Using lactic acid bacteria and packaging with grapefruit seed extract for controlling *Listeria monocytogenes* growth in fresh soft cheese

J. Y. Lim, C. L. Lee, G. H. Kim, Y. J. Bang, J. W. Rhim, and K. S. Yoon*

Department of Food and Nutrition, College of Human Ecology, Kyung Hee University, Seoul 02447, Republic of Korea

ABSTRACT

Various cheese products are involved in outbreaks of listeriosis worldwide due to high consumption and prolonged refrigerated storage. The objective of this study was to determine the efficacy of using lactic acid bacteria and packaging with grapefruit seed extract (GSE) for controlling *Listeria monocytogenes* growth in soft cheese. *Leuconostoc mesenteroides* and *Lactobacillus curvatus* isolated from kimchi were used as a starter culture to make a soft cheese, which was inoculated with a cocktail strain of *L. monocytogenes*. The soft cheese was packed with low-density polyethylene, biodegradable polybutylene adipate-co-terephthalate (PBAT), low-density polyethylene with GSE, or PBAT with GSE and stored at 10°C and 15°C. *Leuconostoc mesenteroides* (LcM) better inhibited the growth of *L. monocytogenes* than *Lb. curvatus*. The PBAT with GSE film showed the best control for the growth of *L. monocytogenes*. When both LcM and PBAT with GSE were applied to the soft cheese, the growth of *L. monocytogenes* was inhibited significantly more than the use of LcM or PBAT with GSE alone. In all test groups, water activity, pH, and moisture on a fat-free basis decreased, and titratable acidity increased compared with the control group. These results suggest that LcM isolated from kimchi and PBAT with GSE packaging film can be used as a hurdle technology to lower the risk of *L. monocytogenes* in soft cheese at the retail market.

Key words: *Listeria monocytogenes*, soft cheese, packaging, lactic acid bacteria

INTRODUCTION

Listeria monocytogenes is a psychotropic and salt-tolerant foodborne pathogen. The CDC (2016) has estimated that about 1,600 people get sick from *Listeria* and about 260 people die from it each year in

the United States. Recently, an outbreak of *Listeria* infection has been linked to deli-sliced meats and cheese in the United States, leading to the hospitalization of 8 people and 1 death (CDC, 2019). The risk of *L. monocytogenes* in dairy products is based on its widespread occurrence in the environment of dairy plants and its ability to survive and grow on dairy products at refrigerated temperatures (Lourenço et al., 2017). Among dairy products, soft cheese is highly perishable with a limited shelf life even under refrigeration due to texture changes and bacteria growth (Di Pierro et al., 2011).

Bacteriocins of lactic acid bacteria (LAB) and grapefruit seed extract (GSE) are well documented to possess antimicrobial effects. During fermentation, LAB produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins (Lindgren and Dobrogosz, 1990). These components not only are desirable due to their effects on food flavor and texture, but also are needed to inhibit undesirable pathogenic organisms (Nettles and Barefoot, 1993).

Grapefruit seed extract is a natural antimicrobial and antioxidant agent that contains tocopherol, citric acid, ascorbic acid, and polyphenolic compounds such as catechins, epicatechin, epicatechin-3-O-gallate, and dimeric, trimeric, and tetrameric procyanidins (Cho et al., 1990; Saito et al., 1998). Grapefruit seed extract shows remarkable consistency in its action against a wide range of organisms including both gram-positive and gram-negative organisms (Reagor et al., 2002). It is generally recognized as safe without human toxicity (Xu et al., 2007). The bacterial growth inhibition effect of GSE can be ascribed to its polyphenols that can penetrate the semipermeable bacterial membrane to react with the cytoplasm or cellular proteins (Corrales et al., 2009). Heggers et al. (2002) have reported that all concentrations of GSE can cause cell death by disrupting the cell membrane of bacteria. The antimicrobial potential is higher because phenolic acids are present in the undissociated form rather than the dissociated form in GSE (Paulus, 2005).

