

Risk Assessment of Fresh Hispanic Cheeses and Technologies to Improve their Safety

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Introduction

The safety of fresh, Hispanic cheeses has become increasingly important to American cheese producers because the demand for these types of cheeses is increasing in recent years. The volume of Hispanic cheese produced in the United States increased from 76 million pounds in 2000 to 193 million pounds in 2008 (Service, U.S. D.o.A-N.A.S. 2008). Due to their high moisture and neutral pH, fresh Hispanic cheeses pose a particular risk for consumers as they are susceptible to bacterial growth even at refrigeration temperatures. The presence of psychrotrophic bacteria such as *Listeria monocytogenes* could lead to potential foodborne outbreaks. Most notably, an outbreak of *Listeria monocytogenes* occurred in 1985 in California from the Hispanic cheese “queso fresco”. This outbreak involved 142 illnesses, and resulted in 48 deaths (Linnan et al., 1988).

Based on this potential for investment in Hispanic cheese production, research is greatly needed to find treatments to control microorganisms in fresh, Hispanic cheeses. *Listeria monocytogenes* is the major concern, related to the safety of fresh Hispanic cheeses, but recently *Salmonella* and *Escherichia coli* O157:H7 also appear to pose significant risk of transmission. Physical, chemical and processing treatments have been assessed for their effectiveness in fresh Hispanic or similar cheeses. Some of these include pasteurization, high pressure processing and irradiation, as well as the use of nisin, caprylic acid, sodium lactate, potassium sorbate, sodium benzoate, and a number of bacteriocin producing microorganisms (Alvarado et al, 2005; Dougle and Stahv, 1994; Johnson and Law, 1999; Kamnetz, 2009; Kasrazadeh and Genigeorgis, 1995; Sandra, Stanford and Goddik, 2004).

Other techniques related to the manufacturing process can also be used to minimize the risks of contamination.

Microbial risks associated with fresh Hispanic cheeses

Fresh Hispanic cheeses provide an optimal environment for pathogen growth because it lacks several inhibitory properties that fermented and ripened cheeses possess. These properties include their relatively high moisture levels, low salt content and high pH (Kasrazadeh and Genigeorgis, 1995). The fresh, Hispanic cheese that has been linked most frequently to outbreaks is “queso fresco”. Queso fresco’s typical composition includes a moisture content of 46 to 57%, and a low salt content of 1 to 3% (Van Hekken and Farkye, 2003). The pH of queso fresco tends to be relatively neutral compared to other cheeses, with a range from 5.8 to slightly less than 7.0.

Another characteristic of fresh Hispanic cheeses that make them susceptible to pathogen survival and growth is the lack of starter cultures used during their manufacture. The absence of starter cultures contributes to the fresh Hispanic cheeses’ neutral pH. Without competition from starter culture bacteria, the growth of pathogenic bacteria in cheeses like queso fresco is not normally inhibited (Kasrazadeh and Genigeorgis, 1995). The lack of inhibitory conditions not only allows the growth of potentially pathogenic organisms, but also leads to queso fresco’s short shelf life (Villar et al., 1999).

In addition to the lack of antimicrobial barriers when compared to other types of cheese, fresh Hispanic cheeses are also at risk for contamination due to the frequent use of unpasteurized milk following traditional artisanal cheese

manufacturing. Pasteurization kills pathogens that are frequently associated with raw milk such as *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter*, and *Mycobacterium bovis* (CDC, 2007). Without a heating step, it is difficult to ensure that milk is free from harmful bacteria before it is used for cheese production. Once the cheese has been produced, the lack of starter cultures, neutral pH, low salt content and high moisture would contribute to the growth of bacteria from raw milk.

Epidemiology

A number of bacterial pathogens have been associated with fresh Hispanic cheese outbreaks. Of the 32 cheese-related outbreaks that occurred between 1973 and 1992, 5 were the result of Hispanic style soft cheeses (Altekruse, Timbo, Mowbray, Bean and Potter, 1998). These pathogens include *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter*, *Shigella* and *Brucella*.

L. monocytogenes is well known as an etiological agent that can be found in cheeses like queso fresco. Although only 0.02% of reported foodborne illnesses are caused by this severe pathogen, it accounts for 27.6% of the deaths attributed to all foodborne diseases (Mead et al., 1999). It is not surprising that so many deaths are attributed to listeriosis, as this infection has a 20-50% mortality rate (Liu, 2008). Between 2001 and 2005 there were on average a total 24 cases of listeriosis per year (CDC 2009).

Another common pathogen associated with Hispanic cheeses is *Salmonella*. In 2006, *Salmonella* was second only to norovirus as the most common source of foodborne outbreaks in United States. It was responsible for 18% of the total number of foodborne disease outbreaks (CDC, 2009). It is estimated that salmonellosis causes a loss of between \$483 million to \$1.4 billion per year for physicians' visits, hospitalizations and death. This estimated total does not include the total loss to the food industry, or loss due to missed time at work (Janda and Abbott, 1998).

Escherichia coli O157:H7 is another pathogen of concern that can be transmitted through cheese. It is believed that the rate of reporting of *E. Coli* O157:H7 infections is higher than other foodborne pathogens because of *E. Coli*'s tendency to induce bloody diarrhea (Mead et al., 1999). Mead (2009) estimated that this pathogen causes an estimated 73,500 illnesses annually, and these illnesses prompt more than 2,000 hospital visits every year (Mead et al., 1999). In 2006, *E. Coli* O157:H7 caused six of the

eleven deaths reported to the CDC that resulted from foodborne illnesses (CDC, 2009).

Although *L. monocytogenes*, *Salmonella* and *E. Coli* O157:H7 pose probably the greatest threats to the safety of Hispanic cheeses, other pathogens have also been associated with Hispanic cheese outbreaks. These illnesses represent a small fraction of illnesses caused by pathogens attributed to cheese consumption. *Campylobacter*, which causes the second greatest numbers of bacterial foodborne pathogen illnesses annually, *Mycobacterium bovis* and *Shigella* have all been attributed to illnesses from contaminated queso fresco (CSPI, 2009; DeDoncker, 2009; Harris et al., 2007; Mead et al., 1999).

Outbreaks in the U.S. Fresh Hispanic cheese has been identified as the source of multiple foodborne outbreaks in the United States (Mead et al., 1999). A selection of the most relevant outbreaks related to Hispanic fresh cheeses is presented in Table 1. The most common etiological agent associated with Hispanic cheese contamination is *Listeria monocytogenes*.

The first major *Listeria monocytogenes* outbreak linked to Hispanic cheeses occurred in 1985 and was due to queso fresco made by Jalisco Products, Inc. in California (Linnan, 1988). More than 98% of the 142 victims were either pregnant or had a preexisting condition when they became infected. The pathogen caused 93 pregnant women, fetuses or newborns to become ill. Of the 48 individuals who died as a result of the infection, thirty were fetuses or newborns, and 18 were immunocompromised adults (Linnan, 1988). This outbreak provided the first evidence of the potential for transmission of listeriosis by fresh Hispanic cheeses.

Additional *L. monocytogenes* outbreaks caused by fresh Hispanic cheeses have occurred since 1985. Twelve Hispanic individuals in North Carolina became infected with *L. monocytogenes* during the fall of 2000 (CDC, 2001). This group included ten pregnant women, one postpartum individual and one 70-year-old immunocompromised man. Five stillbirths, three premature births and two infections in newborns occurred as a result of cheese consumption by their mothers. Homemade, fresh Hispanic cheese made from raw milk was identified as the source of these infections. These products were sold by Latino grocery stores, parking lot vendors, and by door-to-door vendors. North Carolina law prohibits the sale of raw milk and raw milk products, making the production and sale of this cheese illegal (CDC, 2001).

Table 1. Foodborne listeriosis cases linked to consumption of Queso fresco in the U.S.

Year	Serotype	No. of cases (fatalities/ stillbirths)	Food vehicle	State	Reference
1985	4b	142 (48/30)	Queso fresco and Cotija	CA	Linnan et al., 1988
2000	4b	12 (0/5)	Homemade Mexican-style cheese	NC	MacDonald et al., 2005
2003	non-typeable	6 (1/1)	Queso fresco	TX	Gaul, 2007
2004	non-typeable	15 (3/1)	Queso fresco	TX + 7 others	Gaul, 2007
2005	I. non-typeable; II. unknown	I. 9 (n.a); II. 5 (n.a)	Queso fresco	TX + 7 others	Gaul, 2007
2006	non-typeable	8 (0/0)	Queso fresco	TX	Gaul, 2007
2007	non-typeable	5 (0/0)	Queso fresco	TX	Gaul, 2007

^a not available

Between 2004 and 2007, as many as 37 listeriosis cases reported in Texas were linked to the consumption of unpasteurized queso fresco. Most of these cases were in pregnant women or their offspring. The outbreaks resulted in 8 deaths, including 3 non-pregnant adults, 1 infant and 4 fetuses. In addition to those deaths, infection with *L. monocytogenes* induced 11 premature deliveries. The Texas State Department of Health Services believed that the majority of the contaminated queso fresco was purchased in Mexico from flea market vendors (IDCU, 2007). These outbreaks illustrated the complex socio-economical factors influencing the risk of listeriosis.

In summary, *L. monocytogenes* contamination of queso fresco between 1985 and 2007 has led to roughly 191 illnesses and 61 deaths in the United States. The actual number of cases of *L. monocytogenes* caused by fresh Hispanic cheeses is most likely larger than these reported values. Due to the extended incubation period for *L. monocytogenes*, pinpointing the specific foods responsible for infection can be a challenge when victims start to show their first symptoms (Liu, 2008).

In addition to Hispanic cheese contamination by *Listeria monocytogenes*, *Salmonella* has also been the cause of several Hispanic cheese outbreaks. In 1997, two outbreaks of *Salmonella enterica* serovar *Typhimurium* in California were reported affecting 31 confirmed cases in the first outbreak and 79 in the second (Cody et al., 1999). These outbreaks were both attributed to fresh Hispanic cheese sold in local flea markets. Another salmonellosis outbreak occurred in Washington during the same year. From the

54 patients with confirmed cases of *S. Typhimurium* in Washington, 22 participated in a case study to determine the source of the outbreak. At least 17 reported consuming fresh Hispanic cheese within seven days prior to illness. These cheeses were also purchased from local cheese producers (Villar et al., 1999).

