Invited review: Hispanic-style cheeses and their association with *Listeria monocytogenes*

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ABSTRACT

The rise in consumption of Hispanic-style cheeses (HSC), due in large part to the increasing Hispanic population in the United States, has not been met with advances in food safety sufficient to prevent the numerous outbreaks and recalls due to *Listeria monocytogenes*. Hispanic-style cheeses are typically high moisture and have low salt content and low acidity from being subjected to little to no ripening. These conditions necessitate refrigeration to maintain safety and quality, as the majority of traditional extrinsic preservation methods are either ineffective or disrupt the mild sensory attributes of HSC. Unfortunately, the cold-growth of *L. monocytogenes* presents significant problems from post-pasteurization contamination or insufficient pasteurization. In this review, we discuss the factors affecting listerial contamination and growth in HSC, and present current knowledge of *L. monocytogenes* incidence in manufacturing settings and commercial prevalence. Furthermore, we differentiate HSC types by processing methods to aid with interpretation of works involving nonstandardized varieties and, finally, summarize research on intervention methods for eliminating listerial contaminants in HSC.

Key words: Mexican-style cheese, *Listeria monocytogenes*, food safety, antimicrobials

INTRODUCTION

Across the world, cheeses are consumed in numerous styles and broadly acknowledged for their nutritional value. About one-third of the milk produced in United States is used for cheese production, which is reflected in the broad variety of cheese varieties made in the United States, including American types, Italian types, Muenster, Swiss, and Hispanic-style cheeses (HSC; USDA-NASS, 2015). Hispanic-style cheeses

in particular, a category of cheeses referring to those originally developed and manufactured in Mexico and Latin America (Van Hekken et al., 2007), have been increasing in popularity among US consumers over the last 2 decades (Hnosko et al., 2009), which is reflected in the rise in both production and consumption. The HSC have shown an increase in production of 271% and increase in per capita consumption of 188% compared with that in 1996, a growth rate at least 3.5-fold higher than observed for Italian type cheeses, the largest cheese group produced and consumed in the United States (USDA-NASS, 1997, 2016; USDA-ERS, 2015). This trend is likely driven in large part by the growing Hispanic population in the United States, which increased 93.7% between 1996 and 2014, representing an estimated 17% (55 million persons) of the US population (US Census Bureau, 1997, 2015). However, the appreciation for HSC is not limited to Hispanic or Latin populations; the remarkable potential for its consumption by non-Hispanic consumers in the United States has been previously suggested (Clark et al., 2001).

The rise of consumption and production of HSC is expected to continue in the years to come, which has increased the need for proper awareness of the microbial safety concerns of this group of cheeses. It is well known that nonripened cheeses, such as fresh HSC, are prone to foodborne pathogen contamination, notably with *Listeria monocytogenes*. However, most HSC safety research has focused on Queso Fresco, leaving other commercially available varieties unaddressed (Torres-Llanez et al., 2006). Combined with the lack of identity standards for HSC in the United States, this has made it difficult to assess the listeriosis risks of other varieties and there has been no comprehensive assessment of *L. monocytogenes*-associated food safety risk between varieties of HSC.

This is unfortunate, considering the severity of listeriosis and its effect on public health. Infections by *L. monocytogenes* can develop into serious complications, including pneumonia, bacteremia, meningitis, or uterine infections that may result in miscarriage or stillbirth. Although listeriosis is rare, its high fatality rate among young, elderly, pregnant, or otherwise immunocompro-

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mised individuals has led the United States to declare *L. monocytogenes* 1 of 3 zero-tolerance microorganisms in ready-to-eat foods such as HSC. *Listeria* constitutes the greatest source of disease burden in dairy foods, with costs due to medical expenses, productivity loss, and mortality estimated to be between \$60 million and \$2 billion annually (Batz et al., 2012). The majority of dairy-associated listeriosis cases have been linked to HSC and soft-ripened cheeses (Batz et al., 2011). Costs borne by the dairy industry from recalls and outbreaks are unknown; however, individual outbreaks of pathogens in other foods have each been estimated to trigger hundreds of millions of dollars in nonmedical economic losses as well (Hussain and Dawson, 2013).

