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Microbial analysis of commercially available US Queso Fresco

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ABSTRACT

Queso Fresco (QF), a fresh Hispanic-style cheese, is often associated with *Listeria monocytogenes* outbreaks and recalls. Queso Fresco's susceptibility to bacterial contamination is partially due to its high pH and moisture content as well as *Listeria*'s tolerance for the salt content typical for QF. Nine different brands of US QF, 2 packages from 4 different lots (to account for temporal variability), were sampled. The pH, salt content, and moisture content were analyzed in addition to microbial testing including yeasts and molds, coliforms, lactic acid bacteria enumeration, and *L. monocytogenes* counts. The cheeses were also inoculated with a cocktail of 5 food and human isolates of food-borne outbreak-associated *Listeria monocytogenes* strains to evaluate how the differences between brands influenced *Listeria* growth. Three of the cheeses underwent additional genus-level microbial analysis using extracted 16S rDNA, allowing for phylogenetic analysis between bacterial taxa including diversity and relative abundance. We found little variation between the sampled QF pH (range = 6.62–6.86), salt content (1.53–2.01%), and moisture content (43.90–54.50%). Yeasts and molds were below the detection limit of enumeration in all of the cheeses and coliforms were below the detection limit across the first 3 lots, but were detected at varying levels in the fourth lot (>3.0 most probable number/g) for 3 of the brands. *Listeria monocytogenes* was not isolated after enrichment in any of the samples. All cheeses tested positive for the presence of lactic acid bacteria, with only 1 of the cheeses being labeled as produced with added cultures having substantial counts. Fourteen days after inoculation with *L. monocytogenes*, at least 2.5 log10 cfu/g of growth was found for all QF brands stored at 4°C. Microbial genus analysis showed that, among the 3 brands, the microbial community was more similar within brand than when compared with the other 2 brands. *Thermus*, *Anoxybacillus*, and *Streptococcus* accounted for the dominant genera of brands A, B, and C, respectively. These variations within the microbial community may account for sensory differences and help manufacturers determine quality control consistency more readily than culture-based methods.
Key words: *Listeria monocytogenes*, Queso Fresco

INTRODUCTION

The combination of the increasing Hispanic population in the United States (US Census Bureau, 2017) and an increased cultural introduction to the culinary styles of Latin America has increased the purchasing and consumption of Hispanic foods, as reflected by the increased production of Hispanic-style cheese (**HSC**) in the United States (USDA-NASS, 2016). Queso Fresco (**QF**) is a fresh HSC that is traditionally uncultured. Unlike a lot of cheeses, QF possesses a characteristic salt content, high moisture content, and near neutral pH. These characteristics can create a hospitable environment for microbial growth. Despite typically being consumed fresh, QF requires refrigeration to be safely preserved for up to a few weeks.

Dairy products and ready-to-eat foods are the most commonly associated foods with listeriosis (Batz et al., 2011). In particular, listeriosis has been frequently associated with HSC in the United States, with QF being recently recalled in 2014 and 2015 (CDC, 2017a). Unfortunately, *Listeria monocytogenes* can grow in QF under refrigeration, which greatly contributes to the problem (Leggett et al., 2012; Van Tassell et al., 2015). Immunocompromised and elderly adults, as well as children and pregnant women, are generally the groups that are of the greatest concern, with the greatest risk associated with pregnant Hispanic women due to their increased susceptibility and high consumption frequency (CDC, 2017b).

Pasteurization is an effective process of eliminating *L. monocytogenes* from milk. However, *L. monocytogenes* contamination during QF manufacturing and postpackaging are of particular concern, as *L. monocytogenes* has been shown to be persistent in manufacturing settings, including HSC manufacturing facilities (Hnosko et al., 2012; Ferreira et al., 2014), as well as domestic

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Table 1. The distribution of lots for each cheese brand and analysis[1]

Brand	Collected	Selective chemical characterization	Microbial analysis	Lactic acid bacteria	*Listeria monocytogenes* challenge	Microbiome analysis
A	1–4	2, 4	1–4	2	4	1–4
B	1–4	2, 4	1–4	2	4	1–4
C	1–4	2, 4	1–4	2	4	1, 2, 4
D	1–4	2, 4	1–4	2	4	—
E	1–4	2, 4	1–4	2	4	—
F	1–4	2, 4	1–4	2	4	—
X	1–3	2	1–3	2	—	—
Y	2, 4	2, 4	2, 4	2	4	—
Z	4	4	4	—	4	—

[1]Due to availability, 1 to 4 lots were collected, with 2 cheeses per lot, from each brand.

refrigerators (Kilonzo-Nthenge et al., 2008; Macías-Rodríguez et al., 2013). Consequently, antimicrobials that address postpasteurization contamination are needed to completely ensure the safety of QF (Ibarra-Sánchez et al., 2017).

