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Module
on

Microbial Defects In Milk And Milk Products

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DETAILED REPORT

INTRODUCTION

Milk is a natural liquid product that contains almost all the nutrients required for healthy life. The secreted milk in the udder is sterile and later on various extraneous materials get their entry in to it. From the point of *production* to distribution, there are various points from where milk gets contaminated with different microbial and non-microbial substances.

Any accidental entry or introduction of foreign material into the milk is called contamination and the foreign materials are called contaminants. The contaminants that are introduced into the milk can be classified under two major categories:

a) **Physical contaminants:**

Physical contaminants like dirt particles, hair, leaves, rubber and metal particles, paper pieces etc can get entry in to the milk at the time of milking. The dirt particles from air even, unclean udder or body of the cow, unclean utensils and water supply can contaminate milk. The hair of body of cow or of milk can also fall in the milk. The habits of the milker can also add some harmful contaminants like chewing tobacco, or beetle leaves can make entry of the physical contaminants into the milk. At the barn, all the activities of the milker should be scrutinized. The cleaning of the milking equipment should be properly done with a reliable and adequate source of water supply. The dairy barns should be maintained regularly and of good condition. The surrounding area of the barn should be kept clean from the waste materials. The milking premises should be free from the cobwebs and accumulation of the dust particles.

b) Chemical contaminants:

Veterinary, cleaning, agricultural and disinfecting chemicals cancontaminate the milk.

i) Veterinary and agricultural chemicals

The animal treated with any drug or antibiotic can contaminate milk with the residues of drugs. During milking, these chemicals may also be secreted along with milk. During milking adequate precaution should be taken so as to minimize the risk of getting entry of such chemicals into the milk. Such milks should not contain them more than the safety limit approved by the government. Only registered drugs should be used to treat the animal.

ii) Pest contaminants:

Pest in the milking premises can contaminate milk with their feaces, urine, bedding materials, hair etc. at the time of milking or handling of milk. Therefore, the entry of such birds even the flies should be restricted for entry and residing at the dairy farm premises.

iii) Environmental contaminants:

The environment of milking area can make entry of various chemical contaminants into the milk. The disinfectant sprays in the air, chemical substances in the water hypo chlorites, activities of neighboring farms, etc. can be proved to be very harmful.

The detergents should be used with the hot water and effectiveremoval of chemicals from the surface of such equipments should also be carried out with hot water.

Microbial contaminants

i) Contamination by pathogenic bacteria:

The interior of normal udder adds many microorganisms into the milk, i.e., Micrococci, streptococci and some *Corynebacterium.* These bacteria enter through the teat opening and colonize in the interior of udder. The fore milk contains most of them, that's why the fore milk should be excluded during milking

Types of Dairy Foods

The global dairy industry is impressive by large. In 2005, world milk production was estimated at 644 million tons, of which 541 million tons was cows' milk India at 88 million tons, the United States at 80 million tons (20.9 billion gallons), and Russia at 31 million tons. Cheese production amounted to 8.6 million tons in Western Europe and 4.8 million tons in the United

States. The vast array of products made from milk worldwide leads to an equally impressive array of spoilage microorganisms. A short summary of the types of dairy products and typical spoilage microorganisms associated with them is shown in Table 1. & Fig 1

Table 1 Dairy products and typical types of spoilage microorganisms or microbial activity

Food Spoilage	**Microorganism or Microbial activity**
Raw milk	A wide variety of different microbes Pasteurized milk Psychrotrophs, sporeformers, microbial enzymatic Degradation Concentrated milk Spore-forming bacteria, osmophilic fungi Dried milk Microbial enzymatic degradation
Butter	Psychrotrophs, enzymatic degradation Cultured buttermilk, sour cream Psychrotrophs, coliforms, yeasts, lactic acid bacteria Cottage cheese Psychrotrophs, coliforms, yeasts, molds, microbial enzymatic degradation
Yogurt	yogurt-based drinks Yeasts Other fermented dairy foods Fungi, coliforms Cream cheese, processed cheese Fungi, spore-forming bacteria
Soft, fresh cheeses	Psychrotrophs, coliforms, fungi, lactic acid bacteria, microbial enzymatic degradation
Ripened cheeses	Fungi, lactic acid bacteria, spore-forming bacteria, Microbial enzymatic degradation.

