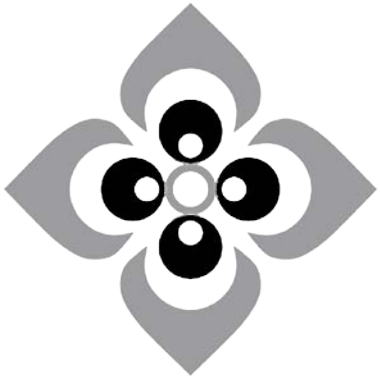

Consortium for Educational Communication

Module
on
Microbiology Of Milk, Testing Of Quality Of Milk And Milk Products

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TEXT

Introduction

Raw milk is a complete food which contains protein, fat, sugars, vitamins and minerals. Cow's milk is the preferred choice for most people. It provides 67 kilocalories and has a protein content of 3.2 grams per 100 millilitres. Milk proteins include casein (about 80%) and whey (about 20%). Whey has a higher nutritional value than casein. Once fat and casein have been removed from milk, it consists mainly of whey, which contains the soluble milk salts, milk sugar and the remainder of the milk proteins. Whey proteins consist of a number of specialised proteins, the most important being beta lactoglobulin (50% of whey) and lactoglobulin. Milk proteins have a high biological value but, unlike egg proteins, they lack sulphur-containing amino acids. The proteins in cow's milk have balanced amino acid profiles and good digestibility, making it the obvious choice when it comes to feeding the family. Casein in cow's milk combines with calcium to form caseinogen. A higher proportion of calcium and casein in cow's milk makes it more difficult to digest than human milk.

Milk is a perishable commodity and spoils very easily. Its low acidity and high nutrient content make it the perfect breeding ground for bacteria, including those which cause food poisoning (pathogens).Even though, raw milk is sterile at secretion, and contamination of milk by microorganisms can take place during milk handling, storage and other pre-processing activities. Bacteria from the animal, utensils, hands, and insects may contaminate the milk, and their destruction is the main reason for processing. This preservation of the milk can be achieved by fermentation, heating, cooling, removal of water, and by concentration or separation of components, to produce foods such as butter or cheese.

Good milk hygiene practices such as maintaining clean and healthy cows, keeping a clean milking environment free of dust and mud, avoid milking if the farmer is suffering from communicable diseases like diarrhoea or typhoid, not mixing colostrum and for milk, washing hands with soap and clean water before milking, washing the udder with warm water and drying the udder with a clean dry cloth and use of clean containers for milking, will improve the quality of raw milk. In addition, cows with mastitis should be milked last and their milk should be discarded and dip teats in an antiseptic solution will further help to reduce contamination of raw milk.

The degree to which milk consumption and processing occurs will differ from region to region. It is dependent upon a whole host of factors, including geographic and climatic conditions, availability and cost of milk, food taboos, and religious restrictions. Where processing does exist, many traditional techniques can be found for producing indigenous milk products. These are more stable than raw milk and provide a means of preservation as well as adding variety to the diet. In addition, the introduction of western-style dairy products and the subsequent setting up of small-scale dairies has provided more choice of dairy products to the consumer.

Quality of milk

The type of animal, its quality, and its diet can lead to differences in the colour, flavour, and composition of milk. Infections in the animal which cause illness may be passed directly to the consumer through milk. It is therefore extremely important that quality-control tests are carried out to ensure that the bacterial activity in raw milk is of an acceptable level, and that no harmful bacteria remain in the processed products.

Average composition (%) of milks of various mammals

Species	*Water*	*Fat*	*Protein*	*Lactose*	*Ash*
Human	87.43	3.75	1.63	6.98	0.21
Cow	87.2	3.7	3.5	4.9	0.7
Goat	87.00	4.25	3.52	4.27	0.86
Sheep	80.71	7.9	5.23	4.81	0.9
Indian buffalo	82.76	7.38	3.6	5.48	0.78
Camel	87.61	5.38	2.98	3.26	0.7
Horse	89.04	1.59	2.69	6.14	0.51
Llama	86.55	3.15	3.9	5.6	0.8

Microbiology: Milk is a complex biological fluid secreted in the mammary glands of mammals. Its function is to meet the nutritional needs of neonates of the species from which the milk is derived. Typically, bovine milk is composed of approximately 87% water, 3.7 - 3.9% fat, 3.2 - 3.5% protein, 4.8 - 4.9% carbohydrate (principally lactose), and 0.7% ash. However, the exact composition of bovine milk varies with individual animals, with breed, and with the season, diet, and phase of lactation.

Fresh milk products refers to liquid milk, Liquid milk is largely heat treated in developed countries, but a small quantity of raw (unpasteurised) milk is still sold in the developed countries(UK). Skimmed and semi-skimmed milk, which are defined by their fat content (0.5%, and 1.5 - 1.8%, respectively), are increasingly important products in the liquid milk market.

Initial Microflora

a) ***Contamination from the udder***

Although milk produced from the mammary glands of healthy animals is initially sterile, microorganisms are able to enter the udder through the teat duct opening.

Gram-positive cocci, streptococci, staphylococci and micrococci; lactic acid bacteria (LAB), *Pseudomonas* spp. and yeast are most frequently found in milk drawn aseptically from the udder; corynebacteria are also common. Where the mammary tissue becomes infected and inflamed; a condition known as mastitis, large numbers of microorganisms and somatic cells are usually shed into the milk. Mastitis is a very common disease in dairy cows, and may be present in a subclinical form, which can only be diagnosed by examining the milk for raised somatic cell counts.

