct effect on the probiotic activity. Since the oat slurry promoted the growth of these LAB, the residual bacterial counts after incubation in artificial digestive juice and the number of bacterial cells attached to intestinal epithelial cells were higher in synbiotic yogurt than in probiotic yogurt.

The number of probiotic LAB in fermented milk products such as yogurt should be maintained at a high viable count until immediately before consumption. In yogurt, the probiotic count should be at least 6–7 log cycles, and yogurt should be consumed at least 100 g/day to ensure the effectiveness of the probiotic [25]. Thus, the number of *L. brevis* SBP49 and *L. acidophilus* SBP55 in probiotic and synbiotic yogurts meets the quality standard of the fermented milk.

Meanwhile, the addition of prebiotic substances has also been shown to improve the sensory characteristics of fermented dairy products and the growth and activity of probiotic microorganisms such as lactobacilli and bifidobacteria in the colon [26]. Some of the sources of prebiotics include breast milk, soybeans, inulin sources (such as Jerusalem artichoke and chicory roots), raw oats, unrefined wheat, unrefined barley, yacon, and non-digestible oligosaccharides such as FOS, galacto-oligosaccharides, transgalactosylated oligosaccharide, isomalto-oligosaccharides, xylooligosaccharides (XOS), soy oligosaccharide, and lactosucrose [27]. Oat, mostly as flakes, is a good substrate for the growth of probiotic strains and a useful material that benefits health because it contains a large amount of β-glucan, a physiologically active substance [28].

β-Glucan has been reported as a prebiotic substance due to its ability to selectively stimulate the proliferation and the activity of probiotic strains and some beneficial residential colon microorganisms such as bifidobacteria after reaching the intestinal tract without digestion [29]. According to many researchers, oats have been reported to be useful as fermentation substrates for growth of LAB in the production of fermented milk products. Meanwhile, lactobacilli are able to produce hetero- or homo-exopolysaccharides (EPS), which play an important role in the physical properties, texture, and mouthfeel of fermented foods. EPS of LAB have been reported to have anticancer, immune function enhancement, and blood cholesterol reduction effect [30]. Among the LAB-producing EPS, *Pediococcus parvulus* 2.6 produced β-glucan belonging to linear and branched polysaccharides. β-Glucan produced by *Pediococcus* and *Lactobacillus* strains has

Table 1 Effect of oat slurry on the resistance to artificial digestive juices and the adhesion to intestinal epithelial cells of *L. brevis* SBP49 and *L. acidophilus* SBP55 used as probiotic yogurt starter

LAB	Content of oat slurry (%)	Viable cell counts (CFU/g)			
		Control	Simulated gastric juice	Simulated intestinal juice	Adhesion to HT-29 cells
L. brevis SBP49	0	$9.5 \pm 2.1 \times 10^8$	$1.2 \pm 2.5 \times 10^7$	$3.6 \pm 0.6 \times 10^7$	$2.7 \pm 2.1 \times 10^6$
	2.5	$3.4 \pm 0.8 \times 10^{9}$*	$2.0 \pm 0.9 \times 10^{8}$*	$4.1 \pm 1.1 \times 10^{8}$*	$3.0 \pm 1.4 \times 10^6$
	5	$5.0 \pm 1.2 \times 10^{9}$*	$1.7 \pm 1.6 \times 10^{8}$*	$3.3 \pm 2.9 \times 10^{8}$*	$4.3 \pm 0.9 \times 10^{7}$*
	10	$8.7 \pm 3.1 \times 10^{9}$*	$4.0 \pm 2.5 \times 10^{8}$*	$5.6 \pm 0.8 \times 10^{8}$*	$7.4 \pm 4.2 \times 10^{7}$*
L. acidophilus SBP55	0	$2.2 \pm 0.6 \times 10^9$	$1.1 \pm 2.0 \times 10^8$	$8.5 \pm 1.1 \times 10^7$	$3.2 \pm 0.7 \times 10^6$
	2.5	$3.1 \pm 1.3 \times 10^9$	$9.9 \pm 0.5 \times 10^7$	$1.0 \pm 1.3 \times 10^8$	$2.8 \pm 0.9 \times 10^{7}$*
	5	$7.9 \pm 1.2 \times 10^{9}$*	$5.4 \pm 3.0 \times 10^{8}$*	$6.6 \pm 3.1 \times 10^{8}$*	$5.8 \pm 2.0 \times 10^{7}$*
	10	$8.6 \pm 3.4 \times 10^{9}$*	$4.3 \pm 0.9 \times 10^{8}$*	$7.2 \pm 2.5 \times 10^{8}$*	$4.0 \pm 1.6 \times 10^{7}$*

