Bactericidal activity of lauric arginate in milk and Queso Fresco cheese against *Listeria monocytogenes* cold growth¹

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ABSTRACT

Lauric arginate (LAE) at concentrations of 200 ppm and 800 ppm was evaluated for its effectiveness in reducing cold growth of *Listeria monocytogenes* in whole milk, skim milk, and Queso Fresco cheese (QFC) at 4°C for 15 to 28 d. Use of 200 ppm of LAE reduced 4 log cfu/mL of *L. monocytogenes* to a nondetectable level within 30 min at 4°C in tryptic soy broth. In contrast, when 4 log cfu/mL of *L. monocytogenes* was inoculated in whole milk or skim milk, the reduction of *L. monocytogenes* was approximately 1 log cfu/mL after 24 h with 200 ppm of LAE. When 800 ppm of LAE was added to whole or skim milk, the initial 4 log cfu/mL of *L. monocytogenes* was nondetectable following 24 h, and no growth of *L. monocytogenes* was observed for 15 d at 4°C. With surface treatment of 200 or 800 ppm of LAE on vacuum-packaged QFC, the reductions of *L. monocytogenes* within 24 h at 4°C were 1.2 and 3.0 log cfu/g, respectively. In addition, the overall growth of *L. monocytogenes* in QFC was decreased by 0.3 to 2.6 and by 2.3 to 5.0 log cfu/g with 200 and 800 ppm of LAE, respectively, compared with untreated controls over 28 d at 4°C. Sensory tests revealed that consumers could not determine a difference between QFC samples that were treated with 0 and 200 ppm of LAE, the FDA-approved level of LAE use in foods. In addition, no differences existed between treatments with respect to flavor, texture, and overall acceptability of the QFC. Lauric arginate shows promise for potential use in QFC because it exerts initial bactericidal activity against *L. monocytogenes* at 4°C without affecting sensory quality.

Key words: lauric arginate, *Listeria monocytogenes*, Queso Fresco, intervention

INTRODUCTION

Queso Fresco (QFC) is a Hispanic-style soft cheese with a refrigerated shelf-life of approximately 30 to 60 d. In the United States, production of QFC is only permitted with pasteurized milk (USFDA, 1987; Sandra et al., 2004; MacDonald et al., 2005). However, there are no further listericidal steps during the cheese-making process, and the cheese curds are processed at approximately 40°C (Sandra et al., 2004). Considering the normal persistence of *Listeria monocytogenes* in the processing environment, QFC may become contaminated during cheese manufacturing or during postprocess packaging. Recently, Moreno-Enriquez et al. (2007) observed a prevalence of *L. monocytogenes* as high as 18.5% in samples that were collected from QFC and its processing facilities located in Mexico. Studies show that QFC is an excellent substrate for the growth of *L. monocytogenes* as well as spoilage bacteria because of its high moisture (45–55%), low salt content, and near neutral pH (Bolton and Frank, 1999; Mendoza-Yepes et al., 1999; Sandra et al., 2004). When *L. monocytogenes* cells were inoculated in QFC, the growth of *L. monocytogenes* increased by 3 logs within 20 d of refrigerated storage (Mendoza-Yepes et al., 1999). A risk-based study conducted by the International Life Science Institute research foundation (Washington, DC) classified QFC as an increased risk factor for listeriosis (ILSI, 2005). In June 1985, a prominent outbreak of listeriosis associated with QFC occurred, which resulted in 142 cases of listeriosis and caused 48 deaths (Linnan et al., 1988).

Different methods such as the use of lactic acid starter cultures or essential oils of citrus and rosemary have been evaluated for their ability to control *L. monocytogenes* in QFC. Mendoza-Yepes et al. (1997, 1999) tested the effectiveness of *Lactococcus lactis* ssp. *diacetylactis* culture and essential oils during QFC preparation and reported the inhibition of *L. monocytogenes* growth for 20 d without decreasing initial counts (Mendoza-Yepes et al., 1997, 1999). However, the use of essential oils as an antimicrobial agent in other food products has been reported to result in decreased consumer acceptability because of unfamiliar flavor and aroma characteristics (Ouattara et al., 1997; Burt, 2004). In another study,

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nisin in combination with caprylic acid was able to inhibit *L. monocytogenes* populations in QFC for 28 d (Diez-Gonzalez et al., 2009). Sensory analysis of QFC samples treated with nisin-caprylic acid indicated potential sensory differences between control and treated samples. Nisin has been effective as an antimicrobial in other soft cheeses such as ricotta and cottage cheese (Ferreira and Lund, 1996; Davies et al., 1997), but different strains of *L. monocytogenes* vary in sensitivity to nisin, and some sensitive strains are shown to develop gradual resistance against nisin (Maisnier-Patin and Richard, 1996).