The microbial population of different foods is influenced by the raw ingredients and environmental factors present during processing (Bokulich and Mills, 2013). The microbial community of industrially produced foods are characterized by a relatively simple community (Ercolini, 2013), which may affect pathogen growth upon contamination (Montel et al., 2014). Principal coordinate analysis has been used in other foods and cheeses to evaluate the microbial communities (Quigley et al., 2012; Aldrete-Tapia et al., 2014; Wolfe et al., 2014). By analyzing the phylogenic differences between the bacteria present across lots, a manufacturer may glean insights into processing consistency in that the lots will cluster based on phylogenetic distances and, thus, possibly reveal process deviations.

The objective of our study was to quantify a selection of chemical characteristics pertinent for microbial growth as well as to compare the microbial communities present in commercially available QF. Multiple QF of different brands and lots were purchased from local retailers in central Illinois, and their pH, salt content, and moisture content were determined. The cheeses were tested for *L. monocytogenes*, coliforms, yeast and molds, and lactic acid bacteria (**LAB**) counts. Bacterial microbiota was examined by profiling the 16s rRNA gene in a subset of cheeses. Finally, the cheeses underwent a *Listeria* growth challenge to determine if any of the differences among the brands would affect *Listeria* growth.

MATERIALS AND METHODS

Cheese Sample Collection

A total of 64 QF samples from 9 commercial QF brands, labeled A through F and X through Z (Table 1), were purchased from different supermarkets in central Illinois over a 7-mo period. All of the QF brands available in central Illinois were sampled. At least 3 of the brands are nationally available in the United States. For brands A through F, we were able to get 4 distinct lots. For brands X through Z, we were unable to acquire 4 distinct lots due to local unavailability, but they are included in the analysis where indicated. Two cheeses were purchased from each lot per brand, and packages were considered to be from the same lot if they shared the same lot number on their packaging. Duplicate cheeses were purchased at the same time before being transported to the laboratory on ice and held at 4°C. Analyses were conducted within 48 h after purchasing.

Selective Chemical Analysis

Queso Fresco samples from the second and fourth manufacturing lots collected were analyzed in duplicate. Three cheese portions were taken from each QF block using a stainless steel spatula by making radial cuts, and then all portions from the same cheese block were cut finely and mixed thoroughly. Moisture content was determined gravimetrically by drying 2-g samples in an oven at 105°C to constant weight (method 926.08; AOAC International, 2012). To determine the salt content, 1 g of sample was homogenized in 10 mL of deionized water, the mixture was centrifuged at 4,000 $\times$ *g* for 30 min at 20°C, and the supernatant was titrated with 0.1 *N* $AgNO_3$ in the presence of K_2CrO_4 (method 935.43; AOAC International, 2012). For pH determination, 1 g of sample was macerated in 10 mL of deionized water and the pH was measured by using a pH meter (Mettler Toledo, Columbus, OH; method 981.12; AOAC International, 2012).

Microbial Analysis

Individual cheese samples were tested for *L. monocytogenes* according to the FDA-Bacteriological Ana-

lytical Manual standard enrichment/recovery method, with some modifications (Hitchins et al., 2017). Briefly, 25-g samples (shaped radial sections) of each cheese were diluted 1:10 in modified *Listeria* enrichment broth (Difco, Franklin Lakes, NJ) supplemented with 1.1 g/L of sodium pyruvate. Homogenized samples were incubated at 30°C for 48 h, followed by selection on PALCAM agar plates (EMD Millipore, Billerica, MA), and incubated at 37°C for 48 h. After incubation, the plates were examined for typical black colonies with a halo (esculin positive), and results were expressed as positive or negative for recovery. Simultaneously, each cheese sample was tested for coliforms, yeast and molds, and LAB counts. Representative 5-g portions (shaped radial sections) of each sample were homogenized with 45 mL of PBS buffer for 1 min with a Stomacher 80 Biomaster (Seward, Bohemia, NY), and serial dilutions were prepared. Coliforms were tested by 3-tube most probable number (**MPN**) method, first by inoculating serial dilutions of cheese samples into lauryl tryptose broth (Difco) followed by confirmation with brilliant green lactose bile (Difco) broth (Feng et al., 2002). Yeasts and molds were enumerated by spread plating onto dichloran rose bengal chloramphenicol agar (Difco) following incubation at 25°C for 5 d (Tournas et al., 2001). Lactic acid bacteria were quantified by enumeration on MRS (Difco) or M17 agar (Difco) and incubated at 37°C for 48 h. The M17 agar and MRS agars were used for isolation and enumeration of lactic streptococci and *Lactobacillus*, respectively. Lactic acid bacteria enumeration data are presented in $\log_{10}$ colony-forming units per gram and coliform data are presented as MPN per gram.

