Korean Journal of Microbiology (2017) Vol. 53, No. 2, pp. 103-117
DOI https://doi.org/10.7845/kjm.2017.7035

pISSN 0440-2413
eISSN 2383-9902

Article

Effect of green tea supplementation on probiotic potential, physico-chemical, and functional properties of yogurt

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요구르트의 프로바이오틱 활성과 물리화학적 및 기능적 특성에 대한 녹차 추출물의 영향

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(Received June 2, 2017; Revised June 21, 2017; Accepted June 26, 2017)

The aim of this study was to evaluate the effect of green tea extract on probiotic potential, physico-chemical and functional properties of yogurt fermented with *Lactobacillus acidophilus* D11 or *Lactobacillus fermentum* D37 strains isolated from *Doenjang*. Probiotic activities such as the resistance to artificial digestive juices and the ability to adhere to epithelial cells were slightly higher in yogurt supplemented with green tea extract than in plain yogurt, which may be attributed to the increase in the number of lactic acid bacteria (LAB) by green tea extract supplementation. Furthermore, the microbiological and physico-chemical properties such as the number of LAB, organic acid production and viscosity were significantly (P<0.05) increased in yogurt added green tea extract compared to plain yogurt fermented with *L. acidophilus* D11. However, the green tea extract did not significantly (P>0.05) affect these properties of yogurt fermented with *L. fermentum* D37 strain. Meanwhile, the antibacterial activities against *Escherichia coli* O157 ATCC 43889, *Salmonella enteritidis* ATCC 13076, and *Salmonella typhimurium* KCTC 2514 and antioxidant activities including total phenol content, radical scavenging ability, and ferric-reducing antioxidant power were significantly higher in plain yogurt fermented with *L. fermentum* D37 than with *L. acidophilus* D11. The antibacterial and antioxidant activities of the yogurt were significantly (P<0.05) increased in proportion to the concentration of green tea extract added to plain yogurt. Consequently, green tea yogurt fermented with *L. acidophilus* D11 or *L. fermentum* D37 was considered to be a useful functional food that can inhibit the growth of pathogenic bacteria and scavenge the free radicals from the body cells.

Keywords: green tea extract, lactic acid bacteria, probiotic

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Bovine milk is an important reservoir for many foodborne pathogens including *Salmonella* spp., *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, *Campylobacter jejuni*, and *Yersinia enterococlitica*. Milk contaminated with these pathogenic organisms is able to cause of serious morbidity and mortality in humans (Lejeune and Rajala-Schultz, 2009). Deterioration of milk is closely associated with inefficient pasteurization temperature, poor packaging material, and improper hygienic management (Tesfay *et al*., 2013). Moreover, milk provides an ideal environment for the survival and growth of many kinds of microorganisms due to the high moisture content, nearly neutral pH, and rich nutrients such as protein, vitamins, and other minerals (Lu *et al*., 2013).

Owing to the characteristics of milk that exhibits great

spoilage potential, the main purpose of milk fermentation using lactic acid bacteria (LAB) is to extend its shelf-life as well as to enhance the nutritive value of milk (Widyastuti *et al*., 2014). LAB have the ability to produce organic acids which is important as preservative agents and generating flavor of the products during fermentation of milk (Gemenchu, 2015). In addition, exopolysaccharides produced by LAB can improve the rheological and textural properties of dairy products (Sanlibaba and Çakmak, 2016). LAB used in the production of fermented milk foods are believed to produce a huge amount of bioactive compounds for human health benefits and to improve dairy food safety and quality (Gemenchu, 2015). Meanwhile, LAB seem to play a key role in maintaining the intestinal homeostasis and stability of gut bacterial community (Servin, 2004). LAB which are often used as starter cultures in food fermentation are classified as Generally Regarded as Safe (GRAS) because of their low adverse effects and toxicity (Avall-Jaaskelainen and Palva, 2005).

Yogurt fermented with different Lactobacilli strains is one of the most popular dairy products as a healthy food due to its multi-functional and therapeutic properties (Shah, 2007). Recent studies have shown that regular consumption of yogurt with live cultures and probiotic strains has been associated with preventing and relieving effects in various types of diarrhea (infantile, traveler's, antibiotic-associated), alleviation of gastrointestinal complaints, reduction of lactose intolerance, lowering serum cholesterol level, anticarcinogenic activity, prevention of urogenital infections, reduction of allergic symptoms, and stimulation of the immune system (Najgebauer-Lejko, 2014).

Meanwhile, many studies suggested that tea components, namely catechins and their derivatives may have beneficial effects on the prevention of cancer, arteriosclerosis, cardiovascular diseases, neural and obesity problems (Najgebauer-Lejko, 2014). Furthermore, tea polyphenols protect the cells and organ systems of the body from damage caused by free radical-induced oxidative stress and have broad-spectrum inhibitory activity against numerous pathogens (Almajano *et al*., 2008). Muniandy *et al*. (2016) demonstrated that the fortification of conventional yogurt with tea extracts improved the antioxidant activity of yogurt during 21 days of storage. The addition of teas or bioactive compounds of tea to yogurt is widely recommended for their health-promoting properties including antioxidant and antimicrobial activities (Jaziri *et al*., 2009).

Hence, the main objectives of this study was to investigate the effect of green tea extract on the physico-chemical and probiotic properties and the antimicrobial and antioxidant activities of yogurt fermented with LAB isolated from a traditional Korean soybean paste.

Materials and Methods

Bacterial isolates and growth conditions

Lactobacillus acidophilus D11 and *Lactobacillus fermentum* D37 that was able to meet the basic requirements for probiotic functions due to high tolerance to acid and bile conditions as well as anticancer, antimicrobial, or antimutagenic activities in previous *in vitro* studies (Lim *et al*., 2006; Lim, 2014) were used as starter for yogurt manufacture in this study. The frozen stock cultures of the two strains were maintained at -20°C in Lactobacilli MRS broth (Difco) containing 20% (v/v) glycerol until further use. Before starting any experiments, the isolates were defrosted and activated aerobically in MRS broth for 24 h at 37°C. To obtain fresh cultures of yogurt starter for the production of yogurt, the LAB were incubated aerobically overnight in MRS broth at 37°C, harvested by centrifugation (7,000 × *g*, 10 min, 4°C), and washed twice with phosphate buffer saline (PBS, pH 7.0).

Preparation of plain and green tea yogurts

Commercially available green tea and whole milk were purchased from a local grocery store in Busan, Korea. An aqueous extract of green tea was prepared following the procedure described by Neffe-Skocińska *et al*. (2015) with slight modifications. Briefly, the green tea leaves were suspended with distilled water in the ratio 1:10 and the mixtures were maintained at 80°C for 30 min, with constant shaking (150 rpm) followed by centrifugation at 12,000 × *g* for 10 min at 4°C. The supernatant was filtered through sterile Whatman paper (No. 4) to remove fine precipitates. The infusion filtered was used as green tea water extract in the making of green tea yogurt. Meanwhile, the preparation of starter culture from

whole milk was carried out using the method described by Shah (2003). Green tea extracts in different concentrations of 0.5, 1.0, 2.0, and 5.0% were added into raw cow milk which was standardized to a solid content of 15% with skimmed milk powder fortification (5%, w/v). The mixtures were pasteurized at 85°C for 15 min and cooled to 40±1°C before inoculation. Then, these samples were inoculated with probiotic starter culture (5%, 1.0×10^6 CFU/g) and mixed thoroughly followed by incubation for 18 h at 42°C. Plain yogurt was prepared in the same manner as previously described without green tea extract (control). Plain and green tea yogurts were prepared on the same day.

