Microbial safety of raw milk cheeses traditionally made at a pH below 4.7 and with other hurdles limiting pathogens growth

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Raw milk acid cheeses are manufactured and consumed in some tropical regions in America, North Africa and in the East Mediterranean countries. For tropical cheeses, little information is available on its microbial safety. This review give some insights of the microbiological safety of some raw milk acid cheeses around the world which are traditionally made at a pH below 4.7 and which contain other hurdles which are generally known as limiting factors for pathogens growth. It is described the occurrence of microbial pathogens in acid cheeses; the intrinsic and extrinsic conditions favoring its survival and growth, including pH, moisture, the presence of lactic acid bacteria and temperature. It is reviewed the published information on outbreaks of human illness linked to consumption of the raw milk cheeses made with acid curds. This information is important at epidemiological level because raw milk cheeses with higher pH values are generally known as vehicles of infection. Cheeses with lower pH values might be considered as a low microbial risk group where pathogen might be at a low level of concern. Microbial safety of these cheeses is due to the bacteriostatic properties given by its manufacture peculiarities. Microbial safety of these cheeses may be enhanced by the usage of good quality raw milk and by following good manufacturing practices in the whole process of cheese making to prevent cross-contamination of the product. Some other strategies used to improve the safety level of this kind of cheeses are mentioned.

Keywords Raw milk cheese, pH, safety, lactic acid bacteria, pathogens

1. Introduction

Cheese has a long history in the human diet [1]. Cheese making evolved centuries ago as a means of concentrating raw milk via acid precipitation of milk [2]. Fermentation of the milk sugars would cause the acidified milk to curdle and the swaying motion would break up the curd and provide solid curd and drinkable whey. The curds would be removed, drained and lightly salted to provide a tasty and nourishing high protein food [1].

Lactic acid bacteria (LAB) were likely the prime agents in producing soured (fermented) milk and dairy products. They are naturally present in the udder, the raw milk of healthy animals and have spread in the dairy environment [3]. Many species of LAB have the Generally Recognized as Safe (GRAS) status and are extensively used in the manufacture of dairy products. The prime property of LAB is the production of acids, especially lactic acid. The inhibitory capacity of this acid lies in its reduction of pH to levels below which bacteria cannot initiate growth. Lactic acid has unique sensory qualities and antimicrobial properties [4]. Lactic acid has a sour and pleasant taste. It has the generally recognized as a safe (GRAS) status by FDA and EC.

1.1 Classification and definition of Acid Cheeses

Currently clotting of the milk in acid curd cheeses is still accomplished by small enterprises around the world by adding acidifying starters (LAB), acids (i.e. lemon juice), and enzymes (rennet). Rennet sets the cheese into a strong and rubbery gel (cheese-like) compared to the fragile and weak gel produced by acidic coagulation alone (i.e. yogurt) [5].

The acid curd cheeses or acid/rennet curd cheeses may be very similar but also rather different in respect of manufacturing protocols, composition and organoleptic as well as mechanical properties. The main factor in the categorization of these cheese is their age.They differ whether they are fresh or ripened, by the moisture content and by their mechanical properties (texture) and other peculiarities of the milk used and the processing, such as the methods of salting or ripening (Fig. 1). However, the classification and definition of cheeses are, in most countries, controlled by a codex or law. An alternative classification of acid cheeses made with raw milk might be done by the hurdles related to microbial safety (Fig 2).

1.2 Fresh cheeses

According to Schulz-Collins and Senge [6], fresh cheeses are generally low in dry matter (DM) and, hence, low in fat and protein and high in lactose/lactate. As most of the calcium is solubilized during the acid coagulation and removed with the whey, fresh cheeses are much lower in calcium than rennet-curd cheeses [6]. They can be divided into various categories, e.g., by the method of coagulation- acid, acid rennet, acid-heat, etc., their consistency- paste, grainy or gel-like, or raw material- milk or whey. Fresh cheeses without additional preservatives can spoil in a matter of days.

1.3 Ripened cheeses

In comparison, most ripened cheeses, are generally high in dry matter (DM) and, hence, high in fat and protein and low in lactose/lactate. Acid-curd ripened cheeses have generally a very strong flavor and odor, and a slightly yellow color and a slightly brittle texture [6]. Ripened cheeses can be divided into two types, mold-ripened cheeses and yellow cheeses. One of the important species for the ripening of yellow cheeses is *Brevibacterium linens* (the reddish-orange "smear bacteria"). It imparts pungent odors and distinctive flavors. *Brevibacterium* is also found on human skin, and structures adjacent to skin and it is partially responsible for body odor. Strains assigned to *Brevibacterium* had not been associated with human infections [7]. This species can grow spontaneously on the surfaces of Harzer acid cheese (Germany) or artisan cheeses, like queso de Poro in Mexico.

During ripening, changes occur in the matrix through the influence of the loss of water and proteolysis. Proteolysis begins with coagulation of the milk in the vat; this is a primary proteolysis, i.e., internal hydrolysis of casein molecules, by the coagulant and indigenous enzymes of milk, such as plasmin. Secondary proteolysis occurs during ripening, by the action of peptidases of microorganisms. Proteolysis weakens the structure of the casein matrix [8].