Packaging materials with antibacterial properties could extend the shelf life of foods and reduce the risk

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*Corresponding author: ksoyon@khu.ac.kr

of pathogens (Appendini and Hotchkiss, 2002). Previous studies have documented the antimicrobial activity of packaging film with GSE (Kanmani and Rhim, 2014; Sogut and Seydim, 2018). Various active food packaging technologies against foodborne pathogens were also reported. The desired antibacterial activity of chitosan-coated, nisin-silica liposomes against *L. monocytogenes* was reported as a promising active packaging material (Cui et al., 2016). Also, improving the anti-*Listeria* activity of polyethylene oxide nanofiber containing nisin-loaded nanoparticles was reported for cheese packaging without an effect on the sensory quality (Cui et al., 2017). Most recently, Lin et al. (2019) tested moringa oil-loaded chitosan nanoparticle for cheese packaging against *L. monocytogenes* and *Staphylococcus aureus* and recommended it for active food packaging materials in the food industry.

The present study aimed to evaluate the antimicrobial effect of LAB isolated from kimchi and packaging film containing GSE on *L. monocytogenes* growth in fresh soft cheese. The synergistic effect of LAB and packaging film with GSE was also tested against *L. monocytogenes*. We hypothesized that packaging films containing GSE could be used to extend the shelf life without causing a quality change of cheese. Thus, microbiological and physicochemical changes of cheese were also evaluated under the conditions of distribution temperature (10°C) and temperature abuse (15°C).

MATERIALS AND METHODS

Preparation of Bacterial Strains

Leuconostoc mesenteroides (LcM, KCTC 13374) and *Lactobacillus curvatus* (LbC, KCTC 3767) isolated from kimchi were used in this study as LAB. All LAB strains were purchased from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea) and stored at -80°C in deMan, Rogosa, and Sharpe (MRS) broth (MB cell, Seoul, Korea) containing 20% glycerol. Each thawed strain (10 µL) was then inoculated into 10 mL of MRS broth and incubated at 30°C overnight at 140 rpm on a rotary shaker. Appropriate dilutions were prepared to reach an inoculation level of 5.0 to 6.0 log cfu/g in fresh soft cheese samples.

Listeria monocytogenes strains (ATCC 15313, ATCC 19111) were purchased from the Korea Culture Center of Microorganisms (KCCM, Seoul, Korea). A strain of *L. monocytogenes* (serotype, 1/2a) isolated from smoked salmon was also used in this research. All strains of *L. monocytogenes* were maintained in tryptic soy broth (TSB; MB Cell, Seoul, Korea) supplemented with 0.6% yeast extract (Oxoid Ltd., Basingstoke, UK). All

strains were stored at -80°C in TSB containing 20% glycerol (Sigma-Aldrich, St. Louis, MO). Each strain of *L. monocytogenes* (10 µL) was then thawed and inoculated into 10 mL of TSB supplemented with 0.6% yeast extract and incubated at 36°C overnight at 140 rpm on a rotary shaker (VS-8480SR, Vision, Daejeon, Korea). Appropriate dilutions of each strain were prepared to an inoculation level of 3.0 log cfu/g. An equal amount of each strain was mixed as a 3-strain cocktail of *L. monocytogenes* for the inoculum onto soft cheese sample.

Preparation of Antimicrobial Packaging Films

Polyethylene was purchased from Freshfield Inc. (Siheung, Korea). Low-density polyethylene (LDPE) was purchased from Hanwha Chemical Co. (Seoul, Korea). The LDPE resins were dried under vacuum at 60°C for 24 h before use. Polybutylene adipate-co-terephthalate (PBAT) was obtained from S-EnPol Co. Ltd. (Wonju, Korea). The PBAT resins were dried under vacuum at 90°C for 72 h before use. Corn starch was purchased from Daejung Chemicals and Metals Co. Ltd. (Siheung, Gyeonggi-do, Korea). Grapefruit seed extract (DF-100) was obtained from Komipharm International Co. Ltd. (Seoul, Korea). Thermoplasticized starch (TPC) was prepared as a carrier of the GSE by mixing corn starch with 20% of glycerol and 40% of GSE as a plasticizer and antimicrobial filler, respectively, at 250 rpm for 10 min using a mixer (Ultra Power Series 4.5-quart stand mixer KSM90, KitchenAid, St. Joseph, MI). The mixture was heated at 120°C for 30 min using an autoclave, spread to cool to room temperature, and then powdered using a blender (DA700-G, Daesung Artron Co., Seoul, Korea) to obtain GSE-incorporated TPC.