Evaluation of probiotic potential

***In vitro* gastrointestinal transit tolerance assay :** The evaluation of probiotic survival in plain and green tea yogurts submitted to simulated gastric and intestinal juices was carried out according to Huang and Adams (2004), with some modifications. Briefly, simulated gastric juices were prepared by suspending pepsin (1:10,000, Sigma), NaCl (125 mM), KCl (7 mM), and $NaHCO_3$ (45 mM) in PBS, adjusting the pH to 2.5 with 1 M HCl or 1 M NaOH, and filtering using a 0.45 μm membrane filter (Millipore Corp.). Simulated intestinal juice was made of NaCl (1.25 M), $NaHCO_3$ (82 mM), $NaHPO_4$ (44 mM), KCl (48 mM), $CaCl_2 \cdot 2H_2O$ (20 mM), $MgCl_2 \cdot 6H_2O$ (5mM), bile salts (17.5 g/L), and pancreatin (5 g/L). The solution was adjusted to pH 8.0 with 1 M NaOH and filtered through 0.22 μm cellulose acetate membranes. Yogurt samples (1 g) were transferred into a sterile test tube containing either gastric or small intestinal juices (9 ml). The mixture was then homogenized by vortex mixer for 10 sec and incubated at 37°C. After incubation in simulated gastric (2 h) and intestinal juice (4 h), aliquots of 1 ml were collected from each tube and serially diluted in PBS (pH 7.0). The residual viable population was determined by plate counting on MRS agar after 48 h incubation at 37°C under aerobic conditions.

***In vitro* adhesion assay :** The human epithelial cell line Caco-2 obtained from the Korean Cell Line Bank (KCLB) was used in the adhesion assay. Cells were routinely grown in Dulbecco's modified Eagle's minimal essential medium (DMEM; HyClone Laboratories Inc.) supplemented with 10% (v/v) heat-inactivated (30 min, 56°C) fetal bovine serum (FBS, GIBCO, Invitrogen Ltd.), glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 units/ml), and streptomycin (50 mg/ml) at 37°C in a 10% CO_2/90% air an atmosphere on glass coverslips placed in six-well tissue culture plates. Cells were plated at a density of 5×10^5 cells/well to obtain confluence. The cell culture medium was changed daily and the cultures were used at post-confluence after 15 days of culture for adhesion assay.

After washing the Caco-2 monolayer twice with sterile PBS (pH 7.0), a 1 g aliquot of each yogurt sample was transferred to post-confluent monolayers of Caco-2 cells (1×10^4 cells/well) in the 24-well multi-dish containing fresh tissue culture medium without antibiotics and incubated at 37°C in 5% CO_2–95% air atmosphere. After incubation for 2 h, the monolayers were washed twice with PBS (pH 7.0) to remove non adherent bacteria and detached from each well by incubation at room temperature for 15 min in the presence of trypsin/EDTA (Gibco). Lysed samples were diluted at least two fold and the adherence capacities were determined by enumerating the adherent bacterial cells on MRS agar plates.

Microbiological and physico–chemical analysis

To enumerate starter strain from plain and green tea yogurts, these samples (10 g) were aseptically homogenized for 3 min in a sterile stomacher bag containing 90 ml of PBS (pH 7.0). Serial 10-fold dilutions of the homogenates were subsequently prepared in the same buffer and spread on the plates containing Lactobacilli MRS agar. After aerobic incubation at 37°C for 48 h, the bacterial colonies that grew on the plates were counted and the number of viable cells in the sample was converted into colony forming unit (CFU) per gram. Meanwhile, the pH value of the samples was measured at room temperature using a pH meter (Fisher Scientific) equipped with a combined electrode. The titratable acidity values of each yogurt sample were determined by titration with 0.1 N NaOH in the presence of 1% phenolphthalein indicator after mixing the sample with 10 ml of hot distilled water (Dave and Shah, 1998). The quantitation of organic acids in yogurt samples was performed by high performance liquid chromatography (HPLC, Shimadzu) method according to Shah and Ravula (2002), with some modifications. One hundred microliter of 15.5 M HNO_3 and 2 ml of 0.009 N H_2SO_4 were added to each sample (6 ml) and they were thoroughly vortexed for 1 min. The samples were then centri-

fuged at 14,000 × *g* for 30 min and the supernatant was then passed through 0.22 μm membrane filter prior to injection into the HPLC system. The operating conditions were an Aminex-HPX 87H column (300 × 7.8 mm; Bio-Rad) at 65°C, 0.01 M H_2SO_4 as mobile phase with a flow-rate of 0.6 ml/min, and UV detection at 210 nm Standard solutions of organic acids (lactic and acetic acid; Sigma-Aldrich) were used to prepare a calibration curves. The amount of acetic acid in yogurt samples was subtracted from the content of acetic acid initially present in MRS broth. The released whey in the yogurt samples was determined using drainage method according to Aryana (2003). Yogurt (300 g) was placed on top of a funnel fitted with a fine (120) mesh stainless steel screen. After 2 h of drainage at 6°C, the quantity of whey collected in a graduated cylinder was considered as the index of syneresis. Apparent viscosity the homogenized samples was measured at room temperature (25°C) using a Brookfield viscometer (Brookfield Engineering Laboratories) equipped with a no. 4 cylindrical spindle. The spindle speed was set to 20 rpm. The viscosity data was collected at 1 min intervals and expressed as centipoises (cP) (Cinbas and Yazici, 2008).

Antibacterial activity of plain and green tea yogurts against milk borne pathogens

The milk borne pathogenic bacteria used in this study were *E. coli* O157 ATCC 43889, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* KCTC 3569, *Salmonella enteritidis* ATCC 13076, and *Salmonella typhimurium* KCTC 2514. An aliquot (20 μl) of each freezer stock of all the indicator strains was transferred into fresh BHI broth (20 ml) and was grown with shaking (150 rpm) at 37°C for 24 h. And then the bacterial cells were harvested by centrifuging (7,000 × *g*, 10 min, 4°C) and resuspended in PBS (pH 7.0). The plain and green tea yogurts were inoculated with concentrations of 1.0×10^6 CFU of the selected pathogenic microorganisms per gram of yogurt and the mixtures were stirred for 30 sec at room temperature and stored in the refrigerator (4°C) for up to 10 days. Samples were taken every 5 days intervals during storage and appropriate dilutions were made and plated in duplicate on selective medium (MRS agar for LAB, Brucella agar for *C. jejuni*, Sorbitol-MacConkey agar for *E. coli* O157, Oxford agar for *L. monocytogenes*, Xylose-Lysine Deoxycholate agar for *S. enteritidis*, Mannitol Salt with Egg Yolk agar for *S. aureus*) to enumerate the number of LAB and pathogens in yogurts. Viable cell counts of *C. jejuni* were determined after incubation at 37°C for 48 h in a microaerobic atmosphere. All other strains were incubated under aerobic conditions at 37ºC for 48 h.

