

INTRODUCTION

Milk has been used since time immemorial, across all cultures, as an integral part of the food pyramid. It has been used right from the raw fluid form to various forms of processed milk like butter, cream and milk desserts, milk powder, cheese etc.

Milk is a nutritious medium, right from meeting complete food requirements of an infant in the form of colostrum, to normal milk which is a rich source of sugars esp. lactose, fat and proteins. Milk is a highly perishable commodity and one of the critical factors determining the keeping quality of milk is its microbiological status. In a healthy animal, milk is sterile in the udder, however microbiological contamination may happen depending on the milking methods and the hygiene standards followed during milking and subsequent storage and transport. There are various types of microorganisms that can be found in milk and related products depending on the hygiene standards followed.

The microorganisms found in milk and milk related products are classified as unfavorable- ones that are responsible for causing spoilage, and favorable- ones that responsible for bringing about desired changes in the products. There are various established methods available to enumerate the type of the microorganisms present along with their quantification. To ensure that the consumer gets a safe product for consumption various bodies like the FDA and Codex have established the product regulatory standards that all the milk producers and processors are supposed to meet across the product marketing chain.

MICROORGANISMS

As defined in Wiley's Encyclopedia, "all living things smaller than 0.1 mm in diameter are classified as microorganisms because they are too small to be seen by the naked eye" (1629).

Types of microorganisms

These can be normally classified into the following categories:

1. Bacteria – These are single celled microorganisms, normally measured in microns and one of the methods of their classification is based on the fundamental shapes that they exhibit: a. Spherical, b. rod and c. spiral. They reproduce asexually by fission and their growth phase is characterized by distinct phases:
 - a. Lag Phase: This represents the phase where the microorganisms become accustomed to the environment. Under less than optimum conditions, the lag phase gets extended.
 - b. Log Phase: The bacteria starts to multiply at a logarithmic rate.
 - c. Stationary Phase: In this stage, the rate of multiplication slows due to the lack of nutrients and also because of the build-up of toxins.
 - d. Death Phase: In this phase, the growth stops and existing cells start to die off.
2. Yeasts - These belong to the group of microorganisms called fungi. These are classified on the basis of morphology, cultural, sexual and physiological characters. Based on their method of reproduction, there are two forms that are of importance in the food industry.

- a. Budding and spore forming yeasts: These are also called as true yeasts and reproduce by producing sexual ascospores.
 - b. Budding or Asexual yeasts: These are also called false yeasts.
3. Molds – These also belong to the class of fungi and are multi-celled and filamentous, the filaments being referred to as hypha. These too can reproduce either sexually or asexually. The sexual reproduction normally happens in the unfavorable conditions by forming spores through the process of nuclear fission, whereas the asexual reproduction happens through either fragmentation or spore production in conidia.
4. Viruses – These are intracellular parasites. They need to contact and invade an acceptable host cell to survive and replicate.

Factors affecting growth

As enumerated by Banwart (102-144), in any food environment, some microbial species will survive and become dominant. Organisms that lack the ability to withstand stresses induced by unfavorable environment will succumb. The conditions that affect the metabolism and multiplication of microorganisms include the following:

1. Nutrients – These are required as source of energy and for synthesis of cellular protoplasm. Microorganisms require a source of carbon and nitrogen, growth factors like vitamins, minerals and water.
2. Moisture – Some microorganisms can remain alive in a dried condition but cannot carry out their normal metabolic activities or multiply without water. They cannot grow in pure water or in absence of water. Water is used to bring nutrients into the cell and to dispel water products. Water is used in chemical reactions that