Many bacterial species are able to cause mastitis infection, but the most common are *Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus uberis* and *Escherichia coli*. These bacteria enter the udder by the teat duct, and Staph. aureus is able to colonise the duct itself. Although the organisms involved in mastitis are not usually able to grow in refrigerated milk, they are likely to survive, and their presence may be a cause of concern for health. Diseased cows may also shed other human pathogens in their milk, including *Mycobacterium bovis, Brucella abortus, Coxiella burnetii, Listeria monocytogenes* and salmonellae. Recently, concerns have also been raised over the presence of Mycobacterium avium var. paratuberculosis (MAP) (the causative organism of Johne's disease in cattle) in milk from infected animals. The outer surface of the udder is also a major source of microbial contamination in milk. The surface is likely to be contaminated with a variety of materials, including soil, bedding, faeces and residues of silage and other feeds. Many different microorganisms can be introduced by this means, notably *salmonellae, Campylobacter spp., L. monocytogenes, psychrotrophic sporeformers*, *clostridia*, and *Enterobacteriaceae*. Good animal husbandry and effective cleaning and disinfection of udders prior to milking are important in minimising contamination.

b) **Other sources of contamination**

Milking equipment and bulk storage tanks have been shown to make a significant contribution to the psychrotrophic microflora of raw milk if not adequately sanitised (1). Exposure to inadequately cleaned equipment and contaminated air are also sources of contamination.

(2). Milk residues on surfaces, and in joints and rubber seals can support the growth of psychrotrophic Gram-negative organisms such as *Pseudomonas, Flavobacterium, Enterobacter, Cronobacter, Klebsiella, Acinetobacter, Aeromonas, Achromobacter* and Alcaligenes, and Gram-positive organisms such as *Corynebacterium, Microbacterium, Micrococcus* and spore forming *Bacillus and Clostridium.*

(3) These organisms are readily removed by effective cleaning and disinfection, but they may build up as biofilms in poorly cleaned equipment. Milk-stone, a mineral deposit, may also accumulate on inadequately cleaned surfaces, especially in hard water areas. Gram-positive cocci, some lactobacilli, and Bacillus spores can colonise this material and are then protected from cleaning and disinfection. Some of these organisms may survive pasteurisation and eventually cause spoilage.

(4) Other, less significant, sources of contamination include farm water supplies, farm workers and airborne microorganisms.

Natural antimicrobial factors

Raw milk contains a number of compounds that have some antimicrobial activity. Their purpose is to protect the udder from infection and also to protect neonates, but

they may also have a role in the preservation of raw milk during storage and transport. Lactoperoxidase is an enzyme found in milk. It has no inherent antimicrobial activity, but, in the presence of hydrogen peroxide (usually of microbial origin), it oxidises thiocyanate to produce inhibitors such as hypothiocyanite. This is referred to as the lactoperoxidase system, and it has bactericidal activity against many Gram-negative spoilage organisms, and some bacteriostatic action against many pathogens. For this reason it has been investigated as a possible means of extending the life of stored milk. Lactoferrin is also found in milk and is a glycoprotein that binds iron so that it is not available to bacteria. The chelation of iron in the milk inhibits the growth of many bacteria. In addition to producing an iron-deficient environment, lactoferrin is thought to cause the release of anionic polysaccharide from the outer membrane of Gram-negative bacteria, thereby destabilising the membrane. Lysozyme acts on components of the bacterial cell wall, causing cell lysis. Gram-positive organisms are much more susceptible to lysozyme than Gram negatives, although bacterial spores are generally resistant. Immunoglobulins of maternal origin are often present in milk, and colostrums is a particularly rich source. These proteins may inactivate pathogens in milk, but their significance in preservation is uncertain.

Processing and its Effects on the Microflora

i) Raw milk transport and storage

In developed countries, raw milk on the farm is usually cooled quickly and stored

in refrigerated bulk tanks at <7 °C prior to collection. Collection by insulated tanker is often on alternate days, or sometimes less frequently, and therefore some of the milk in the tank could be 48 hours old at the time of collection. Temperature control is thus critical to minimise microbial growth, and tanker drivers are usually permitted to refuse milk stored at too high a temperature, or which has an abnormal appearance or odour. Bacterial numbers in the milk may increase during transport, either as a result of contamination from inadequately cleaned tankers or from the growth of psychrotrophic organisms, particularly Pseudomonas spp. Milk temperature and duration of the transport stage are therefore important factors. On arrival at the processing site, the milk is transferred to bulk storage tanks, or silos, prior to processing. The milk may be stored in the silos for 2 - 3 days, and further growth of psychrotrophic bacteria is likely during this period. The degree of growth is dependent on the initial microbial load, and the storage time and temperature. Pseudomonads are the predominant organisms present in stored raw milk, with *Pseudomonas fluorescens, Pseudomonas fragi*, and *Pseudomonas lundensis* being commonly isolated, but Enterobacteriaceae, Flavobacterium, Alcaligenes, and Gram-positive species can also be found. The growth of psychrotrophic bacteria may also be accompanied by the production of heat-stable, extracellular proteolytic and lipolytic enzymes. These enzymes are often capable of surviving pasteurisation and, in some cases, ultra high temperature (UHT) processing, and they may subsequently cause spoilage in the processed milk. A number of techniques have been used to limit the growth of psychrotrophs during raw

milk storage.

ii) **Thermisation**

The most commonly used technique is to apply a mild heat treatment (thermisation), by heating to around 57 - 68 °C for 15 - 20 seconds and then cooling rapidly to <6 °C. This reduces the psychrotrophic population significantly and can extend the storage life of the raw milk by several days. However, thermisation cannot eliminate vegetative pathogens, and is therefore not a reliable control for the hazard. For example, *L. monocytogenes* can survive the process and could then grow during chilled storage.