Within the ripened cheeses, brined cheeses are the most important family of cheeses for East-Mediterranean and neighboring countries [9]. White-brined cheeses (also known as white-pickled cheeses) are the most popular varieties of cheeses manufactured in the North-east Mediterranean area and the Balkans. They are manufactured from ovine, buffalo, bovine and/or caprine milk or from mixtures of these milks. Feta cheese (in Greece), Domiati (in Egypt), Beyaz peynir (in Turkey) and Halloumi (in Cyprus) are the best-known cheeses, while other, less well-known varieties include Batzos from Greece and Brinza from Bulgaria [9]. Other important ripened cheese is queso de Poro, which is a low pH, low moisture and ripened cheese made from raw cow milk in Mexico [10]. It is generally stored and distributed at room temperature.

1.4 Manufacturing

A general procedure of manufacturing raw milk acid cheese is shown in Fig 3. Curd acidification should be accomplished during the early stages of cheese making (curdling, draining). For most of the White Brined Cheese, it is essential that about 24 h after coagulation pH is lower than 5.0, moisture is < 60% and salt-in-moisture content (S/M) is ∼ 2.5% [11]. Lactic acid production during these stages is of vital importance. Too slow or too low acidification may not suppress the growth of some microorganisms (i.e. coliforms) able to cause early gas blowing. This is a defect associated mostly with raw milk cheeses. On the other hand, high curd acidification leads to excessive drainage, lower yield and dry, hard and grainy cheeses without cohesion, especially when goat's or cow's milks are used for cheese-making. The cheeses should be transferred to the cold room (4–5 ◦C) only when their pH attains a value of ∼ 4.6 or lower, moisture level is ∼ 55% and solid/moisture higher than 50% [11].

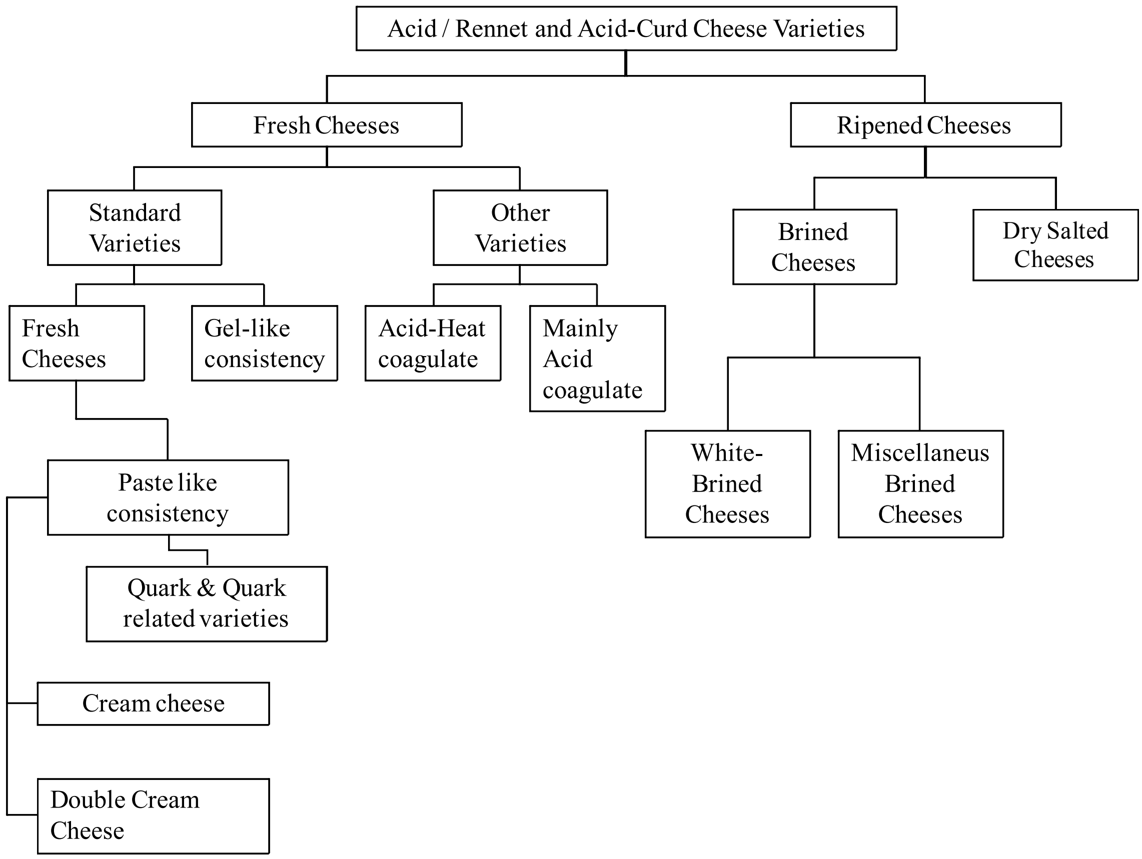

Fig. 1 Classification of Acid Cheeses. Modified of Schulz-Collins and Senge 2004. Ripened Cheeses were divided in two categories: 1) Brined Cheeses, which was subdivided in A) White Brined Cheeses B) Miscellaneous brined cheeses and 2) Dry Salted cheeses.

