**Dairy Microbiology/Theory**

**5th lesson**

**Assist.Prof. Dr. Payman A. Kareem**

**BUTTER**

 The two principal types of butter produced are sweet cream butter and ripened cream butter.

**Initial Microflora**

 Butter is produced from cream, and the cream is the main source of microorganisms in hygienically produced butter, and any organisms likely to be present in raw milk are also likely to be present in cream, including *Clostridium* spp. and *Bacillus* spp.

**Processing and its Effect on the Microflora**

**1- Pasteurization**

 Cream used for butter production is generally pasteurized after separation, and heat treatments of 85 - 95 °C for 15 - 30 seconds are commonly used. Most vegetative bacterial cells and lactic acid bacteria (LAB) are killed by this process, but bacterial spores and some thermoduric organismsmay survive in low numbers.

Raw cream for butter manufacture should be pasteurized as soon as possible after separation, to minimize the potential for growth of Psychrotrophic pseudomonas and other bacteria that produce heat-resistant extracellular enzymes.

**2- Cooling**

 After heat treatment, the cream should be cooled rapidly to 10 – 11 °C and then held for at least 4 hours. This allows the completely liquefied butterfat to crystallize into large numbers of small crystals. This process, known as ageing, allows a stable matrix of fat crystals to develop; this is important for the physical properties of the final product. If cooling is too slow, it is possible that bacterial spores surviving pasteurization could germinate and grow.

**3- Ripened cream butter**

 Ripened cream butter has traditionally been made by inoculating the pasteurized cream with pure or mixed strains of LAB, and then maintaining the temperature at 19- 21 °C until the required level of acidity is reached (usually 4 - 6 hours). The starter cultures consist of a mixture of acid producers (*Lactococcus lactis* subsp. *Lactis* and *Lactococcus lactis* subsp. *cremoris*), and diacetyl-producing species (*Leuconostoc mesenteroides* subsp. *cremoris* and *Lactococcus lactis* biovar *diacetylis*).

**4- Churning**

 Churning involves agitation of the cream at low temperature, which produces fat granules that separate from the aqueous phase of the cream to leave buttermilk. The buttermilk is drained off, giving a doubling of the fat content of the cream. Most of the microorganisms present in the cream are retained in the aqueous phase and are therefore removed in the buttermilk.

**5- Salting and working**

 The concentration of salt in the water phase will then be about 11%, sufficient to inhibit the growth of many microorganisms, but this effect is dependent on an even distribution of the salt. The butter is then worked mechanically both to disperse the salt and water, and to obtain the correct physical structure. The objective is to disperse the water phase into small droplets, preferably between 1- 30 μm in diameter. The microbiological stability of the butter is greatly influenced by this process. If most of the water droplets present are < 10 μm in diameter, any microorganisms within them will not be able to grow and will gradually die off, owing to nutrient depletion and the inhibitory effect of salt or lactic acid (in ripened butter).

**6- Packaging**

 Butter may be packaged either in bulk or in retail size containers. Parchment wrappers are the traditional packaging material, but plastic tubs and laminated foil packs are also common. The packaging should be of good microbiological quality. Bulk butter may be frozen (-30 °C) and stored for periods of up to a year, but good-quality butter stored at chill temperatures generally has an expected shelf life of 6 to 12 weeks. Bulk stored butter may be repackaged prior to sale; this process may cause some redistribution of water droplets, which may affect keeping quality and increase the risk of contamination and subsequent spoilage.

**Spoilage**

**1- Bacterial spoilage**

 Surface taints may develop as a result of growth of *Alteromonas putrefaciens*, and *Pseudomonas putrefaciens* or *Flavobacterium* spp. Such spoilage may be apparent within 7 to 10 days of chilled storage. The surface layers are initially affected, but eventually spoilage is apparent throughout the product. A putrid or cheesy flavor develops due to the breakdown of protein. Rancidity, proteolytic activity and fruity odors may be caused by the growth of *Pseudomonas fragi*.Black discoloration of butter is reported to be caused by *Pseudomonas nigrificans*, *Pseudomonas mephitica* is responsible for a skunk-like odour, and an organism formerly known as *Lactococcus lactis* var. *maltigenes* may be responsible for a 'malty' flavor defect linked to the formation of 3-methylbutanal. Lipolytic spoilage of butter has been associated with the presence of *Micrococcus*.