Assays of antioxidant activity of plain and green tea yogurts

Determination of total phenolic content (TPC) : To remove non-hydrolyzed casein in yogurt, the pH of sample was adjusted to 4.6 using 1 M HCl. The suspension was centrifuged at 10,000 × *g* for 20 min at 5°C, and the supernatant was clarified by passing through a 0.45 μm sterile filter and stored at -20°C until further analysis (Amirdivani and Baba, 2015). TPC assay was carried out according to the method of Shetty *et al.* (2005) with minor modification. Briefly, yogurt extract (1 ml) was transferred 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). 1 N Folin-Ciocalteu reagent (1 ml) was added to each tube and the solution was thoroughly mixed using a vortex and allowed to stand at room temperature for 3 min. 1 N Na_2CO_3 (300 μl) was then added into the reaction mixture, after being thoroughly mixed, the mixture was left to stand for 90 min at room temperature. The absorbance was recorded at 765 nm using UV/Vis spectrophotometer (UV-1601, Shimadzu). Gallic acid (5~60 μg/ml) in ethanol was used as the standard phenolic compound for the quantitative determination of TPC of yogurt samples. TPC was calculated from the calibration curve of gallic acid and expressed as μg of gallic acid equivalent (GAE) per ml of yogurt extract (μg GAE/ml).

1, 1–diphenyl–2–picrylhydrazyl (DPPH) free radical scavenging ability

The DPPH radical scavenging assay was carried out as described previously by Shetty *et al.* (1995). Yogurt extracts (4 ml) were thoroughly mixed with 0.15 mM DPPH reagent (1 ml) followed by incubation at room temperature for 30 min in darkness. The absorbance of the reaction mixture was measured at 517 nm using UV/Vis spectrophotometer (UV-1601, Shimadzu) against distilled water as blank. The DPPH radical scavenging activity was calculated using the following formula: Scavenging

activity (%) = [($blank_{OD}$ − $sample_{OD}$)/$blank_{OD}$]×100. Ascorbic acid was used as positive control.

Ferric-reducing antioxidant power (FRAP) assay

FRAP assay was performed by following the procedure described by Muniandy *et al.* (2016) with some modifications. This method is based on the reduction of colorless ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to blue colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex by the power of the electron donating antioxidants present in the reaction mixture. The FRAP reagent contained 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1. Yogurt extracts (200 μl) were thoroughly mixed with Fe^{3+}-TPTZ reagent (1.8 ml) and the mixtures were incubated at 37°C for 10 min in the dark condition followed by brief centrifugation (1,400 × *g*, 2 min). The absorbance was then measured spectrophotometrically at 593 nm against a reagent blank. Ascorbic acid was used as positive control. Aqueous standard solutions of $FeSO_4 \cdot 7H_2O$ (100~1,000 μM) was used for calibration curve. Results were expressed as mM ferrous ion formed per mg of sample.

Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean±standard deviations (SD). The statistical analysis was carried out using SPSS Statistics version 12.0 software. Data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range test was used to determine significant differences. *P*-value <0.05 was considered to be statistically significant.

Results and Discussion

Effect of green tea extract on probiotic viability in yogurt sample

Table 1 shows the results of measuring the number of bacteria remaining in artificial gastric juice and bile juice after the fermentation of plain and green tea yogurts prepared with the *L. acidophilus* D11 and *L. fermentum* D37 strains. The number of LAB in plain yogurt prepared with *L. acidophilus* D11 was 5.7±0.2×10^7 CFU/g, but it decreased by about 1 log cycle after the exposure to artificial digestive juices. The number of this strain attached to Caco-2 cells was 4.9±0.4×10^4 CFU/g. When 1.0% or more green tea extract was added, the initial number of the strain in yogurt, the resistance to artificial digestive juice, and the adherence to Caco-2 cells were higher than those of plain yogurt. On the other hand, the viable cell counts in green tea yogurt prepared with *L. fermentum* D37 were not significantly (*P*>0.05) different from those of plain yogurt. The resistance to artificial gastric juice and bile juice

Table 1. Effect of green tea extract on the resistance of artificial digestive juices and the adhesion to Caco-2 cells of yogurt starters *Lactobacillus acidophilus* D11 and *Lactobacillus fermentrum* D37

Strain	Concentration of green tea extract (%)	Initial cell counts (Log CFU/g)	Viable cell counts after incubation in simulated gastric juice (Log CFU/g)	Viable cell counts after incubation in simulated intestinal juice (Log CFU/g)	Cells adhered to Caco-2 cell lines (Log CFU/g)
Lactobacillus acidophilus D11	0	$5.7\pm0.2\times10^{7a}$	$2.7\pm1.9\times10^{6a}$	$5.0\pm2.7\times10^{6a}$	$4.9\pm0.4\times10^{4a}$
	0.5	$3.9\pm3.6\times10^{7a}$	$8.4\pm3.3\times10^{6a}$	$6.8\pm3.0\times10^{6a}$	$3.1\pm2.5\times10^{4a}$
	1.0	$8.0\pm2.3\times10^{8b}$	$7.5\pm4.1\times10^{7b}$	$8.8\pm1.5\times10^{7b}$	$6.1\pm0.8\times10^{5abc}$
	2.0	$9.6\pm0.4\times10^{8c}$	$7.6\pm3.8\times10^{7b}$	$8.8\pm5.0\times10^{7b}$	$4.9\pm1.3\times10^{5abc}$
	5.0	$5.5\pm2.0\times10^{8bc}$	$7.4\pm3.0\times10^{7b}$	$9.6\pm2.0\times10^{7b}$	$7.3\pm5.1\times10^{5bc}$
Lactobacillus fermentum D37	0	$2.0\pm2.7\times10^{7a}$	$1.7\pm1.2\times10^{7a}$	$4.6\pm3.4\times10^{6a}$	$1.6\pm1.7\times10^{5ab}$
	0.5	$2.3\pm0.9\times10^{7a}$	$6.9\pm2.4\times10^{6a}$	$7.9\pm3.3\times10^{6a}$	$3.8\pm1.7\times10^{5abc}$
	1.0	$4.7\pm1.4\times10^{7a}$	$8.1\pm0.8\times10^{6a}$	$1.5\pm0.7\times10^{7a}$	$9.3\pm2.0\times10^{5c}$
	2.0	$3.4\pm2.2\times10^{7a}$	$6.3\pm3.7\times10^{6a}$	$8.7\pm5.1\times10^{6a}$	$2.7\pm2.5\times10^{5ab}$
	5.0	$2.2\pm2.4\times10^{7a}$	$2.3\pm0.4\times10^{6a}$	$4.0\pm1.9\times10^{6a}$	$7.2\pm4.5\times10^{4a}$

Data are means±standard deviation from triplicate determinations and means with the different letters in the same column are significantly different (*P*<0.05) as determined by Duncan's multiple range test.

and the adherence to Caco-2 cells were not affected by green tea extract supplementation. Based on the above results, *L. acidophilus* D11 and *L. fermentum* D37 in the plain and green tea yogurts actually fulfilled the basic criteria to be considered as a potential probiotic candidate because of their high resistance to artificial digestive juice and high adherence to Caco-2 cells. The resistance to artificial digestive juices and the adherence to epithelial cells seem to be affected by initial bacterial counts. In particular, green tea extract supplementation promoted the proliferation of *L. acidophilus* D11, which indicated that the probiotic activity of this strain was somewhat higher in this green tea yogurt than in plain yogurt. Therefore, green tea extract supplementation could promote the proliferation of certain LAB, which would exhibit a high survival rate during passage through digestive organs, and increase the number of bacteria adhering to the intestinal epithelium.