Therefore, there is a strong demand in the food industry for antimicrobials that can be used as preservatives in *L. monocytogenes*-susceptible food products (Soni et al., 2010). Lauric arginate (LAE; ethyl *N*-lauroyl-L-arginate hydrochloride) is an FDA-approved, generally regarded as safe (GRAS) food preservative at concentrations up to 200 ppm (USFDA, 2005). The antimicrobial activity of LAE is based on the disruption and instability of the plasma membrane lipid bilayer over a wide pH range (3–7). Efficacy of LAE against *L. monocytogenes* cells has previously been evaluated in ready-to-eat ham and frankfurters, in which LAE appeared to exert listericidal action toward *L. monocytogenes* cells during early storage period (Luchansky et al., 2005; Martin et al., 2009; Taormina and Dorsa, 2009). Currently, there are no reports on the effectiveness of LAE in QFC. The major research goal of the present study was to evaluate the usefulness of LAE for control of *L. monocytogenes* growth in QFC. Research objectives evaluated in this study to attain major research goal were to 1) determine substrate (broth, milk, QFC)-dependent efficacy of LAE toward *L. monocytogenes* cells; 2) determine LAE concentration-dependent inactivation of *L. monocytogenes* in milk and QFC; 3) determine listericidal efficacy of LAE during short-term and long-term storage periods in QFC; and 4) determine the sensory acceptability of QFC samples treated with 200 ppm of LAE.

MATERIALS AND METHODS

Preparation of *L. monocytogenes* Cell Suspensions

All the microbiological components of the present study were performed in a biosafety level II-approved laboratory accessible only to trained research individuals working with human foodborne pathogens. In total, 21 strains of *L. monocytogenes* were used that represent all 13 serotypes; strain ID and source information of these strains are provided in Table 1. All strains were maintained in tryptic soy agar slants at 4°C and cul-

tured in 10 mL of tryptic soy broth (TSB) at 37°C for 18 to 24 h. When needed, a 5-strain mixture of *L. monocytogenes* cells was prepared by mixing an equal volume of the cell suspension containing 10^9 cfu/mL of strains 1 and 2 (serotypes 1/2a) and strains 10, 11, and 14 (serotype 4b). Serial dilutions of these original cell suspensions were prepared in physiological saline (0.8% NaCl) to attain working *L. monocytogenes* concentrations for inoculation purposes.

Source of QFC and LAE

Retail samples of QFC (Cacique Ranchero, Cacique Inc., City of Industry, CA; 340-g round block, 95 mm in diameter and 35 mm thick) that contained no chemical preservatives were obtained from a local grocery store and stored at 4°C (maximum 2 d) before experimentation. From the packaging information, the QFC samples had a shelf life in the range of 45 to 60 d from the purchase date and contained 21% fat, 21% protein, 0% carbohydrate, and 0.8% salt. In addition, QFC samples had water activity of 0.977 measured using Aqua Lab 3TE meter (Decagon Devices Inc., Pullman, WA) and a pH of 6.08 measured using a solid-food pH penetration probe (FlexipHet SS Penetration tip, Cole Palmer, Vernon Hills, IL).

Mirenat-TT lauric arginate was obtained from Vedeqsa Inc. (New York, NY). The Mirenat-TT solution is commercially available and contains 10% active LAE that is dissolved in propylene glycol (solvent) and polysorbate 20 (emulsifier).