iii) **Deep cooling**

As the storage temperature is a key factor for the rate of growth of psychrotrophic spoilage organisms, storing milk at as low a temperature as possible can also extend the storage life significantly. Reducing the storage temperature from 6 °C to 2 °C has been shown to give a 2-day gain in storage life for milk of good microbiological quality.

iv) **Carbon dioxide addition**

There has been some interest in extending the storage life of raw milk by the addition of carbon dioxide at a concentration of 20-30 mM. Three mechanisms are thought to be involved in carbon dioxide inhibition of microorganisms: the first is by the displacement of oxygen; the second is a lowering of the pH of the milk due to the dissolution of carbon dioxide and formation of carbonic acid, particularly for Gram-negative psychrotrophic aerobes; and the third is a direct effect on the metabolisms such as inhibiting the production of enzymes by these organisms. It has also been suggested that the technique could be used to extend the shelf life of pasteurised milk, but concerns have been raised that the use of carbon dioxide addition could allow growth and toxin production by psychrotrophic *Clostridium botulinum*. However, recent work indicates that the risk of botulism is not increased by the use of this treatment.

Following storage, the milk then undergoes further processing.

a) **Separation**

If necessary, the milk is separated into skimmed milk, cream and sediment fractions, using centrifugal separators. The sediment may contain a comparatively high number of microorganisms and must be carefully discarded. The agitation involved may also break up clumps of bacteria, potentially producing an apparent increase in the number of colony-forming units. This process also allows the milk to be standardised to a specified fat content by adding back the correct quantity of cream.

b) **Homogenisation**

The fat globules in milk can coalesce and form a cream layer. Homogenisation reduces the size of the milk fat globules (to an average diameter of <1 μm) by using a pump to force milk through a valve under pressure. The fat globules are then small enough to remain in suspension. This process has little microbiological effect, although clumps of bacterial cells may be broken up. Homogenisers used for pasteurised milk may be linked to the pasteuriser, and run at raised temperature in order to minimise possible microbial contamination. UHT (ultra high temperature) processed milks are homogenised in sterile conditions after heat treatment and before aseptic filling. Effective cleaning and sterilising of the homogeniser are then critical to product safety.

c) **Pasteurisation**

Some form of heat process is commonly applied to milk to ensure microbiological safety, and to extend shelf life. In the UK, the most commonly used process is pasteurisation. Time-temperature requirements for pasteurisation vary between countries, and are often specified in legislation. In the UK, both low-temperature, long time (LTLT, 63 - 65 °C for 30 minutes), and high-temperature, short time (HTST, 71.7 - 72 °C for at least 15 seconds) minimum processes are permitted. However, in practice, the HTST process is now generally used. Recent concern about the possible survival of MAP in pasteurised milk has seen many dairies increasing the length of the HTST process to 25 seconds. Higher processes (such as ultra-pasteurisation at 138 °C for at least 2 seconds) may also be applied to products with high fat and solids content. Plate heat exchangers are the most common method for milk pasteurisation, but it is essential that they are designed, constructed and operated in such a way as to minimise the possibility of recontamination of the pasteurised milk by raw milk. Most commercial pasteurisers are fitted with sensors that continuously monitor the pasteurisation temperature, and are linked to automatic

divert valves. If the pasteurisation temperature falls below a specified value, the valve opens and diverts the under-processed milk away from the post pasteurisation section of the plant and the filling line, into a divert tank. The correct operation of these monitoring systems is critical and should be regularly checked. It is also essential that there are no cross-connections between the raw and pasteurised sides of the process, and this should include separate clean-in place (CIP) systems. It is also usual to maintain a higher pressure in the pasteurised milk to minimise the risk of cross contamination in the heat exchanger. Recontamination of this kind may have serious public health consequences. Accepted pasteurisation processes are designed to reduce the numbers of vegetative microbial pathogens to levels that are considered acceptable, although bacterial spores are not destroyed. Most of the potential psychrotrophic spoilage bacteria are also eliminated. However, certain heat-resistant mesophilic organisms, referred to as thermoduric, are able to survive pasteurisation. Thermoduric species commonly isolated from pasteurised milk include Micrococcus spp., Enterococcus faecium and Enterococcus faecalis, Bacillus

subtilis, Bacillus cereus, and certain lactobacilli. Psychrotrophic strains of these organisms may be able to grow slowly in the pasteurised milk at 5 °C, and, if present initially in high numbers, could eventually cause spoilage. Effective cleaning of the

cooling sections of pasteurisers is important to ensure that these organisms do not build up on surfaces.

d) **UHT or sterilisation processes**

Milk may also be subjected to more severe heat processes sufficient to achieve “commercial sterility”. This may be done by batch heating in closed containers, or continuously with aseptic filling into sterile containers. Both conventional retort sterilisation and UHT processes must achieve a minimum of 3 minutes to ensure product safety. These processes destroy all vegetative cells in the milk, and the majority of spores, although certain very heat-resistant spores may survive. This results in a long shelf life without the need for refrigeration, but also causes organoleptic changes in the milk, such as browning. Conventional sterilisation processes involve heating the milk in thick-walled glass bottles, closed with a crimped metal cap, at about 120 °C for approximately 30 minutes. However, modern large-scale production methods often use an initial UHT treatment prior to filling the container, followed by retorting for a reduced time (10 - 12 minutes), and then a rapid cooling process. This is said to give a product with improved organoleptic properties.