Listeria monocytogenes Growth in QF

Three random shaped radial sections of each QF sample were milled and mixed, and 3 representative 5-g milled portions from each cheese block were collected from the fourth lot of each brand (excluding X due to brand availability) and placed in individual sterile sample bags. Milled cheese portions were inoculated with approximately 4 $\log_{10}$ cfu/g of a cocktail of food and human isolates of food-borne outbreak-associated *L. monocytogenes*, including 1 strain associated with a QF outbreak (Agricultural Research Service Culture Collection Northern Regional Research Laboratory strains B-33104, B33419, B-33420, B-33424, and B-33513). Samples were milled before inoculation to distribute the inoculum among all cheese particles and were not molded back into cheese bricks, as the growth of *Listeria* in QF is independent of the inoculation method (Van Tassell et al., 2017; Ibarra-Sánchez et al., 2018). Inoculated QF milled samples were mixed with a Stomacher 80 Biomaster and stored at 4°C. Cheeses were sampled after storage at 4°C for 0, 7, and 14 d. *Listeria monocytogenes* cells were enumerated by spread plating on PALCAM Listeria-Selective agar supplemented with 20 μg/mL of ceftazidime (VWR International, Radnor, PA). Plates were incubated at 37°C for 48 h.

Cheese Bacterial Microflora Profiling by Illumina 16S rRNA Gene Sequencing

To compare intra- and interlot microbial compositions between brands, total DNA was extracted from 200 mg of cheese (Table 1; 8 samples of A, 8 samples of B, 6 samples of C) using QIAmp DNA stool Mini Kit (Qiagen, Hilden, Germany) with bead-beating (Yu and Morrison, 2004; Barry et al., 2009). The DNA libraries were quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA). The 16S rRNA gene sequencing was performed by the DNA Sequencing Group at the Roy J. Carver Biotechnology Center, University of Illinois, using primers that amplified the V3-V5 variable regions of the 16S rDNA (Liu et al., 2017; Muturi et al., 2017), and the demultiplexed paired reads were stitched using IM-TORNADO pipeline (version 2.0.3.2; Jeraldo et al., 2014).

Further data analysis, including the generation of distance comparison boxplots (Figure 1B), was performed through the QIIME pipeline (Caporaso et al., 2010) and operational taxonomic unit picking was performed against SILVA ribosomal RNA database (release 128; Quast et al., 2013). The phylogenetic distance between sets of bacterial taxa in each cheese sample, including bacterial diversity and abundance, were measured by the weighted unique fraction metric (**UniFrac**). Principal coordinates analysis was performed to summarize the dissimilarity between the microbiota communities of each sample and plotted in R (Lê et al., 2008). The box and whisker plots were created using the UniFrac distances to compare distances within brands (comparing each individual package to all of the other packages of the same brand), between brands (comparison of each individual package to all of the packages from the other 2 brands), and within lots (the distance between an individual package and the other package from the same lot across all brands).

The Chao1, Shannon, and Simpson indices were used to measure the dissimilarity of microbiota community within each brand of cheese (α-diversity). Chao1 was used to evaluate the richness of total bacterial taxa in each brand, the Shannon index was used to evaluate the abundance and evenness of the bacterial taxa, and the Simpson index was used to evaluate the relative abundance of each taxon.

