Addis Ababa University

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Department of Veterinary Microbiology, Immunology and Veterinary Public Health

Food Safety

Undergraduate Program

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Note: Adopted largely from previous lecture note of Dr Fanta Desisa

Contamination of food of animal origin & Milk Hygiene

Learning objectives

- To know the sources and types of food contamination
- To understand the effects of microorganisms on food
- To understand about food spoilage and the cause of spoilage
- Define milk and understand its nutritive value
- Understand the chemical composition

CONTAMINATION OF FOOD

- Refers to the interaction or occurrence of any contaminant particularly micro organisms in food.

- The food that has been corrupted by another substance – either physical, biological or chemical.

Source:

- plants- moulds and yeasts
- animals – GIT contents containing bacteria
- sewage- contains humans and animals GIT content
- water – could be contaminated by soil or sewage
- soil – naturally contain its own bacteria and bacteria of sewage and water
- air – it naturally contains a lot of bacteria, moulds and yeasts

Types of contamination of food

Agent point of view:

Microbial contamination:

- Bacteria (*Campylobacter*, *Clostridium perfringens*, *E. coli*, *Listeria*, *Salmonella*, *Staphylococcus aureus*)
- Fungus (*Aspergillus*, *Fusarium,* aflatoxin)
- Virus (Hepatitis A),
- parasite (taenia saginate, taenia hydatigenia, taenia solium) etc

Non microbial contamination:

o Chemical residues,
o Biological: like bone, hair, insects, and feaces
o Physical: glass metals, wood, string, dirt etc

- **Temporal (time):**

Primary contamination: begins directly from the food animals e.g. Animal infected with anthrax or due to antibiotic treatment

Secondary contamination: results from the environment, other animals, man or water

- **Fermentation :**

Homo-fermentative: which produce only one product e.g. Lactic acid

Hetro-fermentative: which produce more than one products e.g. Contamination with yeast results in CO2 and alcohol

Effect of micro organism on food

- **Food spoilage** is the process of **breaking down of organic matter** like protein, fat and carbohydrate affecting colour, taste, odour, consistency or texture.

- **Carbohydrates**:
  - The break dawn of glucose (glycogen in meat and lactose in milk) results in the production of lactic acid.
  - lactic acid retards bacterial multiplication used in the production yoghurt.

- **Fat**:
  - converted into fatty acids and glycerol
  - It results into rancidity, which imparts unpleasant smell.

- **Protein**: polypeptides, peptides and amino acids with end product of H_2S and NH_3 which give foul smell.

- Food is considered spoiled when it becomes **unfit for human consumption**.

❖ Food spoilage Caused by:

1 *Micro organisms:* bacteria, fungi, arthropod pests, parasitic

❖ Microbial spoilage usually results in an obvious change in physical characteristics such as colour, thickening, odour and flavour degradation

❖ In general MOS in food result in:

- ➢Food spoilage
- ➢Food-borne infections and intoxications
- ➢Economic losses leading to food scarcity

2. *Intrinsic enzymes:*

- refers to the action of enzymes in the living protoplasm of the food
- Enzymes are present in all living cells and their main function is to regulate, accelerate or slow down the chemical changes that take place in the organism

3. Exposure to air: some foods spoiled if left exposed to the ambient temperature or by reacting with the surrounding air (oxygen), such as due to oxidation

4. *Chemicals:* food may come to spoil because of contact to poisonous and toxic chemicals such as contaminated container, pesticide/insecticide spray or other accidental contacts with toxic chemicals that render the food unfit and unhealthy to the consumer.

Detection of food spoilage

❖ **Microbial spoilage can be detected by**

- Organoleptic methods: that is using sense organs (smell, taste, color)
- supportive tests (laboratory tests): determination of *PH*, NH3, volatile fatty acids
- Determination of bacterial load: through counting CFU

NB. Bacterial spoilage is marked by smeary **foul smelling** food where as spoilage due to moulds and yeasts is mostly characterized by **whisker**.

Types of spoilage

- **Slimy:**
  - ✓ It occurs on the outer side casing/covering
  - ✓ In early stage, it can be seen as discrete colonies and later coalesce to form a uniform layer of grey slime
  - ✓ Slime formation is favoured by moist surface and is usually confined to the outer casing.
  - ✓ Removal of this material with hot water leaves the product unchanged.
- **Souring:**

- ✓ takes place beneath the casing
- ✓ The souring results from the utilization of lactose and other sugars by the organisms and the production of acids

- **Greening:** It can occur in fresh, stored and processed meats. It is caused by H_2S and H_2O_2 production.