Results represent means of three independent experiments ± standard deviation

* Significantly different from the control without addition of oat slurry ($p < 0.05$)

been reported to increase their Caco-2 human enterocytes, macrophage immunomodulatory capacity, or survival rate during gastrointestinal passage or technological process [31]. Synbiotics, a mixture of probiotics and prebiotics, enhance the physiological activity of probiotic LAB by prebiotic components such as β-glucan [32]. Similarly, the growth rate of *L. brevis* SBP49 and *L. acidophilus* SBP55 used in yogurt production may also be promoted by β-glucan contained in oat slurry.

The synbiotic yogurt produced with *L. brevis* SBP49 and *L. acidophilus* SBP55 was similar to the number of LAB reported in other studies. The bacterial counts in the synbiotic fermented foods prepared with 18% oat flour and *Lactobacillus plantarum* UFG9 were 2×10^9 CFU/g [33], which were similar to the number of LAB in the synbiotic yogurt of this study. The bacterial counts of *L. plantarum* TK9 in the synbiotic food prepared with the addition of whole oat flour were 2.85×10^9 CFU/g, which was similar to that of *L. acidophilus* SBP55. The viable cell counts of *Bifidobacterium animalis* subsp. *lactis* V9 in the synbiotic food were 3.17×10^8 CFU/g, which was lower than that of *L. brevis* SBP49 [34]. In contrast to our results, the number of LAB in the yogurt prepared with the addition of barley powder was lower than that of the sample without added oat flour because it was known that the LAB were difficult to use starch or protein contained in barley [35].

Probiotic strains are introduced into the intestines through the mouth of the host and should maintain their viability even after exposure to the extreme environments [36]. *L. acidophilus* has a high cytoplasmic buffering capacity and is more resistant than *Bifidobacterium* spp. because of its resistance to changes in cytoplasmic pH under acidic conditions [37]. Some LAB can neutralize pH stress under acidic conditions by activation of proton pumps by ATPase, deamination or amidation to produce ammonium, decarboxylation to induce biogenetic amine production, conversion from glutamine to glutamate, malolactic fermentation, or pyruvate metabolite production from C4 compounds [38]. Since milk has a buffering capacity for the strong acid of gastric juice, the LAB contained in the yogurt show a high survival rate in the intestines [39]. To demonstrate the functionality of probiotic strains, they must not only pass through the digestive tract in a live state but also remain above a certain level during manufacture or storage of the product [40]. Unlike the results of this study, Lim [41] reported that the survival rate of probiotic yogurt starter *L. acidophilus* GK20 (35.56 ± 3.03%) and *L. paracasei* GK74 (35.60 ± 2.96%) in gastric juice was higher than that of *L. brevis* SBP49 and *L. acidophilus* SBP55; however, the prebiotic FOS did not significantly affect the proliferation and probiotic activity of these LAB.

The resistance to bile secreted from the gallbladder in the duodenum is one of the essential requirements of the probiotic strain. Bile acid is an amphipathic molecule with strong antimicrobial activity and acts as a surfactant that destroys biological membranes. It has the ability to affect protein and phospholipids in cell membranes and destroys the homeostasis of cells [36]. The tolerance of LAB to bile acids is caused by the secretion of bile salt hydrolase, and the enzyme has the effect of lowering the serum cholesterol level [42]. Similar to the results of this study, Buriti et al. [43] demonstrated that the tolerance of *L. acidophilus* La-5 to artificial digestive juices was improved by the addition of prebiotic inulin.