The masterbatch for PBAT with GSE was prepared by mixing 5% GSE, 3% of zinc oxide nanoparticles, 8% of calcium stearate, and PBAT resins at 250 rpm for 15 min using a low-speed mixer. Zinc oxide nanoparticles were prepared using the method described by Shankar and Rhim (2019). Briefly, 1,000 mL of 1 mM zinc nitrate was heated to 70°C. Then, 1 M KOH solution was added dropwise with vigorous stirring until the pH reached around 8, followed by continued heating and stirring for 30 min. White precipitates were centrifuged at 970 × g for 15 min at ambient temperature and washed with distilled water 4 to 5 times, followed by washing with ethanol for 2 to 3 times, and drying in an oven at 150°C for 24 h. The PBAT with GSE composite film was prepared with a thermocompression method using a Carver laboratory press (Hydraulic unit, model 3925, Carver Inc., Wabash, IN). About 4 g of the masterbatch was placed between 2 stainless steel plates (1

mm thick, $25.4 \times 25.4 \text{ cm}^2$) coated with Teflon sheets and then pressurized at about 60,000 psi (414 MPa) at 130°C for 3 min. The masterbatch for LDPE film with GSE was prepared by mixing TPC containing 3% GSE and LDPE resins with a weight ratio of 1:10 using a high-speed mixer (600 rpm) for 1 h. For LDPE resin, 3% GSE was the maximum concentration to maintain the packaging film. The mixture was then extruded using a twin-screw extruder (YJC-ST-Ø48/34, Yoojin Eng. Co. Ltd., Siheung, Korea) with a screw speed of 600 rpm. The LDPE-based blend films were prepared using an extrusion blowing method as described previously (Hong and Rhim, 2012). The LDPE masterbatch with TPC was extruded using a single-screw extruder (YJF-faØ50–800L, Yoojin Eng. Co. Ltd.) following the method described by Hong and Rhim (2012). Neat LDPE film was also prepared using the same procedure without TPC. The chemicals we used for packaging film were safe; the FDA proved them as generally recognized as safe, and PBAT is an eco-friendly biodegradable material.

Preparation of Fresh Soft Cheese

Fresh soft cheese samples were prepared with or without LAB according to a modified general cheese manufacturing method (Chandan et al., 2015). Pasteurized whole milk was purchased from a local market and heated to 37°C in a water bath (SB-1200, Eyela, Tokyo, Japan). We added LcM or LbC to milk as a starter culture at 10^5 to 10^6 cfu/mL, mixed for 1 min, and let ferment at 40°C for 1 h. We added 0.02% of rennet (Maysa, Istanbul, Turkey) and 0.2% of citric acid (Weifang Ensign Industry Co. Ltd., Weifang, China) to coagulate the milk. After coagulation, samples were cut to separate the curd and whey. Cooled fresh soft cheese was then pressurized. Figure 1 shows the flowchart of fresh soft cheese manufacturing with processing treatments.

Antimicrobial Effect of LAB and Packaging Film Containing GSE

To determine the antimicrobial effects of LAB isolated from kimchi and packaging film containing GSE on *L. monocytogenes* in fresh soft cheese, 3 different treatment groups were prepared, as shown in Figure 1. For the LAB treatment group, soft cheese was prepared with LcM, LbC, or the mixture of LcM and LbC in a 7:3 ratio. We used this ratio because it was the most effective in inhibiting *L. monocytogenes* and *Salmonella* Typhimurium in dry fermented sausage in our previous work (our unpublished data). Soft cheese samples

prepared with LAB were inoculated with 100 µL of a 3-strain cocktail of *L. monocytogenes* onto the surface of fresh soft cheese at a level of 2.5 to 3.5 log cfu/g using an automatic pipette. Then, 4 films (LDPE, PBAT, LDPE with GSE, and PBAT with GSE) were used as the inner packaging film of soft cheese, which was then vacuum packed with polyethylene. For a synergistic effect of LAB and antimicrobial packaging film, a combination of LcM and PBAT with GSE was also tested. For comparison, a control group that received no treatment was included. They were stored at 10°C (distribution temperature) and 15°C (abused temperature), respectively.

Quality Evaluation

Microbiological analysis and physicochemical characteristics of soft cheese samples were measured at 10°C (distribution temperature) and 15°C (abuse temperature) on d 0, 5, 10, 15, 25, and 30. Physicochemical characteristics including water activity (A_w), pH, titratable acidity, moisture content, and fat were determined in fresh soft cheese samples according to AOAC International, (1995).