- break down substrates to usable molecules. Water activity (a_w) is the index of the availability of water for chemical reactions and microbial growth. In general, for growth, bacteria require a higher a_w than yeasts, and yeasts require a higher a_w than molds.
3. pH: Microorganisms have a minimum, optimum and maximum pH for growth. Microorganisms can grow in a wide pH range. The variations in the pH values for growth may be due to different strains of a species or different species in a genus, the type of substrate, the acid or base used to adjust pH or other factors.
 4. Oxidation-reduction (OR) potential: In microbial cultures, the simultaneous oxidations and reductions are the sources of energy for cell processes. Since energy is needed by the cell to function normally, OR reactions and OR potentials are important. Strictly aerobic organisms grow only in the presence of free atmospheric oxygen. Strictly anaerobic organisms survive only in the absence of free oxygen. Facultative anaerobes can grow with or without free oxygen.
 5. Inhibitory substances: Generally, inhibitors affect microorganisms by acting on the whole cell, cell wall or cell membranes, by interfering with the genetic mechanism of the cell or by binding to the essential nutrients. Some examples of are lysozymes, Avidin, enzyme inhibitors etc.
 6. Temperature: It is one of the most important environmental factors that regulate the growth of microorganisms. Temperature is related to the ability of an organism to grow and survive. It also has an effect on the cell size, metabolic products such as pigments and toxins, nutritional requirements, enzymatic reactions and chemical composition of cells. Each organism has a minimum,

optimum and maximum temperature for growth, based on which they can be classified as psychrophiles (which can grow at low temperatures, the optimum temperature being 15°C), mesophiles (which have an optimum growth temperature of 25°C to 45°C) and thermophiles (which have an optimum growth temperature of 45°C or higher).

Enumeration methods

An estimate of the number of microorganisms in or on foods is needed in order to determine if a product meets the microbial levels expressed in specifications, guidelines, or standards. Microbial count can be used to help predict the shelf life of certain foods. To a limited extent, the microbial numbers might be used to evaluate the potential safety of foods. Hence the enumeration methods serve as a critical tool for quality assurance. (Banwart 11-12).

The samples need to be collected aseptically and diluted to required concentrations. The dilution needed depends on the contamination level of the food. The various tests that are of relevance in food industry are given below, and the method used depends on the purpose of testing:

1. Total Cell Count: One of the quickest ways of determining the microbial load in a system. However, this method does not differentiate between the dead cells and the living cells. It is of use when online adjustments are required in a processing operation to rectify a problem. Direct microscopic counts, coulter counters etc. are the methods used.

2. Total Viable Count: There are many methods, most of which are based on plate count or tube dilution methods. Pour plate count, most probable number (MPN), Tube dilution etc are the methods employed.

3. Estimations based on metabolism: This method uses electrical impedance, acid production and dye reduction tests like resazurin or methylene blue.

4. Measurement of cellular constituents: This is one of the accurate and sensitive methods to estimate the viable bacterial count. For example, luciferase test is used to measure ATP and hence the bacterial count.

Control methods

Just as important the enumeration methods are to ensure the quality of the milk and milk products, control methods also play a crucial role in assuring a safe product to the consumer. The method of control used depends on the type of the microorganism, their initial count and the type of the milk product in question. Generally, the following methods are used either singly or in combination to control the microbiological spoilage of the food products:

1. **Drying:** It is a commonly used method that reduces the water activity of the food and hence the microorganisms cannot grow. Generally sun drying or controlled dehydration at high temperatures is used and is deployed especially for meat products and fruits & vegetables.
2. **High temperature preservation:** In this method, the product is exposed at high temperature, thereby resulting in heat coagulation of proteins and enzymes and killing the microorganisms. However, thermodurics can survive these high temperatures.
3. **Low temperature preservation:** Low temperatures reduce the enzyme activity of the microorganisms, though the microbes may survive for a long time.

- Psychrotrophs can survive these low temperatures and can ultimately spoil the food stored in cold conditions.
4. Radiation treatment: The ionizing radiations like the X-rays and gamma rays break down the DNA structure or may lead to formation of free radicals by ionization of water, thereby breaking the chemical bonds in the microorganisms. Since there is no rise in the temperature of the food, it is also referred to as 'cold sterilization'. The non-ionizing radiations like the UV rays are used for surface treatment.
 5. Use of chemicals: Chemicals used may be bacteriostatic (which prevent the growth of the microorganisms) or bactericidal (which kill the microorganisms). The FDA regulates the use of these chemicals. Nowadays, antibiotics like nisin are also used.
 6. Use of osmotic pressure: The application of osmotic pressure leads to 'plasmolysis' and 'plasmoptysis', which results in the death of the microorganisms. Pickling is a commonly used preservation technique for vegetables.
 7. Change in pH: Since the microorganisms have an optimum pH for growth, any changes from the optimum pH will result in arresting the growth of the microorganisms.
 8. Use of Ultrasound: Non-thermal technologies like ultrasound (waves with a frequency greater than 20KHz) have proven to have bactericidal effects, especially when combined with other methods like mild heating (D'Amico *et al* 556).