The adherence of LAB to epithelial cells is mediated by electrochemical and hydrophobic interactions of cells, steric or passive attraction, and external structures of

bacterial cells such as lipoteichoic acid and exopolysaccharide [44]. LAB strains attached to the epithelial cells effectively induce immune responses and stabilize intestinal mucosa [45]. The adherence of LAB depends on the number of bacterial cells, the components of the buffer solution, the incubation time, the culture medium, the type of intestinal microflora, and the composition of the ingested food [46]. The results of this study are similar to those of Lim [41] reported that FOS addition did not directly affect the adherence of *L. acidophilus* GK20 and *L. paracasei* GK74, although the number of bacteria attached to the intestinal epithelium was high due to the high number of lactic acid bacteria in the mixed culture.

Physicochemical properties of oat-based synbiotic yogurt

Table 2 shows the results of physicochemical properties of probiotic and oat-based synbiotic yogurts. The pH of the probiotic yogurt prepared with *L. acidophilus* SBP55 was 3.90 ± 0.07, which was somewhat lower than that of the samples made with *L. brevis* SBP49 (4.11 ± 0.05). The titratable acidity (1.42 ± 0.03%), β-glucan content (0.67 ± 0.02 g/100 g), syneresis (12.9 ± 0.6%, v/w), and viscosity (1369.5 ± 2.1 cps) of probiotic yogurt prepared with *L. acidophilus* SBP 55 were somewhat higher than those of *L. brevis* SBP49. Meanwhile, the physicochemical properties of the synbiotic yogurt prepared by adding 2.5% or more of oat slurry were significantly differences from those of probiotic yogurt fermented with *L. brevis* SBP49 and *L. acidophilus* SBP55. Since the oat slurry promoted the growth of the LAB, the acidity of the synbiotic yogurt was higher than that of the probiotic yogurt. The organic acid produced from the LAB and β-glucan contained in the oat slurry was found to significantly increase the viscosity of the synbiotic yogurt.

Lee et al. [35] reported that the amount of lactic acid present at pH 3.27–4.53 is ideal for yogurt. The pH of probiotic and oat-based synbiotic yogurts prepared with *L. brevis* SBP49 and *L. acidophilus* SBP55 was within this range. The results of this study were similar to the pH of yogurt added with cereal such as rice, barley, wheat [47]. Grain added into yogurt promotes the organic acid production of LAB, and phosphates and proteins contained in skim milk powder are known to have a pH buffering effect [48]. The pH of oat-based fermented beverages after fermentation with *L. plantarum* UFG9 for 16 h was 3.9, and the amount of lactic acid produced by decomposing glucose and sucrose contained in oats reached about 11 mg/100 g [33].

Yogurt is one of the most popular dairy products that can be obtained through the acidic fermentation of milk with a specific LAB such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* [49]. During the making of yogurt, the lactose in the milk is degraded by the lactase enzyme of LAB and converted to end products, lactic acid and acetaldehyde [50]. Lactic acid lowers the pH of the product and allows some milk proteins to coagulate, thereby making it possible to produce yogurt. When the pH is below 5, the tertiary structure of the casein, a hydrophobic protein, is broken down due to the protonation of its amino acid residues. The denatured protein is reassembled by interaction with other hydrophobic molecules, and the intermolecular interaction of caseins contributes to the formation of the semisolid texture of yogurt [49]. Lactic acid fermentation using LAB is an effective milk preservation method, which improves the texture, flavor, and nutritional value of fermented milk products [51]. In order to improve the quality of yogurt, the acidity of 1.0–1.1% is known to be the most suitable [35], but the acidity of the probiotic and oat-based synbiotic yogurt was somewhat higher than the recommended level. Therefore,

Table 2 Effect of oat slurry on physicochemical characteristics of probiotic yogurt fermented with *L. brevis* SBP49 and *L. acidophilus* SBP55