MIC of LAE Against Different *L. monocytogenes* Strains

Minimum inhibitory concentrations of LAE against different *L. monocytogenes* strains were determined in TSB. Briefly, TSB that contained 200 ppm of LAE was initially prepared and serially diluted (1:1) to yield concentrations of 200, 100, 50, 25, 12.5, 6.2, 3, 1.5, and 0 (control) ppm of LAE in TSB. Two hundred microliters of each of these TSB substrates containing different LAE concentrations was distributed into a 96-well microtiter well plate with 3 replications and inoculated with 10 μ L of approximately 10^5 cfu/mL of each of the freshly grown *L. monocytogenes* strains. These plates were incubated at 37°C for 24 h with shaking at 100 rpm. Following a 24-h incubation, the development of turbidity as an indication of *L. monocytogenes* growth was assessed. The lowest LAE concentration that inhibited *L. monocytogenes* growth was reported as the MIC.

Table 1. *Listeria monocytogenes* strains and serotypes used in this study¹

ID	Strain	Serotype	Source
1	V7	1/2a	Raw milk (FDA)
2	EGD	1/2a	P. Cossart (Institut Pasteur, Paris, France)
3	F4260	1/2b	Human CSF and blood (CDC)
4	V2	1/2c	Human CSF (VICAM)
5	ATCC 19113	3a	Human (ATCC)
6	SLCC 2540	3b	Human CSF
7	SLCC 2479	3c	Unknown
8	ATCC 19114	4a	Bovine brain (ATCC)
9	ScottA	4b	Human clinical (FDA)
10	F4393	4b	Cheese (CDC)
11	F5069	4b	Milk (CDC)
12	F1057	4b	Milk (CDC)
13	F1109	4b	Milk (CDC)
14	ATCC 43257	4b	Mexican style cheese (ATCC)
15	F2385	4b	Epidemic strain, outbreak in California, 1985
16	NRRL 33083	4b	Food (ARS)
17	Murry B	4ab	FDA
18	ATCC 19116	4c	Chicken (ATCC)
19	ATCC 19117	4d	Sheep (ATCC)
20	ATCC 19118	4e	Chicken (ATCC)
21	SLCC 2482	7	Human feces

¹CSF = cerebrospinal fluid; FDA = Food and Drug Administration, Washington, DC; CDC = Centers for Disease Control and Prevention, Atlanta, GA; VICAM = VICAM Inc., Watertown, MA; SLCC = Special *Listeria* Culture Collection (University of Wurzburg, Germany); ATCC = American Type Culture Collection, Rockville, MD; ARS = Agricultural Research Service, Washington, DC.

Efficacy of LAE Against *L. monocytogenes* in Skim Milk or Whole Milk Versus TSB

Samples of TSB, skim milk, and whole milk were evaluated to determine substrate-dependent effects of LAE on quantitative reductions of *L. monocytogenes*. Skim milk (0% fat, 8.5% total solids) and whole milk (3.4% fat, 8.5% nonfat total solids) were obtained from the local grocery store. Both skim and whole milks were studied to see if the fat particles had any influence on inactivation of *L. monocytogenes* by LAE. Samples were first inoculated with a 5-strain *L. monocytogenes* mixed inoculum (~4.0 log cfu/mL). Subsequently, *L. monocytogenes*-inoculated samples were mixed with LAE stock solutions to yield LAE concentrations of 200 ppm in TSB and 200, 400, and 800 ppm in skim milk and whole milk. Control samples received sterile distilled water instead of LAE. Samples were immediately distributed into microcentrifuge tubes (1 mL) and placed at 4°C. *Listeria monocytogenes* cells were enumerated at 0 min and 30 min, and thereafter at 1, 3, 5, 7, 10, and 15 d by spread plating on Palcam agar containing 6 mg/L of ceftazidime (Difco Laboratories, Detroit, MI) and incubating the plates at 37°C for 48 h. When surviving *L. monocytogenes* cells were expected to be low, 1 mL was spread-plated on 4 plates with 250 µL/plate.

