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Effect of modified atmosphere packaging on the growth of spoilage microorganisms and *Listeria monocytogenes* on fresh cheese

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ABSTRACT

Queso Fresco has a limited shelf life and has been shown to support the rapid growth of *Listeria monocytogenes* during refrigerated storage. In addition to improving quality and extending shelf life, modified atmosphere packaging (MAP) has been used to control the growth of pathogenic microorganisms in foods. The objectives of this study were to determine the effects of MAP conditions on the survival and growth of spoilage microorganisms and *L. monocytogenes* during storage of Queso Fresco manufactured without starter cultures. For *L. monocytogenes* experiments, cheeses were surface inoculated at ~4 $\log_{10}$ cfu/g before packaging. Inoculated and uninoculated (shelf life experiments) cheeses were placed in 75-μm-high barrier pouches, packaged under 1 of 7 conditions including air, vacuum, or combinations of N_2 and CO_2 [100% N_2 (MAP1), 30% CO_2:70% N_2 (MAP2), 50% CO_2:50% N_2 (MAP3), or 70% CO_2:30% N_2 (MAP4), 100% CO_2 (MAP5)], and stored at 7°C. Samples were removed weekly through 35 d of storage. *Listeria monocytogenes* counts were determined for inoculated samples. Uninoculated samples were assayed for mesophilic and psychrotolerant counts, lactic acid bacteria, coliforms, and yeast and mold. In general, cheeses packaged under conditions consisting of higher contents of CO_2 had lower pH levels during storage compared with those stored in conditions with lower levels or no CO_2 at all. Similarly, the antimicrobial efficacy of MAP in controlling spoilage microorganisms increased with increasing CO_2 content, whereas conditions consisting of 100% N_2, vacuum, or air were less effective. Mean *L. monocytogenes* counts remained near inoculation levels for all treatments at d 1 but increased ~2 $\log_{10}$ cfu/g on cheeses packaged in air, vacuum, and 100% N_2 (MAP1) conditions at d 7 and an additional ~1.5 $\log_{10}$ cfu/g at d 14 where they remained through 35 d. In contrast, treatments consisting of 70% CO_2 (MAP4) and 100% CO_2 (MAP5) limited increases in mean *L. monocytogenes* counts to <1 $\log_{10}$ cfu/g through 14 d and ~1.5 $\log_{10}$ cfu/g by d 21. Mean *L. monocytogenes* counts increased to levels significantly higher than inoculation (d 0) on cheeses stored in MAP2 on d 21, on d 28 for MAP3 and MAP4, and on d 35 for cheeses stored under MAP5 conditions. Overall, significant treatment × time interactions were observed between air, vacuum, and MAP1 when each was compared with MAP2, MAP3, MAP4, and MAP5. These data demonstrate that packaging fresh cheese under modified atmospheres containing CO_2 may be a promising approach to extend shelf life while limiting *L. monocytogenes* growth during cold storage.
Key words: *Listeria monocytogenes*, cheese, modified atmosphere packaging, Queso Fresco

INTRODUCTION

The Hispanic population in the United States increased by 43% to 50.5 million people between 2000 and 2010, which was 4 times the growth rate of the total US population at 10% (Ennis et al., 2011). With the growing Hispanic population and the incorporation of Hispanic cuisine into the American diet, per capita consumption of Hispanic-style cheese has more than doubled over the same period reaching 0.70 pounds (0.32 kg) in 2010 (Gould, 2017b). Similarly, Hispanic-style cheese production in the United States has grown from 96.3 million pounds in 2000 to more than 266 million pounds in 2016 (Gould, 2017a).

Queso Fresco (**QF**) is a soft, unripened, Hispanic-style cheese with a mild, slightly salty flavor and crumbly texture that is popular in ethnic markets and is often a staple in Hispanic households. Although a formal definition and federal standard of identity do not exist, QF has been generally characterized as having a high moisture content (>50%), low levels of sodium chloride, and low acidity (pH >5.8; Van Hekken and Farkye, 2003). Such conditions provide a favorable growth environment for contaminants including bacterial pathogens (Genigeorgis et al., 1991a; Kasrazadeh and Genigeorgis

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1994, 1995). Without a national standard, considerable variation in composition exists. Depending on the microbial and physicochemical compositions of the cheese, the shelf life is typically less than 1 mo and can be as short as 7 d (Villar et al., 1999; Renye et al., 2008; Bermúdez-Aguirre and Barbosa-Cánovas, 2010).