LAB	Content of oat slurry (%)	pH	Titratable acidity (%)	β-Glucan (g/100 g)	Syneresis (%, v/w)	Viscosity (cps)
L. brevis SBP49	0	4.11 ± 0.05	1.32 ± 0.08	0.42 ± 0.09	10.2 ± 0.1	1235.4 ± 3.0
	2.5	3.95 ± 0.03*	1.48 ± 0.06*	0.56 ± 0.03*	12.0 ± 0.5*	1316.8 ± 2.6*
	5	3.88 ± 0.06*	1.53 ± 0.04*	0.66 ± 0.08*	13.1 ± 0.4*	1400.3 ± 4.7*
	10	3.71 ± 0.05*	1.67 ± 0.02*	0.74 ± 0.09*	14.7 ± 0.6*	1497.5 ± 1.9*
L. acidophilus SBP55	0	3.90 ± 0.07	1.42 ± 0.03	0.67 ± 0.02	12.9 ± 0.6	1369.5 ± 2.1
	2.5	4.00 ± 0.05	1.45 ± 0.02	0.78 ± 0.03*	12.8 ± 0.7	1372.4 ± 3.3
	5	3.61 ± 0.02*	1.73 ± 0.05*	0.84 ± 0.05*	15.5 ± 0.9*	1564.2 ± 4.0*
	10	3.58 ± 0.04*	1.82 ± 0.06*	0.97 ± 0.02*	16.3 ± 0.4*	1633.8 ± 3.6*

Results represent means of three independent experiments ± standard deviation

* Significantly different from the control without addition of oat slurry ($p < 0.05$)

it is necessary to shorten the fermentation time and to use additives that can neutralize the acid in the yogurt. Kim and Ko [47] demonstrated that the acidity of yogurt added with grains is higher than that of the control, because the grains contain some substances required for the lactic acid biosynthesis of LAB. However, our results were somewhat different from the results of Lee et al. [35] who showed that the acidity of yogurt added with 1–3% barley flour was significantly higher than that of the additive-free plain yogurt, but the titratable acidity of the yogurt was rather low when 5–7% of barley flour was added.

Zhang et al. [34] demonstrated that the contents of the soluble dietary fiber and β-glucan were not significantly different between probiotic and oat flour-based synbiotic yogurt fermented with LAB (*L. plantarum* TK9 and *B. animalis* subsp. *lactis* V9); however, the amount of free amino acid in the fermented oat flour was significantly higher than the non-fermented oat flour. This result suggests that the *L. brevis* SBP49, *L. acidophilus* SBP55, and oat slurry have a good potential for the production of novel synbiotic food products containing probiotic LAB and prebiotic β-glucan.

Cereal β-glucan is known to contribute to increase the viscosity and texture of some foods, and this substance is often artificially added as a functional ingredient to manufacture prepared and processed foods [52]. Meanwhile, EPS produced by LAB have been reported to contribute to increasing the viscosity and texture of processed foods as biopolymers [53]. In addition, the several studies have shown that oat β-glucan and EPS produced by LAB can also help improve human health as prebiotic molecules [54]. Nikoofar et al. [55] reported that β-glucan significantly increased the acidity of yogurt by promoting the growth of LAB during the fermentation process. The acidity and viscosity of the synbiotic yogurt prepared from the mixture of probiotic *S. thermophilus* and *L. delbrueckii* spp. *bulgaricus* and prebiotic β-glucan were increased proportionally with inoculation amount of the fermentation starter, β-glucan content, and fermentation time.

The viability of LAB in synbiotic yogurt is higher than that of probiotic yogurt because β-glucan added as a prebiotic ingredient was used as a nutrient for the growth of LAB [56]. The synerese of non-fat set yogurt containing β-glucan was increased by the interaction of polysaccharide with milk protein. In addition, the texture of yogurt containing β-glucan was firmer and stickier than the control (the samples without β-glucan). In contrast to our results, Tudorica et al. [57] demonstrated that the synerese of yogurt containing grain β-glucan was rather reduced compared with the control, which is attributed to the thermodynamic incompatibility between β-glucan and casein as non-interacting polysaccharides. Similar to our results, the viscosity of synbiotic yogurt added with 1% barley flour was not significantly different from that of the control, whereas the viscosity was significantly increased with the addition of barley flour at a higher concentration [35]. Donkor et al. [58] reported that the viscosity of yogurt by *L. casei* L26 was significantly increased by the addition of inulin, similar to the results of this study. Viscosity of yogurt is the crucial factor in yielding desired sensory properties such as mouthfeel and flavor release [33].