Determination of Bactericidal Activity of LAE by Surface Treatment of QFC

Round blocks of QFC samples (approximately 340 g each) obtained from a retail grocery store were brought to the class II biosafety cabinet and the packages were opened aseptically on a sterile cutting board using a knife. Each QFC block was then cut into 6 equal pieces (~46 to 50 g each) and each piece was placed inside a plastic bag (30 cm × 50 cm, Rebel Butcher Supply Co., Flowood, MS). Then, 200 µL of serially diluted *L. monocytogenes* mixed cell suspension was added on top of the QFC sample inside each bag using a 1-mL serological pipette and cells were allowed to attach for 15 min. Subsequently, 1 mL of LAE working solutions (1% and 4% active LAE) prepared in sterile deionized water were added on the surface of *L. monocytogenes*-challenged QFC samples to yield a final LAE concentration of 200 or 800 ppm on a per-gram basis. The control samples were treated with 1 mL of sterile distilled water. Plastic bags containing control and LAE-treated QFC samples were immediately vacuum-sealed (Berkel-250, Louisville, KY) and samples were stored at 4°C. Enumeration of *L. monocytogenes* from control and LAE-treated samples was performed after 0, 1, 7, 14, 21, and 28 d of storage as per *Bacteriological Analytical Manual* protocol (Hitchins, 2002). At each

time point, 3 replicate samples from each treatment (control, 200, and 800 ppm of LAE) were removed from 4°C storage and the vacuum-sealed bags were cut open from the top using sterile scissors. To each of the bags containing QFC samples, 250 mL of peptone water (0.1% peptone containing 0.02% Tween-80) was added and the samples were homogenized for 2 min in a stomacher (model 400C, Seward, London, UK) at 230 rpm. Stomached aliquots or their serial dilutions prepared in physiological saline were subsequently spread-plated on Palcam agar plates. Plates were incubated at 37°C for 48 h for *L. monocytogenes* colony development.

Sensory Analysis

The sensory testing of LAE-treated QFC was performed in Garrison Sensory Evaluation Laboratory (Mississippi State University). Two consumer-based sensory panels ($n = 120$, $n = 60$ per panel) were conducted to evaluate product acceptability and determine if consumers could differentiate between QFC treated with LAE at concentrations of 0 and 200 ppm. Each panel performed both a triangle test and a consumer acceptability test ($n = 60$ panelists per test). The surface of the QFC was exposed to 200 ppm of LAE based on weight; then, the QFC was vacuum-packaged (Ultravac 2100, Koch Equipment, Kansas City, MO) and stored (2 to 4°C) for 24 to 48 h before sensory testing. Only the edges of the QFC were used for sensory testing to ensure that the samples being evaluated were fully exposed to LAE. The edges were cut into square pieces (approximately 2 cm × 2 cm), placed in plastic containers (28-g portion cups, Sweetheart Cup Co., Owing Mills, MD) that were coded with 3-digit random numbers, covered with a lid, and stored under refrigeration conditions (4°C) until evaluation by consumers (within 1–3 h). Consumers evaluated QFC samples for the triangle test first, and once finished, samples for consumer acceptance were presented to the panelists. Panelists evaluated QFC samples in separate booths in a well ventilated and temperature-controlled room under fluorescent light. Water (Mountain Spring Water, Blue Ridge, GA), unsalted crackers (Premium, Nabisco, East Hanover, NJ), and expectorant cups were provided. Panelists were asked to expectorate and rinse their mouths between each sample to remove residual flavors.

The triangle test was performed to determine if consumers could perceive a difference (in taste) between QFC with or without LAE. Half of the panelists ($n = 30$) received a set of 3 QFC samples that contained 2 control samples and 1 sample that was treated with LAE. The other half of the panelists ($n = 30$) received 2 samples that were treated with LAE and 1 control

sample. Panelists were asked to choose which sample was different out of the 3 samples that they received. For the consumer panel, each panelist ($n = 60$) was asked to evaluate the control and LAE-treated sample for overall acceptability and acceptability of texture, flavor, aroma, and appearance using a 9-point hedonic scale, where 1 = dislike extremely, 5 = neither like or dislike, and 9 = like extremely (Meilgaard et al., 2007).

Statistical Analysis

All microbiological experiments were replicated 3 times in a completely randomized design structure, whereas sensory analysis results were based on 2 sensory panels with 60 panelists in each panel. Counts of *L. monocytogenes* were converted into log colony-forming units per gram and analyzed using the SPSS statistical analysis package (version 12.0, SPSS Inc., Chicago, IL) to find mean differences ($P < 0.05$) between control and LAE treatments using a paired-*t*-test. For the triangle test, the number of panelists and correct responses were used to determine if a significant difference ($P < 0.05$) existed among treatments (Meilgaard et al., 2007). A randomized complete block design (replications and panelists as blocks) with 2 replications was used to test the treatment effects ($P < 0.05$) of LAE (0 and 200 ppm) on the overall acceptability and acceptability of appearance, aroma, texture, and flavor of QFC.