MICROBIOLOGY OF MILK AND MILK PRODUCTS

Depending on the health status of the animal to the hygiene standards followed during milking to storage, transport and processing, various types of microorganisms can be found in milk and related products.

Significance of microorganisms in foods depends upon several conditions: (1) the numbers found; (2) the types of microorganisms; (3) the type of food; (4) the treatments to which the food has been exposed; (5) the processing or storage treatments the food will receive; (6) whether the food is to be eaten as is or heated; and (7) the individuals who might consume the food. Microorganisms may have at least one of the four functions in a food. They may have a useful function, cause spoilage, be a health hazard, or be inert (Banwart 49).

Microbiology of Raw Milk

Cow's milk consists of about 87% water and 13% total solids. The freshly drawn milk is slightly acidic, with the pH ranging from about 6.3 to 7.2. The first milk drawn has the highest microbial count, the middle milk has a smaller count and the microbial count of the strippings is the least. It is a general practice to discard the first portions of milk. The temperature of freshly drawn milk is about 38°C, which is very conducive for microbial growth. If the milk is not cooled rapidly to about 16°C, it will spoil very fast. The unsterilised milk ferments rapidly, resulting in formation of lactic acid due to hydrolysis of lactose by *Streptococcus lactis*. In the second stage, the hexoses are fermented to lactic acid. There are various strains of *S. lactis* which have been identified and which result in differences in the flavor produced, rate of acid formation etc. Some of the varieties identified are *S. lactis* var. *maltigenes*, *S. lactis* var. *tardus* and *S. lactis* var.

anoxyphilus. As the acid concentration increases, it eventually causes the precipitation of casein at a pH of about 4.6 to 4.7 and finally results in prevention of growth of the bacteria causing fermentation. After this, molds and yeasts start growing in the soured milk. They utilize some of the acid and produce a corresponding decrease in the acidity. Subsequently the putrefactive bacteria take over.

There are many tests that are done to assess the microbial quality of the fresh milk. These are: 1) the direct microscopic count, which determines the total viable and dead microorganisms in the sample; and 2) the agar plate method, which measures the number of viable microorganisms in the milk.

Practically, the raw milk is stored at a low temperature of less than 16°C to reduce the rate of the bacterial growth. Also, it is critical that the milking is done under hygienic conditions and also that the milking equipments are sanitized before milking since the shelf life of raw milk is proportional to the initial bacterial load. This will help the milk keep well till it is transported for subsequent processing.

Microbiology of Pasteurized Milk

The initial microbial load of raw milk depends on the conditions of milking and also the storage temperatures. In the milk processing plants, the milk is rapidly cooled to less than 4.4°C to maintain quality. It is then pasteurized as whole milk, skimmed milk or low fat milk.

Pasteurization is a process used to kill yeasts, molds, and pathogenic and most other bacteria and to inactivate certain enzymes without greatly altering the flavor. Milk is heated to at least 62.8°C and held for at least 30 minutes. Alternatively, it can be heated to at least 71.7°C and held for at least 15 seconds. Putrefactive microorganisms can

survive pasteurization. Hence the pasteurized milk, which has a shelf life of 2 to 3 days will putrefy rather than develop acidity.

The shelf life of the pasteurized milk depends on the microbiological quality of raw milk and the efficacy of pasteurization. Phosphatase test is done to check the efficacy of pasteurization. Phosphatase is an enzyme present in the raw milk and its thermal resistance is greater than that of the pathogens. A positive test indicates either underpasteurization or contamination with raw milk.

Among the latest developments, microfiltration along with pasteurization has shown a reduction in the microbial count and increase in the shelf life. Raw skim milk was microfiltered at 50°C through 1.4-micron membrane and subsequently pasteurized and cooled to 6°C. In comparison to raw skimmed milk that had a bacterial count of 2400, 3600 and 1475 cfu/ml across three trials, microfiltration reduced the bacterial count to 0.240 0.918 and 0.240 cfu/ml whereas pasteurized microfiltered skim milk had a count of 0.005, 0.008 and 0.005 cfu/ml, demonstrating an average 5.6 log reduction due to combination of microfiltration and pasteurization (Elwell and Barbano E20).