Production of antibacterial substances and in vitro growth control of selected pathogens by LAB in probiotic and oat-based synbiotic yogurts

Table 3 and Table 4 show the amount of antimicrobial substances produced from the LAB in probiotic and oat-based synbiotic yogurts and the antibacterial activity of these yogurts against pathogenic food poisoning bacteria. The yields of lactic acid and acetic acid in the probiotic yogurt fermented with hetero-fermentative *L. brevis* SBP49 were found to be 108.5 ± 1.1 mM and 56.8 ± 1.6 mM, respectively. In particular, the content of lactic acid in synbiotic yogurt prepared with 2.5% or more of oat slurry and *L. brevis* SBP49 was significantly increased compared with that of probiotic yogurt. In addition, acetic acid and hydrogen peroxide production of the LAB were significantly increased with addition of oat slurry. Meanwhile, the content of lactic acid in probiotic yogurt prepared with *L. acidophilus* SBP55 was significantly higher than that of *L. brevis* SBP49 and the content of lactic acid in synbiotic yogurt added with 5% or more of oat slurry was significantly increased. Acetic acid was not detected in the probiotic yogurt produced by the homo-fermentative *L. acidophilus* SBP55, but the amount of hydrogen peroxide produced by the strain was significantly increased by the addition of oat slurry.

On the other hand, the number of pathogenic food poisoning bacteria in probiotic yogurt produced with *L. brevis* SBP49 was much lower than that of *L. acidophilus* SBP55. In particular, the probiotic and oat-based synbiotic yogurts fermented with these LAB effectively inhibited the growth the *S. enteritidis* ATCC 13076 and *S. typhimurium* KCTC 2514. The antibacterial effect against pathogenic food poisoning bacteria was significantly higher in the synbiotic yogurt with oat slurry than the probiotic yogurt. As a rule, the antibacterial activity of probiotic and oat-based synbiotic yogurt was more effective against Gram-negative bacteria than Gram-positive bacteria. The number of pathogenic food poisoning bacteria in these yogurt samples during storage for 5 days was not significantly different from the number of the harmful bacteria in the samples stored for 10 days at 20 °C.

The storage stability of fermented milk is due to the antimicrobial substance produced by LAB used for

Table 3 Effect of oat slurry on the production of antibacterial substances by *L. brevis* SBP49 and *L. acidophilus* SBP55 used as probiotic yogurt starter

LAB	Content of oat slurry (%)	Lactic acid (mM)	Acetic acid (mM)	Hydrogen peroxide (μg/mL)
L. brevis SBP49	0	108.5 ± 1.1	56.8 ± 1.6	7.2 ± 0.2
	2.5	120.2 ± 0.9*	59.4 ± 3.7	11.4 ± 0.7*
	5	131.3 ± 3.1*	65.7 ± 0.8*	13.5 ± 0.3*
	10	134.8 ± 2.9*	70.2 ± 2.2*	13.9 ± 0.2*
L. acidophilus SBP55	0	124.4 ± 0.7	ND	12.8 ± 0.9
	2.5	121.5 ± 1.1	ND	12.0 ± 0.4
	5	145.3 ± 2.4*	ND	15.4 ± 0.6*
	10	150.8 ± 1.9*	ND	17.2 ± 0.8*

Results represent means of three independent experiments ± standard deviation

* Significantly different from the control without addition of oat slurry ($p < 0.05$)

fermentation. Of the antimicrobial substances, organic acids such as lactic acid, acetic acid, and propionic acid produced as end products provide an acidic environment that inhibit the growth of many pathogenic and spoilage microorganisms [59]. During fermentation of LAB in the medium, the homo-fermentative LAB strains produce only lactic acid, whereas hetero-fermentative LAB strains produce various antimicrobial substances such as lactic acid, acetic acid, alcohol, carbon dioxide, formic acid, acetone, acetaldehyde, and diacetyl. The amount of lactic acid depends on the species of the LAB, the constituent components of the culture medium, and incubation conditions [60]. The antimicrobial substance-producing LAB are used as biological preservatives to improve the shelf life and safety of food. Organic acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, and reducing intracellular pH [61]. When the undissociated molecules of the organic acid diffused through the cell membranes of the bacteria, the essential metabolic function of the cell is destroyed by decreasing the cytoplasmic pH [62]. There is a difference in antibacterial activity depending on the amount and type of organic acid produced during fermentation process [60]. Meanwhile, acetic acid produced by LAB contributes to the flavor of fermented foods, while lactic acid increases the preservability and refreshing sourness of yogurt and promotes the digestion of milk protein, the minerals utilization, and the secretion of gastric juice [63].