UHT processes may be direct or indirect. Direct systems inject high-pressure steam directly into the milk to obtain the desired temperature, and then employ flash cooling under vacuum to remove the resulting excess water. Indirect systems utilise heat exchangers and holding tubes. Direct systems are said to give better organoleptic properties, as the heating and cooling processes are very rapid, but they are more complex and expensive to install. UHT processed milk involves preserving milk by holding at a temperature of 140 - 150°C for ١ - ٢ seconds (minimum treatment is 130°C for 1 sec). Heat treatment is usually followed by aseptic filling into sterile cartons or other containers. The maintenance of sterility in filling is vital to prevent recontamination of the treated milk. As with pasteurised milk, it is also vital to ensure that raw milk cannot recontaminate the UHT-treated milk. Certain very heat-resistant spores of mesophilic bacilli, classified as *Bacillus sporothermodurans* are able to survive UHT processes and may subsequently grow in the final product. However, this organism has been shown not to be pathogenic and does not seem to cause any detectable changes to the product. Thermoduric *Bacillus stearothermophilus* are able to survive UHT processes and cause flat-sour spoilage.

Spoilage

a) **Pasteurised milk**

Pasteurised milk provides a very suitable environment for microbial growth and is therefore highly susceptible to microbiological spoilage. Spoilage may result from either the growth of psychrotrophic thermoduric organisms that survive pasteurisation, or post-pasteurisation contamination by psychrotrophs. The latter is considered to be by far the most common cause of spoilage.

b) Thermoduric spoilage

The thermoduric microflora of milk consists largely of Gram-positive sporeformers, mainly Bacillus spp., Clostridium and organisms with heat-resistant vegetative cells, such as Micrococcus, Lactobacillus, Enterococcus, Streptococcus, Corynebacterium and Alcaligenes. Of these, the spore-formers are most important in spoilage, since the other species are not generally psychrotrophic and are unable to grow in refrigerated milk. Several Bacillus spp. contain psychrotrophic strains, notably *B. cereus* and *Bacillus circulans*, which may grow at temperatures as low as 2°C. These organisms may become dominant in milk containing very low numbers of Gram-negative psychrotrophs, but even so, they rarely cause spoilage at <5 °C. However, at slightly higher temperatures (7 - 8°C), *B. cereus* in particular may grow quite rapidly, producing a type of spoilage known as 'bitty cream' or 'sweet curdling', caused by the action of lecithinase on the phospholipids in fat globules. This produces small particles that stick to surfaces. Bitter taints may also be produced as a result of spoilage by Bacillus spp. These organisms are thought to originate from the raw milk, and the level of contamination has been shown to vary with the season, the highest numbers of spores being present between April and September.

b) **Post-process contamination**

The majority of post-process contaminants are Gram-negative bacteria, which may have some resistance to sanitizers and be able to colonise milk contact surfaces downstream of the pasteuriser. Initially, Enterobacteriaceae, such as Enterobacter, Cronobacter, and Citrobacter, predominate, but Gram-negative psychrotrophs, principally pseudomonads, but also Alcaligenes, Klebsiella, Acinetobacter and Flavobacterium, are more important in terms of eventual spoilage. Although these organisms may only contaminate the product in low numbers, they have a competitive advantage over Enterobacteriaceae at low temperatures and may grow rapidly to high levels. Spoilage by Gram negative psychrotrophs usually takes the form of off-flavours, often described as unclean, fruity, rancid or putrid, formed as a result of proteolytic and lipolytic activity. Ropiness and partial coagulation may also occur occasionally. The time for spoilage to occur depends on the numbers and composition of the initial microflora, and the storage temperature. Yeast and mould are also indicators of post-process contamination. Their presence and growth contribute to fruity and yeasty flavours in milk.

c) **UHT or sterilised milk**

Spoilage of UHT-processed products is usually caused by post-process contamination. Spoilage caused by survival of heat-resistant Bacillus spores is rare, unless very large numbers of spores are present initially, although reports of sterility failure caused

by *B. sporothermodurans*, as previously mentioned, are becoming more common. Post-process contamination usually occurs as a result of a failure in the integrity of the aseptic filling system, or, more likely, as a result of packaging defects, such as pinholes or faulty seals. The product may then become contaminated with a variety of environmental organisms and the type of spoilage will be dependent on the nature of the contaminant. A spoilage rate of 1/10,000 units is a realistic target for producers using modern, well operated equipment. A particular problem associated with UHT-processed milk is spoilage by heat resistant, extracellular, proteolytic and lipolytic microbial enzymes. These will have been produced by psychrotrophic organisms growing in the raw milk prior to processing, particularly pseudomonads, Acinetobacter, and Achromobacter, which are then able to survive the thermal process, even though all viable cells have been destroyed. In the course of the long shelf life that these products are given, proteolytic enzymes can cause bitter flavours and gelation, whilst lipases cause the development of rancid flavours.

Pathogens: Growth and Survival

Raw milk

Before the adoption of routine pasteurisation, milk was an important vehicle for the transmission of a wide range of diseases, including typhoid, brucellosis and diphtheria. Pasteurisation and improvements in veterinary medicine have seen a very large reduction in the incidence of such traditionally milk borne diseases. However, raw milk may still contain a very wide range of pathogens, including Salmonella spp. (particularly *Salmonella typhimurium* and *Salmonella dublin*, a virulent serotype in humans), *E. coli* O157, *Listeria monocytogenes* and *Campylobacter* spp. derived from the milk animals, the environment or from farm workers and milking equipment. Pathogens may be present even in hygienically produced milk of generally good microbiological quality. In short, raw milk is a potentially hazardous product, the microbiological safety of which cannot be assured without the use of pasteurisation or an equivalent process.