Types of Spoilage Microorganisms

Psychrotrophs

Psychrotrophic microorganisms represent a substantial percentage of the bacteria in raw milk, with pseudomonads and related aerobic, Gram-negative, rod-shaped bacteria being the predominant groups. Typically, 65–70% of the psychrotrophs isolated from raw milk are Pseudomonas species. Important characteristics of pseudomonads are their abilities to grow at low temperatures (3–7◦C) and to hydrolyze and use large molecules of proteins and lipids for growth. Other important psychrotrophs associated with raw milk include members of the genera Bacillus, Micrococcus, Aerococcus, and Lactococcus and of the family Enterobacteriaceae Pseudomonas (Fig2) can reduce the diacetyl content of buttermilk and sour cream thereby leading to a "green" or yogurt-like flavor from an imbalance of the diacetyl to acetaldehyde ratio. For cottage cheese, the typical Ph is marginally favorable for the growth of Gram-negative psychrotrophic bacteria, with the pH of cottage cheese curd ranging from 4.5 to 4.7 and the pH of creamed curd being within the more favorable pH range of 5.0–5.3. The usual salt content of cottage cheese is insufficient to limit the growth of contaminating bacteria; therefore, psychrotrophs are the bacteria that normally limit the shelf life of cottage cheese. When in raw milk at cell numbers of greater than 106 CFU/ml, Psychrotrophs can decrease the yield and quality of cheese curd.

Coliforms

Like psychrotrophs, coliforms can also reduce the diacetyl content of buttermilk and sour cream, subsequently producing a yogurt-like flavor. In cheese production, slow lactic acid production by starter cultures favors the growth and production of gas by coliform bacteria, with coliforms having short generation times under such conditions. In soft, mold-ripened cheeses, the pH increases during ripening, which increases the growth potential of coliform.

Lactic Acid Bacteria

Excessive viscosity can occur in buttermilk and sour cream from the growth of encapsulated, slime-producing lactococci.(FIG 4) In addition, diacetyl can be reduced by diacetyl reductase produced in these products by lactococci growing at 7◦Cresulting in a yogurt-like flavor. Heterofermentative lactic acid bacteria such as lactobacilli and Leuconostoc can develop off-flavors and gas in ripened cheeses. These microbes metabolize lactose, subsequently producing lactate, acetate, ethanol, and CO2 in approximately equimolar concentrations. Their growth is favored over that of homofermentative starter culture bacteria when ripening occurs at 15◦C rather than 8◦C. When the homofermentative lactic acid bacteria fail to metabolize all of the fermentable sugar in a cheese, the heterofermentative bacteria that are often present complete the fermentation, producing gas and off-flavors, provided their populations are 106 CFU/g . Residual galactose in cheese is an example of a substrate that many heterofermentative bacteria can metabolize and produce gas. Additionally, facultative lactobacilli can cometabolize citric and lactic acids and produce CO2. Catabolism of amino acids in cheese by nonstarter culture, naturally occurring lactobacilli, propionibacteria, and lactis can produce small amounts of gas in cheeses Cracks in cheeses can occur when excess gas is produced by certain strains of Streptococcus thermophilus and Lactobacillus helveticus that form CO2 and 4-aminobutyric acid by decarboxylation of glutamic acid.

Fungi

Yeasts can grow well at the low pH of cultured products such as in buttermilk and sour cream and can produce off-flavors described as fermented or yeasty. Additionally, yeasts can metabolize diacetyl in these products thereby leading to a yogurt-like flavor. FIG 5 Contamination of cottage cheese with the common yeast Geotrichum candidum often results in a decrease of diacetyl content. The low pH and the nutritional profile of most cheeses are favorable for the growth of spoilage yeasts. Surface moisture, often containing lactic acid, peptides, and amino acids, favors rapid growth. Many yeasts produce alcohol and CO2, resulting in cheese that tastes yeasty. Packages of cheese packed under vacuum or in modified atmospheres can bulge as a result of the large amount of CO2 produced by yeast.