The antimicrobial activity of hydrogen peroxide appears to destroy the cellular oxidative action and the molecular structure of cellular proteins. Hydrogen peroxide is produced by various enzymes such as bacterial protein oxidoreductase, NADH peroxidase, NADH oxidase, and glycerophosphate oxidase during the process of oxygen respiration and exhibits strong oxidative action on lipids and proteins, which are the main constituents of the cells [60]. Hydrogen peroxide, organic acids, and secondary metabolites, which are small molecular weight antimicrobial substances, exhibit broad antibacterial activity against harmful bacteria such as *Salmonella* spp., pathogenic *E. coli*, *Clostridium* spp., and *Helicobacter pylori* [64]. Furthermore, the survival rate of *E. coli* and *Bacillus subtilis* was reduced by about 80% by treatment with β-glucan derivatives (2000 μg/mL); thus, the polysaccharides had its own antibacterial effect [12].

These findings were partially consistent with those of Lee et al. [35] who reported that the content of lactic acid and acetic acid in synbiotic yogurt prepared by mixing probiotic strains of *L. acidophilus* KCTC 3140, *L. delbrueckii* subsp. *bulgaricus* KCTC 3635, and *S. thermophilus* KCTC 5092 and barley flour (1–3%) was higher than that of the control without addition of barley flour. When barley flour was added at a concentration of 5–7%, and the content of organic acid in synbiotic yogurt was rather decreased. Meanwhile, the results of this study are similar to those of Paik and Ko [48] who demonstrated that lactic acid content in the yogurt was significantly increased by addition of brown rice. Similarly, Donkor et al. [58] showed that the viable cell counts of the fermentation starter in synbiotic yogurt supplemented with inulin were about 1 log cycle higher than that of the control. Besides, the amount of antimicrobial substances produced by LAB during yogurt fermentation was significantly increased by addition of prebiotics. When *B. bifidum* Bb12 and *L. plantarum* 0407 were cultured in the medium supplemented with oligofructose and XOS, the inhibitory effect of the LAB strains against *E. coli* and *Campylobacter jejuni* was remarkably increased [65]. The antibacterial activities of probiotic *Lactobacillus kefranofaciens*, *Candida kefir*, and *Saccharomyces boluradii* on pathogenic bacteria such as *E. coli*, *S. aureus*, *Salmonella paratyphi* A,

Table 4 Effect of oat slurry on the antibacterial activity of *L. brevis* SBP49 and *L. acidophilus* SBP55 used as probiotic yogurt starter