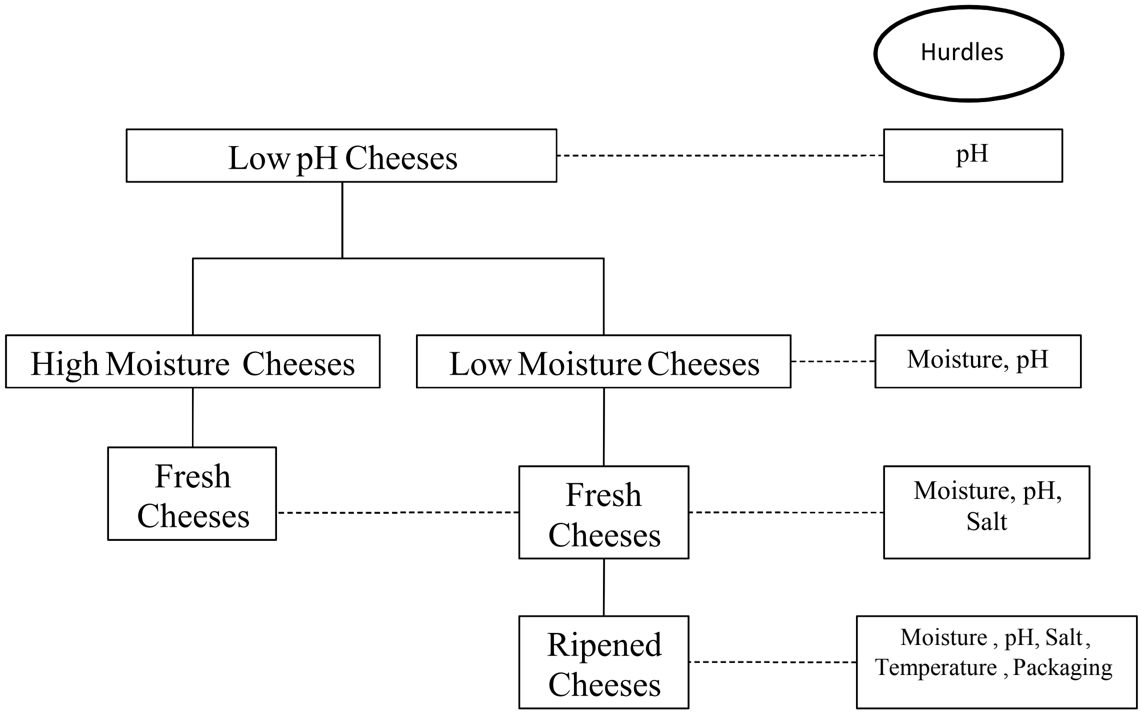

Fig. 2. Simplified scheme of classification of raw milk acid cheeses by microbial hurdles.

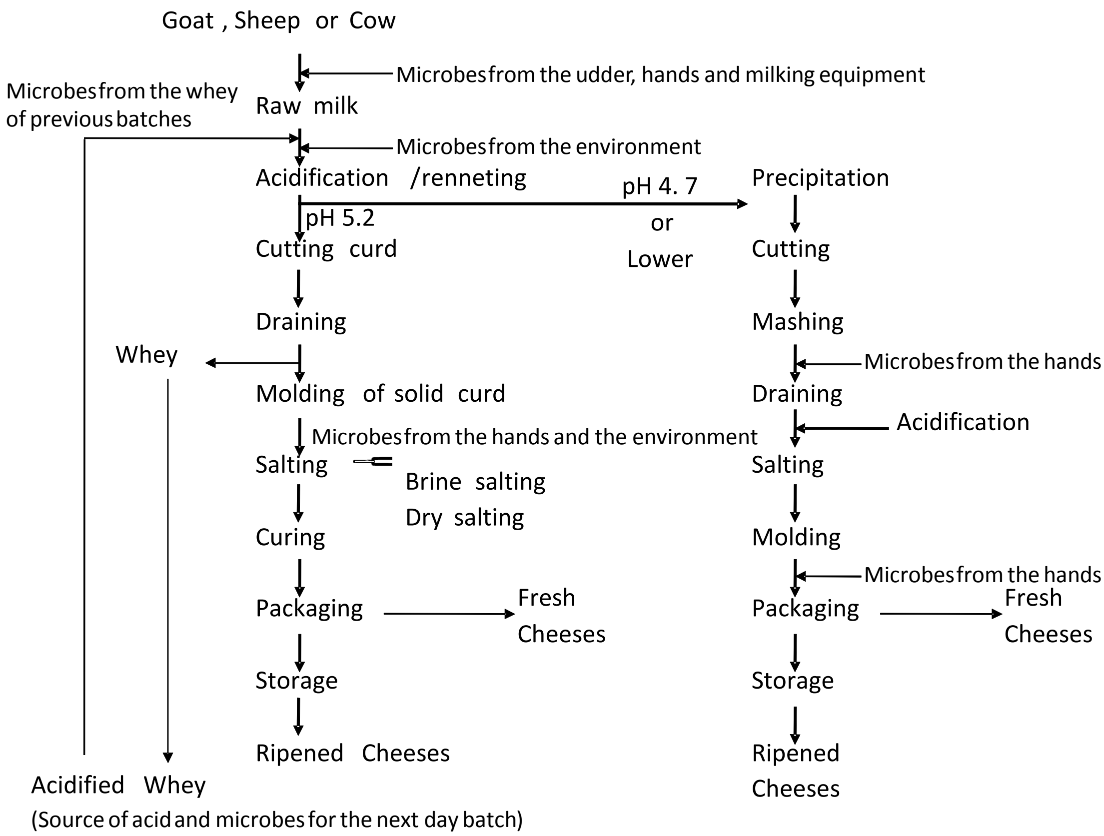

Fig. 3 Schematic illustration for the manufacture of traditional raw milk acid cheese.

