Microflora and bacteria in limburger cheese development

Limburger Cheese

1. Introduction

Limburger cheese gets its name from the country it originated in, Limburg, which is now divided amongst Germany, Belgium, and the Netherlands. [1] Limburger cheese is made from pasteurized cow's milk and has a 42% fat content. [1] This cheese is typically more mild due to the milk that is used in the production process. [1] The texture of this cheese can be characterized as creamy, crumbly, firm, and smooth. [1] However, this cheese is best known for its highly stinky aroma resembling that of foot odor. [1]

Limburger cheese is rind washed. Due to the regular rind washings, the exterior of the cheese is covered with a thin pale, orange-brown rind. [1] Starting out, the Limburger cheese is firm yet crumbly; this phase is very short before the cheese becomes chalky and soft. [1] This chalky and soft characteristic takes place after about six weeks. [1] The cheese becomes creamier and smoother at about two months. [1] Then at three months, the cheese acquires its stinky aroma. [1] The exterior portion of the cheese is the thin rind, while the interior of the cheese is a soft and yielding straw colored paste. [1]

2. Microflora

Initially, the microflora of bacterial smear surface-ripened cheeses appear to be very similar at the beginning of the ripening phase; however, the bacteria that becomes present at the end of the ripening stage is what sets the cheeses apart. [2] The limburger cheese aroma is due to smear ripening with solutions of bacteria. [1] “ Early studies on the microflora of Limburger showed that when the pH of the cheese surface rises to 5. 85, due to the growth of the yeasts, the growth of B. linens commences. [2]”

3. Yeast

Yeast can establish significant interactions with the bacteria present on the surface and lactic acid bacteria. [2] Smear surface cheeses contain the most bacteria on the surface of the cheese such as the rind and play main roles in the final characteristics and attributes. [2] Yeasts and molds initially dominate the surface post manufacture because they are acid tolerant and salt tolerant, but at the end of the ripening period, bacteria of the genera Brevibacterium, Arthrobacter, Micrococcus and Corynebacterium are the dominant microorganisms. [2] Although the yeast play an important role in the smear ripening process, very small yeast cells are present once the cheese ages. [4]

Yeast also play an important role in the bacteria smear ripened cheeses. The yeasts interact with the molds and bacteria including on the surface and the lactic acid bacteria. [2] The yeasts also help contribute to the flavor, texture, and aroma to the cheeses. [2]

The first thing to develop on the external part of the smear ripened cheeses is yeast. [2] “ Studies on the evolution of yeast populations on the surface of cheeses such as Limburger(El-Erian, 1972)have shown that they reach the highest number of 10^{8} – 10^{9} cfu/g of smear after about 7 days of ripening. After that time, a stable value of the yeast population, has a decrease in their number, as shown in Limburger cheese, have been reported. However, the general trend is the yeast domination during the early stages of ripening, followed by bacterial domination of the surface flora. [2]"

4. Bacteria

Clostridium licheniforme and Paraplectrum foetidum are two other bacteria that have been studied and found on the surface of the smear ripened Limburger cheese. [4] After research by Weighmann he came to discover that the two prior bacteria were aiding in breaking down the lactic acid in the cheese and making the medium more alkaline. [4] Weighmann also discovered that a red bacteria that would cover the smeared surface of the cheese, was anaerobically permitting the growth of *P. foetidium.* [4] Weighmann also credited the aroma and flavor of the Limburger cheese to the bacterium *P. foetidium.* [4]

5. Chemical Compounds

Smear surface ripened cheeses happen to ripen faster and have more of an intense flavor profile than other cheeses. [2] The reason for the fast ripening and the strong flavoring is due to poor syneresis properties such as, the method of curing involved which allowed the creation of favorable optimal development for the microbiological community. [2]

The two main microorganisms present in Limburger cheese is *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *lactis.* These two microorganisms are responsible for providing distinct flavors and textural attributes. [3] Limburger cheese is coagulated by rennet, then goes through an external bacterial ripening stage. [3] Limburger is a semi soft cheese with a moisture content ranging from 39-50% with 50% being the maximum moisture content allowed in this specific cheese. [3] This cheese is surface ripened by bacteria and yeast. [3] Due to the salting method that takes place during the ripening of Limburger, salt tolerant microorganisms grow very well on the surface at this time. [4]

Background

There are 17 species of the *Listeria* genus; however, only *Listeria monocytogenes* is pathogenic to humans. [6] L. *monocytogenes* is can be fatal to humans due to its widespread diffusion in the environment and food. [6] Ready-to-eat foods have been traced as being the main vehicle for transmission of Listeria through contamination somewhere in the food chain. [9]