**2- Fungal spoilage**

 Molds are still important spoilage organisms for butter, and mold growth may produce surface discolorations and taints. A number of genera have been associated with spoiled butter, including *Penicillium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Geotrichum*, *Alternaria*, and *Rhizopus*. Yeasts may also cause spoilage of butter. Lipolytic species such as *Rhodotorula may* grow on the surface at chill temperatures and may tolerate high salt concentrations. Other yeasts associated with spoilage include *Candida lipolytica*, *Torulopsis*, and *Cryptococcus*.

**Pathogens: Growth and Survival**

**1- *Staphylococcus aureus***

 Outbreaks of staphylococcal food poisoning have been associated with butter.The presence of staphylococcal enterotoxin A was demonstrated in both butters. It appeared that the enterotoxin had formed in the cream used to make the butter and was carried over into the finished product.

Reduction of the salt content to 0 - 1% allowed the population to increase by a factor of ten in 14 days at 23 °C. Therefore a combination of poor hygiene, low salt concentration (or inadequate salt dispersal), and temperature abuse could allow growth of *Staph. aureus* in stored butter.

**2- *Listeria monocytogenes***

 *L. monocytogenes* has been shown to grow slowly in butter made from contaminated cream at 4 or 13 °C, and to survive for several months in frozen butter without any appreciable decrease in numbers. *Listeria* will not survive cream pasteurization, but it is a very common environmental contaminant in dairy settings, and effective cleaning and hygiene procedures are necessary to prevent recontamination. Surveys of the incidence of *Listeria* in dairy products have not isolated it from butter.

**3- *Campylobacter***

 An outbreak of *Campylobacter jejuni* enteritis was associated with garlic butter prepared on site. The survival of *Campylobacter* in butter, with and without garlic, was later investigated.



  **Figure 1 Production of butter**

**Dairy Microbiology/Theory**

**6th lesson**

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**CREAM**

**Definitions**

Cream is defined as that part of cows’ milk rich in fat that has been separated by skimming.

There are a number of different types of cream, usually classified according to their fat content:

**Minimum fat content (%)**

1. Half cream ≥12

2. Sterilized half cream ≥12

3. Single cream or cream ≥18

4. Sterilized (or canned) cream ≥23

5. Whipping cream ≥35

6. Whipped cream ≥35

7. Double cream ≥48

8. Clotted cream ≥55

**Initial Microflora**

The initial Microflora are essentially those of the raw milk (influenced by Microflora on the cow’s udder, milk-handling equipment, and storage conditions) from which the cream is made.

**Processing and its Effect on the Micro flora**

**1*- Storage and transport of raw milk***

The high fat content of cream means that it is more susceptible to spoilage by extracellular lipases produced by Psychrotrophic *Pseudomonas* spp. and other organisms in raw milk.

These enzymes can survive heat treatment, and therefore it is preferable to minimize the refrigerated storage time of raw milk for fresh cream production, and process as soon as possible after collection.

**2*- Separation***

Separation is the concentration of the fat globules and their removal from the milk. Traditionally, this used to be done by skimming, but centrifugal separators are now used in commercial dairies. Centrifugal separators of the disc stack type are commonly used in modern operations.

To minimize damage to fat globules, separation is ideally carried out at a temperature of 40 - 50 °C a temperature at which rapid microbial growth is possible. Therefore, higher temperatures (55 – 63 °C) are often recommended; viscous creams are generally produced using these high temperatures. Some separators used to produce high-fat creams (40% fat content) are able to operate at 5 °C, at which temperature significant growth will not occur.

Separators are therefore set to give a slightly higher than required fat content and whole or skimmed milk is then added to give the correct value. Standardization is often carried out at about 40 °C and there is therefore a risk of rapid microbial growth if the process is not carried out quickly.

