Chapter 2

Quality of Milk: Grading of Milk-Dye Detection Test, Platform Test, Sensory Evaluation, Milk and Public Health, Common Milk Borne Disease, Spoilage-Causes and Prevention, Adulterants and Their Detection

Objective

In this chapter, we will learn

- *Grading of milk based on certain rapid test*
- *Public health significance of milk*
- *Detection of adulterants in milk*

Introduction

Milk testing and quality control is an essential component of any milk processing industries. Milk being made up of 87% water is prone to adulteration by middleman suppliers and farm levels. Moreover, its high nutritive value makes it an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic production and storage at ambient temperatures. For making good quality dairy products, good quality raw milk and other required materials are indispensable. Therefore, for assuring the quality of raw milk, certain basic quality parameter tests are to be carried out at various stages of transportation of milk from the producer to the processor and finally to consumer. Milk quality control is the use of approved tests to ensure the application of approved practices, standards and regulations concerning the milk and milk products. The tests are designed to ensure that milk products meet accepted standards for chemical composition and purity as well as levels of different micro-organisms.

Testing milk and milk products for quality and monitoring those milk products, processors and marketing agencies adhere to accepted codes of practices costs money. The rationales are:

a) **Milk producer:** The milk producer expects a fair price in accordance with the quality of milk she/he produces.

b) **Milk processor:** The milk processor who pays the producer must assure himself/ herself that the milk received for processing is of normal composition and is suitable for processing into various dairy products.

c) **Consumer:** The consumer expects to pay a fair price for milk and milk products of acceptable to excellent quality.

d) **Public and Government agencies:** These have to ensure that the health and nutritional status of the people is protected from consumption of contaminated and sub-standard foodstuffs and that prices paid are fair to the milk producers, the milk processor and the final consumer.

All the above-is only possible through institution of a workable quality testing and assurance system conforms to national or internationally acceptable standards.

1) Grading of Milk-dye Detection Test

a) Methylene Blue Reduction Test

The dye reduction tests are based on the bacterial dehydrogenase activity. The dehydrogenases are a group of enzymes capable of transferring of hydrogen atoms from a substrate to a biological acceptors or redox sensitive dyes. Aerobic group of organisms use oxygen as electron acceptor. Thus during their growth and metabolism consume dissolve oxygen from the media and decrease the medium redox potential. The larger the initial number of microorganisms and higher the growth and metabolic rate, there will be faster the reduction of redox potential in the medium. Rate of oxygen consumption and duration of exhaustion are varying proportionately to the initial number of microorganisms in the system. A number of dyes can change their colour/ appearance in response to the change in redox potential of the medium and are called redox sensitive dyes, e.g. methylene blue, resazurin etc. In general, the redox dyes are in coloured state at higher redox potential (e.g. at oxidized redox potential, methylene blue remains as blue coloured form) and at reduced redox potential they becomes colourless or leucoform (e.g. at reduced redox potential methylene turns into colourless or leucoform).

Milk inside the udder is devoid of oxygen and remains at reduced redox potential. During milking, dumping, cooling, etc. a lot of oxygen is incorporated in milk and remains as dissolved state and thus, the redox potential of milk is increased as high as 0.3 V. At this redox potential, methylene blue exists as blue coloured (oxidized form). Bacterial growth in milk reduces the redox potential to a level of 0.06-0.10 V and at this redox potential, methylene blue is reduced and changes in leucoform or colourless state.

The methylene blue reduction test is based on the fact that the color imparted to milk by the addition methylene blue will be disappeared. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism cause the color to disappear. A certain quantity of milk and methylene blue dye is taken and mixed in a air tight container and incubate the system, the microorganisms in milk will grow and consume oxygen and thus, the milk oxidation and reduction potential will goes down to reduce the methylene blue dye to leuco-form or colourless state. The total time taken to reduce the methylene blue dye is called methylene blue reduction time (MBRT), which is inversely related to the initial microbial load in the milk sample. Higher MBRT indicates the lower initial microbial load in milk and vice-versa. The reasons for the oxygen consumption are the bacteria. Though certain species of bacteria have considerably more influence than others, it is generally assumed that the greater the number of bacteria in milk, the quicker will the oxygen be consumed, and in turn the sooner will the color disappear. Thus, the time of reduction is taken as a measure of the number of organisms in milk although actually it is likely that it is more truly a measure of the total metabolic reactions proceeding at the cell surface of the bacteria. However, the methylene blue reduction test has lost much of its popularity because of its low correlation with other bacterial procedures. This is true particularly in those samples which show extensive multiplication of the psychrotropic species.