2. Microbes and sources of microbes in raw milk cheeses

Milk is an excellent medium for growth of microorganisms, as it provides rich nutrients (proteins, fats, lactose, vitamins and minerals) for microbes, it is high in moisture, and has neutral pH. Milk is exposed to microorganisms during collection, storage, transportation, and processing.
Microorganisms are important in raw milk cheeses for three principal reasons:

- LAB and other microorganisms may contribute in the preservation of milk and in the production of desirable flavor and physical characteristics [12]. For raw milk cheeses there is a need for knowledge of the natural biodiversity of microorganisms, their role, and their interactions [8].
- Pathogens or their toxins may constitute health hazards.
- Spoilage microorganisms or their metabolites may cause spoilage.

2.1 Microbes with a specific role

Some microbes can produce many flavorful products and can also preserve milk and milk products [13]. Microbes in raw milk cheese which are generally considered safe are LAB, some species of yeasts and molds are safe and are related to flavor production. For some cheeses, other important bacteria are the propionic acid bacteria and *Brevibacteria* as well [14].

2.1.1 Lactic acid bacteria

LAB forms a natural group of Gram-positive, nonmotile. non-sporeforming, rods and coccus-shaped organisms that ferment carbohydrates and higher alcohols to form chiefly lactic acid. They are oxidase negative and catalase negative [15, 16]. Important genera for dairy products are *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Pediococcus*, *Bifidobacterium* [17]. LAB may withstand a low pH because when grown in acidified medium, they maintain a higher pH in the cytoplasm than the culture medium [18]. Higher internal pH is accomplished by pumping out protons (H^+ ions) via ATPases [17]. LAB are naturally present in raw milk andraw milk products. In traditional cheese making they are transferred from old batch to new batch via whey addition. Selection of the beneficial natural flora in milk, such as lactobacilli, streptococci and lactococci naturally occur in good batches and in many instances decrease the numbers of bacterial pathogens [2]. In optimizing the manufacturing process, the direct addition of selected LAB as starter cultures in pasteurized milk is a common practice [2]. As a drawback, the sensory characteristics of the original cheeses are loss partially, because there are some microorganisms important in the flavor formation of artisan cheeses that are not added as starters. At the beginning of ripening, LAB starter counts are high, usually 10^8-10^9 CFU/g, and decrease regularly by two or more log cycles during ageing, whereas adventitious microorganisms, which are initially at a level often <10^3-10^4 CFU/g, grow during ripening [8]. The starter flora is responsible for acid development during cheese manufacture. The majority of the strains belongs to the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Enterococcus*. The main species used as starters for Feta cheese are *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus helveticus* and *L. delbrueckii*. The main LAB isolated from Jben cheese are *L. plantarum*, *L. rhamnosus*, *L. paracasei*, *L. brevis*, *L. buchneri*, *Lactococcus lactis*, *Lc garvieae*, *Lc raffinolactis*, *Lc pseudomesenteroides*, *Lc mesenteroides*, *Lc citreum*, *Eterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus saccharominimus* and *Streptococcus* sp. [19]. In queso Tabasco, the main acidifying bacteria found were *Lactobacillus fermentum* and *Lactobacillus pentosus* [20]. According to Hayaloglua [21], LAB species isolated from Turkish white cheese were, Lc. *lactis* subsp. *lactis* being the predominant species at the beginning of ripening, and enterococci (*E. faecalis* and *E faecium*) were the second most numerous group. *Lactococci* declined during ripening, while *Lactobacillus* species increased, being *Lb. casei* and *L. plantarum* the predominant species.

2.1.2 Yeast

Yeasts are eukaryotic micro-organisms classified in the kingdom Fungi. They are much larger than bacteria. Yeasts play an essential role in the preparation of certain fermented dairy products such as kefir, kefir-derived raw milk cheeses and in the ripening of many cheeses. The high numbers of yeasts in some acidified dairy products may be attributed to their ability to tolerate low pH values and low water activities; and to grow at low temperatures, to assimilate lactose and organic acids such as succinic, lactic, and citric acid; and to tolerate high salt concentrations and cleaning compounds and sanitizers [23]. The predominance and growth of some yeast species has been related to the ripening process due to their ability to produce extracellular proteases and lipases and grow well at 5°C [24]. Because yeast are recovered at the end of the fermentation stage, it is suggested that they play a secondary role in the aroma developement in Lben, a low pH high moisture product from Morocco [25].

The occurrence of yeasts in yogurt can cause spoilage (up to 10^6-10^7 CFU/g) [24]. The public health significance of yeasts in foods, including dairy products, has been considered by most health authorities to be very minimal [24]. Yeast in high numbers (10^6-10^7 CFU/g) in Jben (low pH-low moisture cheese) is not matter of concern to the safety of the product [25]. *Candida famata, Kluyveromyces marxianus, Candida diffluens* and *Rhodotorula glutinis* were the most frequency isolated species [23]. The sources of yeast and surface bacteria of smear cheeses are the following: milk, cheese brines, the air of ripening rooms, ripening shelves, and the human skin [26]. High numbers of yeasts are frequently observed on processing equipment, and in the air of the processing environment [22], such as wooden tables used for dry salting the cheese blocks, since wood is an ecological niche for some species such as *Dek. anomala* [22]. Dominant yeasts of brine from Greek feta cheese were *Saccharomyces cerevisiae*, *Candida famata*, *Torulaspora delbrueckii* and *Pichia membranaefaciens*. It has been suggested that spoilage yeasts may have a role in protecting *L. monocytogenes* from acid in industrial type pilot cheese by metabolizing milk proteins, raising the pH and potentially assimilating organic acids of cheese [27].