Spore-Forming Bacteria

Raw milk is the usual source of spore-forming bacteria in finished dairy products. Their numbers before pasteurization seldom exceed 5,000/ml however, they can also contaminate milk after processing. The most common spore-forming bacteria found in dairy products are Bacillus licheniformis, B (Fig 6). Cereus, B. subtilis, B. mycoides, and B.megaterium.In one study, psychrotrophic B. cereus was isolated in more than 80% of raw milks sampled. The heat of pasteurization activates (heat shock) many of the surviving spores so that they are primed to germinate at a favorable growth temperature .Coagulation of the casein of milk by chymosin-like proteases produced by many of these bacilli occurs at a relatively high pH .

Crome reported that lactose-fermenting B. circulans was the dominant spoilage microbe in aseptically packaged pasteurized milk. Bacillus stearothermophilus can survive ultra-high-temperature treatment of milk. This bacterium produces acid but no gas, hence causing the "flat sour" defect in canned milk products. Spores are concentrated in cheese curd, so as few as one spore per milliliter of milk can cause gassiness in some cheese. An example of gassing caused by C. tyrobutyricum in Swiss cheese is shown in (Fig. 7). Occasionally, gassy defects of process cheeses are also caused by C. butyricum or C. sporogenes. These spores are not completely inactivated by the normal cooking treatment of process cheeses. Therefore, they may germinate and produce gas unless their numbers are low.

Microbiological Spoilage of Dairy Products

Table 2 Causes of gassiness in different types of cheese

Organism	Cheese affected	Time to defect
Coliforms	Raw milk pasta filata cheese	Early blowing
Yeasts	Raw milk Domiati (Egyptian), Camembert, blue-veined, Feta	Early blowing
Lactobacillus fermentum	Provolone, mozzarella	Late blowing

Other Microorganisms

Eubacterium sp., a facultative anaerobe that is able to grow at pH 5.0–5.5 in the presence of 9.5% salt can cause gassiness in Cheddar cheese. An unusual white-spot defect caused by a thermoduric *Enterococcus faecalis* has occurred in Swiss cheese. This bacterium is inhibitory to propionibacteria and *Lactobacillus fermentum*, resulting in poor eye development and lack of flavor in the cheese as well.

Enzymatic Degradation

An indirect cause of dairy product spoilage is microbial enzymes, such as proteases, phospholipases, and lipases, some of which may remain active in the food after the enzyme-producing microbes have been destroyed. Populations of psychrotrophs ranging from 106 to

107 CFU/ml can produce sufficient amounts of extracellular enzymes to cause defects in milk that are detectable by sensory tests. Adams, Barach, and Speck (1975) reported that 70–90% of raw milk samples tested contained psychrotrophic bacteria capable of producing proteinases that were active after heating at 149◦C (300◦F) for 10 s. Others have verified this observation.

Extracellular proteases can affect the quality of milk products in various ways,

But largely by producing bitter peptides. Thermally resistant proteases have caused spoilage of ultra-high-temperature (UHT) milk. In addition, phospholipases can be heat stable. Experimentally, phospholipase production in raw milk can result in the development of bitter off-flavors due to the release of fatty acids by milk's natural lipase Heat-stable bacterial lipases have been associated with the development of rancid flavors in UHT milk *Pseudomonas fluorescens* is the most common producer of lipases in milk and milk products, but lipases can also be produced by Gram-negative psychrotrophic bacteria. Products that may be affected by residual lipases include UHT milk, butter, some cheeses, and dry whole milk. The release of short-chain fattyacids, C4 through C8, results in the occurrence of rancid flavors and odors, whereas the release of long-chain fatty acids results in a soapy flavor. Oxidation of free unsaturated fatty acids to aldehydes and ketones results in an oxidized flavor and fruity off-flavor results from lipolysis of short-chain fattyacids by *Pseudomonas fragi* followed by esterification with alcohols.

Sources of Spoilage Microorganisms

Contamination of Raw Milk

The highly nutritious nature of dairy products makes them especially good media for the growth of microorganisms. Milk contains abundant water and nutrients and has a nearly neutral pH. The major sugar, lactose, is not utilized by many types of bacteria, and the proteins and lipids must be broken down by enzymes to allow sustained microbial growth. In order to understand the source of many of the spoilage microflora of dairy products, it is best to discuss how milk can first become contaminated,via the conditions of production and processing.