TYPES OF HISPANIC-STYLE CHEESES

Hispanic-style cheeses comprise a large and diverse group, albeit generally unstandardized. At least 30 different varieties are recognized in countries such as Mexico (Villegas de Gante, 2004). Currently there are 63 HSC-producing plants in the United States (USDANASS, 2015), manufacturing varieties including, but not limited to, Queso Fresco, Cotija, Oaxaca, Panela, Chihuahua, Queso Blanco, Asadero, Añejo, Manchego, Adobera, and Ranchero (CMAB, 2016). The HSC show considerable variation in shape, size, texture, moisture, and flavor due to differences in cheesemaking procedures such as curd setting methods, pressing, and ripening (Villegas de Gante, 2004). For further information on characteristics and production aspects of artisan cheeses most commonly produced in Mexico, see González-Córdova et al. (2016). Generally, HSC are characterized by their high moisture and are consumed shortly after manufacture. To help better understand the subtleties differentiating them, Figure 1 delineates several major processing steps that result in 11 select HSC types. The diversity among HSC allows for several ways to classify them but, for practical purposes, these cheeses can be divided into 2 main groups according to their degree of ripening: fresh and aged.

Fresh Hispanic-Style Cheeses

Most of the HSC are fresh (unripened), generally characterized by being soft, high moisture, having a mild fresh milk flavor, and being ready for consumption immediately after manufacture (e.g., Queso Fresco, Queso Blanco, Panela, and Ranchero). Moreover, *pasta filata* varieties (e.g., Oaxaca, Asadero, and Adobera), which involve kneading or stretching the curds much like mozzarella, are also considered fresh cheeses. As with all cheeses, HSC undergo varied manufacturing processes that differentiate styles and that may or may

not include a light “cook” step, curd milling, kneading, or pressing. Distinctive characteristics and manufacturing properties of these cheeses are summarized in Table 1.

Aged Hispanic-Style Cheeses

Aged HSC are hard or semi-hard cheeses subjected to ripening, leading to some degree of biochemical transformation of the curd, which affects numerous sensory characteristics. The degree of ripening of aged HSC is usually less than 1 mo, matching the preference of Hispanic consumers. Further description of distinguishing characteristics of prototypical aged HSC are outlined in Table 2. Aging of these cheeses, although less extensive than that of other aged varieties, may contribute to fewer food safety concerns than with fresh HSC, due to decreases in moisture content and pH.

FOOD SAFETY

Several steps can influence pathogen contamination, survival, or growth during manufacture of HSC. The source and microbial quality of milk, adjustment of fat content, milk homogenization, pasteurization, use of starter cultures, coagulation, extensive curd manipulation, hand stirring, salting, whey removal, milling, molding, and storage conditions may all contribute to increase the probabilities of cheese contamination (Ryser, 2007; Fernandez Escartin, 2008).

Traditionally, consumer preferences in Hispanic populations have led to a large proportion of HSC being made from raw milk (Villegas de Gante, 2004; Torres-Vitela et al., 2012), which may contain pathogens such as *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* (FDA, 2015). Consumption of raw-milk fresh cheeses constitutes a significant public health threat, evidenced by the number of outbreaks and recalls due to foodborne pathogen contamination.

Foodborne outbreaks caused by consumption of various cheeses have been tied to *Salmonella* spp., *Staphylococcus aureus*, *E. coli* O157:H7, and *L. monocytogenes* (Gould et al., 2014). It is recognized that HSC provide favorable conditions to support the growth or survival of several foodborne pathogens, including *Campylobacter* spp., *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes*, and their consumption has even caused illness due to norovirus infection. However, with the exception of *L. monocytogenes*, HSC consumption represents no more than 0.02 to 0.5% of the outbreaks due to these pathogens (CDC, 2015a). However, about 1 of every 5 listeriosis outbreaks have been traced to HSC (CDC, 2015a), highlighting the fact that such fresh cheeses support *L. monocytogenes* growth to high levels, as

Figure 1. Main cheese-making steps for manufacturing Hispanic-style cheeses.

refrigeration is generally the only post-manufacturing hurdle to pathogen growth (ILSI, 2005).

Physicochemical Characteristics as Risk Factors for *L. monocytogenes*

Listeria monocytogenes is able to survive in or on several food products and can grow as long as the conditions provided in the food matrix are favorable. Based on published data, fresh HSC that support the growth of *L. monocytogenes* include Queso Fresco (Genigeorgis et al., 1991a; Leggett et al., 2012; Van Hekken et al., 2012; Leong et al., 2014; Van Tassell et al., 2015), Queso Blanco (Uhlich et al., 2006; Leong et al., 2014), Panela, and Ranchero (Genigeorgis et al., 1991a). Often some *pasta filata* cheeses, such as Oaxaca, are considered low risk for pathogen carriage due to manipulation in hot water during manufacturing (thermoplastification). However, laboratory stud-

ies have shown that thermoplastification in water at 70°C is insufficient to kill food-borne pathogens such as *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* when pasteurized milk inoculated with those pathogens is used for Oaxaca cheese manufacturing (Fernandez Escartin, 2008).