Microbiological Analysis

For bacteria enumeration during storage, fresh soft cheese (10 g each) were aseptically transferred into sterile filter bags and homogenized in 40 to 90 mL of 0.1% (wt/vol) sterile peptone water for 2 min using a stomacher (Interscience, Paris, France). Subsequent decimal dilutions in peptone water were then plated onto MRS agar for LAB and incubated at 30°C for 48 h or plated onto PALCAM agar for *L. monocytogenes* and incubated at 37°C for 48 h. Serial decimal dilutions of samples were plated in duplicate onto aerobic count plates and *Escherichia coli* (coliform) count plates (Pet-rifilm 3M, Seoul, Korea), respectively.

Water Activity, pH Measurement and Titratable Acidity

The A_w of cheese was measured with an A_w meter (AwTherm, Rotronic, NY). Measurement of pH was carried out (Orion 3-star pH-Benchtop, Thermo) by immersing a probe into 90 mL of distilled water containing 10 g of fresh soft cheese samples. Contents were constantly stirred until stable pH measurements were recorded.

To determine titratable acidity, 10 g of fresh soft cheese sample was homogenized with 90 mL of distilled water at 40°C using a stomacher (Stomacher, Inter-

Figure 1. Manufacturing flowchart of fresh soft cheese and processing treatments. Lactic acid bacteria (LAB) was added before the coagulation process. GSE = grapefruit seed extract.

science, France) and filtrated. Filtrate (25 mL) was collected and titrated with 0.1 N standard NaOH. Phenolphthalein was used as an indicator. The titratable acidity was expressed as lactic acid using the following formula:

$$\text{Total titratable acidity (\%)} = \frac{0.1 \text{ N NaOH (mL)} \times 0.1 \text{ N NaOH factor} \times 0.009}{\text{Sample (g)}} \times 100.$$

Moisture, Fat, and Moisture on a Fat-Free Basis

For moisture analysis, each 2-g cheese sample was weighed in a 5-cm diameter flat-bottomed metal dish. Each sample was placed in the oven at 100°C and dried to constant weight (~3 h). The sample dish was removed from the oven, cooled, and weighed. Expressed loss in weight was calculated as moisture content using the following formula:

$$\text{Moisture content (\%)} = 100 - \text{solid content (\%)}.$$

For fat analysis, cheese sample (1 g) was weighed into a tall-form beaker. Then, 9 mL of H₂O and 1 mL of NH₄OH were added. The sample was mixed until smooth. It was then warmed at 40°C until casein was well softened. The sample was neutralized with 10 mL of HCl for 5 min. The solution was cooled and transferred to a fat-extraction flask. The used tall-form beaker was rinsed out successively with 10 mL of alcohol, 25 mL of ether, and 25 mL of petroleum ether (boiling range: 30–60°C), which was transferred to a fat-extraction flask and mixed thoroughly. It was let to stand until the upper liquid was practically transparent. The ether solution was decanted into another weighed flask. Lip and stopper of extraction flask were washed with a mixture of equal parts of ether and petroleum ether. Washings were then added to the weighing flask. Extraction was repeated twice using 15 mL of ethers each time. Solvents were evaporated completely on an evaporator (N-1200A, Eyela, Shanghai, China) at 50°C. The fat was dried to constant weight in an oven at 100°C. The cooled flask was weighed without wiping immediately before weighing.

Using the data (moisture content and fat in cheese) obtained above, moisture on a fat-free basis was calculated as follows:

$$\frac{\text{Weight of moisture in the cheese}}{\text{Total weight of cheese} - \text{weight of fat in cheese}} \times 100.$$

Statistical Analysis

Each experiment was repeated at least twice at different times, with 2 replicates per treatment. For each replication, at least 2 to 4 measurements of each parameter were performed. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Because our data had a normal distribution with unequal variances, a significant difference among samples was determined by Welch's ANOVA, followed by Duncan's multiple range tests at $P < 0.05$.