L. monocytogenes is also a dangerous pathogen, due to its ability to withstand and grow in low temperature environments and high salt concentrations. [6] " Compounds used to inhibit growth of this pathogen include organic acids, fatty acids, antioxidants, sodium nitrite, smoke, spices and herbs. [9] " Another reason *L. monocytogenes* is a dangerous pathogen is because " some strains are able to survive for long periods of time under adverse environmental conditions and persist in niches in food processing equipment, and associated drains, walls and ceilings. [9]" However, most large outbreaks are contracted through errors in food processing plants. [9]

There have been multiple different studies done showing if *L. monocytogenes* can survive through the process of pasteurization or not. " An early study by Bearns and Girard showed the *L. monocytogenes* may be able to survive pasteurization if present in fresh milk at concentrains of more than 5 x 10 [4] organisms / ml. Conversely, Bradshaw and collaboratorsfound that *L. monocytogenes* could not withstand pasteurizing temperatures. [7]" The only way the pathogen would be able to withstand the pasteurization would be if it resides within leukocytes. [7] Another way *L. monocytogenes* can withstand pasteurization is through its ability to form inside of biofilms. [9] Overall, L. monocytogenes is more likely to be contracted through the consumption of raw milk. [6]

" L. monocytogenes infects normally sterile parts of the body such as the liver, spleen, cerebral spinal fluid and blood. [9]" Most cases of this pathogen result in being hospitlaized. [9] For healthy adults symptoms mainly include diarrhea and fever, for pregnant women symptoms include fever, diarrhea, abortion or still-birth, and symptoms in newborns include sepsis, pneumonia or meningitis. [9]

Listeria monocytogenes in Semi-Hard Cheeses

The growth potential of *Listeria monocytogenes* on semi-hard cheeses, such as Limburger, was generally lower than the other types of cheeses. [5] The range of *L. monocytogenes* growth potential ranged from 0. 1 to 1. 4 log units at 7 °C and from 0. 0 to 3. 0 log units at 14 °C. [5] " Overall, increased outgrowth of *L. monocytogenes* was noted when inoculation was performed on the cheese slicing surface compared to the cheese rind. [5]" There is a relatively large variation in the presence of *L. monocytogenes* in different cheeses. [5] The storage temperature and the cheeses type play a role in the growth potential of *L. monocytogenes.* [5]

Although, semi-hard cheeses have a lower growth potential of obtaining *L. monocytogenes,* it has the possibility to grow in numbers through post-processing contamination, storage, or handling of the cheese up to 14 days notwithstanding the presence of high numbers of indigenous lactic acid bacteria in these cheeses. [5] It has been tested that the most frequent cases of cheese-related outbreaks with *L. monocytogenes* occurs in the post-pasteurization contamination. [8] In Europe, human foodborne infections of *L. monocytogenes* were identified as the vehicle for the soft and semi-soft cheeses. [5]

" It was also found that semi-hard cheeses will not support growth, but only enable survival of L. monocytogenes. The growth potential in soft and semi-soft cheeses, on the other hand, is noted to be substantially higher than in semi-hard cheeses. Thus, it can be concluded that soft and semi-soft cheeses present indeed a higher concern with regard to listeriosis. [5]"

L. monocytogenes was the most resistant of all bacteria tested. [8] It survived in the interior of the semi-hard cheese for more than 90 days of ripening. [8] *L. monocytogenes* grew profusely on the surface of the semi-hard cheeses. [8] Even after the intense brining and ripening at elevated temperatures for at least 2 months *L. rnonocytogenes* , at the time of commercial ripeness was still present. [8] The optimal water activity in the milk, curd, and cheese for the pathogen, *L. monocytogenes* is 0. 998-0. 999. [10]

Smeared ripened cheeses made from raw cow’s milk have also been identified as a vehicle for *L. monocytogenes.* [5] This is where most of *L. monocytogenes* begins and stays in the pre-production of cheese because this pathogen can get into the biofilms and stay there for longer periods of time. [5] Dairy farms is where this pathogen can be introduced if proper hygiene and sanitation is not taken place, but this pathogen can also have an outbreak in post-production or further distribution. [5] However, “ the epidemiology of cheese-related outbreaks demonstrates that surface-ripened soft cheeses generally represent a greater risk for the transmission of pathogens than do other cheeses. [8]”

“ *L. monocytogenes* can occur during storage, display or slicing when the bacterium colonizes the environment, equipment, utensils and crates. It was also expected that more *L. monocytogenes* growth would occur on the cheese slicing surface than on the cheese rind. [5]” The growth of *L. monocytogenes* is not supported by hard cheeses, however, hard cheeses may support the survival of *L. monocytogenes.* [5] “ Moreover, some studies demonstrated that L. monocytogenes will die during ripening of hard cheeses. [5]"