The minimum required level of probiotic bacteria to provide health benefits when consumed should be maintained at least 10^7 CFU/ml(g) in the fermented foods (Marhamatizadeh *et al*., 2013), therefore the plain and green tea yogurts fermented with *L. acidophilus* D11 and *L. fermentum* D37 strains were suitable for the criteria of viable cell count in fermented milk. Although the yogurt prepared with *L. fermentum* D37 strain was not affected by green tea extract, the number of viable cells was significantly increased when green tea extract was added to yogurt prepared with *L. acidophilus* D11 strain. Plant ingredients containing polyphenols were found to enhance the viability of probiotics in fermented dairy products (Baba *et al*., 2014). As well as the chemical structure and concentration of polyphenols, the bacterial species and strains play an important in the susceptibility of the probiotic strains to phenolic compounds (Tabasco *et al*., 2011).

Meanwhile, the selection criteria for probiotic microorganisms are the resistance to gastric acid, bile acid, and digestive enzymes and the adhesion ability to intestinal epithelial cells (Zárate *et al*., 2002). Unlike our results, Ranadheera *et al*. (2012) reported that the viability of *L. acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12, and *Propionibacterium jensenii* 702 remained largely unaffected at the end of *in vitro* gastric transit at pH 3.0 and pH 4.0 in plain and fruit yogurts, while the viability of these strains were abruptly reduced during *in vitro* gastric transit (pH 2.0). The acid resistance of *Lactobacillus casei* in commercial fermented milk has been reported to be influenced by the presence of food additives, storage time and temperature (Vinderola *et al*., 2011). The specific nutrients present in food products may also protect the viability of probiotics in the harsh gastrointestinal environment (Lavermicocca, 2006). The survival of probiotic LAB in the gastrointestinal tract is known to be influenced by the acidity of gastric juice, the concentration of bile salts, the activity of bile salt hydrolase, and the probiotic species and strains. Many probiotic strains can withstand in harsh acidic environment of the stomach and affect the microecology and metabolism of the mammalian host after reaching the large intestine in a living state. Moreover, there are evidences *in vivo* that some LAB strains attach to intestinal mucosal cells, thereby blocking the harmful effects of pathogenic bacteria in the body (Corr *et al*., 2009). Similar to these results, the bacterial counts of *L. acidophilus* D11 and *L. fermentum* D37 were relatively stable to bile acid, so these strains are likely to survive passage through the gastrointestinal tract and thus have greater metabolic effects. Similar findings were reported by Shokryazdan *et al*. (2014) who found that *L. acidophilus* HM1 and *L. fermentum* HM2 and HM3 strains showed high bile salt tolerance (0.3%). Ranadheera *et al*. (2012) demonstrated that dairy foods such as ice cream, plain and stirred fruit yogurts were generally found to improve the acid and bile tolerance of the probiotics.

Furthermore, the *in vitro* adherence of probiotics was found to be influenced by the carrier food matrix as well as strain specific, bacterial concentration, buffer composition, incubation time, growth medium, normal intestinal microbiota, and digestion (Ouwehand and Salminen, 2003). Although the considerable number (10^5–10^6 CFU/g) of probiotics present in goat's milk ice cream, plain and stirred fruit yogurts adhered to Caco-2 cells, the strains in fruit yogurt showed the highest adhesion ability (Ranadheera *et al*., 2012). Certain probiotic strains may have strong adhesive capacities due to a variety of physiological and biochemical properties such as the presence of mucus-binding pili on cell wall that promotes the adhesion process (Kankainen *et al*., 2009). Adherence of LAB to intestinal mucosa is considered as an important characteristic for the selection of probiotic strain (Shewale *et al*., 2014). LAB showed low ability to adhere to intestinal epithelial cells have a short residence time in the body, so it is difficult to exert

therapeutic benefits in the intestines. Lower adhesion levels have been observed for probiotics when exposed to low pH prior to adhesion assays (Marcinakova *et al*., 2010). Milk containing 1.5% fat was previously reported to significantly reduce the adhesion ability of *Lactobacillus* GG and *Lactobacillus reuteri* ING1 compared to non-fat milk (Ouwehand *et al*., 2001). Probiotic strains attached to intestinal epithelium stimulate the interaction between gut microbes and host through immunomodulation and maintain normal barrier function of the colonic mucosa by inhibition of enteric pathogen adherence (Servin, 2004).

Microbiological and physico-chemical properties of plain and green tea yogurts

The microbiological and physico-chemical characteristics of yogurts with added 0 to 5% green tea extracts are shown in Table 2. The viable counts of *L. acidophilus* D11 and *L. fermentum* D37 in yogurts without the added green tea extracts were $5.7\pm0.2\times10^7$ CFU/g and $2.0\pm2.7\times10^7$ CFU/g, respectively. No significant differences were observed between these two yogurts. Green tea extract (1.0, 2.0, and 5.0%) supplementation enhanced the viability of *L. acidophilus* D11 in yogurt; however, this extract did not significantly affect the growth of *L. fermentum* D37. The pH value of green tea yogurt with *L. acidophilus* D11 was significantly ($P<0.05$) lower than that of with *L. fermentum* D37. As the concentration of green tea extracts was increased, the pH value of yogurt fermented with *L. acidophilus* D11 was gradually decreased; however, the pH value of yogurt fermented with *L. fermentum* D37 was not affected by green tea extract supplementation. The titratable acidity of plain yogurt prepared with *L. acidophilus* D11 was almost similar to that prepared with *L. fermentum* D37. The titratable acidity of plain yogurt prepared with *L. acidophilus* D11 was significantly ($P<0.05$) increased with the addition of green tea extracts; however, the yogurt prepared with *L. fermentum* D37 was not affected by green tea extract supplementation. The amount of lactic acid in plain yogurt fermented with *L. acidophilus* D11 was 60.51±0.34 mM, and it was significantly ($P<0.05$) increased when 1% or more green tea extract was added. However this strain did not produce acetic acid during the fermentation process.