LAB	Content of oat slurry (%)	Inhibition (%)											
		B. cereus ATCC 11778		*E. coli* O157 ATCC 43889		*L. monocytogenes* KCTC 3569		*S. enteritidis* ATCC 13076		*S. typhimurium* KCTC 2514		*S. aureus* ATCC 6538	
		5	10	5	10	5	10	5	10	5	10	5	10
L. brevis SBP49	0	8.1 ± 0.7	5.6 ± 1.1	12.5 ± 1.6	10.4 ± 0.9	6.7 ± 0.8	5.9 ± 0.4	21.3 ± 2.7	19.4 ± 0.6	26.1 ± 2.1	27.4 ± 1.4	13.9 ± 2.6	12.9 ± 2.5
	2.5	11.5 ± 2.1*	9.2 ± 1.4*	11.4 ± 0.9	10.7 ± 0.1	7.1 ± 1.6	8.2 ± 0.9	27.4 ± 0.9*	28.7 ± 1.3*	28.4 ± 3.0	25.1 ± 2.6	12.7 ± 1.3	10.7 ± 1.6
	5	13.4 ± 1.0*	14.5 ± 2.2*	18.7 ± 2.1*	16.9 ± 1.3*	9.3 ± 2.3	10.1 ± 0.2*	31.5 ± 1.6*	29.7 ± 0.6*	32.4 ± 2.7*	31.8 ± 0.9*	14.2 ± 0.5	15.7 ± 4.0
	10	14.7 ± 0.9*	16.4 ± 3.0*	20.3 ± 3.2*	21.8 ± 3.0*	14.7 ± 1.6*	15.7 ± 0.6*	33.0 ± 2.5*	30.7 ± 2.5*	35.1 ± 3.0*	36.1 ± 1.8*	20.4 ± 0.5*	19.1 ± 2.7*
L. acidophilus SBP55	0	4.8 ± 0.7	3.9 ± 0.6	8.3 ± 0.4	9.7 ± 1.1	4.4 ± 0.3	5.7 ± 1.0	14.2 ± 0.7	19.4 ± 1.5	16.8 ± 2.0	18.1 ± 1.7	14.7 ± 2.2	17.1 ± 1.6
	2.5	4.1 ± 0.3	3.7 ± 0.6	7.8 ± 1.3	8.8 ± 1.6	3.9 ± 0.6	5.9 ± 1.1	15.4 ± 0.6	17.8 ± 0.7	17.2 ± 1.6	19.7 ± 2.4	15.4 ± 1.2	19.4 ± 2.5
	5	8.2 ± 1.6*	9.3 ± 1.1*	13.4 ± 2.4*	15.7 ± 2.4*	5.5 ± 1.9	5.1 ± 0.9	20.9 ± 1.3*	20.0 ± 0.8	22.4 ± 2.4*	26.0 ± 1.3*	16.4 ± 0.8	18.4 ± 2.3
	10	10.5 ± 2.2*	11.4 ± 2.7*	16.8 ± 3.1*	20.6 ± 1.3*	9.7 ± 1.4*	11.9 ± 1.8*	23.7 ± 3.5*	25.4 ± 2.6*	26.7 ± 2.7*	28.4 ± 4.1*	23.1 ± 3.7*	25.4 ± 2.9*

Results represent means of three independent experiments ± standard deviation

* Significantly different from the control without addition of oat slurry ($p < 0.05$)

Shigella dysenteriae, and *Vibrio cholerae* were elevated by addition of barley grain extract [66].

TPC and free radical scavenging ability of probiotic and oat-based synbiotic yogurts

TPC and DPPH and ABTS free radical scavenging activities of probiotic and oat-based synbiotic yogurts are shown in Table 5. TPC and free radical scavenging activity in probiotic yogurt prepared with *L. acidophilus* SBP55 were somewhat higher than those of *L. brevis* SBP49. TPC in synbiotic yogurt prepared with *L. acidophilus* SBP55 and 2.5% oat slurry was 240.43 ± 12.41 μg GAE/mL, which was significantly higher than that of probiotic yogurt. In addition, DPPH and ABTS free radical scavenging abilities were significantly higher in the synbiotic yogurt prepared by addition of oat slurry than the probiotic yogurt prepared only with *L. acidophilus* SBP55. However, the DPPH radical scavenging activity of the synbiotic yogurts supplemented with 10% oat slurry and *L. brevis* SBP49 or *L. acidophilus* SBP 55 was lower than that of ascorbic acid (70.28 ± 1.73%) and BHA (87.11 ± 2.04%). As a result, the antioxidant capacity of synbiotic yogurt was higher than that of probiotic yogurt due to the synergistic effect of probiotic LAB and oat slurry containing antioxidant.

ROS such as peroxides, superoxide, hydroxyl radical, and singlet oxygens are by-products or intermediates produced during normal metabolism. The formation and the elimination balance of ROS are required to maintain normal physiological functions for human health. Excessive ROS accumulated in the body cause damage to important macromolecules such as lipids, proteins, and nucleic acids and therefore lead to serious diseases such as cancer and heart disease [67]. Fortunately, some LAB and dietary antioxidants improve human health by blocking the attack of free radicals. The antioxidant activity of LAB results from enzymatic and non-enzymatic defense systems. In particular, the antioxidant enzymes of LAB play an important role in defense against ROS. Superoxide dismutase removes the toxins of superoxide anions and glutathione peroxidase scavenges hydrogen peroxide and hydroxyl radicals. On the other hand, several LAB exert non-enzymatic defense mechanisms such as reducing power and metal ion chelating ability to prevent excessive oxidative stress [68].