**3- *Homogenization***

Homogenization may be carried out before or after heat treatment, but, from a microbiological point of view, homogenization before heat treatment is preferred. Homogenization after heat treatment helps to reduce problems with rancidity caused by lipases present in the milk, and some producers therefore choose this approach. UHT-treated cream is normally homogenized after heat treatment.

**4*- Heat treatment***

High-temperature, short time (HTST) processes are almost universally used in modern dairies, but higher temperatures than those used in HTST processing are often applied, both to achieve a longer shelf life, and to overcome the protective effect of the high fat content.

Another method for sterilization is the Autothermal Thermophilic Aerobic Digester (ATAD) friction process where the milk is initially preheated to 70 °C and subsequently heated to 140 °C for 0.54 seconds. This process can be used for creams containing 12 and 33% fat.

**5*- Cooling and packaging***

Pasteurized cream should be cooled as soon as possible after heat treatment to a temperature of 5 °C or less, to prevent growth of thermoduric organisms, and then be packaged quickly. Most cream for retail sale is now packed in plastic pots sealed with metal foil lids. This type of packaging generally carries very low levels of microbial contamination. However, as with pasteurized milk, the hygienic operation of the filling process is essential to prevent post-pasteurization contamination.

**Spoilage**

Cream usually receives more severe heat processes than milk, and the post-heat treatment microbial population therefore consists almost entirely of relatively heat-resistant species. Aerobic spore-forming bacteria survive pasteurization, and Psychrotrophic strains of *Bacillus cereus* may cause 'sweet curdling' and ‘bitty cream’.

Heat-resistant lipases produced by Psychrotrophic bacteria growing in the raw milk may also survive high-temperature processing and cause spoilage in UHT cream. The high fat content of cream means that lipolytic species, such as *Pseudomonas fluorescens* and *Pseudomonas fragi*, are a particular problem. Psychrotrophic members of the Enterobacteriaceae are also sometimes involved. Yeasts and molds are rarely implicated in the spoilage of cream.

**Pathogens: Growth and Survival**

In practice, to overcome the protective effect of the higher fat content, cream usually receives a more severe heat treatment than milk.

**1*- Salmonella spp.***

Salmonellae (*Salmonella typhimurium* and *Salmonella enteritidis*) will not survive the heat treatment applied to cream, and therefore their presence is likely to be due to post-pasteurization contamination. The cells are likely to survive for extended periods in contaminated cream, but growth is not possible unless significant temperature abuse occurs. Storage at temperatures below 5 °C will prevent multiplication.

**2- *Listeria monocytogenes***

**3- *Yersinia spp.***

*Yersinia enterocolitica* is a common contaminant of raw milk, although the majority of the strains isolated are not pathogenic to humans. The organism is heat-sensitive and does not survive pasteurization, but is capable of Psychrotrophic growth.

**4*- Staphylococcus aureus***

Although *Staph. aureus* can often be isolated from raw milk, and is a common cause of mastitis in cows, it does not survive pasteurization, and cases of staphylococcal food poisoning from pasteurized dairy products are now uncommon. It may be introduced into cream as a post-process contaminant, particularly from infected food handlers.

**5*- Verotoxigenic Escherichia coli (VTEC)***

VTEC, particularly *Escherichia coli* O157, have been found in raw milk and have caused serious outbreaks of infection associated with consumption of raw or inadequately pasteurized dairy products.

**6*- Viruses***

Viral hepatitis is the most likely viral infection to be associated with dairy products. The cause of the outbreak was handling of the cream by an infected cook during preparation.

**Dairy Microbiology/Theory**

**7th lesson**

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**Microbiology of Concentrated and Dry Milk**

**Introduction**

Fluid milk and whey are perishable dairy products that require proper cooling and handling to maintain their freshness and quality. However, milk and whey solids may be preserved for future use by various methods, the most common of which is concentration by removing water, using either heat or membrane methodology, followed by drying. Dairy products commonly manufactured through the use of one or more of these processes are evaporated milks, condensed and sweetened condensed milks, dry milks, condensed whey products, and dry whey products.