Table 2.1. Grading of milk: Standard MBRT and quality chart for milk (BIS standard)

Grade of milk	MBRT (h)
Very good	5 and above
Good	3 and 4
Fair	1 and 2
Poor	½ or less

b) Resazurin Test

Resazurin test is the most widely used test for hygiene and the potential keeping quality of raw milk. Resazurin is a dye indicator. Under specified conditions Resazurin is dissolved in distilled boiled water. The Resazurin solution can later be used to test the microbial activity in given milk sample.

Resazurin can be carried out as:

i. 10 min test.

ii. 1 hr test.

iii. 3 hr test.

The 10 min Resazurin test is useful and rapid, screening test used at the milk platform. The 1 hr test and 3 hr tests provide more accurate information about the milk quality, but after a fairy long time. They are usually carried out in the laboratory.

Table 2.2. Readings and results (10 minute resazurin test)

Resazurin disc No.	Colour	Grade of milk	Action
6	Blue	Excellent	Accept
5	Light blue	Very good	Accept
4	Purple	Good	Accept
3	Purple pink	Fair	Separate
2	Light pink	Poor	Separate
1	Pink	Bad	Reject
0	white	Very bad	Reject

2) Platform Tests

a) Organoleptic Tests

The organoleptic test permits rapid segregation of poor quality milk at the milk receiving platform. No equipment is required, but the milk grader must have good sense of sight, smell and taste. The result of the test is obtained instantly, and the cost of the test are low. Milk which cannot be adequately judged organoleptically must be subjected to other more sensitive and objective tests.

Protocol

1. Open the can/ container of milk.
2. Immediately smell the milk.
3. Observe the appearance of the milk.
4. If still unable to make a clear judgement, taste the milk, but do not swallow it. Spit the milk sample into a bucket provided for that purpose or into a drain basin, flush with water.
5. Look at the can lid and the milk can to check cleanliness.

Obervations/Judgement

Abnormal smell and taste may be caused by:

- Atmospheric taint (e.g. barny/ cowy odour).
- Physiological taints (hormonal imbalance, cows in late lactation- spontaneous rancidity).
- Bacterial taints.
- Chemical taints or discolouring.
- Advanced acidification (pH < 6.4).

b) Indicator Test

- The acidity developed in milk due to bacterial activity is measured in terms of pH value as indicated by special indicator dyes, e.g. brom thymol blue and brom-cresol purple.

c) Sediment Test

- Milk is passed through a funnel containing a filter disc and the amount of dirt and dust collected is compared visually or by weight. The test indicates the gross impurities and dirt in milk as a result of unhygienic conditions of production.

d) Clot on Boiling (C.O.B) Test

The test is quick and simple. It is one of the old tests for too developed acid in milk (pH < 5.8) or abnormal milk (e.g. colostral or mastitis milk). If a milk forms clot on raising the milk temperature to boiling indicate the increasing the acidity of milk or abnormal milk composition (colostral milk). Such milk cannot be heat processed and must therefore be rejected.

e) Alcohol Test

The test is quick and simple. It is based on instability of the milk proteins when the levels of acid and/or rennet are increased and acted upon by the alcohol. Also increased levels of albumen (colostrum milk) and salt concentrates (mastitis) results in a positive test i.e. coagulation of milk upon mixing with alcohol.

f) Alcohol-Alizarin Test

The procedure for carrying out the test is the same as for alcohol test but this test is more helpful. Alizarin is a colour indicator changing colour according to the acidity of milk (Table 2.3). The Alcohol-Alizarin solution can be bought readymade or be prepared (0.4 g alizarin powder in 1 lit of alcohol (61%)).