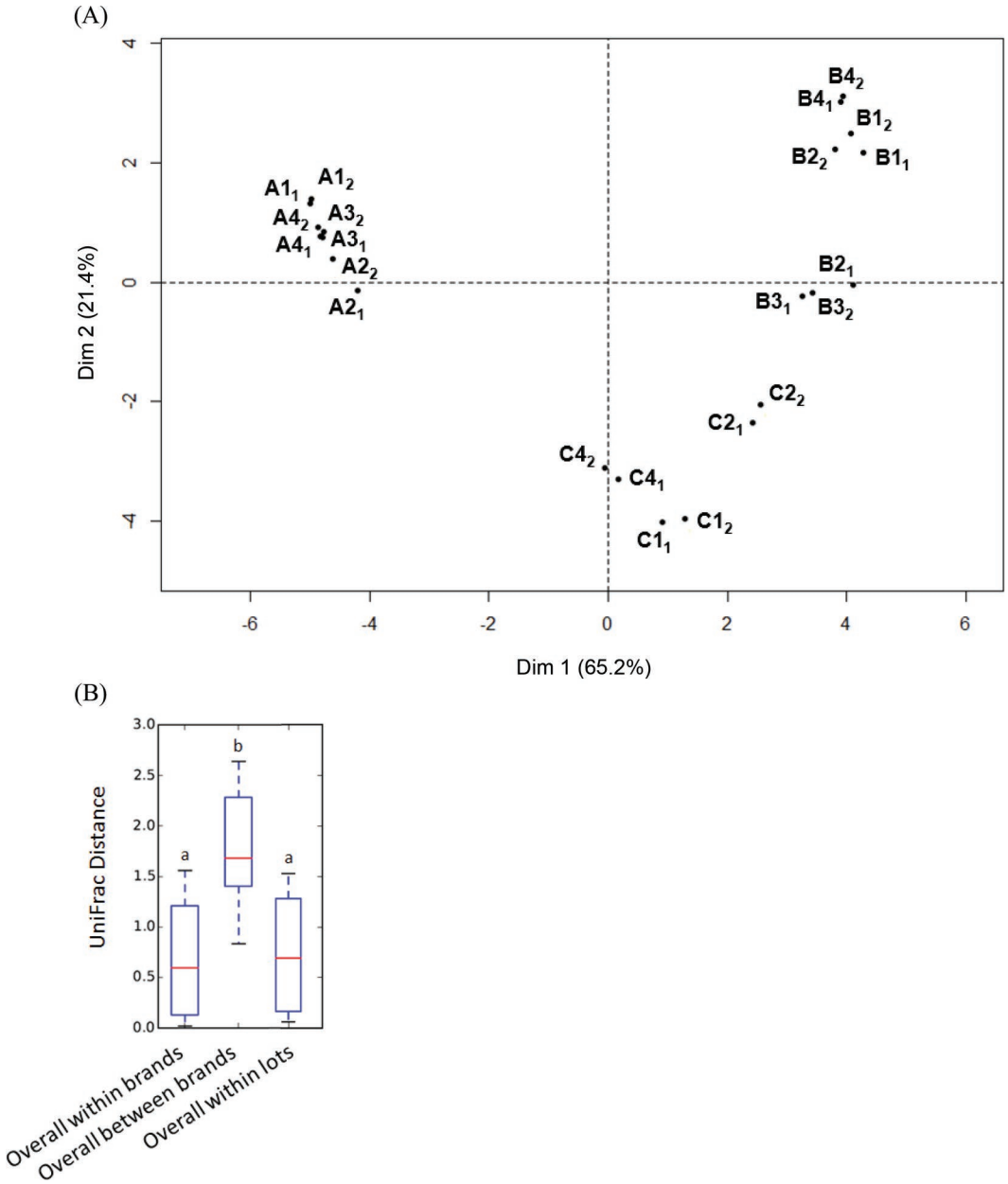

Figure 1. The microbial communities in the sampled Queso Fresco. (A) Principal coordinate analysis plot with 2 dimensions (Dim 1 and Dim 2) was generated based on weighted unique fraction metric, which represents the diversity and abundance of bacteria. The capitalized letters (A–C) represent the brands, numbers 1 to 4 represent different lots, and the subscripts (1,2) indicate different packages of cheese taken from the same lot. (B) Weighted unique fraction (UniFrac) metric distances describing the dissimilarity within each brand, between all 3 brands, and within lot. Boxes: top = third quartile; bottom = first quartile; midline = median. Whiskers: top = maximum; bottom = minimum. Lowercase letters (a,b) show statistical significance ($P < 0.05$). Color version available online.

Statistical Analysis

The Kruskal-Wallis analysis with Dunn's multiple comparison test was used to compare the α-diversity indices of the microbiota for brands A, B, and C. Analyses were performed using GraphPad Prism version 7.03 (GraphPad Software, La Jolla, CA).