2.2 Microbial pathogens

According to U.S. Food and Drug Administration (12), and EEC directive 92/46 [8], the principal pathogens of concern associated with milk and processed milk products are *Salmonella* spp., *L. monocytogenes*, *S. aureus*, pathogenic *E. coli*. Many of the common enteric pathogens such as *Salmonella*, *Escherichia coli* O157: H7 and *Campylobacter* are carried in the intestinal tract of ruminants, including domestic animals used in milk production, e.g. cows, sheep and goats [28]. Effective cleaning procedures, including removing faecal material from udders prior to milking and good manufacturing practices during cheese making process can reduce the risk [28].

2.2.1 *L. monocytogenes*

L. monocytogenes is a Gram positive rod, oxidase negative, catalase positive without pigmented colonies [29].It is also the causal agent of human Listeriosis an atypical foodborne disease and it is one of the most dangerous pathogens to the food industry [28, 30].

2.2.1.1 *Habitat*

It is widespread in nature and has been isolated from soil, dust, food products for humans—both of animal and vegetable origin, feed, water, and sewage, and it can be carried by almost any animal species, including asymptomatic humans [30]**.** *Listeria* spp. most commonly gain access to the milk from the cows' udder during milking. Animals are most likely to become colonized through consumption of the organism in grass or feed and, in particular, silage. As with enteric contaminants, *Listeria* spp. can gain entry to the milk from faecal contamination of the udder [31]. Once introduced into the milking equipment, *L. monocytogenes* can readily colonize these moist environments [31]. *L. monocytogenes* is found commonly in wet areas of dairy plants, such as floor drains, conveyers, floors and stainless steel equipment and has been isolated from brine solutions used for cheese [32].

2.2.1.2 *Adaptations of Listeria* to the environment

L. monocytogenes is a psychotropic and salt tolerant bacterium, and may grow at pH 4.5 to 9.6 or at a_w values as low as 0.92, while certain strains or serotypes may show increased adaptive responses to food-related stresses. Thus, controlling contamination and growth of *L. monocytogenes* during cheese manufacture, ripening, and storage is an important safety concern and consumer demand [27].

2.2.1.3 *Occurrence in cheese*

The occurrence of *Listeria* spp. in raw milk acid cheeses is variable. It has never been reported for Feta cheese [33]. A survey on the occurrence of pathogens in commercial Moroccan dairy products showed that *L. monocytogenes* was frequently detected in the fresh products made with raw milk (low pH-high moisture) but not in ripened cheese [34]. Due to their overall lower hurdle effect compared to hard cheeses, soft cheeses show a higher incidence and potential for survival/growth of *L. monocytogenes*. Accordingly, soft cheeses have been implicated in the most fatal listeriosis outbreaks in total or proportionally to the numbers of reported cases of illnesses [27]. Rudolf and Scherer [35] reported that 6.4% of European red-smear cheese samples were contaminated with *L. monocytogenes*. *L. monocytogenes* was found more frequently in high-moisture cheese.

2.2.2 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive cocci, oxidase negative and catalase positive. It forms clusters showing pigmented colonies when grown in nutrient agar. It often forms characteristic clumps resembling bunches of grapes [29]. Staphylococcal intoxication (staphylococcal food poisoning) results from ingestion of enterotoxins, synthesized during growth of *S. aureus* in foods. Enterotoxin production is most common amongst *S. aureus* isolates of human origin and there is a strong correlation with production of the enzyme coagulase [36].

2.2.2.1 *Habitat*

Staphylococci are predominantly of animal origin, although isolation of some species may be made from environmental sources. Some strains of *S. aureus* are able to colonize equipment and the factory environment. Cleaning schedules should take account of this possibility [37]. They may be present as part of the normal microbiota of humans and other animals. *S. aureus* might be carried on skin and nasal cavities of 30% of the healthy human population [36]. *S. aureus* is frequently found in milk at low levels. It often occurs in the udder of a cow with mastitis or subclinical mastitis. *S. aureus* can gain access to milk either by direct excretion from udders with clinical or subclinical staphylococcal mastitis or by contamination from the environment during handling and processing of raw milk [37]. When the udder is infected, *S. aureus* is excreted in the milk with large fluctuations in counts ranging from zero to 10^8 CFU/ml [37].

2.2.2.2 *Occurrence in cheese*

S. aureus was isolated from a Turkish White cheese, "Beyaz Peynir" during a ripening. This is a brined cheese variety with a soft or semi-hard texture and a salty and acid taste [20, 37]. In Lben (low pH and high moisture dairy product), coagulase positive *S. aureus* was detected in numbers above 10^3 CFU/ml [25]. High numbers of *S. aureus* are generally associated with the presence of toxin ($>10^6$ CFU/g of cheese or ml of whey).

Cheese vats with low acidity for a long time might be a risk, because *S. aureus* might grow at a level high enough to produce the enterotoxins [13]. When LAB are used as starters to make cheese, it is reduced the possibility of toxin-infections with S. *aureus* [38]. However, a small number of outbreaks caused by *S. aureus* have been attributed to the use of contaminated starter cultures. Rapid acid production by active starter cultures could prevent *S. aureus* to grow to the high numbers required for enterotoxin production [12].