Table 2.3 Colour Indicator for Alcohol-Alizarin test

Parameter	Normal milk	Slightly acid Milk	Acid milk	Alkaline Milk
pH	6.6 – 6.7	6.4 – 6.6	6.3 or lower	6.8 or higher
Colour	Red brown	Yellowish-brown	Yellowish	Lilac
Appearance of milk	No coagulation no lumps	No coagulation	Coagulation	No coagulation

f) Measurement of Titratable Acidity (% Lactic Acid)

Bacteria that normally develop in raw milk produce more or less of lactic acid. In the acidity test the acid is neutralised with 1/9 N NaOH. The amount (ml) of alkali is measured and titratable acidity is calculated (% lactic acid). Fresh milk contains in this test also “natural acidity” which is due to the natural ability to resist pH changes .The natural acidity of milk is 0.16 - 0.18%. Higher acidity signifies developed acidity due to the action of bacteria.

g) Gerber Fat Test

The fat content of milk and cream is the most important single factor in determining the price to be paid for milk supplied by farmers in many countries.

Also, in order to calculate the correct amount of feed ration for high yielding dairy cows, it is important to know the butterfat percentage as well as well as the yield of the milk produced. Further more the butterfat percentage in the milk of individual animals must be known in many breeding programmes.

Butterfat tests are also done on milk and milk products in order to make accurate adjustments of the butterfat percentage in standardised milk and milk products.

h) Inhibitor Test

Milk collected from producers may contain drugs and/or pesticides residues. The significant amounts in milk may inhibit the growth of lactic acid bacteria used in the manufacture of fermented milk, besides being a health hazard. The milk sample is evaluated for starter culture activity test, where after heat treatment, milk is fermented with selected lactic starter culture and the developed acidity is measured. The control milk sample (free from inhibitory substances) is run as control and from the acidity quality of milk is judged accordingly.

3) Sensory evaluation

Understanding of the principles of sensory evaluation is necessary for grading milk. Five primary senses are used in the sensory evaluation of dairy products: sight, taste, smell, touch and sound. The greatest emphasis, however, is placed on appearance (colour e.tc.), taste and flavour.

Soon after milk is received on the platform of dairy the lid of can is opened and milk is stirred up with a plunger to test for smell whether pleasant or unpleasant. Then it is tested for appearance (colour e.tc.), taste and flavour.

a) Colour

Observe the colour of the milk. If abnormal in colour, it should be held over for subjection to confirmatory tests.

b) Odour and Taints

Smell the milk in the container immediately after removing the lid. In case of foul or abnormal smell, hold over the milk for subjection to confirmatory tests.

The sense of taste

Taste buds, or receptors, are chiefly on the upper surface of the tongue, but may also be present in the cheek and soft palates of young people. These buds, about 900 in number, must make contact with the flavouring agent before a taste sensation occurs. Saliva, of course, is essential in aiding this contact. There are four different types of nerve endings on the tongue which detect the four basic "mouth" flavours -**sweet, salt, sour, and bitter**. Samples must, therefore, be spread around in the mouth in order to make positive flavour identification. In addition to these basic tastes, the mouth also allows us to get such reactions as coolness, warmth, sweetness, astringency, etc.

The sense of smell

We are much more perceptive to the sense of smell than we are to taste. For instance, it is possible for an odouriferous material such as mercaptain to be detected in 20 billion parts of air. The centres of olfaction are located chiefly in the uppermost part of the nasal cavity. To be detectable by smell, a substance must dissolve at body temperature and be soluble in fat solvents.

Note: The sense of both taste and smell may become fatigued during steady use. A good judge does not try to examine more than one sample per minute. Rinsing the mouth with water between samples may help to restore sensitivity.

Milk grading techniques

Temperature should be between 60-70° F (15.5-21° C) so that any odour present may be detected readily by sniffing the container. Also, we want a temperature rise when taking the sample into the mouth; this serves to volatize any notable constituents.

Noting the odour by placing the nose directly over the container immediately after shaking and taking a full "whiff" of air. Any off odour present may be noted.

Need to make sure we have a representative sample; mixing and agitation are important.

Agitation leaves a thin film of milk on the inner surface which tends to evaporate giving off odour if present.

During sampling, take a generous sip, roll about the mouth, note flavour sensation, and expectorate. Swallowing milk is a poor practice.

Can enhance the after-taste by drawing a breath of fresh air slowly through the mouth and then exhale slowly through the nose. With this practice, even faint odours can be noted.

Milk has a flavour defect if it has an odour, a foretaste or an aftertaste, or does not leave the mouth in a clean, sweet, pleasant condition after tasting.