Prevention of Food Contamination from animals(primary source)

- Good housing and supply of uncontaminated feed and water.
- Testing animals and birds for pathogens and culling the carriers.
- Using good quality water for washing the carcases.
- Hair removal.
- Removal of Contaminated parts
- Proper cleaning of the udder before milking

MILK AND MILK HYGIENE

Nutritive values of milk

- Milk is defined as the lacteal secretion of the mammary glands of a mammal obtained by the complete milking of healthy cows to feed offspring
- It is a well balanced diet as it contains almost all the essential nutrients to sustain life
- Recognized as "**nature's single most complete food**" or "the most **nearly perfect food**"
- However, it is not 'the perfect food" because it is not an entirely dependable source of all vitamins (vitamins C and D present in minute amount) and **does not contain sufficient iron.**

- Why milk deserves this reputation is that it is the one food specifically prepared by nature for the young of mammals

- Moreover, it is: -

  - Palatable
  - Wholesome
  - Digestible and it assimilates very easily
  - A good growth promoter (Child feeding)

- As a food, milk serves the following broad purposes:
  - Growth,
  - Reproduction,
  - Supply of energy,
  - Maintenance and repair and
  - Appetite satisfaction

- The requirements of these categories vary with the individual

- In some instances not all the stated functions of the food need to be served.

  e.g. Adults no longer require food for growth whereas infants do

COMPOSITION OF MILK

- The principal contents of milk include: Water, Fat, Protein, Milk sugar (lactose), Minerals (ash) and Vitamins
- The food value of milk depends upon its milk fat and milk solids-not-fat content
- The total solids (TS) in milk (i.e. fat + protein + lactose + ash or minerals + vitamins) minus the fat content are generally referred to as Total Solids-Non-Fat (TNF) or milk Solids Not Fat (TS- fat = SNF).
- On average, normal cow milk must contain SNF amount 8.54% and TS 13.46%.
  - SNF(8.54 %) = Protein 3.21% (variable from cow to cow and breed)
    = Lactose 4.58%
    = Ash and vitamins 0.75%
  - TS = Fat + SNF= 4.92 + 8.54 = 13.46 %

- There are two types of milk depending on the lactation stage: Colostrum and Non-colostral milk.
- Colostrum is the milk, which is secreted during first week of lactation
- Cholesterol is component of colostrum and it tends to produce intestinal disturbances in children
- For this reason, milk obtained within 15 days before and 5 days after calving, the period during which cholesterol is produced, should be excluded.

Table 2. Average composition of milk from different spp of animals

Mammals	Water (%)	Fat (%)	Lactose (%)	Protein (%)	Ash (%)
Cow	86.54	**4.92**	4.58	3.21	0.75
Water Buffalo	76.89	12.46	3.74	6.03	0.89
Goat	87.88	3.82	4.54	3.21	0.55
Camel	87.61	5.38	3.26	2.98	0.7
Sheep	80.82	6.86	4.91	6.52	0.89
Bitch	78.88	8.56	4.09	6.82	1.08
Cat	81.63	3.33	4.91	9.08	0.51
Human	88.3	3.11	7.18	1.19	0.21

MILK FAT (BUTTER)

- It is the second largest component of milk and is of major commercial value
- It plays important role in human nutrition being a good source of energy and essential fatty acids
- It supplies an energy value of **9.3** calories / gram of fat
- This is higher when compared with energy value derived from protein and lactose
- Moreover, milk fat plays an important role in the flavor and physical properties of milk

- Fats present in the milk in the form of **fat globulins** and these fat globulins play important role in some operations such as milk separation, churning of cream and cheese production

- Lecithin, cholesterol, and carotene are some of the important substances associated with milk fat

- Milk fat is responsible for yellow coloration of normal colostrums and this is made by the carotene of the milk fat

- Fat is soluble in ether and ethyl alcohol and it absorbs odor from surrounding,
- Its specific gravity may vary from **0.936-0.946** at 15^{o}C and melting points at 20-29 ^{o}C
- Fat hydrolysis to fatty acid + glycerol by lipase
- Oxidation of fat that gives **rancidity** are some the possibly chemical changes in milk fat
- These changes are usually characterized by **offensive smell and unpleasant odor**

PROTEINS

- Milk protein is valuable to human since it contains all **essential amino acids (**about 20 amino acids among which 8 of them are essential)
- It has nutritive value more than that derived from meat and egg
- Milk proteins are :
  - ✓ **casein(**76–80% of the total milk proteins)
  - ✓ **whey** proteins(lacto albumin, Lacto globulin, immunoglobulin and enzymes …….. comprising roughly 20–24%

PROTEINS. . .