RESULTS AND DISCUSSION

Selective Chemical and Microbiological Analysis

Cheese pH, salt content, and moisture content were all determined from 2 different lots from each brand (Table 1). The sampled cheeses showed little variation

for these attributes (Table 2). In regards to the cheeses made with cultured milk or added cultures (brands A, D, X, and Z), it has been shown that lactic acid-producing starter cultures can lower the pH of Latin-style cheeses during cold storage (Campagnollo et al., 2018). We can only extrapolate the relative age of the cheeses from the labeled expiration dates where, of the aforementioned cheeses, the cheeses from brand A were the farthest from its expiration date and the cheeses from brand X were the closest; however, the pH for brand X was 0.14 higher than brand A. Our results might suggest that the starter cultures used in the analyzed QF were poor acid producers in QF. Overall, the weight percent of NaCl and moisture content (1.53–2.01 and 43.90–54.50%, respectively) showed little variation among all collected brands. Queso Fresco is reported to have significant variation depending upon the manufacturer, with approximate values of pH ≥6.1, 1 to 3% salt content, and 46 to 57% moisture content (Van Hekken and Farkye, 2003), yet the sampled cheeses in our study were less variable despite being produced by 9 different manufacturers. It is important to note that there is no standard of identity for QF in the United States (Ibarra-Sánchez et al., 2017).

The cheeses all tested positive for the presence of LAB on both M17 and MRS agar (Table 2). Brands A, D, X, and Z were labeled as made with cultured milk, and only B was labeled as containing cultures. The labels did not specify the identities of the cultures. However, of these cheeses (A, D, X, Z, and B), only cheese D had high cell counts on M17 and MRS plates. Additionally, brands A, B, X, and Z all had fewer cell counts than the cheeses that did not label the addition of any cultures. The LAB cultures have been shown to lyse rapidly in cheese (Fox et al., 2017) and, additionally, the amount of salt and the cooking temperature can influence their lysis, resulting in lower starter culture cell counts from the initial inoculum (Hnosko et al., 2008; Fox et al., 2017). On the contrary, adventitious LAB (LAB that were not intentionally added) lyse slowly, are more tolerant to cooking temperatures, and grow rapidly in high moisture cheeses (Fox et al., 2017). The differences in the presence of added LAB cultures and adventitious LAB may account for the observed LAB cell counts, with high cell counts in noncultured cheeses and low cell counts in cultured cheeses (except brand D). Levels of LAB between 2 and 9 $\log_{10}$ cfu/g on M17 and MRS agar have been reported in other industrial HSC made with and without cultures added (Saxer et al., 2013). Cheese manufacturers may add attenuated LAB cultures (slow growing, no lactic acid production, but enzyme producers; Bevilacqua et al., 2017), as in brand B, to their cheeses in the hopes of flavor development, which may be the case for QF. Commercial companies such as International Media and Cultures (Denver, CO) and SACCO (Cadorago, Italy) offer attenuated cultures that can be used for QF manufacture and other Mexican cheeses.

Yeasts and molds were below the detection limit of enumeration (<2 $\log_{10}$ cfu/g) for all of the cheese samples from all 4 lots of each cheese. Using the MPN method to quantify the coliforms from all 4 lots, the cheeses in lots 1 to 3 all resulted in <3.0 MPN/g. In the fourth lot, only the cheeses from brand Y (>1,100 and 240 MPN/g) and brand Z (240 and 23 MPN/g), as well as 1 of the cheeses from brand A (9.2 MPN/g), had detect-

Table 2. Chemical characterization and microbial analysis of 9 different brands of Queso Fresco[1]

Brand	pH	NaCl content (%)	Moisture content (%)	Lactic acid bacteria M17[2] ($\log_{10}$ cfu/g)	Lactic acid bacteria MRS[3] ($\log_{10}$ cfu/g)
A[4]	6.63 ± 0.00	1.61 ± 0.05	51.49 ± 2.18	3.96 ± 0.68	3.46 ± n/a[5]
B[4]	6.67 ± 0.00	1.53 ± 0.04	46.82 ± 0.61	4.41 ± n/a[5]	—[6]
C	6.75 ± 0.06	1.67 ± 0.09	49.88 ± 0.2	4.57 ± 0.41	4.15 ± 0.25
D[4]	6.74 ± 0.04	1.81 ± 0.01	48.69 ± 1.13	9.88 ± 0.05	9.66 ± 0.03[7]
E[7]	6.74 ± 0.11	2.01 ± 0.05	48.51 ± 0.16	7.25 ± 0.16	7.28 ± 0.19[7]
F[7]	6.71 ± 0.08	1.72 ± 0.01	50.65 ± 0.23	8.32 ± 1.63	6.88 ± 0.21[7]
X[4]	6.77 ± 0.01	1.84 ± 0.02	54.50 ± 0.52	5.48 ± 0.52	5.58 ± 0.46
Y	6.85 ± 0.00	1.57 ± 0.02	43.90 ± 0.26	—[6]	6.69 ± 0.27
Z[4]	6.86 ± 0.01	1.83 ± 0.01	46.09 ± 1.62	4.74 ± 0.07	4.65 ± 0.33

[1]Values are means ± SEM.