RESULTS AND DISCUSSION

Antimicrobial Effect of LAB on *L. monocytogenes* in Fresh Soft Cheese

Antimicrobial effects of LcM, LbC, and the mixture of LcM and LbC on populations of *L. monocytogenes* in soft cheese at 10°C and 15°C are shown in Figure 2. At 10°C, there was no significant ($P > 0.05$) difference in the lag time of *L. monocytogenes* between LbC and the control. However, LcM and the mixture of LcM and LbC (7:3) significantly ($P < 0.05$) extended the lag time up to 3.6 and 2.5 times, respectively. The maximum population density of *L. monocytogenes* in soft cheese containing LcM or the mixture of LcM and LbC was lowered to 23.1 or 25.9%, respectively, compared with that of the control. At 15°C, significant ($P < 0.05$) extension of lag time and lower of maximum population density were observed in all treatment groups compared with the control. However, the specific growth rate was not significantly ($P > 0.05$) affected by the addition of different types of LAB. Coelho et al. (2014) prepared fresh cheese from pasteurized cow's milk inoculated with bacteriocin-producing LAB and *L. monocytogenes*. They found that all LAB strains inhibited the growth of *L. monocytogenes* during storage at 4°C for up to 15 d. Aymerich et al., (2019) also reported that *Lactobacillus sakei* CTC494 inhibited the growth of *L. monocytogenes* in smoked salmon after 21 d of storage at 8°C.

Several antimicrobial compounds produced by LAB have been identified and characterized individually for their inhibitory potential against spoilage organisms (Crowley et al., 2013). These compounds range from simple organic acids and primary metabolic products to very complex compounds derived from bioconversions or peptide synthesis, as well as protein cleavage. The most essential and best-characterized antimicrobials produced by LAB are lactic acid and acetic acid. They are bioactive in their protonated form at a low pH (Arena et al., 2016).

Figure 2. The effect of lactic acid bacteria on the growth of *Listeria monocytogenes* in fresh soft cheeses at 10 and 15°C. Control = ●, *Leuconostoc mesenteroides* (LcM) = ◆, *Lactobacillus curvatus* (LbC) = ▼, and the mixture of LcM and LbC at 7:3 ratio = ×.

Antimicrobial Effect of Packaging Film with GSE on *L. Monocytogenes* in Fresh Soft Cheese

Fresh soft cheese was prepared individually and packed with different packaging film: LDPE, LDPE containing 3% GSE (LDPE with GSE), biodegradable PBAT, and PBAT containing 5% GSE (PBAT with GSE). Overall, packaging films containing GSE had a better performance delaying the growth of *L. monocytogenes* in soft cheese than those without GSE (Table 1). At 10°C, there was no significant difference in lag time, and maximum population density of *L. monocytogenes* in soft cheese between LDPE with GSE and PBAT with GSE groups, but the specific growth rate was well controlled in soft cheese packed with PBAT with GSE. On the other hand, the longest lag time was observed in soft cheese packed with PBAT with GSE at 15°C. Wang and Rhim (2016) reported that the antibacterial activity of the packaging film was de-

pendent on the type of polymer matrix, which affected the releasing rate of the GSE. If the polymer matrix more tightly entraps the GSE, the diffusion rate of the GSE from the film decreased, consequently resulting in lower antibacterial activity. The PBAT packaging is a biodegradable material with low water vapor permeability, high flexibility, and good processability (Witt et al., 2001). Poly(lactide)-PBAT-GSE composite film exhibited a high potential for an active food packaging application with antibacterial properties (Shankar and Rhim, 2018). A slightly better antimicrobial effect of PBAT with GSE than LDPE with GSE was observed in the present work. The antimicrobial effect of GSE against *L. monocytogenes* has also been reported in previous studies. Pork loin packed with the edible *Gelidium corneum*-gelatin blend film containing 0.08% GSE showed a decrease in populations of *L. monocytogenes* of 1.05 to 1.14 log cfu/g compared with the control after 4 d of storage at 4°C (Hong et al., 2009).

Table 1. Effect of packaging on the kinetic parameters of *Listeria monocytogenes* in fresh soft cheese at 10°C and 15°C

Treatment ¹	10°C			15°C		
	LT ²	SGR ³	MPD ⁴	LT	SGR	MPD
Control	18.74 ^c	0.03 ^b	7.75 ^a	12.86 ^d	0.08 ^a	7.87 ^a
LDPE	34.44 ^a	0.04 ^a	7.51 ^a	25.64 ^c	0.07 ^b	7.39 ^{bc}
LDPE with GSE	29.58 ^{bc}	0.03 ^b	6.89 ^b	27.12 ^b	0.05 ^c	7.43 ^{bc}
PBAT	8.30 ^d	0.03 ^b	7.57 ^a	9.90 ^e	0.05 ^c	7.50 ^b
PBAT with GSE	23.25 ^{bc}	0.02 ^c	6.76 ^b	34.92 ^a	0.07 ^b	7.27 ^c

^{a-e}Mean values (n = 4) in the same column with different superscripts are significantly different ($P < 0.05$).