On the other hand, the amount of lactic acid and acetic acid in plain yogurt fermented with hetero-fermentative *L. fermentum* D37 LAB was 50.56±3.52 mM and 39.75±1.81 mM acetic acid, respectively. However, the production of lactic acid and acetic acid in yogurt fermented with *L. fermentum* D37 was not affected by green tea extract supplementation. The viscosity of plain yogurt was significantly higher with *L. acidophilus* D11 than with *L. fermentum* D37. Unlike *L. fermentum* D37 strain, the viscosity of yogurt fermented with *L. acidophilus* D11

Table 2. Effect of green tea extract on the microbiological and physico-chemical properties of yogurt fermented with *Lactobacillus acidophilus* D11 and *Lactobacillus fermentrum* D37

Strain	Concentration green tea extract (%)	Viable cell counts (Log CFU/g)	pH	Titability (%)	Lactic acid content (mM)	Acetic acid content (mM)	Syneresis (%, v/w)	Viscosity (cps)
Lactobacillus acidophilus D11	0	$5.7\pm0.2\times10^{7a}$	4.90 ± 0.03^{cd}	0.62 ± 0.02^{abc}	60.51 ± 0.34^{cd}	ND	6.0 ± 0.3^{bc}	1068.55 ± 1.46^{e}
	0.5	$3.9\pm3.6\times10^{7a}$	4.92 ± 0.08^{cd}	0.77 ± 0.05^{c}	53.82 ± 0.75^{abc}	ND	7.7 ± 0.2^{d}	1085.84 ± 2.59^{f}
	1.0	$8.0\pm2.3\times10^{8b}$	4.81 ± 0.04^{bc}	0.92 ± 0.03^{d}	66.11 ± 0.32^{d}	ND	7.9 ± 0.5^{d}	1105.17 ± 3.06^{g}
	2.0	$9.6\pm0.4\times10^{8c}$	4.66 ± 0.10^{ab}	1.03 ± 0.09^{de}	78.20 ± 0.29^{e}	ND	8.4 ± 0.2^{d}	1123.24 ± 2.05^{h}
	5.0	$5.5\pm2.0\times10^{8bc}$	4.59 ± 0.06^{a}	1.10 ± 0.08^{e}	81.49 ± 0.07^{e}	ND	8.1 ± 0.7^{d}	1159.75 ± 0.76^{i}
Lactobacillus fermentum D37	0	$2.0\pm2.7\times10^{7a}$	5.11 ± 0.02^{e}	0.64 ± 0.03^{abc}	50.56 ± 3.52^{ab}	39.75 ± 1.81^{a}	5.0 ± 0.3^{a}	1038.16 ± 3.06^{c}
	0.5	$2.3\pm0.9\times10^{7a}$	5.16 ± 0.12^{e}	0.59 ± 0.10^{ab}	52.67 ± 4.14^{abc}	46.28 ± 0.94^{b}	5.7 ± 0.3^{bc}	1040.25 ± 2.52^{c}
	1.0	$4.7\pm1.4\times10^{7a}$	5.25 ± 0.05^{e}	0.52 ± 0.04^{a}	59.36 ± 2.96^{cd}	44.17 ± 2.13^{ab}	4.9 ± 0.7^{a}	1009.36 ± 0.86^{a}
	2.0	$3.4\pm2.2\times10^{7a}$	5.07 ± 0.07^{de}	0.66 ± 0.07^{abc}	58.90 ± 6.80^{abcd}	41.51 ± 3.06^{ab}	5.3 ± 0.2^{ab}	1048.71 ± 1.09^{d}
	5.0	$2.2\pm2.4\times10^{7a}$	5.20 ± 0.07^{e}	0.70 ± 0.08^{bc}	46.01 ± 4.43^{a}	41.32 ± 1.55^{ab}	6.1 ± 0.4^{c}	1015.43 ± 2.04^{b}

Data are means±standard deviation from triplicate determinations and means with the different letters in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.
ND, Not detected.

strain was significantly (P<0.05) increased when green tea extract was added. From the above results, the effect of green tea extract on the growth of LAB was different depending on the strain. Especially, the microbiological and physico-chemical properties of yogurt fermented with *L. acidophilus* D11 strain were significantly (P<0.05) increased by the addition of 1.0% and 2.0% of green tea extract, whereas the pH and syneresis of the yogurt were decreased. However, the addition of 5% green tea extract resulted in a slightly lower number of bacteria than 1% added.

Najgebauer-Lejko *et al.* (2011) have shown that 5~15% of green tea added to yogurt resulted in higher *Lactobacillus delbrueckii* subsp. *bulgaricus* counts when compared with plain yogurt. A recent study has found that the green tea extract may be effective to maintain the viability of some *Lactobacillus* spp. (López de Lacey *et al*., 2014), probably due to α-glucosidase activity of bacteria that may consumed in sugars from glycosylated flavonoids as an energy source (Schneider *et al*., 1999). The flavonoids (such as quercetin-3-O-rutinoside and kaempferol-3-O-rutinoside) of green tea infusions are known to contain rhamnose in their structural formula (López de Lacey, 2014). The polyphenolic compounds in green tea extracts can also stimulate the growth and/or activity of yogurt bacteria (Jaziri *et al*., 2009). Since the pH reduction rates of herbal-yogurts containing peppermint, dill, and basil were faster than that of plain yogurt during fermentation, the presence of herbs appeared to enhance the metabolic activity of yogurt bacteria (Amirdivani and Baba, 2011).

Our results are only partially consistent with Nikjooy and Hashemi (2015) demonstrated that there was no significant difference in syneresis of the yogurt samples fortified wild thyme extract at three levels (0.5%, 1.3%, 1.5%), respectively, while the addition of this extract resulted in significant changes in pH value and titratable acidity of yogurt. Michael *et al.* (2010) found that the gradual decrease in the pH of yogurt added with plant phenolic extract suggests an increase in the buffering capacity of yogurt, thus it may be beneficial for the growth of yogurt starter cultures. Additionally, Muniandy *et al.* (2017) reported that higher pH values (P<0.05) were shown in tea yogurts than plain yogurt, and inclusion of green, white, and black tea extracts did not affect significantly (P>0.05) the average viable cell counts of *Lactobacillus* spp. compared to plain yogurt.

On the other hand, higher acid production was observed in tea yogurts (0.79~0.99%) than plain yogurt (0.70~0.91%). Meanwhile, the viable cell counts (2.1×10^8~6.2×10^8 CFU/ml) of the LAB and titratable acidity (0.792~0.881%) of drinkable yogurts added with green tea powder (0.5~2.0%) were somewhat lower than those of green tea yogurt fermented with *L. acidophilus* D11 (Jung and Park, 2005). The titratable acidity for the herbal yogurts including peppermint, dill, and basil was higher than plain yogurt during fermentation (Amirdivani and Baba, 2011). The titratable acidity which reflects the total amount of hydrogen ions present in yogurt samples is believed to be related to the proliferation of LAB during fermentation (Eissa *et al*., 2010). Thus the organic acids produced by LAB in yogurts were reported to be linearly related with the accumulation of titratable acidity (Billard *et al*., 2007). According to previous studies, the growth rate of LAB and the acidity of yogurt were affected by the concentration of green tea extract, thus the time to reach the desired acidity was shortened as the concentration of green tea extract increased (Marhamatizadeh *et al*., 2013).