Some of the common microorganisms seen in milk are given in the table below:

Microorganism	Source	Effect
<i>E. coli</i>	Wash water	O157:H7 is a pathogen
<i>Alcaligenes viscosus</i>	Wash water	Ropy or Slimy milk
<i>Clostridium perfringens</i>	Manure, soil	'Stormy' fermentation of milk
<i>Streptococcus lactis</i>	Manure	Souring

Microbiology of Butter

Butter is prepared normally by souring the cream and then churning it to separate fat. Butter is composed of milk fat, water, casein, lactose and salt. Butter cultures consist of:

1. Microorganisms producing high acidity like *Streptococcus lactis* and *Streptococcus*

cremori; and 2. Microorganisms that impart the flavor and aroma like *Leuconostoc dextranicum* and *Leuconostoc citrovorum*.

The sources of contamination in butter are the equipments used, the quality of wash water, air contamination, factory personnel and the packaging materials used. External contamination of butter can lead to problems like (Salle 567-568):

1. Rancidity: The rancid odor is due to the hydrolysis of glycerides to glycerol and fatty acids like butyric and caproic acid. The microorganisms responsible include the species of *Pseudomonas*, *Micrococcus*, *Penicillium*, *Cladosporium* and *Serratia*.
2. Fishiness: This results from high acidity and also due to decomposition of lecithin that results in the formation of trimethylamine. It is caused by the microorganism *Proteus ichthyosmius*.
3. Off flavors: Off flavor is produced by the growth of foreign microorganisms. Some of these defects are malty flavor caused by *Streptococcus lactis* var. *maltigenes*, yeasty flavor caused by *Torula cremoris* and *Torula sphaerica*, cheesy flavor caused by *Lactobacilli* and other organisms and metallic flavor caused by various organisms including a strain of *S. lactis*.

Due to the presence of salt in butter, it becomes an unfavorable medium for microbial growth. However, it is critical that external contamination is avoided to ensure that the keeping quality of butter does not deteriorate.

Microbiological analysis of butter usually includes the following tests: total bacterial count, yeasts and molds, coliform estimation and estimation of lipolytic bacteria.

Microbiology of Cheese

Cheese making is based on the coagulation of casein from milk by acidification, which is achieved by addition of lactic acid producing bacteria. There are many processes used in cheese making and hence there are various types of cheese available in the markets. The first phase of cheese preparation is fermentation of lactose by using lactic acid bacteria that results in the formation of an acid curd. Subsequent processing depends on the type of cheese required. Cheese is broadly classified into the following two categories:

1. Hard Cheese: This is prepared by subjecting curd to a high pressure so as to remove the whey as much as possible. This gives a product that does not soften during the ripening process. Examples of hard cheese are Cheddar cheese, Swiss cheese etc.
2. Soft Cheese: This is prepared by allowing the whey to drain from the curd without any external pressure. Hence these tend to have more moisture than the hard cheese. Examples of soft cheese are Roquefort cheese, Camembert cheese etc.

Many microorganisms are responsible for the characteristic aroma and flavor of different types of cheese. Some of the desirable microorganisms isolated from cheese are *Lactobacillus bulgaricus* and *L. helveticus*, commonly used as a starter to make cheese, *Streptococcus lactis*, *S. cremoris*, *S. thermophilus*, *Leuconostoc citrovorum*, *L. dextranicum*, *L. casei*, *L. plantarum* and *Propionibacterium shermanii* among others.

The common problems seen in cheese as reported by Salle (1954) are 'Swollen Cheese', which is a result of lactose fermentation that results in formation of gas along with lactic acid and the gas causes the cheese to swell and it may eventually burst. The causative organism for this problem generally is *Aerobacter*. The other common problem is the surface discoloration that is caused by molds like *Penicillium casei*, *Cladosporium*

herbarum and *Monilia niger*, *Lactobacillus brevis* var. *rudensis* causes the appearance of rusty spots in cheddar cheese.