Characterization of Flavour Defects

Lipolytic or hydrolytic rancidity

Rancidity arises from the hydrolysis of milkfat by an enzyme called the lipoprotein lipase (LPL). The flavour is due to the short chain fatty acids produced, particularly **butyric acid**. LPL can be indigenous or bacterial. It is active at the fat/water interface but is ineffective unless the fat globule membrane is damaged or weakened. This may occur through agitation, and/or foaming, and pumping. For this reason, homogenized milk is subject to rapid lipolysis unless lipase is destroyed by heating first; the enzyme (protein) is denatured at 55-60°C. Therefore, always homogenize milk immediately before or after pasteurization and avoid mixing new and homogenized milk because it leads to rapid rancidity.

Some cows can produce spontaneous lipolysis from reacting to something indigenous to the milk. Late lactation, mastitis, hay and grain ratio diets (more so than fresh forage or silage), and low yielding cows are more suseptible.

Lipolysis can be detected by measuring the acid degree value which determines the presence of free fatty acids. Lipolytic or hydrolytic rancidity is distinct from oxidative rancidity, but frequently in other fat industries, rancid is used to mean oxidative rancidity; in dairy, rancidity means lipolysis.

Characterized: soapy, blue-cheese like aroma, slightly bitter, foul, pronounced aftertaste, does not clear up readily

Oxidation

Milk fat oxidation is catalysed by copper and certain other metals with oxygen and air. This leads to an autooxidation reaction consisting of initiation, propagation, termination.

RH — R + H **initiation** - free radical

R + O2 —— RO2 **propagation**

RO2 + RH — ROOH + R

R + R — R2 **termination**

R + RO2 — RO2R

It is usually initiated in the phospholipid of the fat globule membrane. Propagation then occurs in triglycerides, primarily double bonds of unsaturated fatty acids. During propagation, peroxide derivatives of fatty acids accumulate. These undergo further reactions to form carbonyls, of which some, like aldehydes and ketones, have strong flavours. Dry feed, late lactation, added copper or other metals, lack of vit E (tocopherol) or selenium (natural antioxidates) in the diet all lead to spontaneous oxidation. It can be a real problem especially in winter. Exposure to metals during processing can also contribute.

Characterized: metallic, wet cardboard, oily, tallowy, chalky; mouth usually perceives a puckery or astringent feel

Sunlight

Often confused with oxidized, this defect is caused by UV-rays from sunlight or flourescent lighting catalyzing oxidation in unprotected milk. Photo-oxidation activates riboflavin which is responsible for catalyzing the conversion of methionine to methanal. It is, therefore, a protein reaction rather than a lipid reaction. However, the end product flavour notes are similar but tends to diminish after storage of several days.

Characterized: burnt-protein or burnt-feathers-like, “medicinal”-like flavour

Cooked

This defect is a function of the time-temperature of heating and especially the presence of any “burn-on” action of heat on certain proteins, particulary whey proteins. Whey proteins are a source of sulfide bonds which form sulfhydryl groups that contribute to the flavour. The defect is most obvious immediately after heating but dissipates within 1 or 2 days.

Characterized: slightly cooked or nutty-like to scorched or caramelized

Transmitted flavours

Cows are particulary bad for transmitting flavours through milk and milk is equally as susceptible to pick-up of off flavours in storage. Feed flavours and green grass can be problems so it is necessary to remove cows from feed 2-4 hrs before milking. Weeds, garlic/onion, and dandelions can tranfer flavours to the milk and even subsequent products such as butter. Barny flavours can be picked up in the milk if there is poor ventilation and the barn is not properly cleared and cows breathe the air. These flavours are volatile so can be driven off through vacuum de-aeration.

Characterization: hay/silage, cowy/barny

Microbial

There are many flavour defects of dairy products that may be caused by bacteria, yeasts, or moulds. In raw milk the high acid/sour flavour is caused by the growth of lactic acid bacteria which ferment lactose. It is less common today due to change in raw milk microflora. In both raw or processed milk, fruity flavours may arise due to psychrotrophs such as *Pseudomonas fragi*. **Bitter or putrid** flavours are caused by psychrotrophic bacteria which produce protease. It is the proteolytic action of protease that usually causes spoilage in milk. **Malty** flavour is caused by *S. lactis* var. *maltigenes* and is characterized by a corn flakes type flavour. Although more of a tactile defect, **ropy** milk is also caused by bacteria, specifically those which produce exopolysaccharides.