In a challenge study, goat milk was inoculated with an enterotoxinogenic *S. aureus* strain to a final concentration of 1×10^4, 1×10^5 and 1×10^6 CFU/ml. Thereafter, a raw goat milk lactic cheese was made (low pH, 37-50% dry matter content). Staphylococcal counts in inoculated in goat milk, declined markedly after the draining period and the organism even disappeared from some cheeses by the end of ripening stage [39].

2.2.3 *Salmonella*

Salmonella spp is a Gram negative, non sporing rods, oxidase negative, and catalase negative without producing pigmented colonies [29]. It is one of the most prevalent pathogens in the food industry. Studies about this microorganism date up to 100 years and have been the causative agent on several outbreaks of foodborne diseases particularly in dairy products. Most of species are pathogenic [40, 41, 12].

2.2.3.1 *Habitat*

The primary habitat of *Salmonella* spp. is the intestinal tract of animals and humans. *Salmonella* food poisoning results from the ingestion of foods containing appropriate strains of this genus in significant numbers [29]. Raw milk is an important vehicle for salmonellae causing human infection [41, 3, 12]. *Salmonella* spp. causes illness by means of infection. They multiply in the small intestine, colonizing and subsequently invading the intestinal tissues, producing an enterotoxin and causing an inflammatory reaction and diarrhea.

2.2.3.2 Occurrence in cheese

Although analysis of commercially made cheeses rarely results in isolation of salmonellae, these species may grow during cheese manufacture and can survive in various cheeses for more than 60 days [12]. *Salmonella* spp. was detected in traditional Jben (low pH-intermediate moisture) at frequencies of 10% [25].

2.2.4 *Escherichia coli* O157: H7

E. coli O157:H7 it is a Gram negative rod, oxidase negative, catalase negative without producing pigmented colonies [29]. Most of foodborne outbreaks of *E coli* O157:H7 have been associated with the consumption of foods contaminated with cattle feces [40, 3, 12].

2.2.4.1 Habitat

It is commonly isolated from feces of cattle and other farm animals; it also has been isolated from raw milk and dairy products [12]. Because *E coli* O157 has been found regularly in healthy cattle feces, this animal is known to be an asymptomatic transmitter [42].

2.2.4.2 Occurrence in cheese

There are no reports of *E. coli* O157:H7 isolated from unpasteurized milk [39]. However, there is a growing concern, because *E. coli* O157:H7 is relatively an acid tolerant microorganism [12]. The presence of viable *E. coli* O157:H7 is of concern to all manufacturers of raw lactic milk cheeses for several reasons. First, this pathogen is infectious even at low doses. Second, experiments *in vitro* have shown that *E. coli* O157:H7 colonies exposed to mildly acid conditions develop an adaptive tolerance response (ATR) to acid environments and become acid-tolerant to even lower pHs during subsequent exposures [39].

3. Factors limiting pathogens growth

The survival and growth of pathogens in cheese depend on the many factors, including variations in pH, a_w, salts, the presence of competing microbiota, and the temperature and biochemical changes during ripening. The microbiological quality of the milk and the good manufacturing practices will also contribute to the safety of the final product, especially in cheeses where milk is not pasteurized [13]. Each limiting factor will be described in the following lines.

3.1 pH

Most of bacteria grows optimally at a pH close to neutrality [29]. Acid curd cheeses might have a longer storage life and higher safety standards than neutral pH cheeses. A low pH decrease the growth rate of most of microbial pathogens and microbial spoilage. Among the pathogenic bacteria affected in their growth rate by the effect of low pH are species of the genus *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus,* and the pathogenic strains of *Escherichia coli* such as the enthomorragic (ECEH), *E. coli* enteropathogenic (ECEP) *E. coli* enterotoxigenic(ECET), *E. coli* enteroinvasive (ECEI) y *E. coli* O157:H7 [41]. For instance growth of *L. monocytogenes* has been reported at pH values ranging from 4.0 to 9.6. Lag phase and generation time increase considerably as pH decreases to below 6.5. *L. monocytogenes* can tolerate pH values up to 4.1 depending on other factors such as moisture content, storage temperature, and the presence of starter cultures [40, 43, 44, 29]. *E. coli* O157: H7 appear to be more acid-resistant, being able to withstand pH values as low as 3.0. Consequently, there is a potential risk that some VTEC strains, and especially strains of E. coli O157:H7, could survive the low pH associated with the cheese manufacturing process [28].

3.2 pH and temperature

Absence of growth and decrease in cell viability may be observed in *L. monocytogenes* at pH ≤5.5, when other environmental conditions are not optimal for survival [46]. By using a simulation model (USDA Pathogen Modeling Program), it was possible to figure out what would be the reduction time of *L. monocytogenes* exposed to low pH (pH = 4.7) at various temperatures (Figure 3); additional conditions in the model were sodium chloride 2.0%, aw 0.989, and lactic acid 1.5%. The higher the temperature, the faster the reduction time.