- Casein:

- ✓ considered as the specific predominant protein of milk
- ✓ It is white in color and enables milk to have normal whitish color
- ✓ Insoluble in water and is found only in milk
- ✓ It exists in milk in combination with calcium phosphate and this combination causes precipitation

- Addition of acids (e.g. citric acid, lactic acid), enzymes (e.g. rennin, pepsin) and alcohols and application of heat coagulates casein and hence casein is used in cheese production.

Milk sugar (Lactose)

- Is the principal carbohydrate of milk
- Lactose or milk sugar exists mainly in milk in two forms as: galactose + glucose (Lactose= glucose + galactose).
- It is almost found only in milk in nature and the amount present in milk depends up on the health of the udder, nutritional status and breed of the milk-producing animal.
- Lactose contents of the milk is increased by over feeding of carbohydrates, especially soluble carbohydrate and decreased by mastitis infection of udder.
- When bacterial enzymes act on milk sugar, it leads to lactic acids production.

- ❖ lactic acid is used for fermentation in yoghurt preparation
- ❖ Lactic acid affects milk constituent such as casein, mineral (Ca^{++})
- ✓ when lactic acid acts on casein, milk is coagulated (precipitated)
- ✓ Take the calcium from calcium phosphate and thus throw the insoluble casein out of the solution as curd
- ❖ Even though, lactose doesn't taste as sweet as an equal amount of sucrose, it imparts or gives fresh milk its normal sweet flavor and taste, and constitutes as one of the major solid constituents of milk

Enzymes

- enzymes are proteins or combination of protein and coenzymes
- Enzymes are usually very specific in their action
- In most cases their power to act is destroyed by heat and as a result, each enzyme has a different critical temperature
- The pH also may limit their activity
- Milk contains different types of enzymes such as peroxidase, phosphatase and lipase

Peroxidase

- Is the most abundant enzyme found in milk
- The test of milk for the presence of peroxidase are sometimes made to ascertain whether hydrogen peroxide has been added (used usually as preservative) or whether the milk has been subjected to sever heat treatment
- Heat treatment of milk such as at a temperature of 80^{o}C for 3 ½ minutes, 73.5 ^{o}C for 28 minutes or 70 ^{o}C for 150 minutes will destroy this enzyme
- The test for peroxidase is not useful for proving pasteurization

Phosphatase

❖ Two types of this enzyme are found in milk,

✓ Alkaline phosphatase which is most active at pH ≥9.6 and

✓ Acid phosphatase which shows its greatest activities around pH 4.0

❖Alkaline phosphatase is destroyed in milk by pasteurization and thus a test for the absence of alkaline phosphatase is widely used to ascertain whether milk has been properly pasteurized

Lipase

- Enzyme lipase seems to be present in all normal milk, but its quantity varies greatly
- Late lactation milk has higher lipase content than normal fresh milk and thus hydrolytic rancidity is common in it
- Homogenization, repeated warming and cooling, the presence of copper or iron ion and exposure to air or direct sunlight initiate lipolysis causing irreversible flavor and odor changes which adversely affect product quality

- Homogenization of the milk exposes a greatly increased fat globule surface area
- Pasteurization destroys activity of lipase, if not pasteurized before or immediately after homogenization, raw homogenized milk will rapidly become rancid because of activity of lipase on the greater fat surface

Ash

- Incineration of milk leaves the ash, a residue which equals about 0.7-0.8% of the weight of the milk
- The ash includes all of the mineral elements of the milk, such as potassium, sodium, calcium, magnesium, chlorine, phosphorus and sulfur in relatively large amounts
- Iron, copper, zinc, aluminum, manganese, cobalt, and iodine present in small amounts while traces of silicon, boron, titanium, vanadium, rubidium, lithium and strontium have been reported as present

- Milk is a rich source of calcium and phosphorous
- Both of these elements assist in the formation of bones and teeth of growing children
- Since milk is deficient in iron, an exclusive milk diet may cause anemia due to iron deficiency

Vitamins

- Milk is good source of vitamin A, B1 (thiamine) and B2 (riboflavin)
- Contains small amount of vitamin C (ascorbic acid), vitamin D and niacin (B3)
- Vitamin A, D, E and K are fat-soluble, so they tend to be present in milk products in proportion to the fat content
- Vitamin C and several vitamins of the B-complex are water-soluble
- Heat is detrimental to vitamin B and C
- Pasteurization destroys ≥ 10% of the B and up to 50% of the C.