In 2007, four cases of *S. Typhimurium* were attributed to the consumption of queso fresco made with raw milk in Pennsylvania. The contaminated queso fresco was produced by an unlicensed manufacturer, and was sold to consumers in a grocery store in a largely Hispanic neighborhood (CDC, 2007). Although Pennsylvania allows the sale of fresh raw milk, it only allows cheeses made with raw milk that have been aged for more than 60 days to be sold. Hispanic fresh cheeses cannot comply with this requirement because their shelf-life is relatively short, typically lasting less than a month (Villar et al., 1999). Very recently, several cases of salmonellosis that occurred in May of 2009 in Utah have been attributed to homemade Hispanic cheeses like queso fresco (UDAF, 2009).

Another pathogen with a high risk potential for causing queso fresco outbreaks is *Escherichia coli* O157:H7. During the spring of 2004 in Washington, three individuals became infected with *E. Coli* O157:H7 after consuming queso fresco made with unpasteurized milk (CSPI, 2009). Although few outbreaks of *E. Coli* O157:H7 have been linked specifically to queso fresco, the risk for future outbreaks is relatively high.

According to the Center for Science in the Public Interest, unpasteurized milk has been responsible for 252 *E. Coli* O157:H7 infections since 1992 (CSPI, 2009; Lynch, Painter, Woodruff, and Braden, 2009; Olsen, MacKinon, Goulding, and Slutsker, 2000). All but ten of these cases have occurred since 2000. Because unpasteurized milk is often used for queso fresco production, unpasteurized queso fresco could potentially become a frequent vehicle of *E. Coli* O157:H7 transmission.

A number of outbreaks caused by pathogens other than *L. monocytogenes*, *Salmonella* and *E. Coli* O157:H7 have been linked to queso fresco in recent years (CSPI, 2009). One of those outbreaks occurred in California in 2003 when 11 individuals became ill with *Campylobacter* spp. after consuming contaminated queso fresco (CSPI, 2009). Another *Campylobacter jejuni* outbreak involved 3 cases that developed diarrhea after eating queso fresco during April of 2009 in Illinois (DeDoncker, 2009). *Shigella* spp. illnesses have also been attributed to queso fresco. Two individuals became infected with *Shigella* in 2005 in California. Additional illnesses occurred in 2005 in Texas when two individuals became infected with *Brucella* spp. after consuming unpasteurized queso fresco (CSPI, 2009).

Although these cases have not been confirmed, recent studies suggest that *Mycobacterium bovis* from fresh Hispanic cheese was a likely source for a number of human tuberculosis cases that occurred in California in 2005 (Harris et al., 2007).

Outbreaks and Prevalence of Contamination in Latin American Countries: Outbreak detection in most Latin American countries is not as advanced as it is in the United States. Because of this difference in identification methods, it is probable that many foodborne outbreaks go undetected. While the actual number of illnesses from contaminated cheese is not known, some outbreaks associated with pathogens from cheese have been reported. One major listeriosis outbreak occurred in Santiago, Chile in 2008, resulting in 119 illnesses and 5 deaths. This outbreak was attributed to soft cheese made by Chevrita Company (2009. Listeriosis outbreak).

Although few outbreaks have been reported, some studies have found that pathogens can be found in cheese manufacturing facilities in Latin America. In Brazil, a type of cheese very similar to queso fresco called minas frescal was sampled to determine the prevalence of *Listeria monocytogenes* in different Brazilian cheeses. Results showed that 7 of the 17 homemade minas frescal samples, or 41.17% of the samples tested positive for *L. monocytogenes*.

Three of the thirty-three manufactured minas frescal and ricotta samples of 3.03% tested positive for *L. monocytogenes* (Silva, Hofer and Tibana, 1998). Serotype 4b was found in the homemade and manufactured minas frescal, while serotype 1/2a was found in homemade minas frescal samples (Silva, Hofer and Tibana, 1998). Studies of queso fresco in Sonora, Mexico reported the detection of *L. monocytogenes* in 3 to 10% of the cheese samples taken. Because the average citizen of Sonora consumes roughly 4.6 kg of queso fresco a year, it is very likely that a number of illnesses may occur without being linked to an outbreak (Moreno-Enriquez et al., 2007).

Recalls

In the event of a foodborne outbreak, the food implicated in the infections is often recalled from the market to prevent additional cases. Food recalls can also be triggered by detection of the presence of foodborne pathogens by microbiological testing, even in the absence of human cases. The FDA and state regulatory agencies are credited with identifying over half of the products that are recalled due to pathogen contamination. Dairy products, including queso fresco, were the most commonly recalled category of food products between 1993 and 1998 (Wong, Street, Delgado and Knontz, 2000).

Between October of 1993 and September of 1998, 61% of all food recalls due to microbial organisms were because of *L. monocytogenes* contamination (Wong, Street, Delgado and Knontz, 2000). *L. monocytogenes* continues to be a source of contamination in products that are recalled. In February of 2004, Peregrina Cheese Corporation (PCC) of Brooklyn, New York recalled pasteurized queso fresco products suspected of harboring *L. monocytogenes* (FDA, 2004). The recall was issued after PCC received a positive test for *L. monocytogenes* in their product during a routine test. Five years later, in January of 2009 and March of 2009, PCC recalled queso fresco products again due to *L. monocytogenes* contamination (FDA, 2009).

Although no illnesses are known to have resulted from these products, they could have been the source of a listeriosis outbreak (FDA, 2009). Similar to PCC's recalls, Quesos Mexico LLC, of Brooklyn, New York, issued a recall for its queso fresco products in 2007 (FDA, May 31, 2009). In June of 2009, Torres Hillsdale Country Cheese in Reading, Michigan recalled two of their fresh Hispanic cheese products; queso fresco and queso requeson (FDA, June 22, 2009). These recalls cause significant losses to the food industry (Liu, 2008).

Characteristics of pathogenic microorganisms associated with Hispanic cheeses

Many types of pathogenic bacteria have been associated with Hispanic cheeses. These organisms, including *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter* spp., *Brucella* spp., *Shigella* spp and *Mycobacterium bovis*, share a number of similar characteristics. Most of these bacteria are aerobic or facultative anaerobic microorganisms. All but *L. monocytogenes* and *M. bovis* are Gram-negative bacteria. Because these microorganisms must also be able to survive in fresh, Hispanic cheeses, they all share the ability to grow in near-neutral pH. The symptoms of infections from most of these pathogens include either gastroenteritis, bloody diarrhea, fever, or a combination of these. The three most predominant types of victims of infection are the young, elderly and immunocompromised (Wareing and Fernandes, 2007).

Listeria monocytogenes: *Listeria monocytogenes* is a Gram positive, non-sporulating, rod-shaped bacterium (Bell and Kyriakides, 1998). The bacterium is a mesophilic bacterium that prefers growing between 30 to 37°C, but it is also considered a psychrotroph because it is able to grow relatively well at temperatures between 0 and 10°C. Based on its overall metabolism, *L. monocytogenes* is considered a facultative anaerobic microorganism. At room temperature, or 20 to 25°C, *Listeria* is motile by its peritrichous flagella. These flagella become inactivated when the *Listeria* is exposed to body temperature, or 37°C (Liu, 2008). The bacteria poses a threat to human health because it is well suited for growth in foods as well as in the human body

Listeria typically infects immunocompromised, elderly, pregnant and newborn individuals (FAO/WHO, 2004). Signs of infection include a variety of symptoms that range from mild gastroenteritis and headache to more serious conditions such as septicemia, meningitis and encephalitis (Liu, 2008). These afflictions may lead to spontaneous abortions and to death. The microorganism usually infects at-risk individuals who consume contaminated foods. After consumption, the bacterial cells may enter the intestinal wall tissue, and eventually enter the bloodstream. The two major organs of the human body that are affected by *L. monocytogenes* infections are the central nervous system and the bloodstream. It is also common for the bacteria to travel through the pregnant uterus into fetuses (McLauchlin, 1987).

The mortality rate from infection is extremely high, ranging from 20 to 30%, but surprisingly, it is estimated that 2-10% of the population carries *L. monocytogenes* in

their intestines without ever becoming sick (FAO/WHO, 2004; Mead et al., 1999). Sometimes pregnant women do not experience any symptoms, although their babies may become seriously infected. It is not uncommon for abortions, premature deliveries and infected newborns to occur after pregnant women consume contaminated foods, but it is uncommon for pregnant women to die as a result of listeriosis (FAO/WHO, 2004; Liu, 2008). Almost all of the reported outbreaks have included cases of stillbirth and abortions.

One possible explanation for the numerous *Listeria* carriers never become ill is that they were not exposed to a high enough dose. A minimum infectious dose has not been officially recognized, but the foods that have been identified as the sources of outbreaks had levels of *L. monocytogenes* that ranged from 10² CFU/ml/g to 10⁶ CFU/ml/g (99). It is estimated that the actual infectious dose depends on the health of the person.

At-risk individuals may not need to consume a large dose in order to become infected. For example, a fetus can become deathly ill with listeriosis while its mother exhibits few symptoms because the fetus cannot tolerate as many bacteria as the mother. As a result of the uncertainty and variability related to its infectious dose, the United States as well as other countries have adopted a zero tolerance for *Listeria monocytogenes* in ready-to-eat foods since the 1980's (Liu, 2008).

L. monocytogenes is a unique foodborne pathogen because of its long incubation period. Frequently, symptoms do not appear until several weeks after consumption of contaminated food, and in some cases the incubation periods can last as long as 90 days (Liu, 2008). This time lapse between infection and diagnosis makes it difficult to determine the source of *L. monocytogenes* to which a victim was exposed.

One characteristic of *L. monocytogenes* that makes it particularly concerning is its presence on dairy farms and in dairy processing facilities. Fox et al found that 17 (18%) of the 91 environmental samples taken from the farms of dairy suppliers in the raw milk cheese industry tested positive for *L. monocytogenes* (Fox, O'Mahony, Clancy, Dempsey, O'Brien, Jordan, 2009). These positive samples originated from fecal samples, yard water runoff and yard dust.