¹Packaging treatments: LDPE = low-density polyethylene; LDPE with GSE = LDPE containing 3% grapefruit seed extract; PBAT = polybutylene adipate-co-terephthalate (biodegradable); PBAT with GSE = PBAT containing 5% grapefruit seed extract.

²LT = lag time (h).

³SGR = specific growth rate (log cfu/h).

⁴MPD = maximum population density (log cfu/g).

Red algae film containing 1% GSE reduced populations of *L. monocytogenes* by 0.85 log cfu/g in a cheese after 15 d of storage at 4°C (Shin et al., 2012).

Antimicrobial Synergistic Effect of LAB and PBAT Film with GSE on *L. Monocytogenes*

Soft cheese was made with LcM and packaged with PBAT with GSE to investigate the antimicrobial synergistic effect of LAB and packaging film. At both 10°C and 15°C, the addition of LcM strain, PBAT with GSE packaging, or the combination of LcM with PBAT with GSE significantly affected lag time and maximum population density of *L. monocytogenes* (Figure 3). However, the specific growth rate of *L. monocytogenes* was the most affected by the addition of LcM or the combination of LcM with PBAT with GSE packaging. At both 10°C and 15°C, significantly longer lag time and lower maximum population density of *L. monocytogenes* were observed in soft cheese made with LcM and packed with PBAT with GSE film compared with the control. This result indicated that the addition of LcM as a starter could be a key to control *L. monocytogenes* growth in soft cheese. The most effective antimicrobial effect against *L. monocytogenes* was observed in soft cheese made with LcM and packaged with PBAT with GSE in this work. Compared with the control, lag time values at 10°C and 15°C were extended to 1.7 and 3.6 times, respectively. Specific growth rate values at 10°C and 15°C also decreased by up to 66.7% and 75%, respectively. The lowest maximum population density value was observed at both temperatures, indicating that the isolated LcM strain from kimchi can be used as a starter culture, along with PBAT with GSE film packaging to control *L. monocytogenes* in soft cheese. Furthermore, the group of soft cheese containing LcM

and packed with PBAT with GSE had the lowest population of coliforms and total aerobic bacteria during 30 d of storage (data not shown).

Lactic acid bacteria can produce various antimicrobial compounds such as lactic acid, acetic acid, diacetyl, ethanol, hydrogen peroxide, reuterin, acetaldehyde, acetoin, carbon dioxide, and bacteriocins during fermentation processes. These natural compounds could be used as biopreservatives to inhibit pathogenic, nonpathogenic, and spoilage microorganisms, thus extending the shelf life of products and reducing the use of antibiotics (Özogul and Hamed, 2018). Abedi et al. (2013) tested the antibacterial effect of *Lactobacillus delbrueckii* ssp. *bulgaricus* on *E. coli* using disk diffusion and spot-on-lawn methods. The inhibition zone of *E. coli* by *L. delbrueckii* was 21.1 mm with the spot-on-lawn method. It was 9.1 mm with the disk diffusion method. The LAB isolated from kimchi inhibited not only the growth of *Salmonella* Typhimurium and *L. monocytogenes* (our unpublished data), but also the growth of pathogenic *E. coli* (Choi et al., 2018).

Hurdle Effect of LAB and PBAT Film with GSE on Microbiological and Physicochemical Changes of Soft Cheese During Storage at 10°C

Water activity is one of the most important physicochemical parameters. It is beneficial for controlling the ripening process for the final organoleptic properties and safety standards of cheese (Saurel et al., 2004). The initial Aw of soft cheese is usually 0.96 to 1.00 (Marcos and Esteban, 1982). As shown in Figure 4, the Aw of the fresh soft cheese was significantly ($P < 0.05$) higher in the PBAT with GSE group over the storage period at 10°C. The Aw values of LcM and LcM with PBAT with GSE groups decreased from 0.979 to 0.951

Figure 3. The effect of lactic acid bacteria and packaging on the growth of *Listeria monocytogenes* in fresh soft cheeses at 10 and 15°C. Control = ●, *Leuconostoc mesenteroides* (LcM) = ◆, biodegradable polybutylene adipate-co-terephthalate (PBAT) with 5% grapefruit seed extract (GSE) = ▼, both LcM and PBAT with GSE = ×.