The mammary glands of many very young cows yield no bacteria in aseptically collected milk samples, but as numbers of milkings increase, so do the chances of isolating bacteria in milk drawn aseptically from the teats. The stresses placed on the cow's teats and mammary glands by the very large amounts of milk produced and the actions of the milking machine cause teat canals to become more open and teat ends to become misshapen as time passes (Fig. 8). These stresses may open the teat canal for the entry of bacteria capable of infecting the glands.

Factors Affecting Spoilage

Spoilage of Fluid Milk Products

The shelf life of pasteurized milk can be affected by large numbers of somatic cells in raw milk. Increased somatic cell numbers are positively correlated with concentrations of plasmin, a heat-stable protease, and of lipoprotein lipase in freshly produced milk. Activities of these enzymes can supplement those of bacterial hydrolases, hence shortening the time to spoilage.

The major determinants of quantities of these enzymes in the milk supply are the initial cell numbers of psychrotrophic bacteria, their generation times, and their abilities to produce specific enzymes, and the time and temperature at which the milk is stored before processing.

Spoilage of Cheeses

Factors that determine the rates of spoilage of cheeses are water activity, pH, salt to moisture ratio, temperature, characteristics of the lactic starter culture, types and viability of contaminating microorganisms, and characteristics and quantities of residual enzymes. With so many variables to affect deteriorative reactions, it is no surprise that cheeses vary widely in spoilage characteristics. Soft or un ripened cheeses, which generally have the highest pH values, along with the lowest salt to moisture ratios, spoil most quickly. In contrast, aged, ripened cheeses retain their desirable eating qualities for long periods because of their comparatively low pH, low water activity, and low redox potential. Fungal growth in packaged cheeses was found to be most significantly affected by the concentration of CO2 in the package and the water activity of the cheese .Cheddar cheese exhibiting yeast spoilage had a high moisture level (39.1%) and a low salt in the moisture-phase value (3.95%. Roostita and Fleet (1996) determined that the properties of yeasts that affected the spoilage rate of Camembert and blue-veined cheeses were the abilities to ferment/assimilate lactose, produce extracellular lipolytic and proteolytic enzymes, utilize lactic and citric acid, and grow at 10◦C.

Prevention and Control Measures

Prevention of Spoilage in Milk

In the early days of development of the commercial dairy industry, milk was produced under much less sanitary conditions than are used today, and cooling was slow and inadequate to restrict bacterial growth. Developments during the first half of the twentieth century created significant reductions in the rate of spoilage of raw milk and cream. Rapid cooling and quick use of raw milk are accepted as best practices and can affect the spoilage ability of Pseudomonas spp. present in milk. Pseudomonads that had been incubated in raw milk for 3 days at 7◦C (44.6◦F) had greater growth rates and greater proteolytic and lipolytic activity than those isolated directly from the milk shortly after milking. As the quality of raw milk improved, so did that of pasteurized milk. Heating of milk to 62.8°C (145°F) for 30 min or to 71.7°C (161°F) for 15 s kills the pathogenic bacteria likely to be of significance in milk as well as most of the spoilage bacteria. A shelf life of 21 days and beyond can be attained with fluid milk products that have been heated sufficiently to kill virtually all of the vegetative bacterial cells and protected from recontamination. Ultra-pasteurized milk products, heated at or above 138°C for at least 2 s, that have been packaged aseptically can have several weeks of shelf life when stored refrigerated. Ultra-high-temperature (UHT) treatment destroys most spores in milk The addition of carbon dioxide to milk and milk products reduces the rates of growth of many bacteria (Dixon & Kell, 1989). King and Mabbitt (1982) demonstrated improved keeping quality of raw milk by the addition of CO2.