Although no positive samples originated from raw milk samples taken from these farms during this study, the average frequency of contamination for raw milk with *L. monocytogenes* was as high as 10% (Fox, O'Mahony, Clancy, Dempsey, O'Brien, Jordan, 2009). Higher

incidences of contamination have also been detected. During a study of two dairy processing plants in Northern Ireland, Kells and Gilmour found that 22.2% of raw milk samples tested positive for *L. monocytogenes*. About 7% of the equipment sampled and 40% of the environmental samples also tested positive for *L. monocytogenes* (Kells and Gilmour, 2004).

Salmonella: *Salmonella* are Gram-negative, rod shaped, facultative anaerobic bacteria (Wareing and Fernandes, 2007). The genus *Salmonella* belongs to the family Enterobacteriaceae, and includes two species; *S. enterica* and *S. bongori* (Jay, Loessner, and Gold, 2005). These species are divided into 2,463 currently recognized serotypes, or serovars, most of them belonging to *S. enterica* (Popoffa, Bockemuhl, and Brenner, 2000). Common illnesses attributed to *Salmonella*'s multiple serotypes include enteric (typhoid) fever and non-typhoid gastroenteritis.

Typhoid fever is a serious condition characterized by fever, abdominal pain and watery diarrhea that typically results from infections of *Salmonella enterica* serovar *Typhi* (D'Aoust, 1994). The underlying cause of typhoid fever is the invasion of *Salmonella* cells to different organs which often leads to death. Non-typhoid gastroenteritis is typically less severe than typhoid fever, but it is probably the most common bacterial foodborne disease in the U. S. The symptoms of non-typhoid gastroenteritis involve diarrhea, fever, nausea and vomiting. Many *Salmonella* serovars cause non-typhoid gastroenteritis, but the most frequent cause of outbreaks and sporadic cases are: *Enteritidis*, *Typhimurium*, Heidelberg, and Newport (CDC, 2009).

Salmonella has a smaller infectious dose than the estimated infectious dose for *Listeria monocytogenes*. In recent years studies have shown that the infectious dose of *Salmonella* can be as small as 10 colony forming units (CFU), which is much less than the infectious dose of 10^6 CFU as previously believed (Todd, Greig, Bartleson and Michaels, 2008; Wareing and Fernandes, 2007). At risk groups including immunocompromised individuals, such as HIV patients, as well as infants and the elderly are more likely to develop *Salmonella* infections after consuming minimal bacteria cells (D'Aoust, 1991).

The number of cells that an individual is exposed to influences the incubation period of the infection. Although *Salmonella*'s incubation period in most hosts ranges from 6 to 48 hours, the range can extend beyond 48 hours to over 168 hours if the patient consumed less than 10^3 cells. The median incubation period for an outbreak of

171 cases of salmonellosis in Japan was 168 hours for the estimated dose of 12 CFU per person. During another *Salmonella* outbreak in Japan, 100 individuals consumed an estimated average of 3.33×10^5 CFU per person, and the median incubation period was 22.5 hours (Abe, Saito, Kasuga, and Yamamoto, 2004). A clear correlation seems to exist between the exposure and incubation period for salmonellosis infections.

After infection and the initial onset of symptoms, the non-typhoid gastroenteritis symptoms can last for as long as 10 days (Nataro, Cohen, Mobley, and Weiser, 2005). After this initial period, roughly 30% of patients with salmonellosis develop irritable bowel syndrome (IBS), which results in chronic, periodic diarrhea. Many of the cases of IBS subside with time, however, for some they do not. In a study of 38 individuals who were infected with *Salmonella enterica*, five of the patients who developed IBS claimed that their condition affected their daily life even in after a year since infection (Janda and Abbot, 2006). Women over the age of 40 are the most likely to develop IBS after *Salmonella* infection.

Besides developing IBS, in a few rare cases, gastroenteritis may develop into life-threatening and serious conditions like meningitis, cardiac inflammation and arthritis (D'Aoust, 1994). Salmonellosis's mortality rate is less than 1% for healthy individuals infected with *Salmonella*, although the disease is responsible for the largest number of deaths associated with foodborne illness. The CDC estimated that 1.3 million cases, 15,600 hospitalizations and roughly 550 deaths are caused by *Salmonella* infections resulting from food contamination in the United States, every year (Mead et al., 1999; Wareing and Fernandes, 2007).

Salmonella is a natural inhabitant of the gastrointestinal tract of many animals, including livestock such as dairy cows. Because of this, contamination of raw milk with *Salmonella* is not uncommon. One study estimated that 1.5-8.9% of raw milk samples contain *Salmonella* (D'Amico, Groves and Donnelly, 2008). Although *Salmonella* is killed during pasteurization, it is possible for milk to become recontaminated before it is used to make fresh Hispanic cheeses. Typically, recontamination occurs as the result of improper handling.

Cross contamination from other items in a kitchen and poor personal hygiene can lead to *Salmonella* contamination (Yadav, Grover, and Batish, 1993). Hand washing is particularly important to prevent the spread of the disease because after a patient's symptoms subside, it is common for the individual to act as an asymptomatic carrier of *Salmonella*. Typically, adults carry the

microorganism for an average of 5 weeks after infection symptoms subside (D'Aoust, 1991).

Children under the age of five carry the bacteria even longer, for an average of 7 weeks. *S. Typhimurium* can stay in a child's intestinal tissue for up to a year after the initial infection (Janda and Abbot, 2006). In addition to carrying *Salmonella* for a longer amount of time than adults, children also shed more of the bacteria in their feces than adults. Children who are asymptomatic carriers of *Salmonella* often shed between 10^6 and 10^7 CFU per gram of feces (D'Aoust, 1991).

Escherichia coli O157:H7: *Escherichia coli* is a Gram negative, nonsporeforming, facultative anaerobic bacterium. Like *Salmonella*, *E. Coli* belongs to the family Enterobacteriaceae. This microorganism normally inhabits the normal gastrointestinal tract of humans, other mammals and birds in small numbers (Janda and Abbott, 1998; Sussman, 1985). Humans are not born with *E. Coli* in their intestines, however, it colonizes infants' intestines soon after birth but their numbers rarely reach more than 10^6 CFU/g feces in healthy adults (Sussman, 1985). The majority of *E. Coli* strains are mostly harmless commensal bacteria, but some strains are capable of causing serious infections and even death (Nataro and Kaper, 1998). *E. Coli O157:H7* is a pathogenic serovar responsible from hemorrhagic colitis and other complications (Boyce, Swerdlow and Griffin, 1995).

Hemorrhagic colitis is a common symptom of *E. Coli O157:H7* infections. Individuals with hemorrhagic colitis often experience nausea, bloody diarrhea, abdominal pain, and occasionally fever (Saunders et al., 1999). Some patients experience bowel movements up to thirty times a day while infected. It is also believed to be the number one cause of bloody diarrhea in the U. S. because roughly 90% of cases of *E. Coli O157:H7* infections, victims experience bloody diarrhea.

Fever is not very common with infection, and only accompanies about a third of the cases patients with bloody diarrhea (Janda and Abbott, 1998). *E. Coli O157:H7* infections can lead to even more serious illnesses. Roughly 6% of victims develop either hemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) (Boyce, Swerdlow and Griffin, 1995). *E. Coli O157:H7* is the leading cause of HUS, responsible for roughly 50% of cases. The mortality rate for children with HUS is less than 10%. While HUS is more common in children, TTP is more common in adults (Su and Brandt, 1995).

This pathogen poses a serious risk to human health because it produces a number of virulence factors. Shiga-like toxins are the most distinctive *E. Coli O157* virulence factor (Varnam and Evans, 1991). These toxins are capable of injuring the tissues of the intestines, as well as other organs (IOM, 2002). Another characteristic of *E. Coli O157:H7* that makes it a concern to human health is its ability to attach to epithelial cells with specific fimbrial antigens (Varnam and Evans, 1991).

In addition to shiga toxins, serovar O157:H7 produces a variety of other virulence factors such as intimin, enterohemolysin and proteins involved in adhesion to the intestinal lining cells. Among foodborne pathogens, this bacteria has one of the greatest tolerance to acidity which enhances the ability of this organism to survive the low pH of the stomach and eventually colonize the large intestine (Diez-Gonzalez and Russell, 1999). These characteristics give *E. Coli O157:H7* an extremely small infectious dose, which has been estimated to be less than 1,000 CFU (Todd, Greig, Bartleson and Michaels, 2008). The incubation period for *E. Coli O157:H7* is on ranges from 1 to 5 days, and illness typically lasts fewer than 10 days (Janda and Abbott, 1998).

An estimated 62,400 illnesses, 1,840 hospitalizations, and 52 deaths occur annually in the United States as a result of *E. Coli O157:H7* infections contracted from contaminated food (Mead et al., 1999). The leading source of *E. Coli O157:H7* in food is ground beef, but many outbreaks have also been related to a variety of other foods such as milk, fresh produce, apple cider and water (Bell et al., 1994). The most common route of transmission for *E. Coli O157:H7* is through fecal contamination of food, but this organism can also be transmitted directly from person to person or from animals to humans (Armstrong, Hollingsworth and Glenn Morris, 1996). *E. Coli O157:H7* is a unique foodborne pathogen because their natural reservoirs are cattle (Borczyk, Karmali, Lior, and Duncan, 1987).

According to multiple studies in cattle populations, it has been estimated that during the summer months serovar O157:H7 can be isolated from the feces of an average of 30% of beef cattle (Elder, et al., 2000). This prevalence is typically greater during the summer months and is correlated to the increase in human cases in late summer and fall seasons. Because cattle are the natural niche of enterohemorrhagic *E. Coli* the majority of outbreaks are related to ground beef, but there have been several documented cases of disease due to milk and dairy products especially if they were not pasteurized (Heuvelink et al., 1998; Upton and Coia, 1994). Since serovar O157 is shed in the feces of cattle, manure can

easily contaminate the udder and eventually the milk. According to the CDC, there has been a total of 10 outbreaks due to contaminated milk and an outbreak linked to queso fresco made from raw milk (CDC, 2009).