RESULTS AND DISCUSSION

Antimicrobial Effectiveness of LAE Against Different Strains of *L. monocytogenes*

In this study, the bactericidal efficacy of LAE was demonstrated against *L. monocytogenes* survival as a function of strain or serotype sensitivity, substrate composition, and long-term storage at 4°C. The MIC of LAE did not vary with serotype, and all *L. monocytogenes* strains were equally sensitive to LAE. The growth of all *L. monocytogenes* strains was inhibited by LAE at a concentration of 25 ppm in TSB. In previous studies, *L. monocytogenes* strains V7 and ScottA demonstrated antimicrobial tolerance to sodium lactate, sodium diacetate, sodium benzoate, potassium sorbate, and nisin compared with other strains or serotypes in broth (Castellano et al., 2001; Neetoo et al., 2008). However, we did not observe variation in strain or serotype sensitivity to LAE because V7 and ScottA showed sensitivity similar to all other strains. The antimicrobial activity of LAE is based on the disruption and instability of the plasma membrane (Rodriguez et al., 2004). Based on the results of the current study, we

can assume that differences, if any, in surface properties of the *L. monocytogenes* strains may not be substantial enough to provide tolerance to LAE. We used a 5-strain cocktail of *L. monocytogenes* in a subsequent validation assay for LAE efficacy in milk or QFC. Serotypes 1/2a and 4b, which belong to lineages I or II, are the most important with regards to clinical and food safety risks (Piffaretti et al., 1989; Nadon et al., 2001; Borucki et al., 2003), hence the 5-strain mixture contained these strains.

Antimicrobial Effectiveness of LAE in TSB, Skim Milk, and Whole Milk Against *L. monocytogenes*

The efficacies of LAE in TSB, skim milk, and whole milk against *L. monocytogenes* reduction for 15 d at 4°C are presented in Figure 1. In the absence of LAE (untreated controls), *L. monocytogenes* increased to 8.6 log cfu/mL in TSB and to 8.1 log cfu/mL in skim and whole milk in 15 d at 4°C from the initial inoculum level of 4 log cfu/mL (Figure 1, panels A, B, and C). Laurel arginate at 200 ppm was found to be highly effective in TSB: the original 4 log cfu/mL of *L. monocytogenes* was completely reduced in 30 min and remained undetectable for 15 d at 4°C (Figure 1A). The effects of LAE against *L. monocytogenes* in whole and skim milk are shown in Figure 1B and 1C. The addition of 200 ppm of LAE to skim and whole milks reduced ($P < 0.05$) *L. monocytogenes* counts by only 1.6 and 1.3 log cfu/mL, respectively, compared with untreated samples after 24 h (Figure 1B and 1C). No further decrease in *L. monocytogenes* counts was observed with 200 ppm of LAE in skim or whole milk, but *L. monocytogenes* counts remained lower by about 1 log cfu/mL compared with control samples after 15 d at 4°C. These studies reveal that a much higher concentration of LAE is required in milk compared with TSB to be effective against *L. monocytogenes*. Other studies reported that a 2- to 100-fold higher concentration of antimicrobial agents is generally required in food compared with that in broth media (Burt, 2004). For example, Mendoza-Yepes et al. (1997) reported that a mixture of essential oils from rosemary, sage, and citrus at 40 ppm was effective at inhibiting *L. monocytogenes* growth in tryptic soy broth, whereas a similar inhibitory effect in QFC required a concentration of 2,500 ppm. Jung et al. (1992) demonstrated that nisin at a concentration of 50 IU/mL was very effective in brain-heart infusion broth, resulting in 4 to 6 log cfu/mL reductions in *L. monocytogenes* populations compared with <1 log cfu/mL reductions in cream samples containing 12.7% fat. The possible mechanisms for the reduced activity of LAE in milk samples when compared with the broth system may be as follows: 1) the greater availability