CONCENTRATED AND DRIED MILK PRODUCTS

Concentrated and dried milk is produced for direct sale to the consumer, but are particularly important as food ingredients, providing a source of milk solids in a variety of other products. The removal of water from fresh milk gives advantages in terms of reduced storage and transport costs, convenience in use, and, in some cases, a useful extension to shelf life. Bulk condensed milk is an important source of milk solids in confectionery, bakery products, ice cream, concentrated yoghurt and other products, and is manufactured in large quantities for this purpose. It may be made from whole, skimmed, or reduced fat milks, depending on the end use. Most bulk condensed milk is made by evaporation, and the degree of concentration is usually within the range 2.5:1 to 4:1, depending on usage.

Sweetened condensed milk may be made from whole or skimmed milk either in bulk as a food ingredient, or in small cans or tubes for direct sale to the consumer. When made from whole milk, it should contain at least 8% fat and 28% total milk solids; when made from skim milk it contains at least 0.5% fat and 24% milk solids.

The initial microflora of concentrated milk is that of the raw milk from which it is produced. Sugar used in sweetened condensed milk may be an additional source of yeasts and moulds and bacterial spores, including thermophilic spores.

Concentrated milk

Raw milk is given a heat treatment approximating that of pasteurisation. It is then concentrated at a low temperature, followed by standardisation, homogenisation and pasteurisation before packaging. Pasteurisation is usually done at 79.4°C for 25 sec; the product is stored at 10°C to prevent growth of thermoduric bacteria and any post-pasteurisation contaminants.

Pathogens: Growth and Survival

Concentrated milks are not normally regarded as high-risk products, principally because of the relatively severe heat treatments used in their manufacture. As with other pasteurised and UHT processed milks, the main concern for condensed and evaporated milk is post heat-treatment contamination by pathogens. Dried milks, however, are the subject of considerable concern, particularly in view of their widespread use in infant foods. There have been a number of outbreaks of foodborne disease associated with dried milk powders.

Sweetened condensed milk

The sweetened condensed milks ensures that only osmophilic and osmotolerant organisms are able to grow. Canned products may be spoiled by slow growth of osmophilic yeasts, particularly *Torulopsis* spp., which enter the product after heating and may produce sufficient gas to cause blown cans. If sufficient oxygen is present in the headspace, or the can has a small pinhole leak, moulds such as *Aspergillus* and *Penicillium* spp. may grow as ‘buttons’ on the surface of the product.

Condensed/evaporated milk

1. ***Listeria* spp.**

The fate of L. monocytogenes has been studied in these products. The organism declined during storage in sweetened condensed milk at 21 °C, but the population remained stable at 7°C. In evaporated milk, growth was recorded at both temperatures.

***2. Clostridium* spp.**

A study of the incidence of clostridia in sweetened condensed milk showed that about 40% of the samples contained >100 cfu/100 g. These contaminants were identified mainly as *Clostidium butyricum* and *Clostridium perfringens*. However, the w^a of these products is too low to allow the germination of spores and vegetative cell growth.

3 *Staphylococcus aureus*

Although there are no reported cases of foodborne disease associated with canned sweetened condensed milk, its aw of 0.85 is very close to the minimum value that would allow *S. aureus* to grow, although toxin production would be inhibited. However, bulk products with much lower sugar contents might be at risk if they become contaminated. Therefore, adequate hygiene is an important control.

Dried milk

Although dried milk products have been implicated in a number of foodborne disease outbreaks, these have usually been the result of post-pasteurisation contamination by pathogens. Foodborne pathogenic bacteria are unable to grow in dried milk powders, but may survive for long periods.

1. **Salmonella spp.**

There have been several significant salmonellosis outbreaks associated with dried milk powders, and Salmonella contamination has come to be regarded as a serious potential hazard in these products. In 1964 to 1965, a nationwide outbreak occurred in the USA associated with non-fat milk powder produced at one plant, but then agglomerated (instantised) at a number of other locations. This outbreak produced reports of infection throughout the USA and led to a major United States Department of Agriculture (USDA) investigation of the incidence of Salmonella contamination in milk drying plants. It was found that contamination was widespread in both product and environmental samples, and this finding gave rise to a number of improvements in hygiene, sanitation and process control.

2. ***Staphylococcus aureus***

Contamination of dried milk powders with staphylococcal entero-toxins was a significant problem in the 1950s, and several outbreaks were recorded, often caused by growth and toxin production in the concentrated milk prior to drying. Improvements in temperature control and hygiene prior to drying have largely eliminated this problem. However, in 1986, several outbreaks were reported in Egypt associated with imported non-fat dried milk. Analysis of samples showed no viable pathogens, but staphylococcal enterotoxins A and B were found at concentrations high enough to cause illness.

3. ***Listeria monocytogenes***

No cases of listeriosis associated with dried milk products have been reported. However, the ubiquity of Listeria spp. in dairy plants and other wet processing areas, and the cases of listeriosis linked to other dairy products suggest that contamination of dried products is likely. The survival of *L. Monocytogenes* during spray drying and storage of product has been investigated. Spray drying was found to give a small reduction in numbers, and the viable count continued to decline during storage, but viable *L. monocytogenes* could still be isolated from some samples after 12 weeks

4. ***Bacillus spp.***

Bacillus cereus has been found to be a common contaminant in dried milk. Although there have been many reports of *B. cereus* food poisoning associated directly with dried milk consumption, in 2005, milk powder contaminated with *B. licheniformis* and

B. subtilis was the cause of an outbreak in Croatia involving 12 children. Reconstituted milk that was held for 2 hours prior to consumption, without boiling,as identified as the cause. *B. cereus* spores can survive for many months in dried milk powders, and rapid growth has been shown in reconstituted powders at ambient temperature.