Trace components of milk

Somatic or body cells

- Body cells or leucocytes, originating from the udder, are always found in fresh milk, even if milk is drawn from healthy quarters

- California Milk Test is used to estimate number of leucocytes and thus, evaluate the quality of milk

- The number of somatic cells may vary considerably, but it is generally lower than 100,000per ml [$10x10^4$ cells/ ml of milk)

- Counts of 500,000 and more are an evidence of abnormalities, usually being an indication of mastitis. Milk from seriously diseased udder (e.g.sever mastitis) may contain millions of cells (> $1x10^6$)
- If the milk of large number of animals is mixed, the infected milk of one or more animals will be diluted with milk of healthy animals and it becomes more difficult to detect the abnormality.

Micro-organism

- Milk may contain some bacteria even incase aseptically drawn
- They may be originated from the teat cannel of the udder (theses are usually the slowly multiplying and almost none pathogenic MOs) or from milkers hand and outside udder
- Generally, microorganisms could be of two types: -
  - **Unfavorable microbes** (pathogenic micro organisms or microbes that cause food spoilage
  - **Favorable micro organisms** (microbes which bring favorable changes in flavor and appearance of milk and such microbes are beneficiary and thus, are usually carefully handled and propagated). E.g. Starter culture.
- Functions of starter culture :
  - produce acid,
  - produce desired characteristics of flavor or aroma
  - prevent the growth of unwanted microorganisms
- Such Micro-organisms are genus streptococcus and include; *Str. lactis, Str. cremoris and Str.lactis sub spps diacetilactis.*

Gases

- The principal gases in milk are CO_2, O_2 and N_2
- They are introduced into the milk during the process of milking and handling.

Water

- This is the principal constituent(app.87%) of milk and is the medium in which all constituents are in solution or in suspension

- In some countries, such as Ethiopia, where the production and distribution of milk is not properly controlled, the adulteration of milk by the addition of water is commonly practiced

Physico-Chemical Properties Of Milk

Physical Properties of Milk

- The major physical properties of milk are color, taste, flavor, specific gravity, freezing point and boiling point

- These are influenced by the composition of milk

- They are also a great help in the processing and testing of milk for adulteration

❖ **Color**: - ranges from bluish-white to golden yellow

- Carotene (Vitamin A) of the fat in the milk that gives a golden

- Riboflavin (B2) of the whey that gives a bluish color

❖ **Taste**: - sweet due to presence of lactose

❖ **Flavor (smell): -** pleasant due to unsaturated fatty acid in the milk, but after few minutes it disappears

✓ Milk may absorb odors from utensils, equipment and atmosphere

✓ The bad odors are foreign to good milk and their presence considered as a defect

❖ **Specific gravity:** is the ratio of the weight of a volume of a material compared to the weight of the same volume of pure water

OR

❖ Ratio of density of milk to density of water

- Specific gravity of milk is ranges between 1.0295- 1.0350 which usually determined at 20 ^{o} C

- The specific gravity of milk fat is between **0.936 and 0.946**
- This is important to determine adulteration of milk or removal of fat butter from milk

- Milk with a lower fat content has higher specific gravity than milk with higher fat content

- **Freezing points**: - freezing point of milk is almost a constant value and freezes at -0.55 to -0.53 ^{o} C and is a suitable indicator for detection of dilution of milk with water

- An increase in freezing point indicates the presence of added water in the milk.

- **Boiling point**: - freshly drawn milk boils at about 100.17 ^{o}C
- Osmotic pressure/strength of solution affects both the freezing point and the boiling point of a solution

- As the strength or concentration of a solution increase, its freezing points diminish and its boiling point increases

Chemical Properties Of Milk

PH: - indicates the strength of the acid condition of the milk

- Normal fresh milk has a pH of 6.5-6.8, average 6.6 ,which indicates that the milk is slightly acidic

- **Titratable acidity**: - measures the total acidity, but not the strength of acidity

- Titratable acidity is acidity of milk demonstrated by titrating a given amount of milk with an alkaline such as NaOH

- It indicates the consumption of NaOH necessary to shift the pH-value from the average normal PH value (6.6 ± 0.1 which is corresponding to fresh milk)

Hygienic milk collection, transportation and processing

Dr. sara Amanuel

Introduction

- Milk from udder is normally sterile
- It contains protein, lipid, lactose, minerals etc which is Ideal medium for rapid proliferation of harmful micro-organism
- As a result it needs to be protected from all possible sources of contamination
- To that end, **Good hygienic practices** should be applied throughout the food chain for safety and suitability of milk
- Production to consumption of milk should be subjected to a **combination of control measures**, and these measures should be shown to achieve appropriate level of **public health protection**