Other Pathogens: *Campylobacter* spp., *Brucella* spp., *Shigella* spp and *Mycobacterium bovis* are four pathogens that have been implicated in Hispanic cheese outbreaks. These microorganisms have not been linked to same number of Hispanic cheese outbreaks as *Listeria monocytogenes*, *Salmonella* and *E. Coli* O157:H7, but they have the potential to cause future outbreaks. *Campylobacter* is one of the leading causes of food poisoning in the United States (Mead et al., 1999). The species *C. jejuni* is responsible for approximately 90% of human illnesses from *Campylobacter* (Janssen et al., 2008).

Like *Salmonella* and *E. Coli* O157:H7, *Campylobacter* is a Gram negative, non-spore forming bacteria, although it has curved or a spiral shape instead of a rod shape (Adams and Moss, 2008). Most types of *Campylobacter* are monotrichous or amphitrichous, as defined by the presence of only one polar flagellum or two flagella at opposite ends of the microorganism (Penner, 1988).

The incubation period for *Campylobacter* is one to seven days after consumption of an infectious dose as small as 500 CFU. Infection can be asymptomatic, or can cause symptoms like fever, headaches, bloody diarrhea, abdominal pain and nausea. Immunocompromised individuals are at a higher risk for contracting *Campylobacter* infections and for developing more severe symptoms than healthy individuals (Janssen et al., 2008).

Brucella are Gram negative, rod shaped bacteria. Although animals are the primary hosts for *Brucella*, some species of this zoonotic microorganism are also pathogenic to humans. The three most common species that cause human infections are *B. melitensis*, *B. abortus* and *B. suis* (Halling and Young, 1994). *B. melitensis*, which originates from goats, causes the largest number of human brucellosis cases. Consumption of raw meats or dairy products made from infected animals products is the typical route for infection (Corbel, 1997).

Identifying human brucellosis infections tends to be difficult, as the incubation period of *Brucella* can last several months (Halling and Young, 1994). Women and people between the ages of 25 and 64 appear to be most at risk for developing this disease, and infections are characterized by symptoms like persistent fever, chills and headaches (Johnson, 1990; Luna-Martinez and

Mejia-Teran, 2002). Although this disease is not currently a major problem in the United States, the problem is endemic in Mexico.

During 2002 an average incidence of 3,500 cases per year were reported, although the actual number of illnesses is believed to be much higher. It is estimated that only about 30% of all brucellosis cases are diagnosed and reported. Of the reported cases, 98% are believed to result from consumption of contaminated dairy products (Luna-Martinez and Mejia-Teran, 2002). In the United States, the majority of brucellosis cases occur in Texas and California among Hispanic populations, most likely due to the illegal importation of unpasteurized dairy products from Mexico (Georgios Pappas, Akritidis, Bosilkovski, 2005).

Shigella are Gram negative rods, and belong to the same family as *E. Coli* O157:H7 and *Salmonella*. Infectious doses of virulent strains *S. dysenteriae* and *S. flexneri* have been found to be as few as 10 CFU (Doyle, 1990). Symptoms of infection like bloody diarrhea, fever and abdominal pain often manifest 1 to 7 days after initial exposure to the pathogen. More serious conditions can develop in elderly individuals, children and people with compromised immune systems (100). In 1990, an average between 15,000 and 20,000 shigellosis cases were reported annually in the United States. Because *Shigella* is only known to infect humans and primates, this pathogen is spread most commonly via contact among humans and from fecal contamination of food (Doyle, 1990).

Mycobacterium bovis is another microorganism suspected to be the cause of Hispanic cheese related illnesses. Unlike *Campylobacter*, *Brucella* and *Shigella*, *Mycobacteria* are Gram positive. They have a somewhat curved shape similar to *Campylobacter*. Infection from *M. bovis* is the number one cause of milk-borne tuberculosis. Cows infected with *M. bovis* may develop lesions in their udders or other infections that cause them to produce milk contaminated with the pathogen (Ryser, 1998).

Although *M. bovis* contamination of milk is no longer the public health issue it once was, it is estimated that between the years 1912 and 1937 roughly 65,000 individuals died from tuberculosis after drinking contaminated milk (Garbutt, 1997). Tuberculosis from milk consumption can cause lesions in the oropharynx and intestinal tract to develop. From there, the disease can spread throughout the body and cause a number of other conditions including lesions in the kidneys and genitourinary tract, kyphosis (or hunchback), and meningitis (Ryser, 1998).

Classification and characteristics of Hispanic cheeses

Hispanic cheeses can be classified as fresh, fermented or processed. These classifications are based on a number of characteristics including manufacturing process, physiological characteristics as well as organoleptic characteristics. Fresh Hispanic cheeses are typically made without a starter culture, and can be characterized by their high moisture contents, high pH values and white color (Farkye and Vedamuthu, 2002; Van Hekken and Farkye, 2003).

Unlike fresh cheeses, fermented cheeses are aged before consumption, and they also have lower pH (Tunick et al., 2008). Processed cheeses differ from fresh and fermented cheeses mainly by their manufacturing process. Unlike other Hispanic cheeses, processed cheeses are stretched and shaped into particular shapes during their manufacturing process. (Alba, Staff, Richter, and Dill, 1991).

Fresh cheeses

Fresh cheeses are the most popular types of cheese in many Latin American countries. In fact, roughly 80% of the cheese consumed in Mexico is fresh, white cheese (Jimenez-Guzman, Flores-Jajera, Cruz-Guerrero, and Garcia-Garibay, 2009). The three most common fresh, Hispanic cheeses are “queso fresco”, “queso panela” and “queso blanco”. One distinguishing attribute of these cheeses is that they do not melt or run when heated, making them great for uses like stuffing enchiladas and other Hispanic foods. Fresh Hispanic cheeses are typically characterized by their high moisture contents, high pH and white color, as well as their lack of a starter culture (Farkye and Vedamuthu, 2002; Van Hekken and Farkye, 2003).

Starter cultures in cheeses are used for a variety of reasons. Not only do starter cultures contribute to the sensorial characteristics of cheese, they also affect fermentation which improves the safety and extends the shelf life of many types of cheeses. Typical starter cultures include bacteria, molds or yeasts as well as mixtures of these three. Although the majority of cheeses are made using starter cultures, some cheeses, like fresh, Hispanic cheese, are produced without them (Tamime, 2002). Queso fresco is one such cheese that does not normally use a starter culture during manufacture.

Queso fresco can be produced using a starter culture, but is generally made without one. Queso fresco is produced by the addition of rennet to part-skim or whole milk. After the milk coagulates, it is cut into curds. These curds

are then cooked briefly and are mostly drained before salt is added. Remaining excess whey is then drained and the cheese is ground to produce smaller curds before packaging. This production process is very similar for other unripened soft Hispanic cheeses like panela (Farkye and Vedamuthu, 2002). Without a starter culture, unripened cheeses like queso fresco are more susceptible to pathogen growth because of their higher average pH.

Another common type of fresh Hispanic cheese is queso panela. This cheese is known for its characteristic basket weave design that is created from its traditionally used basket molds that give it its shape (Graber, 2006). Queso panela is a “slightly pressed, mild and moist cheese, with a sweet, fresh milk flavour” (Jimenez-Guzman, Flores-Jajera, Cruz-Guerrero, and Garcia-Garibay, 2009). It is somewhat like fresh mozzarella, and is often sliced and used in sandwiches or with fruit (2008, New Product Review).

Another widespread type of fresh, Hispanic cheese that does not require a starter culture is queso blanco. “Queso blanco” literally means “white cheese” and identifies a large group of cheeses. Traditionally, queso blanco type cheeses are produced by direct acidification of cow milk with acidic fruit juice and vinegar, but citric, lactic, acetic, tartaric and phosphoric acids can also be used. In the traditional method of manufacture milk is heated to above 80°C and coagulated with acid. The whey is then drained, and the curd is salted while it is still hot. Finally, the curds are pressed and packaged (Parnell-Clunies, Irvine, and Bullock, 1985b). Because of disagreeable traits that it can produce, homogenized milk is not recommended for use for queso blanco production (Parnell-Clunies, Irvine, and Bullock, 1985a). This cheese has been described as having a mild, “clean” flavor, as well as a “distinct dry, crumbly texture and grainy mouthfeel” (Hill, Bullock, and Irvine, 1982; McGregor, Tejookaya, and Gough, 1995).

Physiochemical properties: The ranges of the physiochemical properties for the major fresh, Hispanic cheeses queso blanco, queso fresco and queso panela vary because no standards of identify currently exist for these Hispanic type cheeses in the U.S. (Tunick et al., 2008). Queso blanco, queso fresco and queso panela have similar physiochemical properties. Because of its generic name, queso blanco has come to identify a large group of cheeses. One source identified ranges of 40-60% moisture, 7-38% fat, 17-25% protein, 1.2-3.5% salt and pH levels of 4.9-6.3 as the characteristic physiochemical properties of queso blanco (Hill, Bullock, and Irvine, 1982).

The optimal pH for the most accepted flavor of queso blanco ranges from 5.2-5.3 (Hill, Bullock, and Irvine, 1982). Queso fresco typically has a moisture content of 47-52%. One study found that consumers prefer queso fresco with a salt content ranging from 1.4-2.4% and a pH of 5.4-6.1 (Clark, Warner, and Luedecke, 2001). Queso panela typically has a higher average moisture content than queso blanco and queso fresco, with 53-58% moisture. Additionally, queso panela has a protein content of 18-20%, a salt content of 1.3-1.8 and a range of pH's between 5.6-6.4 (Van Hekken and Farkye, 2003).

Standards of identity: Despite the growing demand for Hispanic cheese in the United States, the FDA has not set standards of identity for Hispanic cheeses. The Dairy Processing and Products Research Unit with the USDA is currently working with the Centro de Investigacion en Alimentacion y Desarrollo to identify standard characteristics including the “chemical, physical, functional, rheological, and textural properties of Hispanic-style cheeses” (Tunick et al., 2008).