5. ***Cronobacter and Enterobacter spp.***

Cronobacter and Enterobacter spp. are not normally regarded as foodborne pathogens, but there have been a number of sporadic outbreaks of neonatal meningitis caused by Cronobacter (Enterobacter) sakazakii associated with dried milk consumption, with fatality rates as high as 30 – 80%. A powdered infant formula contaminated with C. sakazakii was responsible for outbreaks among infants: one involving nine infections and two deaths in 2004, in France.

CREAM

According to the UK Food Labelling Regulations 1996, cream is defined as that part of cows' milk rich in fat that has been separated by skimming or otherwise and which is intended for sale for human consumption. Cream is often perceived as a luxury item, and therefore purchasing patterns are different from those that apply to milk. For this reason, the required shelf life is longer than for milk, and therefore heat processes are usually greater for cream than for milk.

The spoilage of cream is generally similar to that described for liquid milk products. However, because of the difference in purchasing patterns, cream is often required to have a longer shelf life than milk (up to 14 days for pasteurised cream), and containers may be opened and then used by the consumer over several days. The keys to obtaining sufficient shelf life are the microbiological quality of the raw milk, good hygiene in processing, and effective temperature control during distribution and

storage.

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 pasteurisation, and psychrotrophic strains of Bacillus cereus may cause 'sweet curdling' and 'bitty cream'. Other, more heat-resistant species, such as *Bacillus licheniformis, Bacillus coagulans*, and *Bacillus subtilis*, may survive sterilisation and even UHT processes, and may cause bitterness and thinning in sterilised creams. *Bacillus pumilus* and *Bacillus sporothermophilus* are now recognised as potential contaminants in cream, primarily carried over from raw milk. Heat-resistant lipases produced by psychrotrophic bacteria.

The keeping quality of cream is greatly affected by the introduction of post process contamination. Psychrotrophic bacteria such as pseudomonads may contaminate pasteurised cream during processing and are important spoilage organisms. The high fat content of cream means that lipolytic species, such as *Pseudomonas fluorescens* and *Pseudomonas fragi*, are a particular problem. Yeasts and moulds are rarely implicated in the spoilage of cream. Few yeasts are able to ferment lactose, but species such as *Candida lipolyticum* and *Geotrichum candidum* may occasionally spoil bakers' whipping cream where sucrose has been added. In addition to this Viral hepatitis is the most likely viral infection to be associated with dairy products. In 1975 in Scotland, an outbreak of hepatitis A infection occurred associated with cream consumption. The cause of the outbreak was handling of the cream by an infected cook during preparation.

BUTTER AND DAIRY SPREADS

Butter is a water-in-oil emulsion typically consisting of at least 80% fat, 15 - 17% water, and 0.5 - 1% carbohydrate and protein. Reduced-fat dairy spreads have a milk fat content of about 50 - 60%. Low-fat dairy spreads contain 39 – 41% fat, and very low-fat spreads have <30% fat. These have a much higher water content than butter or reduced-fat spreads. Where the fat content is below about 20%, these products tend to form a continuous water phase and become oil-in-water emulsions.

Butter is produced from cream, and the cream is the main source of microorganisms in hygienically produced butter.

Spoilage

Bacterial spoilage: Modern hygienic manufacturing methods mean that bacterial spoilage of butter is much less common than in the past. However, defects caused by microorganisms do occasionally occur. Surface taints may develop as a result of growth of *Shewanella 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 flavour develops due to the breakdown of protein. Rancidity, proteolytic activity and fruity odours may be caused by the growth of Pseudomonas fragi and, occasionally, *Pseudomonas fluorescens*. Black discoloration of butter is reported to be caused by Pseudomonas nigrificans (1), 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' flavour defect linked to the formation of 3-methylbutanal. Lipolytic spoilage of butter has been associated with the presence of Micrococcus.

Fungal spoilage: Moulds are still important spoilage organisms for butter, and mould 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*.

Dairy spreads

There is little information on spoilage of spreads. In theory, the aqueous phase of some low-fat spreads would allow the growth of spoilage bacteria, such as pseudomonads, but in practice the majority of problems are the result of mould growth. Generally, the same genera are involved as for butter spoilage. Preservatives such as sorbic acid help to prevent mould growth, but some species, including *Penicillium* spp. and *Trichoderma harzianum*, are able to convert preservatives to other compounds, which may result in tainting.The yeast *Yarrowia lipolytica* and bacteria *Bacillus polymyxa* and *E. faecium* have also been reported to be important spoilage organisms in a low-fat dairy spread.

Cheese

Cheese is a stabilised curd of milk solids produced by casein coagulation and entrapment of milk fat in the coagulum. The water content is greatly reduced, in comparison with milk, by the separation and removal of whey from the curd. With the exception of some fresh cheeses, the curd is textured, salted, shaped, and pressed into moulds before storage and curing or ripening. There are said to be approximately 1,000 named cheeses throughout the world, each produced using a variation on the basic manufacturing process. Most of these varieties fit into one of three main categories according to their moisture content, and method and degree of ripening:

Soft cheese: High moisture (55 - 80%)

a) Fresh, unripened (cottage cheese, Ricotta, Quarg, Fromage Blanc, Neufchatel, Mozzarella)

b) Surface mould-ripened (Brie, Camembert)

Semi -soft / semi-hard cheese (Moderate moisture (41 - 55%)

a) Surface smear ripened (Limburger, Munster, Tilsit)

b) Ripened by bacteria (Caerphilly, Lancashire, St Paulin)

c) Blue-veined, internally mould ripened (Stilton, Roquefort, Gorgonzola)

Hard / low moisture cheese (Low moisture (20 - 40%)

a) Ripened by bacteria, with eyes (Emmental, Gruyere)

b) Ripened by bacteria, no eyes (Cheddar, Edam, Cheshire)

c) Very hard (Grana (Parmesan), Asiago, Romano)

Spoilage

Microbial spoilage of cheese can be caused by both bacteria and fungi, but the type of spoilage depends very much on the characteristics of individual cheese varieties. Both visual and organoleptic defects may result, either on the surface of the cheese or internally.