[2]M17 agar is recommend for use in isolation, enumeration, and cultivation of lactic streptococci in dairy products.

[3]de Man, Rogosa, and Sharpe (MRS) agar is recommended for use in isolation, enumeration, and cultivation of *Lactobacillus*.

[4]Labeled as cultured milk or added cultures.

[5]One cheese sample had enumerable cell counts. n/a = not available.

[6]Enumeration data unavailable.

[7]Potassium sorbate added.

able coliforms. Others reported values for the coliforms present in pasteurized milk QF from Mexico, with an average of 4.6 $\log_{10}$ cfu/g (Renye et al., 2008). The microbial limits for QF are defined in Mexico in the Norma Oficial Mexicana as ≤100 cfu/g of coliforms and ≤500 cfu/g of yeasts and molds (Secretaria de Salud, 2010); however, in the United States, the microbial guidelines for cheeses are suggested by the National Committee on Microbial Criteria for Foods as <100 cfu/g of coliforms and <10 cfu/g of *Escherichia coli* (NACMCF, 2015). We found no discernable differences in the fourth lot of brand Y compared with the other cheeses, despite having the highest coliform count. The association between coliforms and cheese characteristics, such as pH and water activity, have been previously analyzed (Trmčić et al., 2016), but these associations have only been established for categories of cheeses and not for specific types of cheese. Additionally, specific coliform counts for QF are not reported within US sampling studies, making within-US comparisons unavailable; however, reputable manufacturers would want to be cognizant of any coliform issues. The fourth lot coliform variation could have been due to postprocessing contamination or mishandling as well as the time year the milk was collected (Gillespie et al., 2012). Additionally, all 64 cheeses sampled were negative for *L. monocytogenes* after enrichment.

Listeria Challenge

To determine if the addition of cultures (brands A, B, D, and Z) or preservatives (brands E and F), by some QF brands collected, affected postmanufacturing *L. monocytogenes* contamination, the cheeses were inoculated with a cocktail of *L. monocytogenes* strains. This cocktail, including strains associated with foodborne illness outbreaks, grew on all of the sampled cheeses during storage at 4°C, (n = 16, 8 duplicate cheeses). All cheeses were contaminated with a similar initial inoculum of *L. monocytogenes* cocktail (Table 3). After 7 d of cold storage, *L. monocytogenes* grew at least 1 $\log_{10}$ cfu/g in all QF samples. Fourteen days after inoculation, we found approximately a 1 log difference between the highest *Listeria* population (brand D, 7.27 ± 0.05 $\log_{10}$ cfu/g) and the lowest (brand C, 6.19 ± 0.08 $\log_{10}$ cfu/g) in the cheeses. The highest *Listeria* population was found in 2 cheeses labeled as having added cultures. Potassium sorbate, a chemical additive used in brands E and F, did not have any apparent effect on *Listeria* growth. However, even with a 50-d difference in expiration date, the cell counts of *Listeria* were similar between the cheese farthest from its expiration date (brand A) and the cheese closest to its expiration date (brand Y).

Greater growth would be expected if the temperature fluctuated to a higher temperature or if the cheeses were examined after a longer time (Mendoza-Yepes et al., 1999). A higher concentration of *L. monocytogenes* in the consumed food product will result in more illnesses (Buchanan et al., 1997), highlighting the need for antimicrobials that can, at a minimum, prevent *L. monocytogenes* from growing over QF shelf life. It is not surprising that the sampled QF were a suitable substrate for *Listeria* growth, considering that approximately 20% of listeriosis outbreaks are associated with high-moisture HSC (Ibarra-Sánchez et al., 2017). We have recently determined that combined treatment of nisin and PlyP100 (an endolysin) can dramatically reduce the *Listeria* population during refrigerated storage, suggesting that a preservative approach could dramatically reduce the number of listeriosis outbreaks (Ibarra-Sánchez et al., 2018). Many studies have analyzed *L. monocytogenes* growth to different chemical compositions in Mexican style cheeses and QF (Genigeorgis et al., 1991; Bolton and Frank, 1999); however, due to the lack of variation in the assessed chemical characteristics of the sampled QF in our study, little can be extrapolated in regard to whether any differences in these factors account for the observed differences in fold change (Table 3). It would be interesting to manufacture QF with greater variation, within the acceptable limits of QF compositional identity, to ascertain the effect that these factors have on *L. monocytogenes* growth. These results not only highlight the high-risk factor associated with contamination of QF, but also demonstrate