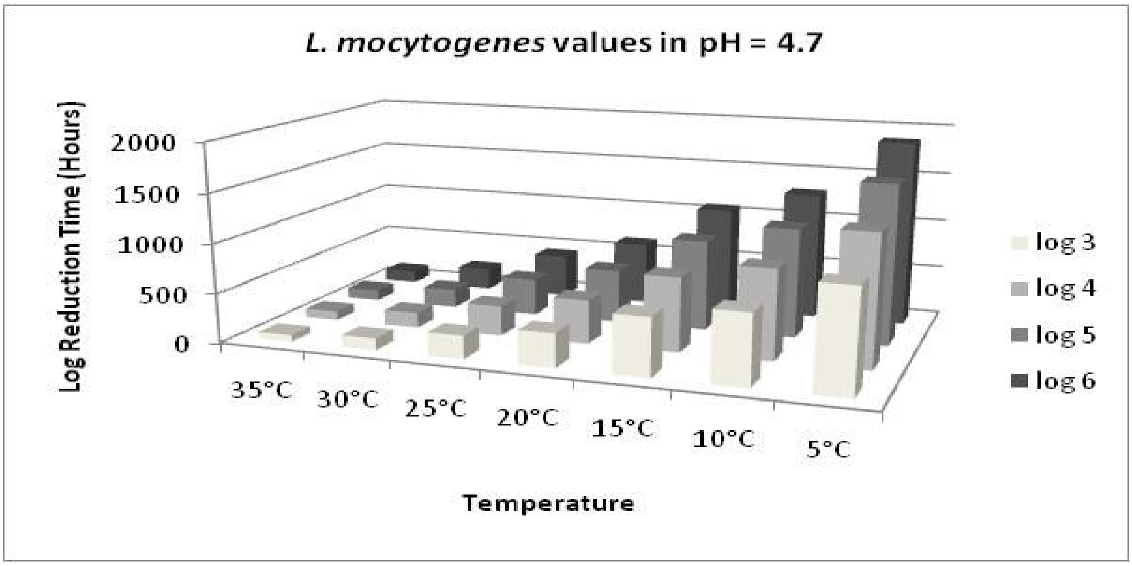

Fig. 4 Exposure time, needed to decrease *L. monocytogenes* populations 3 log units (i.e., 3 D-values) at different storage temperatures, as determined by the USDA Pathogen Modeling Program. (USDA. Pathogen Modeling Program [version 7.0]). http://ars.usda.gov

By using The USDA Pathogen Modeling Program, it was also possible to simulate what would be the reduction time of *L. monocytogenes*, *S. aureus*, *Salmonella* and *E.coli* by varying pH and temperature. High temperatures were related with a fast reduction time for pH values between 4 and 5.2. The longer reduction time was associated with high pH and low temperature.

In order to confirm such predictive information derived from a mathematical model, it is necessary to carry out challenging experiments to examine survival of the pathogens during the manufacture and ripening of raw milk cheeses as described by Vernozy-Rozand and colleagues [39]. A source of variation could be the temperature of ripening. Such information could help to know whether the actual temperatures for ripening of some regional cheeses specialties like queso de Poro in Mexico are the best for microbial safety. Queso de poro is usually ripenened and stored at room temperature.