Fungal spoilage

Although the growth of moulds on the surface or in the body of some cheese varieties is essential for ripening, mould growth is generally not desirable. Mould spoilage is usually unpleasant in appearance, and may result in musty taints and odours. Moulds are also responsible for liquefaction of the curd. There is also the possibility of mycotoxin production in some cases. Moulds commonly involved in cheese spoilage include members of the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Fusarium*, *Monilia* and *Alternaria.* Effective hygiene is important in the control of mould spoilage in cheese, particularly in ripening rooms, and rigorous cleaning procedures are needed to prevent the accumulation of mould spores. Yeast may also proliferate on the surface of ripened cheeses, especially if the surface becomes wet, causing slime formation. Yeasts most frequently isolated from spoiled cheese include *Candida* spp., *Yarrowia lipolytica*, *Pichia* spp., *Kluyveromyces marxianus*, *G. candidum* and *Debaryomyces hansenii*.

Bacterial spoilage

In fresh cheeses with a sufficiently high pH, such as cottage cheese, bacterial spoilage may occur. This is likely to be caused by Gram-negative, psychrotrophic species, such as pseudomonads and some coliforms. These organisms may contaminate the product through water used to wash the curd. *Pseudomonas* spp., *Alcaligenes* spp., *Achromobacter* spp. and *Flavobacterium* spp. are the psychrotrophic bacteria of concern. *Pseudomonas fluorescens*, *Pseudomonas fragi* and *Pseudomonas putida* cause bitterness, putrefaction and a rancid odour, liquefaction, gelatinisation of curd, and

slime and mucous formation on cheese surfaces. *Alcaligenes viscolactis* is responsible for ropiness and sliminess in cottage cheese, and *Alcaligenes metacaligenes* for 'flat, flavourlessness' in cottage cheese. Psychrotropic *Bacillus* spp. cause bitterness and proteolytic defects.

Bacteria may also cause spoilage by the production of internal gas in the cheese, resulting in slits, small holes or blown packs. This may happen in fresh cheese, early in the ripening phase ('early blowing'), or well into the ripening stage ('late blowing'). Early blowing is usually caused by members of the Enterobacteriaceae, but other organisms, such as Bacillus spp., are sometimes involved. The problem can be effectively controlled by adequate hygiene and process control in manufacturing. Late blowing, which may occur after 10 days in varieties such as Gouda, or after several months in some Swiss cheeses, is caused by clostridia that are able to produce butyric acid from lactate. Late blowing sometimes also occurs in Cheddar. Species commonly involved are *Clostridium butyricum, Clostridium tyrobutyricum* and *Clostridium sporogenes*, spores of which survive pasteurisation and can be present in cheese milk. Contamination of milk with these organisms is often seasonal (*C. tyrobutyricum* is more prevalent in winter). Small, irregular slits may also sometimes appear in 3- to 6-week-old Cheddar, and this 'intermediate blowing' is thought to be associated with the presence of non-starter gas-producing lactobacilli.

Microbiological tests:

Various microbiological tests performed in dairy industry can be broadly categorized in to following groups:

- Direct enumeration of Total Bacterial Count e.g. Direct Microscopic Count
- Estimation of number of viable bacterial cells e.g. Standard Plate Count
- Assessing the microbial metabolic activities e.g. Dye Reduction Test
- Detection of specific Contaminants e.g. Coliforms, Pathogens
- Estimation of biochemical changes or metabolites formed in dairy products as a result of microbial growth e.g. acidity, gas production, toxin production etc.

i) Direct Microscopic Count (DMC) Method

The DMC method enables rapid enumeration of bacterial cells along with their study of morphology of the total bacterial count in milk and cream with minimum equipment. It consists of examination of stained films of a measured volume of milk or milk product (0.01 ml) spread over 1 cm^2area and dried on a glass slide under microscope. Somatic cells, shapes and arrangement of bacterial cells present in films can be easily and rapidly visualized and recorded. The microbial morphology and arrangement give the clue to possible cause of high count while high somatic cell indicates udder infection

e.g. mastitis. For determination of average number of bacterial cells or clumps of cells about 5 to 50 microscopic fields are scanned (fewer the number of cells, more fields to be scanned). The diameter of a field is measured with the help of a stage micrometer to calculate microscopic factor (MF). The DMC/ml is then calculated as follows:

DMC/ml=N x MF

Where N= Average number of cells per field

MF= Microscopic Factor

MF= Area of Smear/Area of Microscopic x 1/Volume of milk (0.01 ml)

=10,000/3.1416 x r2

This technique is very useful for screening of milk supplies on the receiving platform of a dairy plant as well as for grading of milk. However, the limitation of this method is that both dead as well as viable cells are counted.

ii) Standard Plate Count (SPC) Method

In this method a known quantity of milk sample is diluted to known degree and equal portions of each dilution is poured in to a petriplate followed by addition of nutrient agar medium, a technique known as pour plate method. The medium is allowed to solidify after mixing the contents by gentle rotation of the plate. The organisms present in the sample are expected to be trapped in the agar gel. The plates are subsequently incubated at 37^{o}C for 48 to 72 hours. In principle each organism is expected to take up a separate position in the medium and grow in to a mass of cells of a size sufficient enough to be counted by naked eyes, recognized as a colony forming unit (cfu). Hence, a colony count performed at this stage represents number of viable bacteria present in the given volume of milk sample. The major limitations of this method is that it is time consuming and only those bacteria which are capable of growing under given set of growth conditions (medium, incubation temperature and period) and forming colonies can be counted. Determination of microbiological quality of milk and milk products invariably involves performing different plate counts. These include SPC, the