Meanwhile, the antioxidant properties of diverse cereal grains inhibit the formation of the radical cations and prevent the peroxidation of the soybean oil and L-a-phosphatidylcholine liposome oxidation [69]. In particular, the outer layer of oats has been known to stabilize edible oil and fat against rancidity due to the presence of a variety of antioxidants such as vitamin E, phytic acid, phenolic compounds, flavonoids, and sterols [70]. Oat flour can

Table 5 Effect of oat slurry on the antioxidative activity of *L. brevis* SBP49 and *L. acidophilus* SBP55 used as probiotic yogurt starter

LAB	Content of oat slurry (%)	Total phenolic content (μg GAE/mL)	DPPH assay (%)	ABTS assay (%)
L. brevis SBP49	0	69.23 ± 10.12	28.51 ± 0.17	11.31 ± 0.66
	2.5	113.91 ± 7.85*	46.94 ± 0.35*	20.06 ± 0.94*
	5	223.72 ± 13.40*	52.15 ± 0.06*	27.63 ± 0.28*
	10	357.12 ± 7.94*	65.12 ± 0.48*	40.82 ± 1.02*
L. acidophilus SBP55	0	81.85 ± 9.63	39.48 ± 0.55	19.19 ± 2.06
	2.5	240.43 ± 12.41*	57.85 ± 0.13*	34.15 ± 1.45*
	5	319.14 ± 9.13*	60.69 ± 0.94*	43.09 ± 0.77*
	10	449.54 ± 15.04*	76.74 ± 0.61*	54.71 ± 0.34*

Results represent means of three independent experiments ± standard deviation

* Significantly different from the control without addition of oat slurry ($p < 0.05$)

reduce the risk of radicals in biological systems by acting as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules, and activators of antioxidative defense enzyme systems. Oat extracts showed the antioxidant activity against low-density lipoprotein (LDL) and R-phycoerythrin protein oxidation in the oxygen radical absorbance ability test [69].

The results of this study were similar to those of Madhu et al. [71] who noted that the number of the LAB in probiotic yogurt prepared with *L. plantarum* CFR 2194 or *Lactobacillus fermentum* CFR 2192 was significantly increased by addition of prebiotic FOS. Additionally, DPPH radical scavenging activity of synbiotic yogurt (85%) was significantly higher than that of the control (72%), and the highest TPC was also detected in synbiotic yogurt. Therefore, the prebiotic FOS added during yogurt production helps to improve the functionality of the probiotic strain. Similarly to our results, Lee and Kang [72] noted that DPPH radical scavenging ability of the synbiotic yogurt (77.93–87.66%) prepared by adding chitooligosaccharide (COS) was significantly higher than that of probiotic yogurt (60.04%) fermented with *Lactobacillus bulgaricus* LB-12 and *S. thermophilus* St-36. DPPH radical scavenging ability and ferric reducing ability of probiotic lactobacilli were significantly increased when mixed with prebiotic XOS [73].

In conclusion, *L. brevis* SBP49 and *L. acidophilus* SBP55 in the probiotic and oat-based yogurts showed strong resistance to artificial digestive juices and good adhesion ability to intestinal epithelial cells. Although the probiotic yogurt was produced by LAB of different species, there were no significant differences in the physicochemical properties of the probiotic yogurt and the probiotic potentials of *L. brevis* SBP49 and *L. acidophilus* SBP55 present in the sample. The probiotic activities, viscosity, and acidity of probiotic yogurt fermented with *L. brevis* SBP49 were significantly increased when oat slurry was added at 2.5% or more, whereas those of probiotic yogurt made with *L. acidophilus* SBP55 were increased in the yogurt prepared with the addition of 5.0% or more oat slurry. The production ability of antimicrobial substances such as lactic acid and hydrogen peroxide and the antioxidant activity were higher in probiotic yogurt prepared with *L. acidophilus* SBP55 than *L. brevis* SBP49. Although the antibacterial and antioxidant activities of these probiotic strains were significantly increased in the synbiotic yogurt prepared by adding the oat slurry, the amount of oat slurry required for increasing the activities varied depending on the species of LAB. Therefore, the oat-based synbiotic yogurt prepared by the probiotic strain and cereal grain contained prebiotic substances is highly valued as a functional food that can protect human body from pathogenic bacteria and ROS.

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