**1-Condensed milk**

Condensed milk may be manufactured using either whole or skim milk. Typically, milk is pasteurized and then concentrated by heat in an evaporator until the product contains 40–45% total solids. Following concentration, the product may be dried or distributed for use as concentrated milk. Most condensed whole milk is used as an ingredient in chocolate/confectionery, bakery, or dairy (frozen dessert) industries.

These products are not commercially sterile and, when intended for shipment as an ingredient, they immediately are cooled and continuously held at temperatures below 7°C. Microorganisms surviving the heat treatments usually are thermoduric or thermophilic types. Under proper handling and storage conditions, these organisms grow slowly, if at all, and are not expected to create keeping quality problems. If spoilage occurs, it usually is attributed to post-heating contamination.

Psychrotrophic bacteria, yeasts, or molds may cause spoilage if product is held for unusually long periods or under improper storage conditions.



**2-Sweetened condensed milk**

The primary difference between condensed and sweetened condensed milks is addition of sugar. Sweetened condensed milk is preserved by addition of sugar, which reduces water activity to a point inhibitory to most microorganisms. The increased milk solids content also decreases the water activity. However, osmophilic yeasts of genus *Torulopsis* may grow to caus e gas formation. They also cause coagulation and produce fruity flavours. Other microorganisms that grow in sweetened condensed milk are micrococci and molds such as *Aspergillus* and *Penicillium sp*. Which grow on the surface of sweetened condensed milk were sufficient air and oxygen is available. These microorganisms are the most common cause of spoilage of sweetened condensed milks. Their presence is indicative of unsanitary post-pasteurization conditions.

**3-Evaporated milks**

Evaporated milk, like other processed canned foods, originated with the experiments of the French scientist Nicholas Appert. Appert, whose work on food preservation began in 1795, was the first person to evaporate milk by boiling it in an open container and then preserving it by heating the product in a sealed container. Fifty years later, another French scientist, Louis Pasteur, laid the scientific foundation for heat preservation through demonstrations that food spoilage could be caused by bacteria and other microorganisms.



**A-Products and Processing**

Evaporated milk is a canned whole milk concentrate to which a specified quantity of vitamin D has been added and to which vitamin A may be added. A typical processing scheme for evaporated milk begins with high quality, fresh whole milk to which vitamins, emulsifiers, and stabilizers are added. The product is then pasteurized, concentrated under reduced pressure in an evaporator, homogenized, cooled, and standardized to the composition desired in the final product. After cans are filled and sealed, they are sterilized in a three phase continuous system consisting of preheater, retort, and cooler and then labelled and packed for shipment.

**B. Microbiology**

Because of the heat processes and packaging used to manufacture evaporated milks, the product is commercially sterile. This means that the product is free of all microorganisms of public health significance and does not show microbial defects during its intended shelf life under normal conditions of handling, storage, and distribution. Whereas vegetative cells do not survive evaporated milk processing, and absolute sterility is obtained in most cans, small numbers of non-pathogenic spores occasionally may survive the heat treatment and, depending on the microorganism and its previous growth and heat exposure, subsequently may germinate. The microorganisms were identified as *Bacillus stearothermophilus*, *B. licheniformis*, *B. coagulans*, *B. macerans*, and *B. subtilis*. These bacilli reduced the pH to 4.7 to 5.3 and produced acid and cheesy odours/ flavours and dark colour milk. The sources of these contaminants were studied during the processing of evaporated milk. *Bacillus coagulans* and *Bacillus licheniforms* were isolated from all raw milk samples used in the processing and from some canned evaporated milks. Species of the genus *Bacillus* (i.e., *cereus*, *coagulans*, *megatherium*, *stearothermophilus*, and *subtilis*) earlier were implicated in evaporated milk spoilage. It has been demonstrated that B*. cereus* naturally present in raw milk, reported no evidence that this organism would cause intoxication in healthy adult humans at levels less than 105/mL. *Enterococcus faecium* and *Bacillus subtilu* were isolated from an acid-coagulated evaporated milk. Both acid and gas production that resulted in ruptured cans were noted in spoiled evaporated milk.