Moreover, the syneresis of plain yogurt fermented with *L. acidophilus* D11 was significantly greater than green tea yogurt, which has been reported the similar result by Joung *et al.* (2016). In the case of supplementation of herb extracts, the best water holding capacity was obtained in herbal yogurt including *Nelumbo nucifera* leaf (Joung *et al*., 2016). The acidification process of the yogurts supplemented herb extracts allowed obtaining gels with increased firmness, lower permeability, and finer protein networks and improved whey drainage (Ozer *et al*., 2007). Furthermore, LAB strains are known to synthesize short chain fatty acids, vitamins, and EPS which contribute to the texture and viscosity of yogurt during the fermentation process (Curk *et al*., 1996). Unlike green tea yogurt fermented with *L. acidophilus* D11, the drinking yogurts produced by *S. thermophilus* exhibited the highest viscosity in control sample without black and green tea extracts (Ünal *et al.*, 2016).

Meanwhile, green tea extract did not significantly affect the growth and organic acid production of *L. fermentum* D37. This result was in accordance to the findings of Jaziri *et al*. (2009) who showed that the presence of green and black teas at 2.0% to 4.0% (w/v) did not significantly (P<0.05) influence the

growth of the yogurt bacteria (*S. thermophilus* and *L. bulgaricus*) and the pH and lactic acid levels of the final products. The green tea infusion had no impact on the level of *S. thermophiles* and *Bifidobacterium lactis* BB-12 in bio-yogurts, and its effect on the count of *L. acidophilus* LA-5 depended on the concentration and probiotic milk type (Najgebauer-Lejko, 2014). Reconstituted skim milk containing green tea powder (0.5~2.5%) did not significantly stimulate growth and acid production of *S. thermophilus* and *Lactobacillus casei*, whereas the growth and acid production of *L. acidophilus* were slightly enhanced by the addition of green tea powder (Jung *et al*., 2005).

Survival of the foodborne pathogen in yogurts in the presence and absence of green tea extract

Table 3 shows the inhibition of pathogenic food poisoning bacteria artificially inoculated on plain and green tea yogurts prepared with the *L. acidophilus* D11 and *L. fermentum* D37 strains. The antimicrobial activity against *S. aureus* (30.5±5.1%), which was fermented with *L. acidophilus* D11, was the highest, followed by that against *L. monocytogenes* (26.3±4.5%), *E. coli* O157 (12.5±0.9%), *S. enteritidis* (8.3±0.9%), and *S. typhimurium* (5.8±0.6%). As the concentration of green tea extracts was increased, the inhibition rate against pathogenic food poisoning bacteria was gradually increased; it was increased by about 7~17% when 5% green tea extract was added. On the other hand, the antimicrobial activity against *E. coli* O157, *S. enteritidis*, and *S. typhimurium* was significantly higher in plain yogurt prepared with *L. fermentum* D37 than in that prepared with *L. acidophilus* D11; however, the inhibition rate against *L. monocytogenes* was low. A similar level of inhibition against *S. aureus* was observed. The antimicrobial effect of green tea yogurt prepared with *L. fermentum* D37 was significantly higher compared with that of plain yogurt. Furthermore, when 5% green tea extract was added, the antimicrobial activity of green tea yogurt was increased by 4~9%. The synergistic effect of inhibition was relatively low. Based on the these results, the antimicrobial activity of plain yogurt against pathogenic food poisoning bacteria was different depending on the fermentation strains. The *L. acidophilus* D11 strain showed a higher antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria; however, the inhibitory effect of the *L. fermentum* D37 strain against *S. aureus* and *S. enteritidis* was higher than against *S. typhimurium* and *L. monocytogenes*. These results confirmed that the green tea extract added to produce of yogurt was not related to the growth of *L. fermentum* D37 strain, whereas the growth of *L. acidophilus* D11 was accelerated by the extract. It is presumed that the green tea extract promotes the growth and the production of antimicrobial substances of certain LAB, thereby increasing the antibacterial activity of the LAB.

Our results were in accordance with the findings of Lee *et al.* (2006), who showed that some LAB were not severely affected by tea phenolic compounds in opposition to other pathogenic bacteria. Specifically, green tea does not affect the growth of

Table 3. Effect of green tea extract on the antibacterial activity of yogurt fermented with *Lactobacillus acidophilus* D11 and *Lactobacillus fermentrum* D37

Strain	Concentration of green tea extract (%)	Inhibitory effect (%) against pathogenic bacteria during storage at 4°C									
		E. coli O157		*S. enteritidis*		*S. typhimurium*		*L. monocytogenes*		S. aureus	
		5	10	5	10	5	10	5	10	5	10
Lactobacillus acidophilus D11	0	12.5±0.9^{a}	13.4±1.7^{a}	8.3±0.9^{a}	6.4±2.5^{a}	5.8±0.6^{a}	4.4±1.3^{a}	26.3±4.5^{cd}	21.7±4.3^{c}	30.5±5.1^{a}	22.6±3.7^{a}
	0.5	16.1±1.1^{ab}	14.2±3.2^{a}	9.0±1.2^{a}	6.7±1.5^{a}	6.3±1.2^{ab}	5.8±0.9^{ab}	23.9±5.2^{c}	29.8±3.2^{d}	28.9±3.6^{a}	26.1±1.3^{a}
	1.0	24.6±2.0^{def}	20.3±1.8^{b}	11.7±3.0^{a}	15.0±2.0^{b}	7.8±1.7^{abc}	8.9±2.7^{bc}	31.2±0.8^{de}	28.7±1.4^{d}	33.7±8.0^{a}	32.9±2.5^{c}
	2.0	25.8±1.8^{ef}	29.0±0.6^{cd}	13.2±5.2^{a}	19.3±2.9^{c}	8.2±0.3^{abc}	10.3±0.6^{cd}	34.7±0.5^{ef}	31.4±5.0^{d}	35.0±6.0^{a}	36.8±4.1^{cd}
	5.0	29.5±3.1^{f}	33.0±4.4^{d}	15.3±2.1^{ab}	20.0±1.9^{c}	12.1±1.4^{cd}	14.7±1.3^{e}	39.9±1.9^{f}	37.3±2.6^{e}	40.5±2.7^{a}	38.6±3.3^{d}
Lactobacillus ferementum D37	0	17.6±4.9^{abc}	15.3±0.9^{a}	20.4±3.2^{bc}	16.7±2.5^{bc}	10.7±2.7^{abcd}	9.8±2.5^{c}	15.7±2.4^{ab}	10.2±2.1^{a}	31.2±6.8^{a}	26.3±1.6^{a}
	0.5	19.3±2.6^{bcde}	22.4±1.8^{b}	22.1±2.7^{c}	20.6±3.4^{c}	12.3±3.2^{cd}	13.4±0.9^{de}	13.9±0.6^{ab}	11.6±2.1^{ab}	30.2±0.5^{a}	27.1±2.8^{ab}
	1.0	18.0±0.7^{abcd}	15.3±4.7^{a}	23.4±2.6^{cd}	25.1±1.8^{d}	9.9±2.2^{bcd}	15.2±2.1^{e}	12.5±0.9^{a}	13.2±0.4^{ab}	28.3±1.8^{a}	26.9±4.0^{ab}
	2.0	21.4±3.3^{bcde}	27.0±1.4^{c}	27.0±0.8^{cd}	30.4±2.0^{e}	11.4±1.3^{abcd}	13.7±1.1^{e}	11.8±1.8^{a}	11.8±2.0^{ab}	35.1±3.7^{a}	32.1±1.3^{bc}
	5.0	23.9±1.6^{cdef}	30.6±2.2^{cd}	29.2±1.7^{d}	35.1±2.2^{f}	14.5±3.1^{d}	16.7±3.4^{e}	20.0±3.8^{bc}	16.8±3.6^{bc}	37.4±5.5^{a}	35.2±3.3^{cd}