Figure 4. Comparison of microbiological and physicochemical changes of fresh soft cheese with different treatments during storage at 10°C. *Leuconostoc mesenteroides* (LcM) = ◆, biodegradable polybutylene adipate-co-terephthalate (PBAT) with 5% grapefruit seed extract (GSE) = ▼, both LcM and PBAT with GSE = ×.

and from 0.984 to 0.938, respectively. In general, pH values of the soft cheese gradually decreased regardless of treatment due to lactic acid formation in cheese. Whitley et al. (2000) reported a positive correlation of pH values with the increase of LAB.

The titratable acidity is important in the cheese-making process because it plays a fundamental role in all phases of milk coagulation, including the aggregation of para-casein micelles and the reactivity of rennet. The titratable acidity also influences the rate of syn-thesis and determines the suitability of milk for cheese making (De Marchi et al., 2009). In the production of premium cheeses, milk with low acidity (hypoacidic milk) is generally considered unsuitable for cheese making because of its adverse effects on the rheology of acid-rennet curd and textural properties of cheese paste (Formaggioni et al., 2001). At 10°C, initial titratable acidity values were 0.35 to 0.52%, and final titratable acidity values in LcM, PBAT with GSE, and LcM with PBAT with GSE increased up to 1.15, 1.55, and 1.48%, respectively, after 30 d. These results were related to the decrease in the pH of soft cheese due to increased LAB populations during 30 d of storage. Previous studies have also shown that titratable acidity values of Ricotta cheese and Minas fresh cheese increased with increasing days of storage (Buriti et al., 2005).

Cheese moisture and fat content are generally 29.2 to 79.0% and 4.4 to 34.9%, respectively (Chandan et al., 2009). Mozzarella cheese, as a soft cheese, has a

54.1% moisture and a 21.6% fat content (Chandan et al., 2009). Based on the Korean industry standards, soft cheese has >67% moisture on a fat-free basis. In this study, moisture, fat content, and moisture on a fat-free basis in fresh soft cheese was 52.9 to 56.4%, 14.2 to 18.7%, and 64.9 to 69.4%, respectively. Moisture contents in LcM, PBAT with GSE, and LcM with PBAT with GSE groups decreased from 56.3, 56.4, and 52.9% to 43.5, 47.9, and 44.2%, respectively. During 30 d of storage at 10°C, there was no significant difference in moisture content in PBAT with GSE group for up to 15 d, which seemed to be capable of keeping the moisture well, and moisture on the fat-free basis was maintained in LcM and LcM with PBAT with GSE groups up to 20 d. This tendency was also observed at 15°C (data not shown).

CONCLUSIONS

The risk of *L. monocytogenes* in various cheese is based on its ability to grow on these products at refrigerated temperatures. In this work, we investigated the antimicrobial effects of LcM and LbC isolated from kimchi and packaging film containing GSE on *L. monocytogenes* in soft cheese. Addition of LcM and the mixture of LcM and LbC (7:3) significantly ($P < 0.05$) extended the lag time value. Significantly lower maximum population density and longer lag time values of *L. monocytogenes* were observed in soft cheese packed

with LDPE or PBAT containing GSE. Notably, the lag time of *L. monocytogenes* in soft cheese packed with PBAT with GSE was 2.7 times longer than that of the control, even at the abused temperature (15°C). The PBAT with GSE group was capable of keeping the moisture well at 10°C and 15°C. The most effective antimicrobial effect was observed in soft cheese made with LcM and packaged with PBAT with GSE film. The results indicated that cheese manufacturing plants might be able to use this approach as a form of hurdle technology to control the growth of *L. monocytogenes* at the retail market.

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ORCIDS

- J. Y. Lim <https://orcid.org/0000-0002-1704-3715>
 C. L. Lee <https://orcid.org/0000-0002-0136-4012>
 G. H. Kim <https://orcid.org/0000-0003-3948-6469>
 Y. J. Bang <https://orcid.org/0000-0001-5851-4248>
 J. W. Rhim <https://orcid.org/0000-0003-1787-5391>
 K. S. Yoon <https://orcid.org/0000-0002-7492-4076>