**4-Dry milk**

Development of the dry milk industry stems from the days of Marco Polo in the 13th century. It is reported that Marco Polo encountered sun-dried milk on his journeys through Mongolia and that, from this beginning, dry milk products evolved

**A. Products and Processing**

The primary dry milk products manufactured domestically are non-fat dry milk, dry whole milk, and dry buttermilk. Non-fat dry milk is the product resulting from removal of fat and water from milk. It contains lactose, milk proteins, and milk minerals in the same relative proportions as the fresh milk from which it is made. Non-fat dry milk contains not more than 5% by weight of moisture. The fat content is not more than 1.5% by weight unless otherwise indicated. Dry whole milk is the product resulting from removal of water from milk and contains not less than 26% milk fat and not more than 4% moisture. Dry whole milks with milk fat contents of 26.0 and 28.5% are most commonly produced. Dry buttermilk is the product resulting from removal of water from liquid buttermilk derived from manufacture of butter. It contains not less than 4.5% milk fat and not more than 5% moisture.

**B. Microbiology**

Relatively few species of bacteria have been reported as naturally occurring in dry milks. However, microorganisms and their enzymes can be present and cause problems on use once rehydrated. Microorganisms like the genera *Streptococcus*, *Micrococcus*, *Bacillus*, *Clostridium*, and *Sarcina* comprising the primary microflora of dry milks.

**5- Microbiology of dry whey products**

As drying processes for whey are essentially the same as those for milk. Cultural or direct microscopic (DMC) procedures may be used. If using the latter, it must be understood that most whey processed is derived from cheese manufactured using bacterial cultures; thus, large numbers of viable lactic organisms are present in fresh whey. Except for the more heat-resistant strains of lactic bacteria, these organisms are not expected to survive pasteurization and are not detected by cultural techniques. However, when freshly dried whey is examined by direct microscopic techniques, cells of nonviable bacteria often stain.



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**8th lesson**

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**Probiotics**

*Lactobacillus delbrueckii* subsp. *bulgaricus* could grow in the intestinal tract of humans and displace any putrefying bacteria that are present. Displacement of this group of bacteria was thought to reduce production of toxic compounds that adversely affect the human body, thus enabling humans to live longer. *Lb. delbrueckii* subsp. *bulgaricus* neither survives nor establishes itself in the gastrointestinal tract. However, other species of lactobacilli have been reported to provide some beneficial effects through growth and action in the gastrointestinal tract. This group of bacteria and others are now often referred to as probiotics.

Cultures most often mentioned as probiotics for humans include *Lb. acidophilus, Lb. casei*, and *Bifidobacterium* species. All these species can survive and grow in the intestinal tract, and thus have the potential to provide benefits.

Bacteria normally used as starter cultures for some fermented milk products, such as *Lb. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* used to manufacture yogurt, also may provide benefits, but not through the ability to survive and grow in the intestinal tract. Benefits they provide come primarily from serving as a source of enzymes needed to improve digestion of nutrients in the gut. For example, β-galactosidase is needed for hydrolysis of lactose in the small intestine.

Several benefits are possible from such microorganisms, including control of intestinal infections, control of serum cholesterol levels, beneficial influences on the immune system, improvement of lactose utilization in persons who are classified as being lactose maldigestors, and anticarcinogenic action.

**POTENTIAL BENEFITS**

Most of the potential benefits to be discussed will focus on those applicable to human health or nutrition.

**A. Control of Growth of Undesirable Organisms in the Intestinal Tract**

*Lb. acidophilus, Lb. casei*, and species of *Bifidobacterium* can inhibit growth of undesirable microorganisms that might be encountered in the gastrointestinal tract. The probiotic bacteria in question all produce large amounts of acid during their growth, because they rely on fermentation to obtain energy for growth. However, the antagonistic action that they produce toward undesirable microorganisms apparently is not caused just by acid produced during their growth. Several of these organisms produce antibiotic-like substances, some of which have been classified as bacteriocin, which may be involved in the antagonistic action toward these pathogens. Bacteriocin, according to the classic definition, are bacterial proteins active against organisms closely related to the producer organism. This may limit the breadth of action of these inhibitory substances produced by probiotic bacteria.