Regarding aged HSC, Chihuahua and Manchego have been shown to allow survival of *L. monocytogenes* during manufacture and ripening, but they did not support its growth (Solano-López and Hernández-Sánchez, 2000). The pathogen was unable to initiate growth in Cotija cheese in a laboratory study (Genigeorgis et al., 1991a). However, the minimal ripening subjected to some commercial Cotija (Villegas de Gante, 2004), in contrast to genuine Cotija, may increase the risk for survival of *L. monocytogenes*.

The survival or growth of *L. monocytogenes* in or on different fresh or aged HSC is not surprising considering their gross physicochemical composition. Fresh

Table 1. Basic characteristics of selected fresh Hispanic-style cheeses

Name	Class	Cheese characteristics	Manufacturing distinction	Reference
Adobera	Fresh- <i>pasta filata</i>	Meltable, sliceable; final format resembles brick-like shape.	Rennet-set, milled texturized, uncooked, non-hot-kneaded, nonstretched, pressed. Industrial manufacture includes the addition of mesophilic culture. No thermoplasticity step during cheese-making, its melting behavior is due to its low pH (near 5.1–5.3).	Villegas de Gante, 2004
Asadero	Fresh- <i>pasta filata</i>	Meltable, shreddable; often confused with Oaxaca.	Rennet-set, uncooked, hot kneaded, stretched, nonpressed. Industrial manufacture includes the addition of thermophilic culture. Cheddarized curd is heated in a small volume of whey to promote plasticity and stretching. Different mold formats (e.g., blocks, balls, or discs).	Villegas de Gante, 2004; Villegas de Gante and Messner Guillen, 2015
Oaxaca	Fresh- <i>pasta filata</i>	Meltable, shreddable; referred to as Hispanic-style Mozzarella.	Rennet-set, uncooked, hot kneaded, stretched, nonpressed. Industrial manufacture can be performed by direct acidification (e.g., acetic acid) or by adding thermophilic culture. Stretching and kneading of acidified curd (near pH 5.1–5.4) in hot water. Typically molded like a ball of twine.	Villegas de Gante, 2004; Hnosko et al., 2009
Panela	Fresh	Soft, sliceable; final format is truncated cone shape; whey often oozes during cold storage.	Rennet-set, generally uncooked, self-pressed. Curd is molded in baskets and cheeses are auto pressed by piling them up on one another. Molding of the curd using a basket gives its particular shape.	Villegas de Gante, 2004
Queso Blanco	Fresh	Soft, mildly acid, sliceable; also named Queso para Freir (frying cheese).	Acid-set, uncooked, pressed. Curd is rapidly set by isoelectric coagulation by adding acetic, lactic or citric acid to hot milk (approximately 80°C).	Villegas de Gante, 2004; Hnosko et al., 2009
Queso Fresco	Fresh	Soft, crumbly; most widespread Hispanic-style cheese in the United States.	Rennet-set, generally uncooked, pressed. Salted curds are milled before pressing to enhance crumbling properties.	Hnosko et al., 2009
Ranchero	Fresh	Soft, crumbly; might be confused with Queso Fresco due to crumbly attributes.	Rennet-set, uncooked, nonpressed. Salt is added directly to dry milled curds and afterward molded in a galvanized metal, plastic, or wooden ring.	Villegas de Gante, 2004

Table 2. Basic characteristics of selected aged Hispanic-style cheeses

Name	Class	Cheese characteristics	Manufacturing distinction	Reference
Añejo	Aged	Crumbly; guajillo chili paste can be used to cover the ripened cheese.	Rennet-set, uncooked, nonpressed. Different degrees of crumbliness can be achieved during curd manipulation (multiple slicing, milling, and kneading steps). Unmolded genuine Añejo cheese is ripened over approximately 1 mo up to 12 mo at room temperature.	Villegas de Gaute, 2004; Hernández-Morales et al., 2010; Hernández Morales et al., 2011
Chihuahua	Aged	Semi-hard, sliceable, meltable; often called Menonita or Quesadilla cheese.	Rennet-set, uncooked or lightly cooked, pressed. Industrial manufacture includes the addition of mesophilic culture. Proper cheddardization (pH near 5.2–5.5) affects texture and ripening. Ripening period lasts from 15 d to 1 mo in cold room.	Villegas de Gaute, 2004
Cotija	Aged	Semi-hard, crumbly, acidic, salty; referred to as Hispanic-style Parmesan.	Rennet-set, uncooked, pressed. Genuine Cotija cheese made with raw milk is subjected to several months of ripening. Commercial Cotija cheeses might have ripening periods no longer than 2 wk or even no aging.	Villegas de Gaute, 2004
Manchego type	Aged	Semi-hard, sliceable, meltable; Hispanic-style version of Protected Designation of Origin Manchego.	Rennet-set, light cooked, pressed. Industrial manufacture includes the addition of mesophilic culture. Ripening period goes from 10 d to less than 1 mo at 10 to 12°C.	Villegas de Gaute, 2004; Hnosko et al., 2009