Data are means±standard deviation from triplicate determinations and means with the different letters in the same column are significantly different (P<0.05) as determined by Duncan's multiple range test.

probiotic LAB, but green and black tea extracts are known to exhibit antibacterial activity of tea extract against many pathogenic microorganisms (Marhamatizadeh *et al*., 2013). Michalczyk and Zawislak (2008) reported that the addition of green tea extracts significantly inhibited the growth of *S. aureus*, *E. coli*, *S. enteritidis*, whereas it had no significant effect on the growth of selected LAB such as *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Lactobacillus rhamnosus*. Chan *et al.* (2011) reported that the green tea extracts inhibited the growth of Gram-positive *Micrococcus luteus*, *S. aureus*, and *Bacillus cereus* and the catechins such as epigallocatechin gallate and epicatechin gallate inhibited the growth of many bacterial species. Bazzaz *et al.* (2016) proposed that the possible mechanisms for the antibacterial activity of the green tea extract are related to the irreversibly damage the bacterial cytoplasmic membrane and the activity of dihydrofolate reductase.

As reported by several researchers, the antimicrobial substances produced by probiotic bacteria exert antimicrobial effect on Gram-positive pathogenic bacteria stronger than Gram-negatives (Jack *et al*., 1995). Specific important mechanisms underlying the antagonistic effects of probiotics against pathogenic microorganisms (i) direct inhibitory effect through production of antimicrobial compounds such as bacteriocins and organic acids or inhibitors of virulence gene expression; (ii) competition for binding sites and limiting nutrients; (iii) enhancement of epithelial barrier function and nonspecific cellular immmue responses; (iv) suppression of proinflammatory cytokines; and (iv) inhibition of virulence gene(s) or protein expression in gastrointestinal pathogens (Corr *et al*., 2009). Zhu *et al*. (2010) demonstrated that bio-yogurt contained the four probiotic bacterial species (*Lactobacillus bulgaricus*, *S. thermophilus*, *L. acidophilus*, and *Bifidobacterium* ssp.) was capable of inhibiting specific periodontal pathogens. Our results were in accordance with Azizkhani and Tooryan (2016) that concluded adding zataria, basil, or peppermint essential oils into probiotic yogurt formulation could improve the potential functionality of the product and also made an inhibitory effect against *L. monocytogenes* and *E. coli*.

Antioxidant activity

Table 4 shows the total phenol content, DPPH radical scavenging ability, and FRAP of plain and green tea yogurts prepared with the *L. acidophilus* D11 and *L. fermentum* D37 strains. The TPC of plain yogurt prepared with *L. acidophilus* D11 was 33.32±11.52 μg GAE/ml and was significantly increased with the addition of green tea extracts; it was about 10 times higher when 5% green tea extract was added. On the other hand, the TPC of plain yogurt prepared with *L. fermentum* D37 was significantly higher than of that prepared with *L. acidophilus* D11. The DPPH radical scavenging ability of plain yogurt prepared with the *L. acidophilus* D11 and *L. fermentum* D37 strains was 3.52±0.06% and 10.21±0.08%, respectively. The DPPH radical scavenging ability of green tea yogurts was significantly increased as the concentration of green tea extracts

Table 4. Effect of green tea extract on the antioxidant activity of yogurt fermented with *Lactobacillus acidophilus* D11 and *Lactobacillus fermentrum* D37

Strain	Concentration of green tea extract (%)	Total phenolic content (μgGAE/ml)	DPPH free radical scavenging ability (%)	FRAP (μM Fe^{2+} E/L)
Lactobacillus acidophilus D11	0	33.32±11.52^{a}	3.52±0.06^{a}	0.12±0.01^{a}
	0.5	116.81±8.96^{b}	14.97±0.19^{c}	1.47±0.05^{b}
	1.0	185.52±2.44^{c}	35.10±0.18^{d}	1.83±0.03^{c}
	2.0	236.97±6.19^{d}	48.96±0.07^{e}	2.49±0.11^{d}
	5.0	319.85±12.54^{e}	69.55±0.21^{g}	2.96±0.10^{e}
Lactobacillus ferementum D37	0	92.48±9.15^{b}	10.21±0.08^{b}	0.20±0.02^{a}
	0.5	169.71±6.47^{c}	56.13±0.17^{f}	2.55±0.12^{d}
	1.0	305.90±4.58^{e}	72.37±0.26^{h}	3.06±0.16^{ef}
	2.0	397.16±7.50^{f}	82.35±0.09^{i}	3.27±0.04^{f}
	5.0	489.56±15.03^{g}	91.55±0.06^{j}	4.16±0.09^{g}

Data are means±standard deviation from triplicate determinations and means with the different letters in the same column are significantly different (P<0.05) as determined by Duncan's multiple range test.

was increased. However, the DPPH radical scavenging ability of green tea yogurt prepared with these LAB was significantly (P<0.05) lower than that of ascorbic acid (93.5±1.6, data not shown). FRAP activity was significantly higher in plain yogurt prepared with *L. fermentum* D37 (0.20±0.02 μM Fe^{2+} E/L) than with *L. acidophilus* D11 (0.12±0.01 μM Fe^{2+} E/L) when the concentration of green tea extracts was increased. The FRAP activity of green tea yogurt prepared with *L. fermentum* D37 (4.16±0.09 μM Fe^{2+} E/L) was significantly higher than of that prepared with *L. acidophilus* D11 (2.96±0.10 μM Fe^{2+} E/L). The TPC, DPPH radical scavenging ability, and FRAP activity, which could affect antioxidant capacity, were different depending on the strains.

Free radicals cause various diseases including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, and AIDS and are the main cause of lipid peroxidation. The antioxidant exerts an antioxidative effect by directly reacting with reactive oxygen species (ROS) and/or by chelating the catalytic metal ions. Phenolics and flavonoids mainly contained in plants are natural antioxdiants, which are actually less toxic than synthetic antioxidants, e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These compounds have also been reported to exert the antioxidant effects based on the absorption or neutralization of free radicals, quenching of singlet and triplet oxygen, and decomposition of hydroperoxides (Kumar *et al*., 2013).