Sources of milk contamination

- Internal factors
  - ❖Udder infection – Mastitis
  - ❖Foremilk – contains large no. of bacteria
- External factors
  - ❖Cow/animal's body
  - ❖Udder and teats
  - ❖Milker – hygiene and habits
  - ❖Method of milking
  - ❖Milking Utensils
  - ❖Milk Storage utensils
  - ❖Feed and water
  - ❖Milking environment

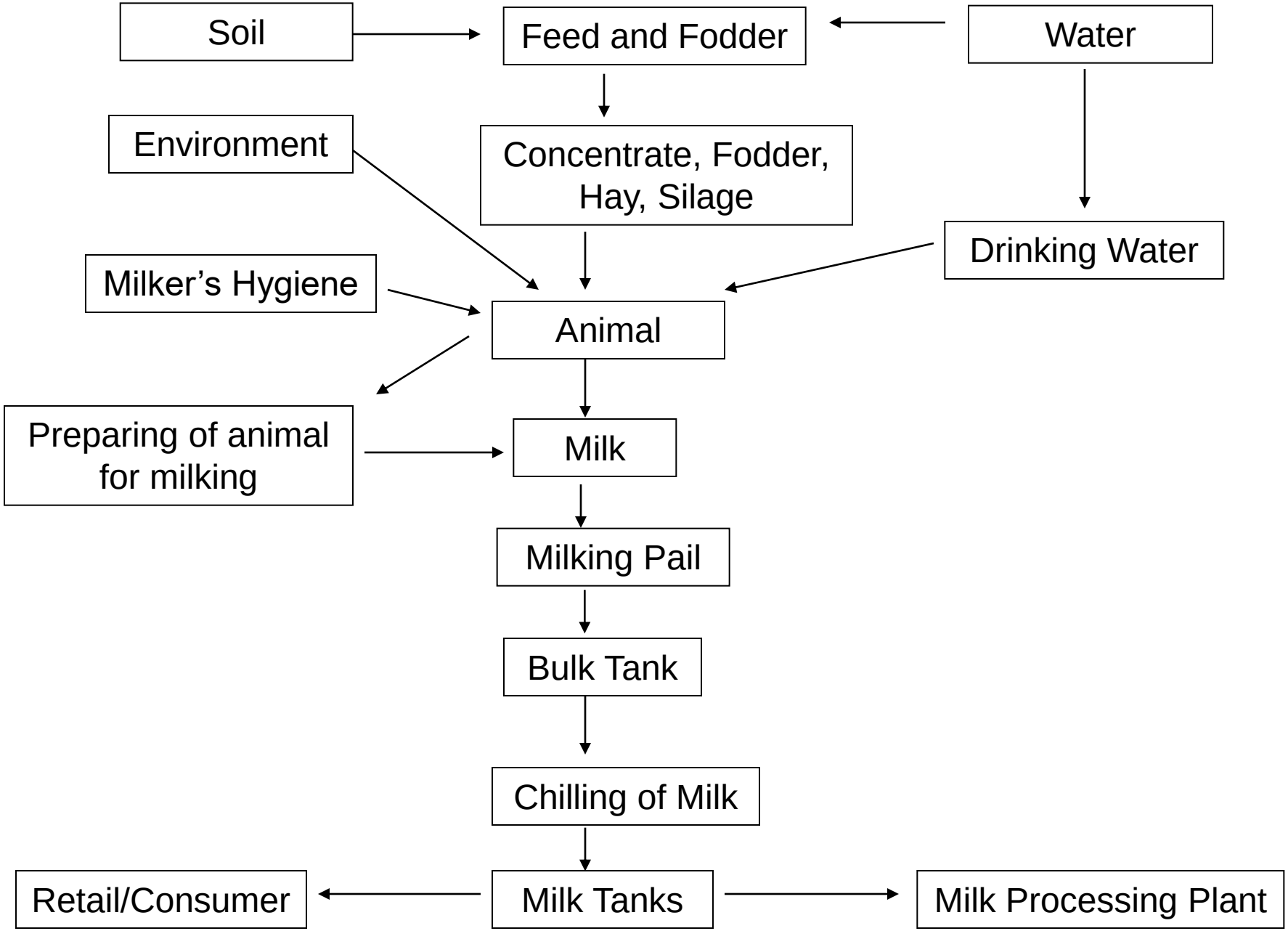


Figure: Source of contamination of milk

Hygienic milk collection

- Milk collection is often one of the first activities of milk producer groups/cooperatives.
- Once the milk from several group members is collected in a central location, the milk can be processed or transported to processing centres or markets.
- Milk should be collected within four hours of milking.
- Hygiene during milk collection is very important for the quality and shelf life of dairy products.
- The farmer could provide containers or the group may provide clean standard milk churns to improve hygiene.