U. S. regulations: Few U. S. regulations effect fresh, Hispanic cheeses. One major regulation important to these cheeses is a mandatory 60-day aging period for cheeses that are produced with raw milk (FDA, 2008). Because of the short shelf life that fresh Hispanic cheeses have, this requirement dictates that cheeses including queso blanco, queso fresco and queso panela, which are typically consumed within the first 60 days after their manufacture, must be produced using pasteurized milk (Villar et al., 1999).

Even if fresh, Hispanic cheeses were aged for 60 days and then consumed, some argue that the 60-day age requirement is inadequate for ensuring the safety of cheeses like camembert (D’Amico, Druart, and Donnelly, 2008). Currently, no U.S. regulations relate specifically to Hispanic cheeses, as these cheeses have not been specified with any standards of identity (Tunick et al., 2008).

Fermented cheeses

Queso Chihuahua is a semi-hard, pale yellow cheese with firmly packed curds. The texture can be compared to that of a brick of cheddar cheese, and the flavor is bland to tangy (Hekken et al., 2006; Hekken et al., 2007). Queso Chihuahua is produced following a cheddar-like processing referred as “cheddarization.” In the United States, queso Chihuahua is sometimes called queso Menonita or queso Chester (Hekken et al., 2006). This cheese is ripened for 1 to 2 months and consumed within the first few weeks after production, and it begins to develop small air pockets as it ages (Tunick et al., 2007). The average moisture of queso Chihuahua is 39.4%, the

average fat content is 32.6% and the average protein content is 25.9%. The cheese is low in salt, with between 1.0 and 1.5% NaCl. The average pH of queso Chihuahua is 5.1 (Tunick et al., 2008).

Queso Manchego is another fermented cheese. This semi-hard cheese is typically ripened for five days before consumption. Mexican Manchego, which is made with cow’s milk, originated from Spanish Manchego, which is made from sheep’s milk (Lobato-Calleros, Robles-Martinez, Caballero-Perez, Aguirre-Mandujano, 2001). According to Mexican standards of identity, Manchego cheese should have a moisture content of no more than 45%, a minimum fat content of 25% (30.5% typical), a protein content of at least 22% (24% average), and a maximum pH of 5.5 (Lobato-Calleros, Velazquez-Varela, Sanchez-Garciab, Vernon-Carterc, 2003; Solano-Lopez, and Hernandez-Sanchez, 2000)

The two most common fermented, hard Hispanic cheeses are Cojita and queso Añejo. These cheeses have the lowest moisture contents out of all of the Hispanic cheeses with only about 20-42% moisture (Van Hekken and Farkye, 2003). Cojita is an aged Mexican cheese, and although it is sometimes soft like feta cheese, it is usually firm like parmesan (CDC, 2008; Clark, Hillers, and Austin, 2004). The composition of Cotija is 35-42% moisture, 23-30% fat, 28-31% protein and a high salt content, usually above 4%. The pH of Cotija cheese is lower than most other Hispanic cheeses, sometimes as low as 4.7 (Van Hekken and Farkye, 2003). Queso Añejo has a moisture content of 43%, a fat content of 23-27% and a salt content of 3%. The pH of queso Añejo is typically 5.7 (Van Hekken and Farkye, 2003).

Process cheeses

Two processed Hispanic cheeses are Asadero and Oaxaca. These cheeses are very similar compositionally. Asadero has a slightly higher average moisture content than Oaxaca of 41 to 49%, compared to Oaxaca’s 40 to 46% (Van Hekken and Farkye, 2003). Asadero contains roughly 18-32% fat, 21-30% protein and 1-2% salt (Alba, Staff, Richter, and Dill, 1991). Oaxaca cheese contains 23% fat and 24% protein (Van Hekken and Farkye, 2003). Both types of cheese can be stretched to create a string or a long, flat strand of cheese. As a final step in manufacturing this strand is wrapped into a ball, similar to a ball of yarn (Alba, Staff, Richter, and Dill, 1991). Asadero has a “slightly tangy, buttery flavor”, whereas Oaxaca has a “sweet milk flavor” (Van Hekken and Farkye, 2003). Asadero, which means “suitable for roasting”, can be kept for up to 60 days at refrigeration temperatures (Davis, 1976; Soto-Cantu, et al., 2008).

Prevalence and growth of foodborne pathogens in fresh Hispanic cheeses

Fresh Hispanic cheeses offer a favorable environment for pathogenic growth. Their high moisture and low salt contents, along with their neutral pH, make cheeses like queso blanco and queso fresco favorable substrates in which pathogens can thrive (Sutherland, Bayliss, and Braxton, 1995). Some of the pathogens that may grow and survive in these Hispanic cheeses are *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, *Campylobacter*, *Shigella*, *Mycobacterium bovis* and *Brucella*. Although most of these pathogens cannot grow at refrigeration temperatures, *Listeria monocytogenes* can, which poses a particular threat to Hispanic fresh cheeses' safety (Kamnetz, 2009).

Listeria monocytogenes

Listeria monocytogenes is known for its ability to survive and grow at refrigeration temperatures. This bacterium grows best between 30 to 37°C, but it is capable of growing at temperatures as low as 0°C (Liu, 2008). When subjected to temperatures above 70°C, its population decreases rapidly. The generation time, or the time that it takes for a bacterium's population to double, is small enough at refrigeration temperatures for *L. monocytogenes* in raw cow milk to allow the population to multiply several times during long term storage. During one study of unpasteurized milk contaminated with *L. monocytogenes*, it took only 25 hours for the population of *L. monocytogenes* to double when incubated at only 4°C.

A similar study conducted with soft cheese made from goat's milk reported a *L. monocytogenes* generation time of 30 to 50 hours when incubated at 4°C (Bell and Kyriakides, 1998). This information indicates that generation times for *Listeria* at cold temperatures are sufficient to produce large numbers of bacteria in foods relatively quickly. This characteristic of *L. monocytogenes* means that foods with short shelf life, such as queso fresco, can harbor a large bacterial count even if it stored at refrigeration temperatures for short periods of time.

The salt content of a food can also affect *L. monocytogenes*' ability to survive. Studies have shown that this bacterium can survive in food with 40% NaCl, and it can grow at a 10% NaCl concentration (Liu, 2008). Another physicochemical property of foods that affects *Listeria monocytogenes* growth is pH. *L. monocytogenes* grows best in neutral environments (Bell and Kyriakides, 1998) and its optimum pH range is 5.2 to 9 (Liu, 2008). Cheeses with pH greater than 5.9 have been identified as excellent media for *Listeria* growth.

When *L. monocytogenes* is subjected to acidic conditions, like cheeses with pH values ranging from 4.0-5.3, the bacterium is markedly inhibited (Bell and Kyriakides, 1998).

The risk of *L. monocytogenes* in Hispanic fresh cheeses is based on its widespread occurrence in the environment of dairy plants and its ability to survive and even grow on these products. A longitudinal study reported that as many as 6% of cheese and 11% of environmental samples from Hispanic fresh cheese plants that used pasteurized milk were positive for *L. monocytogenes* (Kabuki, Wiedmann, and Boor, 2004).

In one of the first studies that investigated the ability of this pathogen to grow on fresh cheeses, Genigeorgis et al. (1991) indicated that *Listeria* could grow in commercial samples of Mexican-style fresh cheeses stored at refrigeration temperatures and it could be present at significant levels after 30 days of storage. In a recent publication, the population of this bacterium in queso fresco increased from 10 to more than 1,000 CFU/g in less than two weeks and it remained at almost 10,000 CFU/g after 12 weeks of storage at 4°C (Lin, Zhang, Doyle, and Swaminathan, 2006). These studies clearly indicate that Hispanic fresh cheeses can be a vehicle for transmission of *Listeria monocytogenes* and stress the importance of developing antimicrobial interventions post-pasteurization.

Salmonella

Salmonella can grow at temperatures from 5 to 47°C. For optimal growth, this microorganism prefers temperatures around 37°C. This bacterium is heat sensitive, and is readily killed by pasteurization (Adams and Moss, 2008). For some serotypes of *Salmonella*, including serovars *Typhimurium* and Dublin, growth was inhibited when contaminated queso fresco is held at 6°C and 8°C, respectively (Bell and Kyriakides, 1998).

Although refrigeration should prevent *Salmonella* growth, a recent survey revealed that only about 37% of people check to make sure the temperature in their refrigerator is at or below 4°C. Shockingly, this study also found that only 11% of refrigerators are at temperatures at or below 4°C (Lagendijk, Assere, Derens, and Carpentier, 2008). That study demonstrated that although *Salmonella* should not grow in Hispanic cheeses that are properly refrigerated, adequate refrigeration does not appear to be guaranteed.

The generation time for *Salmonella* depends on a combination of factors. One study found the generation

times for *Salmonella* growth in tryptic soy broth, or TSB, for combinations of different temperatures, pH values and sodium chloride concentrations. The generation times for *Salmonella* when held at 10°C in sodium chloride concentrations ranging from 0.8 to 4.6% and pH levels ranging from 6.0 to 6.4 were from 8.9 to 18.4 hours. Higher NaCl concentrations resulted in slower generation times (Gibson, Bratchell, and Roberts, 1988). Under favorable conditions the minimal water activity for *Salmonella* was 0.95 (Lanciotti, Sinigaglia, GaustoGardini, LuciaVannini, and Guerzoni, 2001). The optimal pH for *Salmonella* growth lies between 6.5 and 7.5, but it is capable of growing at pH values between 4.5 and 9.0. When subjected to environments with pH of less than 4.1, the survival of *Salmonella* may be compromised (Varnam and Evans, 1991).

***Escherichia coli* O157:H7**

Refrigeration effectively controls the growth of *E. Coli* O157:H7 because this pathogen is generally unable to grow in temperatures below 8°C. Serotype O157:H7 is less heat resistant than most other types of *E. Coli*, and cannot grow above 44-45°C. Similar to other human pathogens, the optimal temperature for *E. Coli* O157:H7 is body temperature or 37°C (Bell and Kyriakides, 1998). *E. Coli* O157:H7 and other pathogenic strains of *E. Coli* are able to grow in a broader range of conditions than *Salmonella*. This microorganism can grow in pH levels as low as 4.4, but prefers a more neutral pH (Adams and Moss, 2000).