HSC are characterized by their high moisture, salt content as low as 1% and not greater than 3%, and near neutral pH (Table 3). Conversely, aged HSC have mid-level moisture, salt content similar to that of fresh cheeses or, in the case of Cotija cheese, slightly higher than 4%, and generally have pH no lower than 5.0 due to their short ripening periods. Additionally, both fresh and aged cheeses require storage at refrigeration temperature. *Listeria monocytogenes* is capable of growth in a wide range of temperatures (1 to 45°C) and pH (4.1 to 9.6), is tolerant of relatively harsh conditions such as high salt concentrations (10%), and can grow in the presence of numerous antimicrobial agents (Adzitey and Huda, 2010). As such, even under refrigeration, physicochemical properties of HSC contribute to increased *L. monocytogenes*-associated food safety risk.

Prevalence of *L. monocytogenes* in Hispanic-Style Cheeses and in Processing Plants

The prevalence of *L. monocytogenes* in commercial or handcrafted HSC varieties is not well understood. This is especially true in the United States, because the limited research into such prevalence has been largely conducted in Mexico (Table 4), where poor epidemiological surveillance also limits comparability of the listerial food safety landscape between countries (Castañeda-Ruelas et al., 2014). The presence of the pathogen has been documented in finished products and marketed cheese samples, showing greater prevalence in fresh cheeses, especially in handcrafted cheeses, although in some aged cheeses sampled (Chihuahua and Manchego), *L. monocytogenes* was not recovered (Table 4). It is important to highlight that only a small portion of HSC varieties have been investigated in surveys of *L. monocytogenes* in Mexico. In addition, US surveys have focused on detecting the presence of *L. monocytogenes* in Queso Fresco or HSC as a group, without distinguishing varieties commercially produced, leading to uncertainty in whether all HSC possess similar risk of *L. monocytogenes* contamination.

The presence of *L. monocytogenes* in HSC at the retail level has clear relevance in the aforementioned published surveys; however, studies that have investigated listerial prevalence in HSC processing facilities are scarce. Results have suggested that *L. monocytogenes* contamination occurs predominantly through post-processing steps (Table 5). One study conducted in New York City found that although isolation frequencies varied between 3 Latin-style fresh-cheese processing plants, *L. monocytogenes* could be isolated from drains, floors, and crates in all plants (Kabuki et al., 2004), suggesting that the multiple functions of crates (transportation of pasteurized milk and finished prod-

Table 3. Composition and pH values of selected Hispanic-style cheeses

Cheese variety	Moisture (%)	Protein (%)	Fat (%)	NaCl (%)	pH	Reference
Adobera	47	23.4	22.5	NA ¹	4.98–7.3	Villegas de Gante, 2004; Torres-Vitela et al., 2012
Añejo	31.8–41.9	21.44–30.3	25–33.8	1.8–2.8	5.1–5.4	Hernández-Morales et al., 2010
Asadero	42.16–53.01	20.74–29.93	18.36–32.23	0.76–1.93	NA	Alba et al., 1990
Chihuahua	35.14–42.4	22.1–27.3	27.3–37	0.78–2.18	5.19–5.42	Saltijeral et al., 1999; Van Hekken et al., 2007; Olson et al., 2011
Cotija	37.3–38.2	28.2–28.5	24–25	4.11–4.63	5.20–5.65	Villegas de Gante, 2004
Manchego type	38.7–45.5	24.41–27.59	24.89–31.91	0.88–2.5	5.09–5.51	Caro et al., 2014
Oaxaca	44.6–53	20–22.8	19.88–24.92	0.7–2.3	4.81–5.41	Caro et al., 2014; Fuentes et al., 2015
Panela	49–59.4	16.24–20.56	15.44–22.16	1.03–2.13	4.92–6.53	Saltijeral et al., 1999; Torres-Vitela et al., 2012; Caro et al., 2014
Queso Blanco	47.02–50	19.6–25	18.2–24.31	2.32–3.3	5.2–6.8	Villegas de Gante, 2004; Uhlich et al., 2006; Leong et al., 2014
Queso Fresco	49.4–58.9	14–19.7	20.5–31	1.02–2.7	5.26–6.77	Tunick and Van Hekken, 2010; Leggett et al., 2012; Tunick et al., 2012; Van Hekken et al., 2012, 2013
Ranchero	50.1–56.9	22.6–25.6	17.4–26.4	0.8–1.8	4.9–5.4	Solís-Méndez et al., 2013

¹NA = data not available.

uct, and storage of finished products in cooler rooms) may contribute to the spread of contaminants from different areas within the plant.