Important points for good hygiene during milk collection are:

1. Use clean containers and equipment
2. Use containers that are easy to clean with a wide opening;
3. keep the milk covered and in the shade;
4. Transport the milk as quickly as possible after milking;
5. Cool as quickly and whenever you can (4°C or below);
6. Try to avoid any delays in milk collection.

Hygienic milk transportation

- Collected milk should be transported and delivered without undue delay, and in a manner that avoids the introduction of contaminants into milk and minimizes the growth of micro-organisms in the milk.
- Milk transport tankers and cans should be designed and constructed :
  - ✓ For effective cleaning and disinfecting
  - ✓ To ensure complete drainage
- Milk transport tankers and cans should not be used to transport any harmful substance.
- Transport temperature and time should be in a manner that minimizes any detrimental effect on the safety and suitability of milk preferably using cooling facility

Hygienic Milk processing

- Milk processing converts liquid milk into dairy products like pasteurized liquid milk, yoghurt, butter, cheese, ghee and so on.
- Reasons for processing are:
  - ✓Processed products attract a higher price
  - ✓Increased keeping time of the product;
  - ✓More distant markets can be accessed;
  - ✓processed products are generally easier to transport
  - ✓Increased quality and hygienic safety;
  - ✓More flexibility in satisfying consumer demands, (make more or less liquid milk, more cheese, etc.)
  - ✓It creates employment
- Milk products can be processed as illustrated in the following figure