The generation time for *E. Coli* O157:H7 is influenced by a number of factors. Sodium chloride concentrations in cheese below 3% were ineffective to slowing the generation time of *E. Coli* O157:H7. Once this concentration increases to more than 3%, growth rates are adversely effected (Sutherland, Bayliss, and Braxton, 1995). All *E. Coli* O157:H7 growth is inhibited at 8.5% NaCl (Bell and Kyriakides, 1998).

The generation time for *E. Coli* O157:H7 in camembert cheese with a pH of 5.5 and NaCl concentration of 3.36% at 15.5°C was 2.09-4.98 hours (Sutherland, Bayliss, and Braxton, 1995). This data indicated that temperature abused camembert, a cheese somewhat similar to queso fresco regarding pH, can provide a suitable environment for *E. Coli* to multiply quickly. The quick generation time could mean that bacteria levels in queso fresco may reach dangerously high levels if the cheese is not properly refrigerated.

Other pathogens

Although *Campylobacter* causes numerous cases of food

poisoning annually, this microorganism is relatively fragile. *Campylobacter* cannot multiply at temperatures below 30°C, so growth in refrigerated Hispanic cheeses is limited. Surprisingly, when held at 25°C the pathogen is more likely to die because of drying than it is to die at 4°C from cold stress. When subjected to temperatures above 48°C *Campylobacter* dies quickly (Stern, 2001). The optimal temperature range for *Campylobacter* growth is 37°C to 42°C (Jacobs-Reitsma, 2000). The optimal pH range for growth is between 6.5 and 7.5 and pH levels lower than 4.9 kill *Campylobacter* (Jacobs-Reitsma, 2000; Stern, 2001). At water activity levels lower than 0.98 and sodium chloride levels higher than 2%, *Campylobacter* dry out and die (Wareing and Fernandes, 2007).

Brucella can survive temperatures below 0°C, and can grow in temperatures ranging from 10 to 40°C (Johnson, 1990; Ryser, 1998). The ability to survive at temperatures as low as 0°C allows *Brucella* to survive during refrigeration. Similar to all other pathogenic bacteria discussed above, pasteurization is very effective to kill *Brucella*. The optimal growth temperature for *Brucella* is 37°C. Once dairy products are contaminated with *Brucella*, the pathogen can survive in those products for long-periods of time such as in goat's cheese for as long as 180 days (Johnson, 1990). *Brucella* requires a relatively neutral pH for growth from 6.6 to 7.4, but it can also grow within pH 5.8 and 8.7, making it suited for growth in cheeses like queso fresco (Halling and Young, 2001).

Shigella have an optimum growth temperature of 37°C, but they can grow over a range of temperatures from 7-46°C (Wareing and Fernandes, 2007). Unlike many of the other foodborne pathogens discussed, *Shigella* requires a relatively small range of pH levels for growth. Between 5.5 and 7 this microorganism is able to grow, and it is killed when held at pH levels lower than 4.5 (Varnam and Evans, 1991).

Mycobacterium bovis has a slow growth rate compared to other pathogens even around its optimal growth temperature of 35°C. It cannot grow in milk held at 4°C in a refrigerator, but it is capable of surviving at this low temperature (Ryser, 1998). One explanation for the slow growth rate of *M. bovis* is its waxy cell wall that prevents the uptake of nutrients from the environment (Adams and Moss, 2000). Despite the fact that milk contaminated with *M. bovis* has caused countless deaths, it is believed that cheeses typically do not provide optimal environments for this microorganism to proliferate (Ryser, 1998).

Antimicrobial technologies for minimizing

microbial risks

Antimicrobial technologies are essential for minimizing the microbial risks of fresh, Hispanic cheeses. These cheeses are becoming more common in the United States due to the increase in the Hispanic population and the popularity of Mexican-style foods. Given their relatively high risk for transmission and growth of foodborne pathogens, the development of antimicrobial treatments to prevent foodborne illnesses is greatly needed. Technologies that prevent pathogen growth can be broken up into multiple categories, including physical, chemical and manufacturing methods to prevent contamination.

Physical methods

Physical methods for antimicrobial treatments are desirable because they do not require the addition of ingredients to the cheese that do not occur naturally. Currently, U. S. regulations require that cheeses which are consumed fresh be made with pasteurized milk (FDA, 2008). Effective pasteurization has been shown to reduce the numbers of bacterial pathogens found in milk to undetectable levels (Johnson and Law, 1999). Other areas of physical treatments that have been explored for Hispanic cheeses or similar cheeses include high pressure processing and irradiation (Konteles, Sinanoglou, Batrinoi, and Sflomos, 2009; Sandra, Stanford, and Goddik, 2004).

Thermal treatments: Currently, the most effective way to prevent the transmission of foodborne pathogens via cheese is through the use of pasteurized milk during manufacture. Pasteurization of milk at 72°C for 15 seconds is an effective way to kill most pathogens that could be found naturally in milk (Johnson and Law, 1999). The reluctance to use pasteurization in cheese making stems from the potential impact of this thermal treatment on texture, flavor and other quality characteristics. These potential changes may be quite relevant in ripened cheeses, but in the case of Hispanic fresh cheeses, pasteurization may have relatively little impact on the flavor and characteristics of cheese. The addition of calcium chloride is one of the few modifications needed to make cheese using pasteurized milk instead of raw milk (Johnson and Law, 1999).

Most of the outbreaks caused by contaminated queso fresco have been due to the use of raw or poorly pasteurized milk, and this practice has led to the belief that fresh Hispanic cheeses require the use of non-pasteurized milk. However, the utilization of raw milk rather than pasteurized milk in those cases was not due to any standard of identity, but to the use of artisanal methods of manufacture frequently used by Hispanic

immigrants and their lack of awareness about the risks of raw milk.

In Yakima County, Washington, a project called The Abuela Project was created to teach members of a Hispanic community about the potential health risks posed by cheeses made with unpasteurized milk. In this project, a number of middle-aged or older Hispanic women taught local community members how to make queso fresco using pasteurized milk (Bell, Hillers, Theo, and Thomas, 1999). These women also educated the community members about the risks of using raw milk.

In addition to teaching community members, the Abuela Project also applied surveys in their community. The surveys indicated that 47% of the individuals had made queso fresco using fresh, unpasteurized milk before The Abuela Project was initiated in their community. After The Abuela Project was established in their community, only 1% of the respondents reported the use of unpasteurized milk (Bell, Hillers, Theo, and Thomas, 1999). This project proven to be successful as it decreased the amount of raw milk cheese being produced, as well as preserved the tradition of making homemade queso fresco. A main contributing factor to this program's success was the active involvement of community members (Bell, Hillers, and Thomas, 1999).

Non-thermal treatments: In addition to pasteurization, other physical methods including high pressure processing (HPP) and irradiation have been explored as methods to kill pathogenic bacteria in fresh, Hispanic cheeses. Sandra et al (2004) conducted research to determine the effect that HPP had on pathogens in queso fresco. Three variations of queso fresco were used during this study, including a control cheese made with raw milk, a cheese made with HPP treated milk, and a cheese that was HPP treated.

The study determined that the HPP-treated milk experienced a 97% reduction in coliforms and HPP-treated cheese experienced a 90% reduction in coliforms when held at 400 MPa for 20 minutes (Sandra, Stanford, and Goddik, 2004). None of these reductions were large enough to achieve the 5 log CFU/g reduction of bacteria that is required for pasteurized milk (Sandra, Stanford, and Goddik, 2004). Another study looked at the effect of HPP-treated Turkish white cheese, which is a white, pickled cheese. That study found that cheese held at 600 MPa for five or ten minutes reduced the counts of *L. monocytogenes* by as much as 4.9 log CFU/g for both pasteurized and raw milk cheese samples (Evrendilek, Koca, Harper, and Balasubramaniam, 2008).

Additional studies have been done with HPP treatments of cheddar cheese. Drake et al. (1997) found no coliforms in cheese that was made with milk that had been HPP treated, although they found coliforms in the milk that was HPP treated. The researchers believe they recovered no coliforms in the cheese made with pressurized milk because the coliforms that survived pressurization were killed during the cheese making process (Drake et al., 1997). Another study of cheddar cheese looked at the effect of high pressure treatments on milk, and found that high pressure treatment caused only a 3 to 4-log cycle reduction of *Listeria innocua*. One explanation for why HPP treatment of milk does not cause a larger log cycle reduction is that the milk fat may form a protective barrier for the bacteria from the high pressure (Kheadr, Vachon, Paquin, and Fliss, 2002).

Although HPP treatments can reduce the population of bacteria found in Hispanic cheeses, these treatments can also influence other characteristics of the cheese as well. Sandra et al (2004) also identified changes in some physiological characteristics of the queso fresco they used for their study of HPP. That study indicated that cheese made with HPP treated milk had a higher protein content, a lower fat content and a higher moisture content than the raw milk control cheese. These changes caused the textural characteristics to differ enough from the control to make the HPP milk cheese product undesirable.

The only detected physiochemical difference between the raw milk control cheese and the HPP treated cheese was that the HPP treated cheese had a slightly higher pH than the control. The researchers concluded that HPP treated cheese had “acceptable sensory attributes, and similar composition to traditional raw milk Queso Fresco” (Sandra, Stanford, and Goddik, 2004).

Irradiation of cheeses has also shown an antimicrobial effect in soft cheeses other than fresh Hispanic cheeses. One study found that 2.5 kGy radiation was able to kill 10^4 CFU/g of *L. monocytogenes* in camembert cheese (Bougle and Stahv, 1994). Another study determined the ability of gamma-irradiation to reduce bacterial counts of *L. monocytogenes* for pre and post-process contamination in feta cheese. This study determined that high levels of irradiation, of 2.5 and 4.7 kGy, were able to reduce the levels of *L. monocytogenes* to undetectable levels in post-process contaminated cheeses. These post-process contaminated cheeses were inoculated with brine containing 10^3 CFU/g, and were held for one month of storage at 4°C.

Cheese samples that were contaminated during pre-processing steps did not experience the same level

of eradication of *L. monocytogenes*, but a significant reduction in total bacterial count was still detected (Konteles, Sinanoglou, Batrinoi, and Sflomos, 2009).