Increasing consumer demand for preservative-free, "clean label" foods favors the use of modified atmosphere packaging (**MAP**) to extend the shelf life of a variety of foods. Modifying the composition of the gases that surround a food product during storage, including N_2 and CO_2, can reduce physiological changes, oxidation reactions, and microbial growth (Mastromatteo et al., 2014). However, extending the shelf life of refrigerated foods raises concerns about the growth of the foodborne pathogen *Listeria monocytogenes*, which can reach high levels rapidly in QF when stored at refrigeration temperatures due to the cheese variety's relatively low acidity and high moisture content (Kozak et al., 2018). According to the Centers for Disease Control and Prevention's National Outbreak Reporting System, 11 listeriosis outbreaks between 2009 and 2015 were linked to cheeses knowingly produced from pasteurized milk, 6 of which were associated with the consumption of Mexican- or Hispanic-style soft cheeses including QF (CDC, 2018). Outbreaks linked to cheese produced from pasteurized milk highlight the need for strategies for controlling *L. monocytogenes* contamination of postlethality exposed ready-to-eat foods.

The potential of MAP to extend the shelf life of different dairy products has been demonstrated (Khoshgozaran et al., 2012). However, MAP is an under investigated approach for extending shelf life and controlling the growth of *L. monocytogenes* on QF and similar cheeses. Given the relatively hospitable composition of QF for supporting microbial growth, this cheese also serves as a worst-case scenario model for other cheeses. Therefore, the objectives of this study were to determine the effects of MAP conditions on the survival and growth of (1) spoilage organisms and (2) *L. monocytogenes* as surface contaminants on QF during refrigerated storage at 7°C.

MATERIALS AND METHODS

Bacterial Strains, Growth Conditions, and Inoculum Preparation

A cocktail of 8 *L. monocytogenes* strains was prepared as previously described (Kozak et al., 2018) using strains associated with outbreaks linked to soft cheeses or isolated from cheese processing environments, which included F5069/ATCC 51414 (milk-related outbreak), CWD 675–3 (Hispanic-style cheese-related outbreak), CWD 1567 (Hispanic-style cheese–related outbreak), Scott A (milk-related outbreak), 2012L-5323 (Ricotta salata cheese–related outbreak), 2014L-6025 (Hispanic-style cheese–related outbreak), DJD 1 (washed-rind cheese–related outbreak), and CWD 193–10 U5–2 (dairy plant food contact surface). The cocktail was serially diluted in Butterfield's phosphate buffer (**BPB**), pelleted through centrifugation (30 min, 4,000 × *g* at 4°C; Thermo Scientific Sorvall Legend X1R, Thermo Fisher Scientific, Waltham, MA), and resuspended in BPB to attain ~7 $\log_{10}$ cfu/mL.

Cheese Manufacture and Analysis

For *L. monocytogenes* growth experiments, QF was manufactured in the University of Connecticut Creamery according to a standard protocol using 50 gallons (189.3 L) of cow milk standardized to 3.5% fat using pasteurized skim milk and cream (Garelick Farms, Franklin, MA) as previously described (Kozak et al., 2018). Briefly, after filling the vat, calcium chloride was added to the milk at a level of 0.02% (vol/vol) and thoroughly mixed. Milk temperature was raised to 32°C and the pH was adjusted to 6.45 with dilute lactic acid (50% vol/vol in sterile water, 85% lactic acid, Sigma-Aldrich, St. Louis, MO). Chymosin (CHY-MAX DCI Star double strength microbial rennet, Dairy Connection, Madison, WI) was added at a rate of 15.83 mL/100 L, and milk was stirred for approximately 45 s to mix. Once desired firmness was reached, the coagulum was cut into 1 × 1 cm curds using sanitized stainless-steel curd knives. Curds were allowed to rest for 5 min and then stirred for 5 min, which was repeated for 3 cycles for a total of 30 min. After an additional 5 min rest, the majority of the whey was removed by draining. Curds were then transferred to standard 20# Wilson hoops lined with disposable cheese cloth and pressed at 68.95 kPa for 20 min. Pressed curds were then removed from the hoops, broken into individual curds, and kosher salt (Diamond Crystal, Cargill, Wayzata, MN) was added to obtain a target salt (as sodium chloride) concentration of ~2% in the final cheese. Salted curds were returned to the hoops and pressed at 68.95 kPa for an additional 75 min. For shelf life experiments, QF was manufactured in the laboratory using a laboratory-scale cheese vat (Labtronix Inc., Philomath, OR) according to the same standard protocol using 6 gallons (22.7 L) of cow milk standardized to 3.5% fat using pasteurized skim milk and cream (Price Chopper, Schenectady, NY). The only other change was that curds from laboratory-scale production were transferred to plastic hoops (15 × 10.5 cm; M605MTF066, Fromagex, Rimouski, Quebec,