Table 3. Growth of *Listeria monocytogenes* in commercial Queso Fresco stored at 4°C

	Cell count[1] ($\log_{10}$ cfu/g)		
Brand	d 0	d 7	d 14
A[2]	3.71 ± 0.14	5.38 ± 0.16	6.70 ± 0.65
B[2]	3.63 ± 0.05	5.00 ± 0.11	7.03 ± 0.08
C	3.69 ± 0.01	4.78 ± 0.04	6.19 ± 0.08
D[2]	3.75 ± 0.06	5.22 ± 0.01	7.27 ± 0.05
E[3]	3.73 ± 0.12	4.86 ± 0.16	6.22 ± 0.22
F[3]	3.72 ± 0.01	5.00 ± 0.17	6.63 ± 0.08
Y	—[4]	5.00 ± 0.00	6.42 ± 0.12
Z[2]	3.70 ± 0.10	4.76 ± 0.15	6.70 ± 0.09

[1]Values are means ± SEM.

[2]Labeled as cultured milk or added cultures.

[3]Potassium sorbate added.

[4]*L. monocytogenes* could not be enumerated accurately due to background bacteria.

the need for manufacturing and antimicrobial interventions to address *Listeria*'s propensity for growth in QF.

Cheese Microbiota Community

To evaluate the microbial communities of randomly selected brands, a subset of cheeses (A, B, and C) were profiled with Illumina MiSeq (Illumina Inc., San Diego, CA), resulting in an average of 15,210 paired-end high-quality reads per sample. Genus-level analysis revealed the most abundant genera for each brand's microbial community. The top 8 genera accounting for greater than 1% of the total abundance in at least 1 of the sampled brands of QF are shown in Table 4. The dominant genera in brand A were *Thermus* (~87.70%) and *Streptococcus* (~8.63%; Table 4). *Streptococcus* and *Thermus* were also the dominant genera in brand C, but made up a lower percentage of the community when compared with brand A (~30.81 and 28.08% respectively). *Anoxybacillus*, a common dairy industry contaminant (Burgess et al., 2009; Goh et al., 2014), had a relative abundance of 13.38 ± 5.06% in brand C. *Anoxybacillus* and *Lactococcus* were the dominant genera (both >6% relative abundance) in brand B. We also found 74, 107, and 83 additional genera detected in brands A, B, and C, respectively. Each of these genera individually accounted for less than 1% of the total relative abundance of each cheese microbiome but collectively constituted approximately 3, 45, and 21% of the total abundance, respectively. *Streptococcus* is often a common starter pair with *Lactobacillus* in the production of pasteurized milk QF (Tunick and Van Hekken, 2010). *Streptococcus* has been shown to increase moisture retention in Manchego-type cheeses (Lluis-Arroyo et al., 2014) and the fresh HSC Panela (Jiménez-Guzmán et al., 2009). *Thermus* has been shown to enter cheese production facilities through hot water systems (Quigley et al., 2016), which may suggest that the dominant bacterial genera from brand A is a contaminant; however, further testing would be required by the cheese manufacturer to determine if this is the case. *Thermus* is often associated with a pink defect in cheeses, but QF shelf life is not long enough for the pink discoloration to be a problem.

Table 4. Percentage of bacterial genera (% total) in the sampled Queso Fresco[1]

Genus	A	B	C
Acinetobacter	0.30 ± 0.09	0.87 ± 0.24	3.41 ± 1.03
Anoxybacillus	0.05 ± 0.02	22.99 ± 13.41	13.38 ± 5.06
Bacteroides	0.02 ± 0.01	3.22 ± 2.54	0.09 ± 0.05
Lactococcus	0.14 ± 0.03	10.06 ± 3.95	0.33 ± 0.12
Mycoplasma	0.01 ± 0.00	6.31 ± 4.11	0.00 ± 0.00
Pseudomonas	0.30 ± 0.19	6.62 ± 4.16	4.94 ± 1.25
Streptococcus	8.63 ± 1.47	4.39 ± 2.60	30.81 ± 8.53
Thermus	87.70 ± 1.44	0.04 ± 0.01	28.08 ± 5.43
Other	2.85	45.5	20.99

[1]A: n = 8, B: n = 8, C: n = 6. Values are means ± SEM. Bacterial genera >1% in at least 1 brand of Queso Fresco.