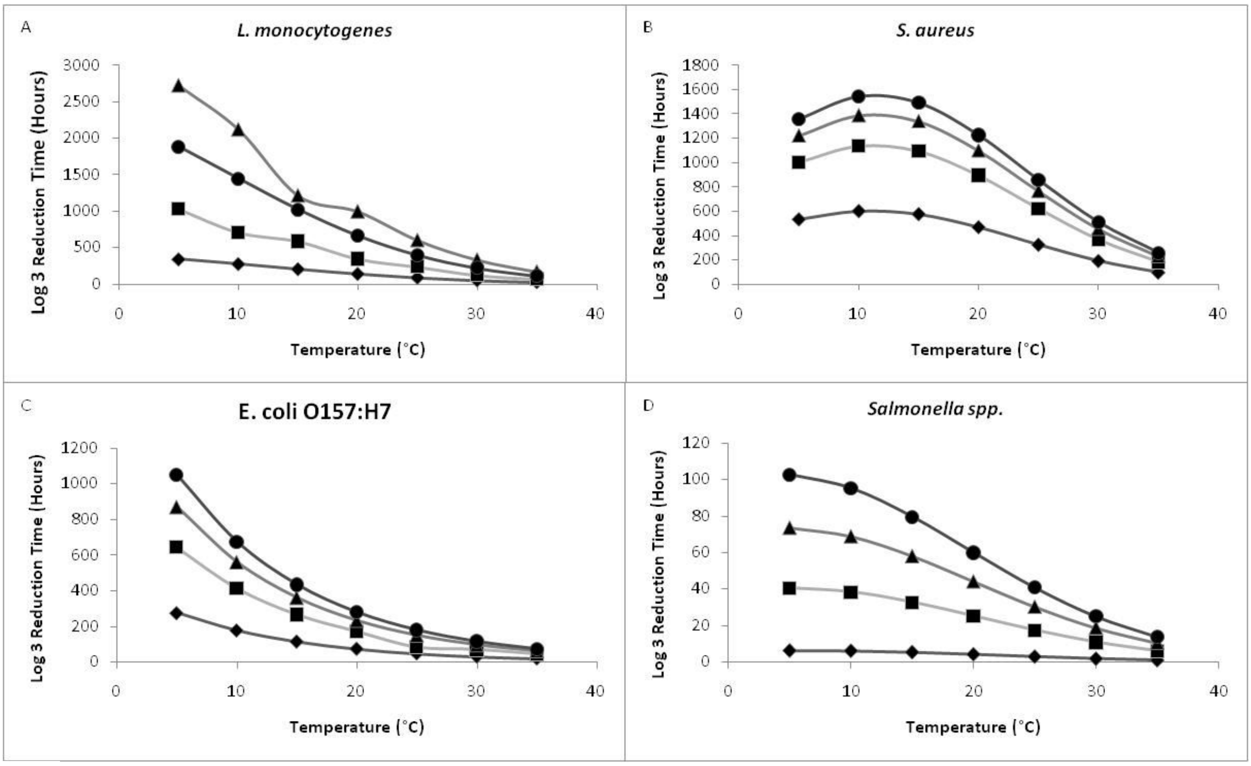

Fig.5 Exposure time, needed to decrease A) *L. monocytogenes*, B) *S.aureus*, C) *E. Coli* O157:H7, D) *Salmonella spp.,* populations 3 logs (i.e., 3 . D-values) at different storage temperatures and different pH concentrations, values obtained with the USDA Pathogen Modeling Program. (From [47] Pathogen Modeling Program [version 7.0]. http://ars.usda.gov. Symbols ♦ph 4.0, ■ pH 4.7, ▲ pH 5.0, ● pH 5.2)

There are some adaptation mechanisms to acid reported for the genus *Salmonella* [48], *Listeria, Staphylococcus aureus* and *E.coli*. Acid-adapted cells of *Salmonella* had increased resistance to inactivation by organic acids commonly present in cheese including lactic, propionic, and acetic acids. Such mechanisms could be enhanced when milk fermentation takes a long time [48]**.**

These observations support the theory that acid adaptation is a mechanism of adaptation that allows *Salmonella spp.*to persist in fermented dairy products and possibly other acidified food.

3.3 Alkalinization of cheese

Some varieties of cheese may have other non-lactic and non-acidifying microbiota such as yeasts, fungi that can alkalize the cheese as *Debaryomyces hansenii* or *Kluyveromyces marxianus* and can allow the growth of *Brevibacterium* in numbers as high as lactic biota. Growth of some pathogens such as Listeria can occur on cheese surfaces [49].

3.4 Other hurdles

In the acid cheeses, the others intrinsic factors like the a_w and the salt content generate adverse conditions for microbial growth, which vary depending on the type of cheese and the level technology used in its manufacture [8]. In general *S. aureus* and *L. monocitogenes* are resistant to salinity (up to 6%) and pH of 4.1 in certain cases [40,43,44]

3.5 Stress adaptation

A stress adaptation occurs when a bacterium exposed to a sub-lethal stress, may become more resistant to subsequent applications of the same stress in a higher intensity or to exposure to other stresses such as temperature, salt content, moisture and a_W conditions [50, 51]. Several foodborne pathogens such as *Salmonella* spp, *L. monocytogenes*, *E. coli* O157:H7 and *S. aureus* have been reported to exhibit an enhanced resistance to severe acid environments after exposure to a mild acid environment. The acid-adapted bacteria may also show increased resistance, or cross-protection, to other environmental stresses.

4. Outbreaks of disease by the presence of pathogens

There is limited published information on outbreaks of human illness linked to consumption of the raw milk cheeses made with acid curds. However there are many reports of outbreaks of foodborne diseases as a result of ingestion of high or mild pH cheeses made of raw milk.

Cheeses are susceptible to contamination during the ripening process, for instance, multiplication of *L. monocytogenes* is associated with increasing pH during ripening of cheeses like Camembert, Brie, Blue and Feta [12]. There is not information regarding to possible outbreaks of salmonellosis in acid cheeses. However Salmonella *spp.*in dairy products caused several outbreaks of foodborne disease in Canada, Europe and USA, in particulary cheeses like Cheddar, Mozzarella, Irish [41, 52] which are mild acidified cheeses.

5. Strategies used to improve the microbial safety level of raw milk cheeses

Hygienic milking practices involving udder and teat cleaning and sanitization can help to reduce contamination of the milk with pathogens like *Listeria* spp. Equally important is the cleaning and sanitization regime applied to the milking and milk storage equipment (31). Rapid acidification in the early stages of the process of raw milk cheeses manufacture is a key factor to effectively control the development of *L. monocytogenes* and other pathogens with low core contamination in raw-milk [49]. Rapid acidification may be accomplished either by adding active starter cultures or acidified whey, or by addition of acid. It is necessary to incorporate the Hazard Analysis Critical Control Point (HACCP) and Microbial Risk Assessment (MRA) plans for prevention of contamination of raw milk cheeses.

6. Conclusion

Raw milk acid cheeses are produced by small enterprises in various countries around the world. The process of manufacture of raw milk acid cheeses is similar in many ways. Microbial risks might be prevented by including hygiene in the milking process and by the rapid acidification of the milk and the rapid reduction of moisture as well. Many microorganisms such as lactic acid bacteria, yeasts, molds and other non-pathogenic bacteria are adapted to survive and grow in such environments. They have a key role in the biochemical and sensory characteristics of each kind of cheese. The lactic acid bacteria group may also have a role inhibiting the growth of pathogenic and spoilage bacteria. Very few reports have shown the occurrence of foodborne pathogens isolated from raw milk acid cheeses, particularly from raw milk acid fresh cheeses. Moreover, to our knowledge~~,~~ raw milk acid cheeses have never been involved in outbreaks of human diseases. It is feasible that the processing protocols to manufacture raw milk acid cheeses create an unfavorable environment for pathogen microbes to thrive at a level that represent a hazard risk for the consumer.

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