Miscellaneous defects

- astringent
- chalky
- chemical/medicinal - disease - associated or adulteration
- flat - adulteration (water)
- foreign
- salty - disease associated
- bitter - adulteration

4) Milk and Public Health

Milk is an excellent medium for the growth of a large variety of bacteria. Bacteria need considerable amounts of nutrient such as water, carbohydrate, fat and other substances for their growth. Milk contains all of these nutrients. Microorganisms are capable of causing deterioration in flavour, physical appearance of milk and transmission of infectious diseases to the consumers. The various organisms get into milk through unhygienic, carelessness and unsanitary practices of the farmers, processors and distributors. Discoloration, sliminess, ropiness, putrefaction, rancidity and many other defects are caused by various microorganisms growing in the milk and milk products. Bacterial contamination of raw and pasteurized milk is considered to be a great problem for dairy milk. The important genera of bacteria normally found in milk are, *Microbacterium*, *Micrococcus*, *Streptococcus*, *Staphylococcus*, *Lactobacillus*, *Bacillus*, *Clostridium*, *Arthrobacter, Actinomyces*, *Coxiella*, *Pseudomonus* etc. Most of these organisms are free living, widely distributed in soil, feeds, cows, buffaloes, goats, dairy utensils etc. Contamination usually occurs at the farm where milk is produced. Contamination of raw milk can arise from several sources including, soiled udders, inadequate cleaned milking equipment, and poor handling and processing of samples. Prolonged or improper holding of dairy products may permit microbial contamination to increase. Bovine mastitis may cause contamination with *Staphylococcus aureus*, *Streptococcus agalactiae, E. coli* and other microorganisms. Poor cleaning of the milking equipment may cause contamination with streptococci, coliforms, or heat resistant *Bacillus* spp. Spoilage of pasteurized or raw milk by proteolytic psychrotrophic bacteria can occur on prolonged storage below 7°C.

5) Common Milk Borne Disease

Pathogenic bacteria in milk and significance

Some micro-organisms may cause food poisoning (pathogenic microorganisms), either by intoxication and/or infection. Intoxication implies the production of poisons in the food prior to its consumption. Infection means the establishment, active growth, and multiplication of such microorganisms in the human body. Often rather large numbers are needed to cause an infection, but sometimes, as in the case of *Salmonella* typhimurium*,* the MID (minimum infection dose) may be as small as one bacterium.

The human pathogens transmitted through milk are classified into food infection and food poisoning groups. In food infection milk act as a carrier of the microorganisms, this

enters in human body through milk. It takes time to a person to become ill and fairly small numbers of microorganisms may suffice to cause illness. In food poisoning, preformed toxins in milk are responsible. Consumers rapidly fall ill. Large numbers of the pathogenic microorganisms are usually needed to cause food poisoning. Hygienic milk production practices, proper handling and storage of milk and mandatory pasteurization has decreased the threat of milk borne diseases such as tuberculosis, brucellosis, and typhoid fever. There has been a number of food borne illnesses resulting from the ingestion of raw milk, or dairy products made with milk that was not properly pasteurized or was poorly handled causing post-processing contamination.

It should also be noted that moulds, mainly of species of *Aspergillus*, *Fusarium* , and *Penicillium* can grow in milk and dairy products. If the conditions permit, these moulds may produce mycotoxins which can be a health hazard.

The following bacterial pathogens are still of concern today in raw milk and other dairy products:

Bacillus cereus
Listeria monocytogenes
Yersinia enterocolitica
Salmonella spp.
Escherichia coli O157:H7
Campylobacter jejuni

Table 2.4. Pathogenic bacteria in raw milk – significances and control

Pathogens	**Disease**	**Control Measures**
Mycobacterium bovis/ M. tuberculosis	Tuberculosis	Clean milk production Low temperature storage Pasteurization Hygienic processing
Brucella abortus/ B. melitensis	Brucellosis	Clean milk production Thermization before low temperature storage for processing Pasteurization Hygienic processing