Chemical methods

Physical methods such as pasteurization can effectively lower bacterial counts to safe levels, but because cheeses are susceptible to post-process contamination and fresh products may sustain the growth of pathogenic bacteria, it is critical to find effective antimicrobial ingredients. Various chemical methods, including the use of generally recognized as safe (GRAS) ingredients, food additives as well as novel antimicrobials have been tested to treat fresh Hispanic cheeses.

Use of existing GRAS ingredients: Bacteriocins is one of the ingredients that can be used to inhibit the growth of pathogenic microorganisms in queso fresco. Bacteriocins are proteinaceous substances produced by certain types of bacteria capable of inhibiting other bacteria. It is important to note that bacteriocin-producing bacteria are protected against their deleterious effects. Because of the bacteriocins’ proteinaceous nature, they are not considered to be antibiotics (Montville, and Kaiser, 1993).

The bacteriocin that has probably been best characterized is nisin. This peptide is produced by certain strains of *Lactococcus lactis* subsp. *lactis* and is effective against a wide variety of Gram-positive bacteria. Gram-negative bacteria are less susceptible to nisin because their cell membrane prevents nisin from forming pores in their cytoplasmic membranes (Stevens, Sheldon, Klapes, and Klaenhammer, 1991). Nisin has been used legally in the United States since 1988 to control the growth of *Clostridium botulinum* in processed cheese spreads (Harlander, 1993). Nisin has also been globally approved as a natural food preservative.

Nisin is the only bacteriocin approved for use in cheese products as an antimicrobial for against Gram-positive pathogenic bacteria (21CFR 184.1538). An enormous amount of research has indicated that nisin is effective for inhibiting *Listeria* in a variety of cheese products, but its utilization in Hispanic cheeses had not been investigated (Delves-Broughton, Evans, and Hugenholtz, 2004).

In addition to nisin, other bacteriocins or bacteriocin-producing microorganisms have been tested against *Listeria* in cheese. Several reports have indicated that lacticin 3147, a bacteriocin produced by a *Lactococcus lactis* starter culture, can inhibit the growth and even reduce viable counts of *L. monocytogenes* in a variety of dairy and food products (McAuliffe, Hill and Ross, 1999;

Ryan, Rea, Hill and Ross, 1996). Similar to nisin, the use of lactacin had never been attempted for Hispanic cheeses. A study completed by Davies et al. (1997) found that the addition of nisin to ricotta-type cheeses inhibited the growth of *L. monocytogenes*. Ricotta-type cheeses are fresh, white cheeses like queso fresco and queso blanco. During this study two amounts of nisin, either 1.25 or 2.5 mg/l, was added to milk before it was made into cheese. These cheeses were inoculated with 10^2 - 10^3 CFU/g of five *L. monocytogenes* strains and stored for eight weeks at 6-8°C.

It was determined that the addition of 1.25 mg/l nisin had a bacteriostatic effect on the growth of *L. monocytogenes* until day 11 in their cheese with a pH of 6.1, and prevented the growth of *L. monocytogenes* until day 26 in their cheese with a pH of 5.8 (Davies, Bevis, and Delves-Broughton, 1997). When 2.25 mg/l nisin was added to the cheese, the *L. monocytogenes* grew after 55 days in the cheese with pH 6.1 and did not exhibit any growth during the 70 days of the study in the cheese with pH 5.8 (Davies, Bevis, and Delves-Broughton, 1997).

Davies et al. (1997) mentioned that the high bactericidal action of the nisin may have resulted from an interaction of the nisin with potassium sorbate that was added to prevent mold growth (Davies, Bevis, and Delves-Broughton, 1997). Buncic et al completed a study that supported this hypothesis of a synergistic relationship between nisin and potassium sorbate (Buncic, Fitzgerald, Bell, and Hudson, 1995).

Work in our laboratory has indicated that nisin was also effective against the growth of *Listeria monocytogenes* in queso fresco. According to one study, the addition of 0.1, 0.3 and 0.5 g/kg nisin to the queso fresco curds lead to a reduction of *L. monocytogenes* numbers by 1.0, 1.7 and 2.9 log CFU/g, respectively, compared to the control. Although final bacteria counts on day 21 of these trials for the cheeses with 0.1 and 0.3 g/kg nisin added reached levels that were similar to the positive control, the final count for the cheese with 0.5 g/kg was 3.0 log CFU/g lower than the positive control (Kamnetz, 2009).

In our initial project funded by the Midwest Dairy Association, we tested the effect of several antimicrobial ingredients to inhibit growth and inactivate *Listeria monocytogenes* in queso fresco. We first evaluated the effect of individual treatments of monolaurin, caprylic acid, nisin, lacticin-producing *Lactococcus lactis*, and a commercial combination of sodium lactate/sodium diacetate (SL/SD) on queso fresco batches that had been inoculated with a mixture of five *L. monocytogenes* and stored at 4°C. After that initial evaluation, we

tested the combined effect of addition of these food ingredients (Kamnetz, 2009). Individual treatments with lacticin-producing *L. lactis*, monolaurin, lactic acid, and combinations of sodium lactate/diacetate had little effect in inhibiting growth.

SL/SD mixtures have been used successfully for ready-to-eat meats, but our results indicated that they were not effective in queso fresco. Treatment with nisin caused an initial count reduction but was not able to stop growth. Increasing concentrations of caprylic acid slowed growth and at 3.2 g/kg almost no population increase was observed. Several combinations of antimicrobial GRAS ingredients reduced the population and inhibited growth of *L. monocytogenes* achieving from 2 to 4 log CFU/g count differences compared to controls. However, none of those treatments were as effective as binary mixtures of nisin and caprylic acid. At a nisin concentration of 0.5 g/Kg combined with caprylic acid (0.8 to 1.6 g/Kg), the initial *L. monocytogenes* population was immediately reduced and remained less or equal to 10 CFU/g during most of the experiments at 4°C.

One method that bacteriocins can be added to Hispanic cheeses is through the addition of microorganisms that produce bacteriocins. This method has been studied by a number of research groups with a different bacteriocin-producing microorganism. In one study, enterococci bacteria like *Enterococcus faecium* and *Enterococcus durans* were tested. Although some enterococci bacteria can elicit bacteremia and nosocomial infections, they can also produce bacteriocins which are effective against *Listeria* spp.

Renye et al. (2009) isolated certain strains of *E. faecium* and *E. durans* from queso fresco and queso Mennonite (also known as queso Chihuahua) which possessed “proteolytic and lipolytic activities” against *Listeria* spp (Renye, Somkuti, Paul, and Van Hekken, 2009). Some believe that *E. faecium* contributes to the organoleptic properties of traditionally made queso fresco, so the addition of this bacteria to cheeses could actually be beneficial to the cheese in multiple ways (Renye, Somkuti, Paul, and Van Hekken, 2009). During another study researchers isolated *E. faecium* UQ31 from queso panela and found that it produced a compound with anti-*Listerial* properties (Alvarado, Garcia-Almendrez, Martin, and Regalado, 2005).

Minas cheese, a Brazilian cheese that is very similar to queso fresco, was used to test the effectiveness of a lactic acid bacteria (LAB) culture type O containing *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* against *E. Coli* O157:H7. In that study

Minas cheese samples were inoculated with 10^3 or 10^6 CFU/g of *E. Coli* O157:H7, and were then stored for 14 days at 8.5°C. The researchers found that within the first 24 hours after inoculation, the cheese without the type O lactic culture experienced a 2 log increase in *E. Coli* and the cheese with the added type O lactic culture only experienced a 0.5 log increase of *E. Coli* (Saad, Vanzin, Oliverira, and Franco, 2001).

Not only was the initial growth in the cheeses different between the two cheeses, the subsequent growth of *E. Coli* O157:H7 in the cheese also differed for the remaining 14 days of the trials. The levels of *E. Coli* in the cheese without the added LAB remained relatively constant, whereas the cheese with the added LAB experienced a slight decrease in the *E. Coli* counts (Saad, Vanzin, Oliverira, and Franco, 2001).

Mendoza-Yepes et al. studied the effect of the starter culture *Lactococcus lactis* subsp. *diacetylactis* on the inhibition of *L. monocytogenes*, *E. Coli* O157:H7, *E. cloacae*, *P. aeruginosa*, and *P. fluorescens* growth in queso fresco (Mendoza-Yepes, Abellan-Lopez, Carrion-Ortego, and Marin-Iniesta, 1999). Samples containing 5 g of cheese and 0.1 mL of bacterial culture containing 10^5 CFU/mL were stored at either 3 or 7°C for 20 to 25 days.

This study found that *L. monocytogenes* did not grow in the samples containing 10^7 CFU/g *Lactococcus lactis* subsp. *diacetylactis* after 22 days of incubation (Mendoza-Yepes, Abellan-Lopez, Carrion-Ortego, and Marin-Iniesta, 1999). *E. Coli* O157:H7 growth was not detected until 17 days after inoculation in the samples containing 10^7 CFU/g *Lactococcus lactis* subsp. *diacetylactis* held at 7°C. They also found that samples with 10^7 CFU/g *Lactococcus lactis* subsp. *diacetylactis* prevented the growth of *P. aeruginosa* at 7°C for 25 days, and that the culture didn't have a great impact on the growth of *E. cloacae* and *P. fluorescens*.

El-Ziney et al. (1998) studied the effect of reuterin, which is an antimicrobial produced by *Lactobacillus reuteri*, on *Listeria monocytogenes* and *E. Coli* O157:H7 in cottage cheese. These researchers held the cheeses at 7°C, and found that by day seven, 50, 100 and 150 units per gram of reuterin reduced the count of *E. Coli* O157:H7 by 2, 3 and 6 log cycles, respectively (El-Ziney and Debevereh, 1998). A higher concentration of reuterin was required to reduce the counts of *L. monocytogenes*. They found that *L. monocytogenes* counts were reduced by 2 and 5 log cycles with the addition of 100 and 150 units of reuterin per gram respectively (El-Ziney and Debevereh, 1998).