Coliform count,and the yeast and mold count. Techniques employed for plating are identical for these tests though method of sampling and media may vary.

iii) Dye Reduction Test

There are certain dyes, which act as oxidation-reduction indicator. Bacteria consume dissolved oxygen during their growth in milk and consequently reduce the OH to a level at which these dyes are reduced and get decolorized. Such dyes can be employed to assess the biochemical activity of bacteria and thus estimate number of bacteria indirectly.

a) Methylene Blue Reduction (MBR) Test

Methylene blue is a dye, which remains blue in its oxidized state and turns colorless on its reduction. This characteristic is put to use for estimation of bacterial load of milk and milk products. When bacteria grow in milk they release hydrogen during respiration, which is simultaneously accepted by methylene blue. As a result, it is reduced to colorless or leuco compound.

The majority of bacteria, both aerobic and facultative present in milk indulge in lowering of oxidation-reduction potential of milk to such an extent that dye gets decolorized. Hence greater the number of viable cells, shorter is the time taken to reduce the dye. The result of this test is expressed in terms of time required for the colour of methylene blue to disappear at incubation temperature of 37^{o}C.

This test renders very useful information on general bacteriological quality of milk in a short period and requires fewer apparatus. Limitations of this technique include suitability only for unheated milk, no indication of type of organisms and incubation temperature favorable only for mesophilic bacteria.

b) Resazurin Reduction (RR) Test

Resazurin is also an OH indicator and hence is liable to be reduced by bacteria. Reduction of blue dye takes place in two stages. First, the dye is irreversibly reduced to resorufin undergoing through a series of colors ranging from blue to lilac, mauve,

purple and pink. During second stage, resorufin is reversibly reduced to a colorless compound, dihydroresorufin.Various colors developed sequencely during reduction of dye can be well compared with a standard resazurin disc with the help of a small apparatus known as resazurin comparator. Results are expressed in terms of standard resazurin disc number ranging from 6 to 0.The time taken for the reduction of dye to a specific stage (disc number) or the color change recorded on completion of incubation after a certain period can be used as a scale for measurement of bacterial activity.The test is carried out at incubation temperature of 370 C for 10 minutes, I hour or till complete reduction.This test finds its application in quick grading of milk (even faster than MBR Test).However, reduction of dye is susceptible to light and confusion may arise in interpretation of results due to the fact that besides bacteria, this dye is liable to be reduced by leucocytes.

c. Coliform Test

The Coliform group of bacteria (*Escherichia, Enterobacter, Klebsella*) includes gram-negative, non-spore forming, aerobic and facultative rods capable of fermenting lactose in to lactic acid and gas. As per American Public Health Association (APHA) method, Coliforms in milk are detected by following scheme:

Presumptive Coliform Test: One ml of milk sample or decimal dilution is poured to sterile plates followed by addition of 10-15 ml of Violet Red Bile Agar (VRBA). The content of plates is by gently rotating and tilting each dish and finally agar is allowed to solidify and incubated at 32^0C for 24 h. Appearance of typically red colonies measuring about 0.5 mm is taken as positive test. Alternatively fermentation tubes of 2% brilliant green lactose bile (BGLB) broth are inoculated with sample (1.0 ml) and production of gas after incubation of 48 h at 32^oC is considered as positive indication for presence of Coliforms in milk.

Confirmed Test: A confirmed test of doubtful colonies form VRBA is carried out by transferring each of five colonies to tubes of 2 % BGLB broth and observing for gas production.

Completed Test: Finally, material from typical colonies on solid media or from BGLB broth tubes showing gas production is streaked on Eosine Methylene Blue Agar. Coliforms form dark colonies or dark centered colonies with colourless peripheries and red colonies on VRBA. Pure cultures so isolated should be able to produce gas in fermentation tubes of lactose broth at 32^oC within 48 h and microscopic examination should reveal only gram-negative, non-spore forming rods.

v. Detection of Pathogens

Milk is a favourable niche for pathogens and various pathogens found in milk have been discussed. In this section, detection of two frequently encountered pathogens viz. Salmonellae and Staphylococci have been discussed. Both of these organisms are enterotoxigenic (produce enterotoxin). Being heat labile, though these organisms may be destroyed during heat treatment, yet their toxins survive National and international microbiological standards have specified limits for them in various dairy products. Staphylococci are gram positive, catalase positive, coagulase positive (coagulate blood plasma) cocci occurring singly or in clusters. The undiluted sample or its decimal dilution of milk/milk product is analyzed by plate count method using selective media (Trypticase soy broth, Baird Parker Agar, Vogel and Johnson agar, Staphylococci medium). Appearance of typical black colonies on agar surface after incubation for 48 h at 37^{o} C is taken as a positive test.

Subsequently, Coagulase test is performed to confirm their presence. Colonies are picked from the agar plates, inoculated into brain heart infusion broth tube and 0.5 ml of coagulase plasma is added before incubation at 37^{o}C for 6 h. The tubes are periodically observed for clot formation as a positive reaction. Doubtful colonies may be further subjected to additional tests such as catalase reaction, anaerobic utilization of glucose and mannitol, susceptibility to lyostaphin etc. The detection scheme for Salmonellae is elaborate. This organism is gram negative may or may not produce H_2S. Presence of other related organisms e.g. *Escherichia, Enterobacter, Shigella* and *Proteus* might prove interfering in interpretation of results. These organisms are differentiated on the basis of reaction they exhibit on slants of Triple Sugar Iron agar and appearance of characteristic colonies on the surface of differential agar media.