Figure 1. Cold growth of *Listeria monocytogenes* in tryptic soy broth, skim milk, and whole milk during 15 d storage at 4°C after adding lauric arginate (LAE) at different concentrations. All food substrates were initially inoculated with the *L. monocytogenes* 5-strain mixture at 4.0 log cfu/mL (mean values $\pm$ SE bars are shown). (A) Efficacy of LAE at 0 ppm (control) and 200 ppm in tryptic soy broth; (B) and (C), efficacy of LAE at 0 ppm (control), 200 ppm, 400 ppm, and 800 ppm in skim milk and whole milk, respectively.

of nutrients in food systems, which can aid bacteria in repairing damaged cells (Gill et al., 2002); 2) intrinsic factors of foods such as fat or protein and antioxidant properties that can neutralize the effects of the antimicrobial (Burt, 2004); and 3) the lower water content in food substrates compared with laboratory media that limits the access of the antimicrobial to the bacterial sites (Smith-Palmer et al., 2001).

With an increase in LAE concentration, there was a proportionally higher reduction in *L. monocytogenes* counts in skim and whole milk (Figure 1B and 1C). Compared with those in control samples, *L. monocytogenes* counts decreased by 1.3 to 2.6 log cfu/mL with the 200 ppm LAE treatment, by 2.2 to 2.5 log cfu/mL with the 400 ppm treatment, and were undetectable (4.7–4.8 log cfu/mL reductions) with the 800 ppm treatment after 24 h at 4°C. Moreover, regrowth was observed in milk samples treated with 200 and 400 ppm of LAE, whereas there was no growth initiation with 800 ppm of LAE during 15 d of storage at 4°C. A listeristatic effect was observed in skim milk with 400 ppm of LAE, in which the *L. monocytogenes* population initially declined and remained below the original inoculum level for 15 d. In the whole milk treatment with 400 ppm of LAE, inhibition of *L. monocytogenes* growth was achieved for only 3 d before steady growth (Figure 1B and 1C). As experienced with other antimicrobial agents, one reason for such behavior could be the decreased lipid solubility of LAE in the whole milk samples compared with the skim milk samples (Burt, 2004).

Effectiveness of LAE Surface Treatment of QFC Against *L. monocytogenes*

During the first 3 wk of QFC storage, *L. monocytogenes* grew from an initial 4 log cfu/g to 8.3 log cfu/g in untreated controls (Figure 2). No further growth was observed in untreated controls after 3 wk. These results confirm that refrigerated storage is ineffective at controlling *L. monocytogenes* and therefore antimicrobial barriers are necessary to decrease or inhibit the growth of *L. monocytogenes* during cold storage (Schmid et al., 2009; Soni and Nannapaneni, 2010). Treatment with 200 ppm of LAE gave an initial reduction of 1.2 log cfu/g in *L. monocytogenes* compared with untreated controls. Overall, *L. monocytogenes* counts were approximately 2 log cfu/g lower over 21 d at 4°C following treatment with 200 ppm of LAE compared with untreated controls. With the 800 ppm treatment, *L. monocytogenes* counts were initially decreased by 3 log cfu/g within 24 h and remained stable for up to 7 d. After 7 d, regrowth of *L. monocytogenes* was observed but the final counts remained 2.3 log cfu/g lower ($P < 0.05$) than the untreated controls during 4 wk of stor-

Figure 2. Cold growth of *Listeria monocytogenes* in Queso Fresco (QFC) after surface treatment with 200 or 800 ppm of lauric arginate (LAE). The efficacy of LAE on QFC was determined over 28 d of storage at 4°C after inoculating with the *L. monocytogenes* 5-strain mixture at 4.0 log cfu/g (mean values $\pm$ SE bars are shown). Control samples were surface treated with a saline (0.8% NaCl) solution instead of LAE.

age at 4°C. In contrast, no surviving *L. monocytogenes* cells were detected in skim and whole milk samples treated with 800 ppm of LAE. Because QFC is semi-solid, the soft cheese curd is not very tightly bound and the surface is rough and porous. Therefore, it is very likely that some *L. monocytogenes* cells did not come in contact with LAE because of inaccessibility or possible penetration of *L. monocytogenes* into the porous region of the cheese.