Pathogens	Disease	Control Measures
Coxiella burnetii	Q fever	Clean milk production Low temperature storage Pasteurization Hygienic processing
Staphylococcus aureus	Enterotoxin	Clean milk production Thermization before low temperature storage for processing Pasteurization Hygienic processing
Escherichia coli	Some serotypes pathogenic for men, faecal contamination	Clean milk production Thermization before low temperature storage for processing Pasteurization Hygienic processing
Listeria monocytogenes	Listeriosis	Clean milk production protocol reduces the initial number in raw milk Pasteurization
Bacillus cereus	Enterotoxin	Clean milk production UHT sterilization of milk Use of bactofugation and microfiltration in processing operation
Clostridium perfringens	Gas gangrene/ survive pasteurization	Clean milk production UHT sterilization of milk Use of bactofugation and microfiltration in processing operation

6) Spoilage-Causes and Prevention

Spoilage Organisms

The microbial quality of raw milk is crucial for the production of quality dairy foods. Spoilage is a term used to describe the deterioration of a foods' texture, colour, odour or flavour to the point where it is unappetizing or unsuitable for human consumption. Microbial spoilage of food often involves the degradation of protein, carbohydrates, and fats by the microorganisms or their enzymes. In milk, the microorganisms that are principally involved in spoilage are psychrotrophic organisms. Most psychrotrophs are destroyed by pasteurization temperatures, however, some like *Pseudomonas fluorescens, Pseudomonas fragi* can produce proteolytic and lipolytic extracellular enzymes which are heat stable and capable of causing spoilage. Some species and strains of *Bacillus, Clostridium, Cornebacterium, Arthrobacter, Lactobacillus, Microbacterium, Micrococcus* , and *Streptococcus* can survive pasteurization and grow at refrigeration temperatures which can cause spoilage problems.

Saprophytic Bacteria in Milk and Significances

According to the main points of attack on the major milk constituents, the saprophytic bacteria are subdivided as follows:

a) Microorganisms degrading milk carbohydrate (lactose) are classified as glycolates, e.g. Streptococci, Lactobacilli, Coliforms

b) Microorganisms degrading proteins are classified as proteolytes, e.g. pseudomonas, enterobacteriaceae, aerobic spore-formers.

c) Microorganisms degrading lipids are classified as lipolytes, e.g. pseudomonas, micrococci, aeromonas, corynebacteria.

The effect of growth of saprophytic bacteria in milk may be important in three ways as follows:

a) The change in milk composition may interfere with manufacture, if a fermentation is involved in the manufacture process, and this may affect the yield and quality of the product, e.g. cheese.

b) The flavour of the raw milk may be adversely influenced (e.g. rancidity) and this may directly affect the flavour of the product e.g. pasteurized milk or cream.

c) Heat-stable bacterial enzymes may continue to act in the product, particularly during long storage, and adversely affect the stability and/ or flavour of cream and UHT milk.

Table 2.5. Saprophytic bacteria in milk - significance and control measures

Group of microorganisms	Representative organisms	Significances	Control Measures
Lactic acid bacteria	*Lactococcus* spp. *Lactobacillus* spp. *Strpotococcus thermophilus*	At room temperature ferment lactose to lactic acid and milk gets sour and become unfit for processing due to the loss of heat stability	Pasteurization of milk to kill mesophilic lactic acid bacteria Storage of milk at low temperature (4°C)
Coliforms	*Escherichia coli* *Enterobacter aerogenes* *Klebsiella* spp. *Citrobacter* spp.	Ferment lactose using hetero-lactic pathway Degraded proteins Produce gas and cause "unclean flavour" in milk Indicator of post processing contamination and hygienic operations	Pasteurization kill all the coliforms Hygienic processing of raw milk Clean milk production protocol reduces the initial number in raw milk
Psychrotrophs	*Pseudomonas,* *Achromobacter,* *Flavobacterium,* *Alcaligenes*	Grow at refrigerated temperature Produce heat stable proteases and lipases which break down the milk proteins and fats Cause off flavour and taste, off colour	Thermization of raw milk before low temperature storage for processing Pasteurization of raw milk Hygienic processing of raw milk Clean milk production protocol reduces the initial number in raw milk
Heat resistant bacteria	*Microbacterium lacticum*, *Micrococcus* spp. Thermophilic streptococci, sproe-froming bacillus: *Bacillus* spp. (*B. cereus, B. subtilis*), *Clostridium* spp. (*Cl. tyrobutyricum*)	Spoil heat treated milk by sweet curdling, off flavour, clumping of fat globules	Clean milk production protocol reduces the initial number in raw milk UHT sterilization of milk Use of bactofugation and microfiltration in processing operation