Microbiological Spoilage of Dairy Products

Table 3 Dairy product heat treatment standards

Treatment	Temperature	Time
Pasteurization of milk	63°C/145°F 72°C/161°F	30min 15s
Ultra-pasteurization of milk	138◦C/280◦F	2s
Ultra-high temperature (UHT)-treated milk	140–150°C/ 284–302°F	2s

Prevention of Spoilage in Cultured Dairy Products

Cultured products such as buttermilk and sour cream depend on a combination of lactic acid producers, the lactococci, and the leuconostocs (diacetyl producers), to produce the desired flavor profile. Imbalance of the culture, improper temperature

Or ripening time, infection of the culture with bacteriophage, presence of inhibitors, and/or microbial contamination can lead to an unsatisfactory. Although cooking of the curd destroys virtually all bacteria capable of spoiling cottage cheese, washing and handling of the curd after cooking can introduce substantial numbers of spoilage microorganisms. It is desirable to acidify alkaline waters for washing cottage cheese curd to prevent solubilization of surfaces of the curd.

Cheesemakers can use the addition of high numbers of lactic acid bacteria to raw milk during storage to reduce the rate of growth of psychrotrophic microbes. For fresh, raw milk, brined cheeses, gassing defects can be reduced by presalting the curd prior to brining and reducing the brine temperature to $<12^{\circ}$c. Pasteurization will eliminate the risk from most psychrotrophic microbes, coliforms, leuconostocs, and many lactobacilli, so cheeses made from pasteurized milk have a low risk of gassiness produced by these microorganisms Most bacterial cells, including spores, can be removed from milk by centrifugation at about 9,000g. The process, known as bactofugation, removes about 3% of the milk, called bactofugate.

Other potential inhibitors of butyric acid fermentation and gas production in cheese are the addition of nitrate, addition of lysozyme cold storage of cheese prior to ripening, direct salt addition to the cheese curd, addition of hydrogen peroxide, or use of starter cultures that form nisin or other antimicrobials The most popular mold inhibitors used on cheeses are sorbates and natamycin.

Sorbates tend to diffuse into the cheese, thereby modifying flavor and decreasing their concentration, whereas very little natamycin diffuse.

Since nearly all mold spores are killed by pasteurization, practices that limit recontamination and growth, although difficult, are vital in prevention of moldy cheeses. Processing times and temperatures used in the manufacture of cream cheese and pasteurized process cheese are able to eliminate most spoilage microorganisms from these products. However, the benefit of the presence of competitive microflora is also lost. It is very important to limit the potential for recontamination, as products that do not contain antimycotics can readily support the growth of yeasts and molds. Sorbates can be added; however, their use in cream cheese is limited to amounts that will not affect the delicate flavor.

Prevention of Spoilage in Other Dairy Products

The high salt concentration in the serum-in-lipid emulsion of butter limits the growth of contaminating bacteria to the small amount of nutrients trapped within the droplets that contain the microbes. However, psychrotrophic bacteria can grow and produce lipases in refrigerated salted butter if the moisture and salt are not evenly distributed. When used in the bulk form, concentrated (condensed) milk must be kept refrigerated until used. It can be preserved by addition of about 44% sucrose and/or glucose to lower the water activity below that at which viable spores will germinate. Lactose, which constitutes about 53% of the nonfat milk solids, contributes to the lowered water activity. When canned as evaporated milk or sweetened condensed milk, these products are commercially sterilized in the cans, and spoilage seldom occurs. Microbial growth and enzyme activity are prevented by freezing. Therefore, microbial degradation of frozen desserts occurs only in the ingredients used or in the mixes prior to freezing.

Conclusion

While the introduction of pasteurization has helped to ensure the safety of dairy products, progress has been slower in preventing the microbial spoilage of cheese and dairy products. Worldwide standardized pasteurization practices would be an effective first step in eliminating or reducing the levels of many spoilage microorganisms. However, preventing post process contamination by spoilage microorganisms and retarding the growth of surviving organisms remain a challenge. Novel technologies and preservatives are needed to prevent the growth of spoilage microorganisms and extend the shelf life of dairy products. Limited applicability of current approved antimycotics such as sorbic acid and natamycin provides a major opportunity to expand the arsenal of preservatives available for today's dairy processor. In addition, studies to determine the interaction of current preservative technologies against spoilage microorganisms are also needed. Improved methods for detecting spoilage microbes, especially the slow-growing psychrotrophs and fungi, could assist in finding the niche environments in processing facilities that lead to post process contamination. The next century will bring many challenges to the dairy processor, but maintaining the quality and shelf life of this highly nutritious food should not be one of them.