Other studies conducted in Mexico have tracked the presence of *L. monocytogenes* through the cheese-making process and environment, across milk samples, processing plants, and retail samples, and suggest that food contact surfaces likely constitute recurrent sources of contamination more commonly than raw or improperly pasteurized milk (Moreno-Enriquez et al., 2007; Rosas-Barbosa et al., 2014). *Listeria monocytogenes* strains were isolated from milk and curd tanks, utensils, baskets, cheese molds, refrigerators, brooms, and other handling surfaces. In positive cheese samples (Panela,

Queso Fresco, and Adobera), common outbreak serovars 1/2a, 1/2b, and 4b were found, suggesting that consumption of these cheeses may increase the risk of listeriosis (Rosas-Barbosa et al., 2014). These studies illustrate that contamination at the processing level represents an important source of *L. monocytogenes* regardless of the pasteurization status of the milk used for making HSC.

Indeed, it has become clear that *L. monocytogenes* persistence in food-associated environments contributes significantly to the prevalence of contaminated food products, recalls, and listeriosis outbreaks (Ferreira et al., 2014). Whole-genome sequencing has become invaluable in US listeriosis outbreak investigations, pro-

Table 4. Prevalence of *Listeria monocytogenes* in some types of Hispanic-style cheeses in Mexico (MX) and the United States (US)

Cheese variety	Prevalence (positive samples/total samples)	Country	Reference
Adobera	12% (12/100)	MX	Torres-Vitela et al., 2012
	18.75% (3/16)	MX	Rosas-Barbosa et al., 2014
Chihuahua	0% (0/40)	MX	Saltijeral et al., 1999
	0% (0/60)	MX	Alcázar Montañez et al., 2006
Manchego type	0% (0/40)	MX	Saltijeral et al., 1999
Panela	15% (6/40)	MX	Saltijeral et al., 1999
	0% (0/60)	MX	Alcázar Montañez et al., 2006
	6% (6/100)	MX	Torres-Vitela et al., 2012
	37.5% (6/16)	MX	Rosas-Barbosa et al., 2014
Queso Fresco	2.75% (48/1,746)	US	USDA-FSIS, 2003
	3.4% (5/149)	MX	Moreno-Enriquez et al., 2007
	9.3% (7/75)	MX	Soto Beltran et al., 2015
	6.25% (1/16)	MX	Rosas-Barbosa et al., 2014
Hispanic-style cheese, variety unspecified	2% (2/100)	US	Genigeorgis et al., 1991b
	0.17% (5/2,931)	US	Gombas et al., 2003
	6.3% (7/111)	US	Kabuki et al., 2004
	1% (2/204)	MX	Kinde et al., 2007

viding significantly greater epidemiological resolution relative to prior methods and allowing for enhanced tracing of foodborne illness back to specific manufacturers (Jackson et al., 2016). Subtyping isolates from the processing environment via whole-genome sequencing could help differentiate sporadic isolates from persistent strains that may pose recurring problems and identify associated process deviations or sources responsible for their introduction to final products (Stasiewicz et al., 2015). To our knowledge, such an approach has yet to be implemented within manufacturing facilities for the identification of persistent contaminants in HSC but could inform intervention strategies for targeting their sources.

Additionally, it is unclear to what extent consumer contamination of HSC contributes to foodborne illness; however, poor food storage and handling, combined with the prevalence of pathogens such as *L. monocytogenes* in home refrigerators, undoubtedly contributes in some capacity (Kilonzo-Nthenge et al., 2008; Macías-Rodríguez et al., 2013). Consumer handling of ready-to-eat foods, particularly improper storage, is a known contributor to elevated risk of foodborne listeriosis (Yang et al., 2006), and susceptibility of HSC to further listerial growth during storage suggests that additional hurdles should be implemented to account for this risk.