7) Adulterants and Their Detection

Modes of Adulteration

- Removal of fat by skimming
- Addition of skim milk
- Addition of water
- Addition of starch and cane sugar for raising density
- Addition of neutralizers and other preservatives to increase keeping quality

a) Detection of skim milk

- Lower fat percentage
- Higher density of milk
- Higher ratio of SNF: fat

b) Detection of starch

- Take 3 ml milk in a test tube.
- Boil the milk sample.
- Cool it and add a few drop of iodine solution (1%).
- Appearance of blue colour indicates the presence of starch.
- Blue colour disappears when the sample is boiled.

c) Detection of cane sugar

- Take 1 ml milk in test tube.
- Add 1ml HCl.
- Add 0.1g resorcinol powder and mix well.
- Place the test tube in boiling water for 5-10 min.
- Appearance of red colour indicates the presence of cane sugar in milk sample.

e) Detection of neutralizers

- Take 1 ml milk in test tube.
- Add 5 ml alcohol.
- Add a few drops of rosalic acid solution (1%) and mix well.
- Appearance of rose red colour indicates the presence of carbonate.

f) Detection of skim milk powder

- If colour of milk by addition of nitric acid turns light violet it indicates mixing of skim milk powder otherwise normal milk shows yellow colour.

g) Detection of gelatin

- Add picric acid and shake well and observe the precipitation which in case is of yellow colour, indicates mixing good amount of gelatin and if is shows light brown colour it indicates mixing in very little quantity.

h) Detection of total solids

This adulteration can be determined by following Richmond's formula:

$T.S. = CLR/4 + 1.2F + 0.14$

Where,
T.S. = total solids in milk (%)
CLR = correct lactometer reading of milk (20°C)
F = fat in milk (%)

i) Detection of water in milk

The water adulteration in milk can be detected by following tests:

A) Nitrate test

- Water from ponds, river, wells and hand pump contains nitrate, therefore, if milk is adulterated with such water this test is positive.

- Treated tap water normally does not contain nitrate.
- This test is not useful in case milk is adulterated with tap water.

B) Lactometer test

Milk has a specific gravity (1.028 -1.032). When it is adulterated with water, the specific gravity of milk is shifted from its normal value. The lactometer test is designed to detect the change in specific gravity of such adulterated milk.

- Mix the milk sample gently and pour it gently into a measuring cylinder (300-500).
- Let the Lactometer sink slowly into the milk. Read and record the last Lactometer degree (ºL) just above the surface of the milk.
- If the temperature of the milk is different from the calibration temperature (Calibration temperature may be 20°C) of the lactometer, calculate the temperature correction.
- For each ºC above the calibration temperature add 0.2ºL; for each ºC below calibration temperature subtract 0.2 ºL from the recorded lactometer reading.
- Milk has specific gravity of 1.026 -1.032 and lactometer reading range of 26.0 -32.0ºL.
- Reading consistently lower than expected indicates the adulterated milk.

C) Freezing Point Determination

Freezing point is the most constant of all measurable properties of milk. Small change in milk composition (adulteration of milk with water) will shift the freezing point of milk from its normal values (-0.54ºC). In case milk is adulterated with water the fat and total solids in milk are reduced causing a rise in freezing point. This can be determined by use of standardized apparatus Hortvet cryoscope.

$$\text{Water added (\%) in milk} = \frac{100\,(T - T_1)}{T}$$

T and T_1 is freezing point of pure and adulterated milk.

REVIEW QUESTIONS

1. What is the need of testing milk and milk products for quality?
2. How is the adulteration of milk with pond water determined?
3. Define spoilage of milk and milk products. How do saprophytic organisms cause spoilage of milk and milk products?
4. Enlist the various methods of controlling spoilage causing and pathogenic organisms in milk and milk products.
5. Enlist the pathogens which are transmitted through milk and milk products.
6. Differentiate between alcohol test and alcohol-allizarine test.
7. Dye reduction test is a good indicator of quality of raw milk – why?
8. Importance of determination of specific gravity of milk.
9. Enlist the various flavour defects observed in milk and milk products.
10. How is the addition of neutralizers in milk detected?