Principal coordinate analysis of the QF microbiota revealed substantial clustering of the microbiota for each brand regardless of lot (Figure 1A). The individual packages (subscript 1 or 2) within each lot were also highly clustered, with only a single package from the second lot of brand B being far from its counterpart. Together, with the selected chemical analyses and microbial culture results, these results show that the cheeses were quite homogeneous within each lot. It is tempting to suggest that microbial community profiling could be used by QF producers as an additional tool to confirm they have a consistent process and identify when there is a production deviation (due to seasonality, milk sourcing, improper cleaning, and so on) when this analysis is more economically feasible for producers. Although it is pointless to pinpoint the current cost of microbial community sequencing, as it is rapidly decreasing, a recent review highlighted how the plummeting cost of sequencing and rising level of data interpretation is resulting in the value of microbial monitoring of food and food preparation systems justifying the expense for manufacturers (Bokulich et al., 2016).

Additionally, we were able to compare the dissimilarities among the brands and lots by measuring the phylogenetic distances between sets of bacterial taxa using UniFrac (Figure 1B). The lots within each brand had less UniFrac distance than between brands, highlighting the temporal consistency of each brands microbial community. Both brands A and B were labeled as having added cultures or being produced with cultured milk; however, the dominant genera from these brands did not indicate that this was a reason for their tight clustering. The overwhelming similarity within brand A was likely due to the consistent dominance of *Thermus* present in each of the samples (Table 4). The α diversity analysis revealed a significant increase in the microbial diversity and richness, as measured by the number of operational taxonomic units, Chao1 (richness of total bacterial taxa in each brand), Shannon (the abundance and evenness of the bacterial taxa), and Simpson (the relative abundance of each taxon) indices of brands B and C compared with brand A (Table 5). It would be valuable for the facility to perform microbial profiling at different locations to determine if the microbial population was endemic to the facility or was the result

Table 5. Alpha-diversity of cheese microbiota[1,2,3,4]

Brand	Chao1	Observed operational taxonomic unit	Shannon	Simpson
A (n = 8)	120.44[a] ± 13.56	59.63[a] ± 5.81	1.35[a] ± 0.07	0.37[a] ± 0.02
B (n = 8)	306.46[b] ± 49.04	182.88[b] ± 25.24	4.41[b] ± 0.63	0.77[b] ± 0.10
C (n = 6)	314.69[b] ± 34.35	163.17[b] ± 20.47	3.60[b] ± 0.32	0.79[ab] ± 0.05

[a,b]Values within columns with dissimilar letters are significantly different ($P < 0.05$) when compared using Dunn's test.

[1]Microbiota from cheese (n = 24, see Figure 1).

[2]Microbial analysis by Illumina 16S rRNA gene sequencing (V3-V5 hypervariable region; Illumina Inc., San Diego, CA).

[3]Rarefaction was calculated based on 3,110 sequences per sample when the maximum number of operational taxonomic units were observed in all groups.

[4]Values are means ± SEM.

of the different raw material suppliers used by each QF producer. In addition, it is unknown how these different microbial communities influenced the sensorial qualities of each brand. Regardless, QF manufacturers can potentially use microbial profiling as an additional tool to confirm a consistent product is being produced.

CONCLUSIONS

This work provides pH, salt content, moisture content, and microbial characterizations for a set of QF. The cheeses showed little variation in their pH, salt content, and moisture content, falling within previously reported ranges. However, the cheeses labeled as having added cultures tested among the lowest for LAB. Yeast and molds tested below the detection limit for all cheeses, and the fourth lot of 3 brands had cheeses that tested positive for coliforms, potentially due to post-processing contamination or mishandling. We showed that all of the sampled cheeses were suitable substrates for *L. monocytogenes* growth. Lastly, microbial profiling over several lots of 3 cheese brands revealed that each QF has a unique microbial fingerprint that is consistent across lots yet distinct from other brands. As the price of sequencing decreases, analyzing the microbial community over lots may help manufacturers determine quality control issues more readily than culture-based methods.

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