According to the results of this study, the antioxidant capacity of green tea yogurt was significantly increased compared to plain yogurt, which is presumably due to the polyphenol compounds contained in green tea. Similar to our results, yogurt with green, white, or black tea before fermentation significantly increased the TPC compared to the control due to the abundant presence of catechin and epicatechin in tea (Muniandy *et al*., 2017). Phenolic compounds could act as antioxidants by donating hydrogen or electron to the free radical to terminate the chain reaction or by chelating transition metal ions (Rice-Evans *et al*., 1997). Meanwhile, this was likely related to milk and polyphenol interaction as reported in previous studies. TPC values in green tea yogurt were estimated to be increased by the degradation of phenolic compounds in milk and green tea as a result of microbial metabolism during fermentation (Blum, 1998). Damin *et al*. (2009) reported that the TPC values in plain yogurt without plain extracts were associated with milk protein breakdown. During the yogurt fermentation process, milk proteins were hydrolyzed by LAB and amino acids with phenolic side chains such as tyrosine were liberated, thereby contributing to the increase in TPC (Korhonen, 2009). In addition, the use of phenolic acids such as ferulic and ρ-coumaric acid during fermentation leads to the production of other phenolic acids such as vanillic and phydroxybenzoic acids (Blum, 1998).

On the other hand, it has been reported that the antioxidant capacity was reduced by the interaction of milk and polyphenol. Namely, there was a strong affinity between the proline groups contained in the milk protein and the hydroxyl groups present in the phenolic compounds. These interactions may lead to the precipitation of phenolic compounds and reduce the antioxidant capacity (Arts *et al*., 2002). Marhamatizadeh *et al*. (2013) reported that when the green tea extract was added to milk fermented with *L. acidophilus*, the oxygen scavenging ability and the redox potential of the growth medium were decreased due to phenolic compounds present in green tea extract. Phenolic compounds are also known to have beneficial effects on the microbiological quality and sensory and functional properties of yogurt product. Furthermore, some LAB used for yogurt fermentation also have antioxidant power, such as reactive oxygen species scavenging, metal ion chelation, enzyme inhibition, and the reduction activity and inhibition of ascorbate autoxidation (Lin and Yen, 1999). During yogurt fermentation, LAB break down milk proteins to produce peptides, free amino acids and fatty acids, and these substances exhibit antioxidant potential (Farvin *et al*., 2010).

Muniandy *et al.* (2016) demonstrated that green, white, and black tea extracts were effective in enhancing the antioxidant capacity of yogurt. Green tea extract has the highest content of polyphenols, followed by white and black tea extract. The higher antioxidant activities in herbal yogurts than plain yogurt were due to the result of the phytochemical contents in the herb and the microbial metabolic activities (Thompson *et al*., 2007). Consequently, the addition of plant extracts to probiotic yogurts may contribute to the improvement of the health function associated with high antioxidant activity (Joung *et al*., 2016). The antioxidant activity of green tea yogurt was significantly

(P<0.05) higher than that of plain yogurt from the beginning of fermentation, which is consistent with higher DPPH radical scavenging ability of yogurt with plant and fruits (Apostolidis *et al*., 2007). Nikjooy and Hashemi (2015) reported that the antioxidative capacity of yogurt increased in proportion to the concentration of wild thyme extract, and the DPPH radical scavenging ability (69%) was maximized when 1.5% of the extract was added. As the amount of tea extract added to yogurt increased, the radical scavenging ability and ferric-reducing power increased. However, the FRAP value was slightly lower after 3 weeks of storage than immediately after fermentation, but there was no statistically significant difference between the samples (Najgebauer-Lejko, 2014). These results are in concordance with recent studies (Muniandy *et al*., 2016) showing that the free radical scavenging ability of yogurt (88~92%) supplemented with tea extract was significantly higher than that of control group (5.84%). Besides, the inclusion of tea extracts into milk reduced (P<0.05) FRAP values to 3.71±0.48 mM Fe^{2+} E/L, 2.85±0.13 mM Fe^{2+} E/L, and 2.14±0.38 mM Fe^{2+} E/L for green tea yogurt, white tea yogurt, and black tea yogurt, respectively. Amirdivani and Baba (2015) reported that the highest FRAP value was found in air-dried green tea-yogurt (12.8 mM Fe^{2+} E/L), followed by steam-treated green tea-yogurt and plain yogurt (9.93 and 0.97 to mM Fe^{2+} E/L, respectively).

During the production of yogurt, the LAB have an increased metabolic activity, which promotes the degradation of the phenolic compounds contained in the green tea extract (Amirdivani and Baba, 2015). Tea catechins and low-molecular polyphenols contribute to high antioxidant power of tea extracts, thus green tea yogurt has higher antioxidant potential than plain yogurt (Zhu *et al*., 2002). In addition, the proteolysis by LAB may release bioactive peptides with antioxidant activity in fermented milk products (Farvin *et al*., 2010). Meanwhile, some LAB possessed significant antioxidative activity, allowing the preservation of catechins from oxidation during yogurt fermentation (Jaziri *et al*., 2009). Consequently, the results of this study indicated that the *L. acidophilus* D11 and *L. fermentum* D37 used for the production of green tea yogurt may be able to exert their function after reaching the intestine. Especially, it was confirmed that their antibacterial and antioxidant activities could be expected to be enhanced by adding green tea extract to plain yogurt. Therefore, green tea yogurt fermented with LAB having probiotic activity is considered to be a functional food for health improvement.

적 요

본 연구에서는 된장으로부터 분리된 *Lactobacillus acidophilus* D11 또는 *Lactobacillus fermentum* D37 균주로 발효시킨 요구르트의 프로바이오틱로서의 가능성, 물리·화학적 및 기능적 특성에 대한 녹차 추출물의 영향을 조사하였다. 인공 소화액에 대한 저항성과 상피 세포에 대한 부착력과 같은 프로바이오틱 활성은 플레인 요구르트보다 녹차 추출물을 첨가한 요구르트에서 다소 높게 나타났는데, 이는 유산균의 수의 증가에 기인하는 것으로 추정되었다. *L. acidophilus* D11로 발효시킨 플레인 요구르트에 녹차 추출물을 첨가한 경우 유산균수, 유기산 함량 및 점도와 같은 요구르트의 물리·화학적 특성도 유의하게 (P<0.05) 증가하였다. 하지만 녹차 추출물은 *L. fermentum* D37 균주 발효시킨 요구르트의 물리·화학적 특성에는 유의한 영향을 미치지 않았다 (P>0.05). 한편, *Escherichia coli* O157 ATCC 43889, *Salmonella enteritidis* ATCC 13076 및 *Salmonella typhimurium* KCTC 2514에 대한 항균 활성 및 총 페놀 함량, 라디칼 소거능 및 철 환원력과 같은 항산화 활성은 *L. acidophilus* D11 보다는 *L. fermentum* D37로 발효시킨 플레인 요구르트에서 현저히 높았다. 게다가 요구르트의 항균 및 항산화 활성은 녹차 추출물의 농도에 비례하여 유의적으로 증가하였다 (P<0.05). 결론적으로, *L. acidophilus* D11 또는 *L. fermentum* D37로 발효시킨 녹차 요구르트는 병원성 세균의 성장을 억제하고 체세포 내에 생성된 자유 라디칼을 제거 할 수 있는 유용한 기능성 식품으로 이용할 수 있는 것으로 판단되었다.

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