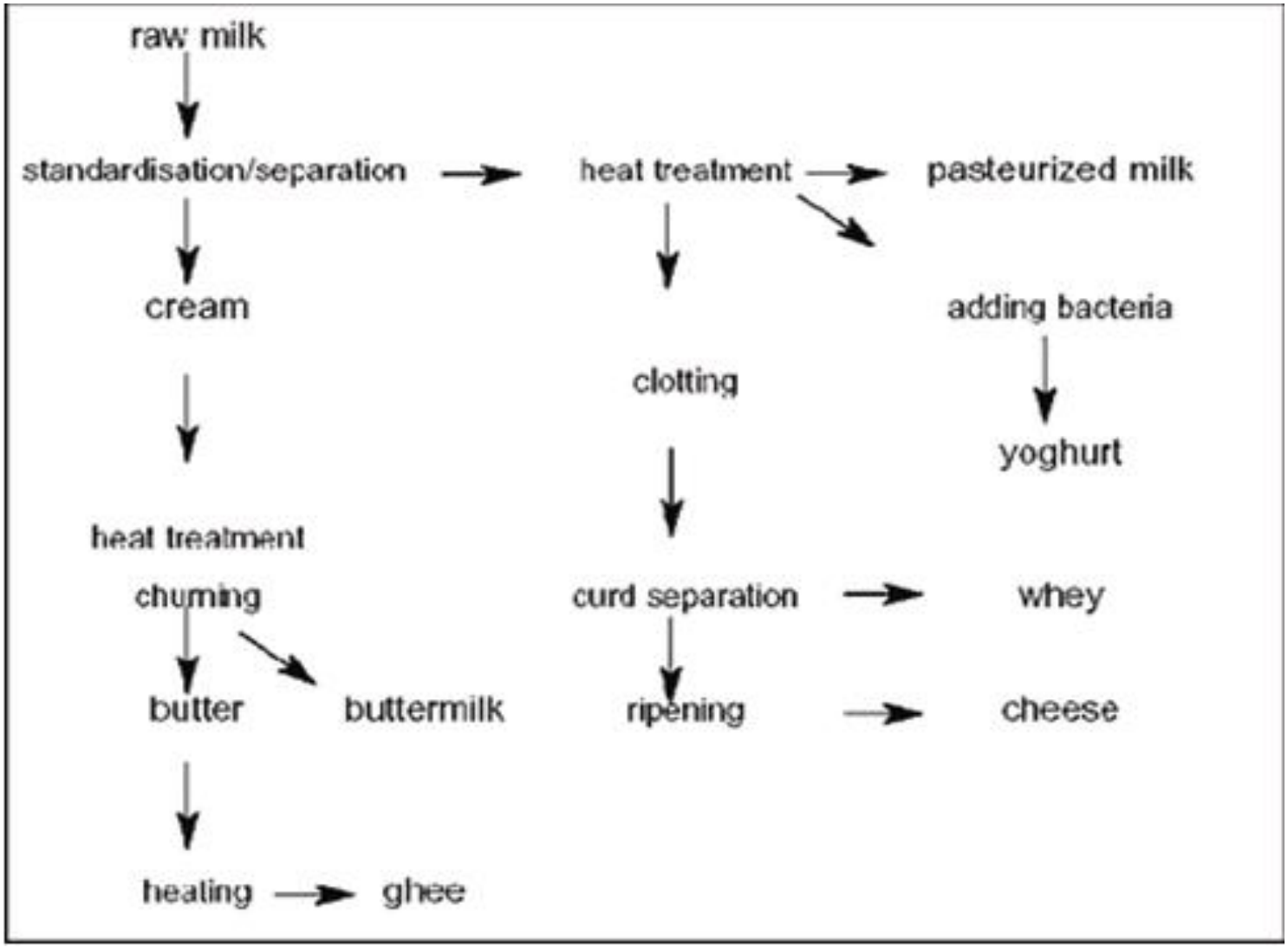


Figure: Milk processing options: Traditional and modern

Key definition:

Standardization: making milk with constant butterfat through partial skimming.

Heat treatment: destroying any potential pathogenic germs by heating to a minimum of 63°C for 30 minutes.

Clotting: changing from liquid to (semi-) solid by adding starters and rennet;

Curd separation: after coagulation the milk is separated into whey and cheese curd;

Ripening: cheese texture becomes homogeneous and develops aroma;

Churning: cream is churned to produce a semi-solid product that becomes butter;

Hygienic practices at Processing plant

- Design and facilities of processing
- Control of food hazards
- Key aspects of hygienic control system
  - Temperature and time control
  - Management of the product within the plant including incoming milk and intermediate products
  - Distribution of the finished products
  - Establishment of shelf-life
  - Specific process steps
  - Microbiological and other specifications
  - Microbiological cross contamination
  - Physical and chemical contamination

Milk Quality Determination

- Milk being made up of 87% of water it is prone to adulteration by unscrupulous and unfaithful individuals
- Moreover, its nutritive value makes it an ideal medium for the rapid multiplication of bacteria
- Hence, in order to make milk and milk products safe and avoid economic loss milk quality control is essential
- **Milk quality control** is the use of approved tests to ensure the application of approved practices, standards, and regulation concerning milk and milk products
- The tests are designed to ensure that milk products meet accepted standards for chemical and purity and as well as levels of different micro organisms.
- Quality control carried out at farm, collection centers and processing plant(through out **milk production chain**)

Milk quality determined by assessing milk samples for:

- Freshness
- Adulteration
- Bacterial content, and
- Milk constituents for payment calculation
- Pasteurization efficiency

Quality control on raw milk

Organoleptic tests

- The organoleptic test permits rapid segregation of poor quality milk at the milk receiving platform/collection center
- 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

Procedure

- Open a can of milk
- Immediately smell the milk
- Observe the appearance of the milk
- If still unable to make a clear judgment, taste the milk, but do not swallow it!!!
- Look at the can lid and the milk can to check cleanliness

Judgment

Abnormal color, smell and taste may be caused by:

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

Sediment Test

- The purpose is to determine the amount of insoluble visible filth or extraneous matter in the milk

Procedure

- From a mixed milk sample force a measured amount of milk through a tester made of stiff cotton or pad discs measuring about 1 inch in diameter whereupon the sediment is collected
- If this is not available a simple filtration through a filter paper can do the same job

Interpretation

✓ **Poor/dirty sediment**-evidence of carelessness/poor handling

✓ **Clean -** may merely represent efficient straining on the farm

Freezing Point Determination

- The freezing point of milk is regarded to be the most constant of all measurable properties of milk
- A small adulteration of milk with water will cause a detectable elevation of the freezing point of milk from its normal average values of -0.54ºC
- Since the test is accurate and sensitive to added water in milk, it is used to detect whether milk is of normal composition and adulterated
- Cryoscope is used for determination of freezing point of milk

Estimation of milk pH

- Milk pH may be obtained using paper strips impregnated with an indicator and PH meter
- Paper strips treated with bromocresol purple and bromothymol blue are sometimes used on creamery platforms as rejection tests for milk

- Bromocresol purple indicator strips change from yellow to purple between pH 5.2 and 6.0, while bromothymol blue indicator papers change from straw yellow to blue-green between pH 6.0 and 6.9

Method

- Immerse the indicator paper or PH meter into milk and read

Interpretation

- The normal milk pH shall range from 6.5 to 6.8

- PH value below 6.5 suggests

  - ✓Microbial contamination
  - ✓Presence of disinfectants in milk
  - ✓Excessive lactic acid production

- PH value greater than 6.8 suggests mastitis?