Listeriosis Outbreaks Associated with Hispanic-Style Cheese Consumption

Each year in the United States, approximately 1,600 persons become seriously ill as a result of *L. monocytogenes* infection, and 260 of these persons die due the infection (CDC, 2014a). During the period from 1998 to 2014, 56 confirmed listeriosis outbreaks were recorded, resulting in 707 illnesses, 520 hospitalizations, and 116 deaths (CDC, 2015a). Of these outbreaks, 11 (19.6%)

implicated HSC, comprising 98 illnesses, 60 hospitalizations, and 5 deaths. Although the majority of listeriosis cases affect the elderly (age ≥ 65 yr; Silk et al., 2012), the greatest risk of listeriosis is shared by pregnant Hispanic women (Pouillot et al., 2012) due to their more frequent consumption of HSC and heightened susceptibility to listerial infection during pregnancy, although socioeconomic factors may contribute as well (Gillespie et al., 2010).

Insufficient pasteurization was the cause of listerial contamination found in the first identified HSC outbreak in the United States. This 1985 outbreak of listeriosis from Mexican-style cheese in Los Angeles County, California, resulted in 142 illnesses and 3 deaths, allegedly from insufficient pasteurization of milk or the introduction of raw milk into pasteurized milk during manufacturing (Linnan et al., 1988). Similarly, between October 2000 and January 2001, a listeriosis outbreak occurred in Winston-Salem, North Carolina, resulting in 13 illnesses, including 5 stillbirths, 3 premature deliveries, and 3 infected newborns. Illnesses were traced to the consumption of Mexican-style cheeses made from contaminated raw milk by unlicensed cheesemakers (MacDonald et al., 2005).

More commonly, however, outbreaks are traced back to the production of fresh cheeses under conditions that allow post-pasteurization contamination from the manufacturing environment, as described above. A 2008–2009 multistate listeriosis outbreak, resulting in 8 illnesses across 5 states, was associated with a Mexican-style cheese manufacturing facility where multiple cheese varieties tested positive for the outbreak strain, suggesting post-pasteurization contamination of pasteurized milk (Jackson et al., 2011). Several other multi-state outbreaks have been linked with HSC recently, resulting in 8 cases and 1 death across 2 states in late 2013 (CDC, 2014b), 5 illnesses and 1 death

Table 5. Occurrence of *Listeria monocytogenes* isolates from Hispanic-style cheese processing plants

Type of sampling site	Prevalence (positive samples/total samples)	Reference
Milk	0% (0/47)	Moreno-Enriquez et al., 2007
	0% (0/16)	Rosas-Barbosa et al., 2014
Curds	10% (3/30)	Rosas-Barbosa et al., 2014
Equipment	19.3% (33/171)	Rosas-Barbosa et al., 2014
Food contact surfaces	1.7% (2/119)	Kabuki et al., 2004
	33.3% (10/30)	Moreno-Enriquez et al., 2007
	29.2% (14/48)	Rosas-Barbosa et al., 2014
Floors	25.7% (19/74)	Kabuki et al., 2004
	6.3% (1/16)	Rosas-Barbosa et al., 2014
Cheeses	6.3% (7/111)	Kabuki et al., 2004
	3.4% (5/149)	Moreno-Enriquez et al., 2007
	17.5% (11/63)	Rosas-Barbosa et al., 2014
Others	11.3% (6/53)	Kabuki et al., 2004
	0% (0/29)	Moreno-Enriquez et al., 2007
	31.3% (10/32)	Rosas-Barbosa et al., 2014

across 4 states through 2014 (CDC, 2014c), and 30 illnesses and 3 deaths in 10 states from the summer of 2010 to 2014 (CDC, 2015b). Strains of *Listeria* have been traced back to environmental samples from the manufacturing facilities for each of these outbreaks, connecting observations of unsanitary conditions with post-pasteurization contamination of the cheeses. In one of these outbreaks, US Food and Drug Administration inspection reports cited particularly egregious violations, including food residue remaining after cleaning procedures, uncovered storage and transfer containers, deteriorated infrastructure that precluded effective cleaning or sanitation, and water leaking from the roof onto equipment, all of which could facilitate contamination of *L. monocytogenes* after pasteurization (FDA, 2016).

INTERVENTIONS

Industry and federal guidance documents provide extensive recommendations for the control of *L. monocytogenes* at the manufacturing level, including facility and equipment design factors, cleaning and sanitation procedures, and environmental and product monitoring techniques, as well as personnel training and food formulation, handling, and storage guidelines (FDA, 2008; IDFA, 2015). Adhering to these standards should minimize opportunities for listerial contamination or growth in HSC manufacturing settings. In practice, however, allowing good manufacturing procedures to remain the primary preservation method for HSC clearly risks insufficient or inconsistent implementation, as described above, resulting in the numerous outbreaks and recalls observed. Although execution of environmental controls for preventing contamination must clearly be improved to adequately address the risks of listerial contamination in many situations, additional interventions and additives have also been sought for the prevention of listerial growth and survival in fresh cheeses.