There is a difference in the amount of *L. monocytogenes* present depending on what type of milk is used. Along with what kind of milk the cheese is being made with, the type of distribution can also have an affect of *L. monocytogenes* growth this includes a local market, small shop, or big retail outlet. [5] One of the last affects of the presence of *L. monocytogenes* is the selection of regions where the samples of the milk is taken from. [5] " It could not be observed from the challenge tests that growth of *L. monocytogenes* in pasteurized cheeses is significantly higher than growth in raw milk cheeses. [5]" It was proven; however, that cheese made from raw milk had a slower growth rate of *L. monocytogenes* compared to that of pasteurized milk. [5] " Mathew and Ryser (2002)observed that heat-injured cells of *L. monocytogenes* were recovered with higher rates in heat-treated or pasteurised milk than in raw milk. [10]"

" This difference in growth potential of *L. monocytogenes* may be explained by the presence of the lactoperoxidase enzyme in raw milk cheese which has bacteriostatic properties in milk and milk-based products. In this study, it was noticed that pasteurized milk cheeses have lower contamination levels of *E. coli* than raw milk cheeses. This is due to the heat treatment used during processing. Therefore, more bacterial competition is expected to be present in raw milk cheeses which may be as well an explanation of the lower growth potential of *L. monocytogenes* in these cheeses Although the prevalence on pasteurized cheese may be lower, if there are opportunities for growth of the pathogen, higher numbers of *L. monocytogenes* may be obtained in pasteurizedcheeses, makingraw milk cheeses and pasteurized cheeses equally important in terms of at risk products for listeriosis. [5]"

L. monocytogenes may not have a large presence in cheese, but it definitely has some sort of presence. " L. monocytogenes had not yet exceeded numbers of 100 CFU/g. As mentioned before, the presence of low numbers of L. monocytogenes in cheese is not an infrequent event. [5]" Once this number rises above a certain point, is when this pathogen becomes dangerous to humans and consumption.

Results

A study ofGould, Mungai, and Behravesh (2014)where 90 outbreaks in the United States attributed to cheese were analyzed. [5] The study showed that 42% of the outbreaks were due to cheese made with unpasteurized milk and 49% due to cheese made with pasteurized milk. [5] Only 12 of these outbreaks were caused by *L. monocytogenes.* [5] Only 4 out of these 12 were involved with unpasteurized milk cheese. [5] The remaining 8 out of the 12 outbreaks were involved with pasteurized milk cheese. [5] Overall, soft raw milk cheese holds the greatest risk for survival and growth of *L. monocytogenes* although the growth potential will ultimately depend upon the actual storage temperature. [5]

More results from this study demonstrate that " higher growth of L. monocytogenes is obtained on a sliced surface of the cheese than on the cheese rind. It was also shown that in both raw and pasteurized semi-soft washed-rind milk cheeses, the L. monocytogenes population increased as the temperature increased. [5]” In specific cheese types, however, in neither the inter-batch nor intra-batch variability was recorded having consistent behavior with *L. monocytogenes* in fermented dairy products such as cheese and milk. [5]

Pasteurized milk was found to favor the growth of L. monocytogenes during cheese making; moreover, there was no growth of L. monocytogenes during cheese making with raw milk. [10] The ripening period of the cheeses revealed that the cheeses made with pasteurized milk had a decrease in numbers of *L. monocytogenes* present on the rind. [10] In contrast, the ripening period of the cheeses made with raw milk revealed *L. monocytgenes* grew in raw milk cheese, but was inactivated in the pasteurized milk cheese. [10] “ In this study, we observed that *L. monocytogenes* grew during the manufacture ofpasteurized milkcheese, whereas during the manufacture ofraw milk cheese, no growth of the pathogen occurred. [10] Morgan et al. (2001)also found that *L. monocytogenes* survived the ripening of soft lactic cheese made with raw milk, whileMargolles et al. (1997)found that *L. monocytogenes* was inactivated during the manufacture, ripening and storage of an artisanal acid-coagulated cheese made with pasteurized milk. [10]” The main difference between the raw milk and the pasteurized milk was pH and lactic acid concentration. [10]

To prevent the spread and contamination of L. monocytogenes, one must ensure the safety of the post-processing contamination and be aware that contamination can occur from the farmer, to retailer, to salesmen. Being aware and taking proper hygiene precautions can limit the risk of listeriosis. 5

Listeria monocytogenes in Raw Bovine Milk

L. monocytogenes can originate inside of an infected cow's ruminant mastitis. [6] This specific case was found in a herd in Italy. [6] Health inspectors went to the dairy farm that the infected bovine milk came from to test to see which cow or cows were infected with *L. monocytogenes.* [6] To test for this, each cow was milked and their milk was tested for the presence of this pathogen. One cow in the herd was found to be producing a persistent excretion of this pathogen. [6] *L. monocytogenes* can stay present in the milk for months after a contamination and does not have any antimicrobial treatment. [6]