The addition of lactic acid to cottage cheese that was stored at 4°C for seven days caused a reduction on the bacterial count of *E. Coli* O157:H7 when the pH was adjusted to 5.18 and 5.27. The cheese experienced a 0.9 log CFU/ml reduction for the cheese at pH 5.18 and a reduction of 0.3 and 0.1 log CFU/ml for two samples with pHs of 5.27 (Guraya, Frank and Hassan, 1998).

After 35 days at 4°C, the cottage cheese with pH 5.18 had a 3 log CFU/ml reduction and the cheeses with the pH 5.27 had reductions of 1.6 and 2.6 CFU/ml (Guraya, Frank and Hassan, 1998). These results suggest that the bacterial counts continued to decrease throughout storage between day 7 and day 35, so for cheeses like queso fresco that have shelf lives of only about 21 days, the addition of lactic acid could maintain lower levels of *E. Coli* O157:H7 during their storage time.

Kasrazadeh and Genigeorgis (1995) studied the effects of different compounds on *E. Coli* O157:H7 in queso fresco and found that the addition of 4.0% sodium lactate had no significant effect in reducing the number of bacteria, while 0.3% potassium sorbate and 0.3% sodium benzoate had a significant effect (Kasrazadeh and Genigeorgis, 1995). All three treatments (4.0% sodium lactate, 0.3% potassium sorbate and 0.3% sodium benzoate) significantly increased the lag time of *Salmonella* spp. when added to the contaminated queso fresco. The lag and generation times increased further with the acidification of the cheese to a pH of about six using hydrochloric, acetic or propionic acids (Kasrazadeh and Genigeorgis, 1994).

Application of novel antimicrobials: One enzyme that can be used as an antimicrobial in cheeses is lysozyme-dextran conjugate. One study tested the effect of a non-modified lysozyme and a lysozyme-dextran conjugate in cheese curds against *E. Coli* and *S. aureus* at a concentration of 10^6 CFU/40g in cheese stored at 4°C. The researchers found that after 21 days, the addition of 400 µg/ml of non-modified lysozyme had reduced the *E. Coli* count of by about 1.5 log CFU/mL as compared to the positive control, and the lysozyme-dextran conjugate lowered the count by about 3 log CFU/mL as compared to the positive control.

During the study with *S. aureus*, neither the non-modified lysozyme or the lysozyme-dextran conjugate had any significant effect in lowering the bacterial counts when compared to the control (Amiri, Ramezani, and Aminlari, 2008). Duan et al. (2007) tested the effect of chitosan-lysozyme films and coatings on mozzarella cheese that was surface inoculated with *L. monocytogenes*. They found a 0.32 to 1.35 log₁₀ CFU/g reduction for different

samples. A higher percentage of lysozyme rather than chitosan had antimicrobial effect (Duan, Park, Daeshel, and Zhao, 2007).

Management

GMP's and sanitation: GMP's, or Good Manufacturing Practices, are procedures that food manufacturers are required to follow to ensure sanitary production. Some of the areas covered in GMP's include food handlers, facilities, equipment, waste removal and water sanitation (Drosinos and Siana, 2007). GMP's require food handlers to be dressed appropriately, be in good health, and follow sanitary hygiene habits. The facilities and equipment used for food production must be suitable for food production (Drosinos and Siana, 2007). GMP's must be followed in order for a manufacturer to implement a successful HACCP program.

In the case of fresh Hispanic cheeses, it is essential that processing plants adhere to strict sanitation practices supported by an active environmental testing program. In fresh Hispanic cheeses made from pasteurized milk, the origin of contamination could involve equipment, processing materials, workers and packaging material. The use of an appropriately design cleaning and sanitation schedule that would prevent the formation of biofilms is critical to minimize environmental contamination. Processing and storage temperature controls could also play an important role in minimizing rapid growth of environmental contaminants.

HACCP: According to the FDA, HACCP (Hazard Analysis and Critical Control Points) is a food safety management system through the "analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product." (FDA 07/20/2009). These comprehensive programs are currently only required in meat, seafood, poultry, and fruit juice production, but they are recommended for nearly all foods. HACCP programs for the dairy industry are followed voluntary. This form of food safety management can be useful for the production of foods with few antimicrobial barriers, like fresh Hispanic cheeses, as a way to minimize the potential risk of contamination of the product.

In a case study of a HACCP program for cheese manufacturing, Drosinos and Siana stated that, "good manufacturing practices (GMPs), sanitation standard operating procedures (SSOPs), water safety control, receiving, storage and shipping control, pest and waste control, supplier control, trace and recall programs,

equipment calibration and employee training" were all essential areas for a successful HACCP plan for cheeses (Drosinos and Siana, 2007).

Once these prerequisite food safety areas are reviewed and deemed appropriate for a food manufacturing facility, the general process for developing a HACCP program follows several steps. First, it is recommended that a diverse group of people form a team to create the HACCP plan. In order for the food safety management system to be effective, the group must be able to look at the cheese manufacturing process from a comprehensive point of view. Then this team should describe the product being made (USDA IFSIS, 1998). For cheeses like queso fresco, where no standards of identity have been identified, it is important that the a company's HACCP states the properties of its specific cheese product (Tunick et al., 2008).

The HACCP team should then create a diagram of the cheese production process which occurs in the facility. From this diagram, the team can more easily complete a hazard analysis of the cheese production and identify steps in the production that could pose potential safety hazards. The HACCP team can identify critical control points, which are steps in the process where additional safety procedures could be implemented to give the producer more control on the safety of the product. Protocols for monitoring the product throughout the process, corrective action plans, and documentation of product information as it flows through the production process are further ways to enhance product safety and improve a HACCP plan's effectiveness (USDA IFSIS, 1998).

Conclusions

A considerable challenge remains to ensure safe, fresh Hispanic cheeses since this type of cheese provides an optimal environment for pathogen growth, and also due to the possibility of the product becoming re-contaminated after the pasteurization process. The economic impact of product recalls, hospitalization and treatment costs of patients during outbreaks have stressed the need for the development of preventive measures to control the spread of pathogenic bacteria in fresh Hispanic cheeses.

A better and deeper understanding of how organisms relate to this food product, use of novel inhibitory techniques, establishment of HACCP systems in the dairy industry, regulated product sampling and testing, along with better detection and surveillance systems to report foodborne disease outbreaks would contribute to controlling foodborne illness related to Hispanic cheeses consumption.

The increasing market for Hispanic cheese in the United States, the still widespread home-based production using unpasteurized milk, and the lack of a standard of identity make the implementation of these tools extremely difficult and may contribute to an increase in the incidence of foodborne diseases. However, the development of new strategies, focused on the use of generally recognized as safe (GRAS) ingredients to control the growth and survival of *Listeria monocytogenes*, *Salmonella*, and *E. Coli* O157:H7, can prove helpful to control foodborne illness while meeting the increasing consumer demand without any loss in sensory and nutritional characteristics.

Research needs

The intrinsic characteristics of fresh Hispanic cheeses make this type of products very susceptible to pathogenic bacteria transmission. Because Hispanic cheeses are minimally processed after pasteurization and are almost completely devoid of any preservation technique other than refrigeration, the survival and growth of *Listeria monocytogenes*, *Salmonella* and *E. Coli* O157 are very likely. Those risks urge the development of novel approaches for control. In our laboratory, we have been working to develop an ingredient-based control with very promising results. The combined use of nisin with caprylic acid against *L. monocytogenes* has proven to be very efficacious, but it may have cost implications and may not have broad spectrum activity.

In addition to GRAS ingredients, other methods of control should be explored. The use of novel ingredients such as bacteriophages has been approved for ready-to-eat meats for control of *Listeria*, but there has been no attempt to apply commercially available cultures for Hispanic products. Several researchers have been investigating the use of natural products in many cases, treatments with spice or herbal extracts have been pursued, but all of these efforts still remain very experimental. More work is needed to confirm their effectiveness and lack of impact on sensory characteristics.

High pressure processing has been used in other dairy products (Drake et al., 1997) with relatively success and its effectiveness in reducing bacterial numbers has been readily proven. If applied to fresh Hispanic cheeses, it would likely control those pathogens and it may not have an impact on quality; however, the cost consequences may be prohibitive. Irradiation is a treatment that has been demonstrated to be highly effective against vegetative cells of pathogenic bacteria, but it has never been tested in Hispanic cheeses. The potential utilization of irradiation should only be deployed if the consumer perception improves. Other non-thermal processing may be needed to improve the safety of Hispanic cheeses.

The specific mode-of-action of generally recognized as safe (GRAS) ingredients to control the growth and survival of pathogenic bacteria is not yet fully understood and further research on the use these ingredients will provide insights that may prove useful for technological applications. In particular, interactions between GRAS ingredients and other food ingredients and additives need to be investigated. Potential synergistic effects could be exploited to maximize the antibacterial activity of the ingredients and to minimize the concentrations to prevent the microbial growth.

Enhancing the safety of ready-to-eat food products may benefit from a fresh look to post-processing contamination and the sanitation practices needed for minimizing pathogen transmission. Research efforts should be devoted to explore technologies that may lead to aseptic handling, molding and packaging of products such as fresh Hispanic cheeses. These enhanced sanitation methods could include the use of antimicrobial coating materials resulting from nanotechnology materials. We have tested in our laboratory a commercially available material that could reduce the count of *L. monocytogenes* more than 4 log CFU/cm² on surfaces previously treated with that material.

Possible secondary or indirect consequences of the use of GRAS ingredients also need to be investigated as the presence of these ingredients might elicit the cross resistance of these pathogens to other challenges. Such an event is well known in *Listeria monocytogenes*. This bacterium has been shown to become more tolerant to mild heat (56°C) after being exposed to ethanol, hydrogen peroxide or low pH. Could a similar phenomenon occur with GRAS ingredients?

The antibacterial activity of nisin, caprylic acid, and cinnamaldehyde against bacterial cells would provide a particularly appropriate model. The extent to which bacteria can survive to the presence of these compounds in Hispanic cheeses is also important for further evaluation; nisin has been shown to lose its activity against *Listeria monocytogenes* with time and the study of how bacteria can become resistant to this hurdle its useful, and it may lead to the creation of solutions to overcome the resistance.

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