Antimicrobial Treatments to Reduce *L. monocytogenes* in Hispanic-Style Cheeses

Relatively few studies have been published on the examination of antimicrobial methods for the preservation of fresh HSC. To date, almost all studies have been carried out using Queso Fresco as an HSC model with *L. monocytogenes* or *Listeria innocua*.

High-pressure processing is often used as a final hurdle to preserve packaged ready-to-eat foods such as sausages, so it was understandably investigated to address pathogens in HSC. While pressures of 500 to 600 MPa can considerably reduce listerial populations in Queso Fresco (>5 log cfu/g), high-pressure processing

cannot prevent regrowth of surviving cells (Tomasula et al., 2014; Hnosko et al., 2012) and compromises protein structure and textural properties in the finished products, resulting in negative effects on consumer sensory preferences (Sandra et al., 2004; Hnosko et al., 2012; Van Hekken et al., 2013).

Organic acids, bacteriocins, and other fermentates, as well as combinations thereof, have been assessed as antilisterial agents for Queso Fresco with limited success. Overcoming the instability and low activity of organic acids near the neutral pH of HSC by combining these combinations with the bacteriocin nisin can improve listerial reduction but are generally bacteriostatic at best (Gadotti et al., 2014; Van Tassell et al., 2015), although they may not diminish consumer acceptability. Queso Blanco manufactured with acetic acid and a commercial bacterial fermentate has also been shown to reduce *L. monocytogenes* by 2 to 3 log cfu/g over a 6-wk shelf life but did not eliminate the pathogen, even at low inoculation levels (Glass et al., 1995). Another promising additive used in meat and poultry—lauric arginate—has been demonstrated to maintain consumer acceptability when applied to Queso Fresco and exhibits moderate reductions in listerial survival (Soni et al., 2010), especially when used in combination with a listerial bacteriophage or a potassium lactate–sodium diacetate mixture (Soni et al., 2012).

Inhibition of surface contamination via antimicrobial packaging materials may also improve protection of HSC, as they have shown promise on meat, produce, and other cheeses (Moreira et al., 2011; Irkin and Esmer, 2015). Edible packaging films of chitosan and lactic acid have been shown to reduce *L. monocytogenes* on the surface of Queso Fresco, enhancing pathogen inhibition further with the grafting of other components within the films (Sandoval et al., 2016); however, significant moisture loss in the cheeses was observed during storage. Effects on HSC quality by such coatings remain to be seen, as do their effectiveness against non-surface contaminants, such as those introduced during curd manipulation. For further discussion on the potential and difficulties of antimicrobial food packaging, see Malhotra et al. (2015).

The quest for antilisterial treatments in HSC, illustrated by the aforementioned studies, is characterized by 2 main gaps. First, even though Queso Fresco is the best known HSC in the United States, it is not necessarily representative of all HSC: different manufacturing processes may imply not only different listerial contamination scenarios, but also that antimicrobial treatments may show different efficacy depending on the cheese variety. Additionally, studies have focused primarily on preservation methods less likely to affect the subtle taste and delicate texture of fresh HSC;

however, confirmation of sensory impact or consumer acceptability is less commonly reported. A food antimicrobial agent should meet 3 important aspects in the food product: (1) its antimicrobial activity should be selective, (2) it should have negligible effect on texture and sensory properties, and (3) it should be safe for human consumption (Oliveira et al., 2012). Therefore, evaluating an antimicrobial with potential application to food industry should not be limited only to its antimicrobial properties.

Cheese Surrogates

The simplest method for examining the effect of antilisterial treatments for fresh cheeses involves contaminating and treating commercially prepared or otherwise finished cheeses. Several treatments have been tested by taking slices of prepared cheese and applying antimicrobials and *L. monocytogenes* to the surface, and these have shown effectiveness at reducing bacterial growth over product shelf life (Soni et al., 2010, 2012; Malheiros et al., 2012). This is a quick and effective method for assessing antimicrobial efficacy on finished cheeses but it may offer a narrow view of antimicrobial treatment: antimicrobial interactions are confined to an environment with limited impact by the cheese matrix itself. Homogenization of samples confers greater incorporation of the components into the product, but the disrupted microstructure may poorly reflect the interactions taking place between contaminants and antimicrobials within commercial applications. Unless the antimicrobial treatment is intended solely for external application, its design should consider the logistics of cheesemaking. In this case, laboratory-made cheeses should be preferred over already prepared samples for studying preservatives, to allow for the cheese model to resemble the most appropriate steps for contamination and antimicrobial addition without altering the manufacturing process.