The sources of contamination in Cheese could be the quality of raw milk used, less ripening period, equipments used, environment and the personnel. The routine cheese microbiological analysis includes enumeration of yeasts and molds, total coliforms and Staphylococci, which is done during manufacturing process and also after curing.

Microbiology of Fermented Milk Products

The consumption of soured milk preparations is widespread as it is associated with having therapeutic values. These are known by different names in different parts of the world. Normally Streptococcus, Lactobacilli and Saccharomyces species are used in starter cultures. The products like yoghurt, curd, kefir etc. come into this category. Nowadays, this category is gaining prominence as many functional foods are being designed using specialized cultures to deliver a specific health benefit.

REGULATION AND STANDARDS

At a national level in the US, US Department of Agriculture has issued the quality standards for various dairy products and can be accessed at www.ams.usda.gov/standards/standair.htm. Also, US FDA also defines the products, their standards and the labeling requirements that can be accessed at www.fda.gov. At an international level, Codex Alimentarius is used as a reference for the standards of milk and milk products, which can be accessed at www.codexalimentarius.net.

CONCLUSION

Microorganisms are ubiquitous and are either desirable or may be pathogenic. Milk and milk products are a good medium for microorganisms to grow. Hence it is very important that proper hygienic practices are followed through the complete processing cycle, right from milking of animals to the final consumption of milk and milk products to ensure a safe product to the consumer.

Use of proper enumeration techniques is a critical part of the quality control process. It is the responsibility of the producers and processors to provide a safe product to the consumer and the national governments should ensure that local environmental conditions are taken into consideration while framing the regulatory standards for milk and milk products.

Though in the past a lot of emphasis was given to protect the consumers from the pathogens and undesirable microorganisms, of late there has been a concerted effort to utilize the desirable microorganisms and design food products for specific health benefits. In a study on cancer patients, Henriksson (81) reported that intake of fermented milk products could be of value in decreasing chronic bowel discomfort following radiotherapy of pelvic malignancies.

Being an excellent growth medium for microorganisms, milk can be transformed into fermented milk products supplying humans with living healthful probiotic bacteria, recognized to influence human health by preventing specific health problems and combating some illnesses like diarrhea. Using targeted dairy cultures, antihypertensive fermented dairy products can be obtained, delivering increased ACE-inhibitory effects. Milk and technologically modified dairy foods are meeting growing consumer demands

for pure products without unnatural additives, thus providing the same benefits as other artificially fortified functional foods (Gallman). For example, Danone's *Lactobacillus casei imunitass* strain is particularly effective in reducing diarrhea in kids. There are designer yoghurts that have probiotic strains, which have been shown to destroy *H. pylori*, the organism responsible for peptic ulcers. Promising ongoing research is being conducted on the use of probiotics for the treatment of *Clostridium difficile* colitis, treatment of irritable bowel syndrome, treatment of intestinal inflammation in cystic fibrosis patients etc.

WORKS CITED

- Banwart, G.J. Basic Food Microbiology. New York: Van Nostrand Reinhold, 1989.
- D'Amico D.J., et al. "Inactivation of microorganisms in milk and apple cider treated with ultrasound." Journal of Food Protection, 69.3 (2006): 556-563.
- Elwell, M.W. and Barbano, D.M. "Use of Microfiltration to Improve Fluid Milk Quality." Journal of Dairy Science, 89 (2006): Suppl 1: E20-30.
- Francis, F.J. "Milk and Milk Products." Wiley Encyclopedia of Food Science and Technology. 2nd ed. 2000.
- Gallman, P.U. "Milk as a source of Nutraceuticals and Dairy based Functional Foods." Internet. Available: http://ift.confex.com/ift/2002/techprogram/paper_10336.htm. 24 Apr 2006.
- Henriksson, R., et al. "Effects if active Addition of Bacterial Cultures in Fermented Milk to Patients with Chronic Bowl Discomfort Following Irradiation." Supportive Care in Cancer, 3 (1995): 81-83.
- Salle, A.J. Fundamental Principles of Bacteriology. New York: McGraw-Hill Book Company Inc., 1973.