Sensory Analysis

Consumers were unable to detect a difference ($P > 0.10$) between the control and LAE-treated QFC samples. Forty-five out of 120 panelists (37.5%) chose the correct QFC, which is slightly higher than the probability of randomly guessing which sample is different (33.3%). In addition, no differences ($P > 0.05$) existed among QFC samples with respect to aroma, flavor, texture, or overall acceptability, with mean acceptability scores between “like slightly” and “like moderately” (Table 2). However, consumers slightly preferred ($P < 0.05$) the appearance of the LAE-treated QFC to the control QFC. Acceptability results indicate that consumers could not differentiate between the samples and did not substantially differ in their liking of the product. Based on sensory results, it is unlikely that

Table 2. Mean hedonic scores for consumer acceptability of appearance, aroma, flavor, texture, and overall acceptability¹ of Queso Fresco cheese surface treated with 200 ppm of lauric arginate (n = 120)

Lauric arginate	Acceptability				
	Appearance	Aroma	Flavor	Texture	Overall
0 ppm	6.9 ^b	6.4 ^a	6.4 ^a	6.0 ^a	6.4 ^a
200 ppm	7.1 ^a	6.3 ^a	6.4 ^a	6.1 ^a	6.5 ^a
SEM	0.07	0.06	0.10	0.08	0.08

^{a,b}Means with the same letter within each column are not significantly different ($P > 0.05$).

¹Consumer acceptability was based on a 9-point scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely).

consumers could detect a sensory difference due to use of LAE in the product.

To date, a minimal number of studies have evaluated the bactericidal efficacy of LAE in food systems. Luchansky et al. (2005) reported a dose-dependent effect of LAE on *L. monocytogenes* reduction in ham with an initial 2 log cfu/sample reduction with 200 ppm of LAE and a negligible inhibitory effect during 60 d of product storage at 4°C. Taormina and Dorsa (2009) determined the short-term effect of LAE as a postlethality treatment of *L. monocytogenes*-challenged frankfurters and noted a ~2 log cfu/package reduction within 48 h. Martin et al. (2009) also demonstrated a ~1 log cfu/cm² reduction in *L. monocytogenes* counts by LAE treatment with no subsequent inhibitory effect and enhanced *L. monocytogenes* control in frankfurters by combining LAE with lactate or diacetate. Similarly, the results of our study in both milk and QFC samples revealed that LAE exhibits lethality toward *L. monocytogenes* cells early during storage, and the subsequent long-term bactericidal effectiveness is dependent on a higher application dose. Currently, there are no reports of sublethal injury of *L. monocytogenes* by LAE. In our studies, no survivors of *L. monocytogenes* were detected for up to 14 d when LAE-treated samples were plated from TSB, skim milk, or whole milk on *Listeria*-selective agar. Future studies are therefore needed to determine if any sublethal injury occurs in *L. monocytogenes* in the presence of low and high concentrations of LAE treatments in TSB or milk and QFC by comparing plating efficiencies on *Listeria* selective agar versus nonselective agar or *Listeria* repair agar containing sodium pyruvate (Donnelly, 2002; Pan and Breidt, 2007; Wu, 2008). In addition, quantitative real-time reverse-transcription PCR using *Listeria*-specific primers to a housekeeping mRNA can be attempted to recover total viable cells of *L. monocytogenes* under these conditions (Tasara and Stephan, 2007).

In conclusion, the FDA-approved dose of 200 ppm of LAE was effective in initially reducing *L. monocytogenes* populations by 1 to 2 logs in milk and QFC. However, there was no subsequent inhibitory effect by LAE;

therefore, *L. monocytogenes* cold growth resumed slowly during long-term storage of QFC at 4°C. However, LAE at 800 ppm was highly effective against *L. monocytogenes* cold growth at 4°C, which was clearly observed in milk possibly because of homogeneous mixing of LAE in milk samples compared with that in QFC. Further studies should be conducted by mixing LAE with other generally regarded as safe antimicrobial agents (such as sodium or potassium lactate and sodium diacetate) to evaluate synergistic or additive effects against *L. monocytogenes* cold growth. In this study, we did not evaluate the effect of LAE on other microflora because LAE was applied as a surface treatment. In further studies, the broad-spectrum efficacy of LAE against *L. monocytogenes* and other native microflora will be investigated in QFC by adding LAE during the salting step within the cheese production process.

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