Incorporation of pathogens on a large scale can be cumbersome for maintaining proper biosafety, so these methods often rely on homogenization of the finished cheeses before inoculation, but modifying cheese manufacturing processes or incorporating novel antimicrobials into pilot-scale cheese production to assess the impact on pathogen growth and survival is not unheard of (Kasrazadeh and Genigeorgis, 1995; Bolton and Frank, 1999). *Listeria monocytogenes* has been shown to survive and grow when incorporated into the production process for soft cheeses under various conditions (Leggett et al., 2012), even when commercial starter cultures are present (Solano-López and Hernández-Sánchez, 2000; Leuschner and Boughtflower, 2002). Screening antimicrobial agents in relatively large batches of cheese can

be costly, although combinations of organic acids and nisin have shown to exhibit inhibitory effects (Gadotti et al., 2014). Coelho et al. (2014) used a much smaller batch size (0.5 L) to assess the antilisterial effects of incorporating bacteriocin-producing cultures into fresh cheeses, which raises the question of what scale is actually necessary for modeling fresh cheese production.

High-throughput, miniaturized cheese manufacturing models have been explored for their use in screening starter cultures and antimicrobials, resulting in cheeses with comparable structure to traditional equivalents despite preparation in 96-well microplates (Bachmann et al., 2009). We recently developed a similar model that now shows promise for screening antimicrobial additives in the production of miniature fresh cheeses (Van Tassell et al., 2015). Such miniaturized cheese models can be adapted to address numerous manufacturing parameters while benefitting from greater replication with minimal capital or resource consumption and improved biosafety management. In this way, antimicrobial treatments can be assessed in situ for intact cheeses under different treatment or contamination scenarios, reflecting commercial applications more appropriately.

Use of a surrogate organism for modeling listerial contamination of cheeses should also be addressed with care. Because of its close phylogenetic relatedness and similar physiology, nonpathogenic *L. innocua* can be used in place of *L. monocytogenes* for experimental work. However, variations in stress response may result in reactions to different antimicrobials that are not comparable between organisms under different environmental conditions. Therefore, caution should be taken in extrapolating results from the use of a surrogate, and validation with *L. monocytogenes* strains is encouraged for any given application. For a thorough review of this subject, see Milillo et al. (2012).

FUTURE DIRECTIONS

Relatively little is known about the association of *L. monocytogenes* with specific varieties of HSC. Most studies approach Queso Fresco as being representative of the category; however, this may not be the case. It is unclear to what extent different manufacturing steps and variations in the finished product affect epidemiologic factors, as our knowledge of *L. monocytogenes* in commercial products is largely outdated and limited to Queso Fresco, especially in the United States. More current knowledge of incidence, particularly in other varieties of HSC, would be informative for risk analysis and preventative purposes.

Advances in monitoring and sanitation in dairy processing plants may contribute significantly to preventing further outbreaks of listeriosis due to HSC, especially

considering the proportion of contamination likely due to post-pasteurization contact with environmentally persistent strains. One such area with room for growth involves the use of next-generation sequencing tools for microbial community profiling, such as already demonstrated in some wineries and artisanal cheese producers (Bokulich and Mills, 2013; Bokulich et al., 2016). More thorough knowledge of typical microbial communities and “abnormal” or “unsafe” deviations therefrom could perhaps help track potential sanitation issues and avoid environmental persistence of *L. monocytogenes*.

Furthermore, the extensive exploration of model cheese systems could promote deeper understanding of the interactions between antilisterial treatments, their targets, and food matrices. Such models could similarly facilitate the screening of combinatorial treatments that may exhibit synergistic mechanisms of antimicrobial action and the optimization of application parameters for their use in processing environments. Novel antimicrobials that may function as enhanced food preservatives include bioengineered bacteriophages or phage endolysins (Van Tassell et al., 2016). It would also be necessary, however, to overcome the confines of laboratory testing and confirm efficacy among the complexities of a commercial manufacturing setting.

As more attention is drawn to the field, collaborative and interdisciplinary efforts will ultimately produce a cost-efficient, consumer-friendly solution to effectively limit *L. monocytogenes* in Hispanic-style cheeses.

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