They would not be expected to have any effect on gram-negative intestinal pathogens. Furthermore, because of their sensitivity to proteolyic enzymes, bacteriocin may not survive the digestive function of the intestines.

Antimicrobial substances, other than bacteriocin, produced by probiotic bacteria, having a role in controlling intestinal pathogens .A low molecular weight nonproteinaeous material produced by a *Lactobacillus* culture was active against a broad range of gram-negative and gram-positive bacteria. These researchers suggested the inhibitory agent to be a short-chain fatty acid other than lactic or acetic.

The ability of lactobacilli or Bifidobacterium to occupy binding sites on the intestinal wall, thereby preventing attachment and growth of enteric pathogens .Most likely the antagonistic actions produced by probiotic bacteria toward intestinal pathogens result from a combination of factors.

**B. Improvement of Immune Response**

Enhancement of the body’s immune response by consuming cells of certain lactobacilli increases resistance of the host to intestinal infections, this action involves activation of macrophages which in turn destroy pathogenic organisms in the body. It also has been suggested that consumption of these organisms is followed by secretion of components into the intestinal tract, which are inhibitory toward certain of the foodborne pathogens. This enhancement of the immune system increases the host defense mechanisms and could be very important for control of foodborne illnesses.

**C. Improvement of Lactose Digestion**

People who lack the ability to digest lactose adequately are classified as lactose maldigestors. (In the past, terms such as ‘‘lactose intolerance’’ or ‘‘lactose malabsorption’’ have been used to describe this condition). The problem results from inadequate levels of β-galactosidase in the small intestine to hydrolyze ingested lactose adequately.

The presence of viable starter cultures in yogurt can be beneficial to lactose maldigestors. Once the yogurt culture reaches the small intestine, it interacts with bile, which increases permeability of the cells of these bacteria and enables the substrate to enter and be hydrolyzed.

Nonfermented milk containing cells of *Lb. acidophilus* also can be beneficial for lactose maldigestors. This organism, unlike the yogurt starter cultures, can survive and grow in the intestinal tract. However, a similar mechanism in improving lactose utilization in lactose maldigestors to that observed for yogurt bacteria is probably involved. β-galactosidase activity of cells of *Lb. acidophilus* is greatly increased in the presence of bile because of increased cellular permeability. As with yogurt cultures, cells of *Lb. acidophilus* do not lyse in the presence of bile, but their permeability is increased permitting lactose to enter the cells and be hydrolyzed.

The level of β-galactosidase activity also varies among strains of *Lb. acidophilus* as well as among commercial yogurt cultures. Therefore, it is important to consider the level of β-galactosidase activity in probiotic or starter cultures to be used for improving lactose digestion in lactose maldigestors. It also is important for the activity to remain high during transportation and storage of such products so that the consumer receives the product containing enough of the enzyme to provide a benefit.

**D. Anticarcinogenic Actions**

*Lactobacillus acidophilus, Lb. casei*, and *Lb. delbrueckii* subsp. *bulgaricus* are species most often mentioned as having potential to provide anticarcinogenic actions. However, for *Lb. acidophilus* and *Lb. casei* growth or action in the gastrointestinal tract seems to be important. Part of the benefit may involve direct effects in inhibiting tumor formation. However, the main effect may result indirectly through inhibiting growth of undesirable bacteria that form carcinogens in the large intestine.

**E. Control of Serum Cholesterol**

Organisms such as *Lb.acidophilus* can potentially reduce serum cholesterol levels in humans. Excretion of bile acids through feces represents one of the major mechanisms whereby the body eliminates cholesterol. This is because cholesterol is a precursor for synthesis of bile acids and many bile acids that are excreted from the body are replaced by synthesis of new ones. Thus, there is a potential for reducing the cholesterol pool in the body. There may be other Probiotic organisms that can help to control serum cholesterol levels. Some of these include *Lb. casei* and *Bifidobacterium* species. *Bi.longum* removes cholesterol from laboratory media much the same as does *Lb. acidophilus* and incorporates part of it into the cellular membrane of this bacterium. Both these organisms also can deconjugate bile acids.