Titratable acidity

Purpose: to determine the freshness of the milk by measuring the concentration of lactic acid in the milk

Procedure

- Pour 25ml milk into 50ml glass beaker
- Add 1 ml 2% phenolphthalein solution into milk containing beaker
- Pour adequate quantity of NaOH(0.1N) into graduated tube and record the reading of NaOH

- Titrate /allow drops of NaOH to fall into the milk-phenolaphtalein solution until the color of milk changes to pink; Shake every time to mix the solution

- Take the reading of NaOH when it turns to pink

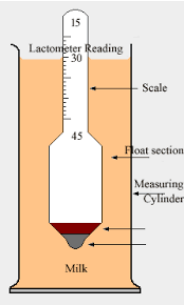
- Subtract the last reading from the first reading to know the amount of NaOH consumed

NB. The number of mls of sodium hydroxide solution divided by 100 express the percentage of lactic acid

Interpretation

- The normal titratable acidity of milk is 0.16-0.18%

- If the acidity is higher than 0.18 %, the milk quality is poor due to bacterial contamination and it cannot be heated and processed



Lactometer test

Principle

- When milk is adultered with water or other materials, the density of milk change from its normal value to abnormal
- The lactometer test is designed to **detect the change in density** of such adulterated milk

Procedure

- 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 (°L) just above the surface of the milk

- If the temperature of the milk is different from the calibration temperature (Calibration temperature usually 20 oC) 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

Examples of Calibration temperature of lactometer 20ºC

Sample	Milk temperature	Lactometer reading	Correction	True reading
No.1	17 ºC	30.6 ºL	- 0.6 ºL	30.0 ºL
No.2	20 ºC	30.0 ºL	Nil	30.0 ºL
No.3	23 ºC	29.4 ºL	+ 0.6 ºL	30.0 ºL

Interpretation

- The relation ship between lactometer reading(L) and specific gravity is

Specific gravity (sg) = (L / 1000) + 1

- The normal sg of milk ranges from 1.0295 to 1.0350

- A specific gravity less than 1.0295 indicates either water is added or SNF has been removed

Clot on Boiling(COB) Test

- It is one of the old tests for too acid milk(pH<5.8)
- If a milk sample fails in the test, the milk must contain many acid or rennet producing microrganisms or the milk has an abnormal high percentage of proteins like colostral milk
- Such milk cannot with stand the heat treatment in milk processing and must therefore be rejected

Procedure

✓ Boil a small amount of milk in a spoon, test tube or other suitable container

Interpretation

☞ If there is clotting, coagulation or precipitation, the milk has failed the test(not fresh not with stand subsequent heating)

☞ Heavy contamination in freshly drawn milk cannot be detected, when the acidity is below 0.20-0.26% Lactic acid

Alcohol Test

- It is based on instability of the 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

Procedure

- The test is done by mixing equal amounts of milk and 68% of ethanol solution in a small bottle or test tube
- For routine testing 2 ml milk is mixed with 2 ml 68% alcohol
- NB: 68 % Ethanol solution is prepared from 68 ml 96%(absolute) alcohol and 28 ml distilled water)

Interpretation

- If the tested milk is of good quality, there will be no coagulation, clotting or precipitation

- The first clotting due to acid development can first be seen at 0.21-0.23% Lactic acid

Bacterial load determination

A. Methylene-Blue Reduction Test

- The purpose is to determine the quality of raw milk before pasteurization by determining the bacterial load

- Principle: the test involves determination of time required for the disappearance of color when methylene blue thiocynate solution is added to raw milk

Procedure

- Add 1 ml of standard methylene blue solution in a test tube with 10 ml of milk

- The sample is mixed and then place either in a hot water bath or in an incubator at 350C-370C

- Observations are made at intervals of 15-20 minutes for an **8** hour period to determine the time required for the disappearance of the blue in the sample

Interpretation

- Milk with a high bacterial content will decolorize the dye quite rapidly whereas milk with a low bacterial content retains the blue color for several hours
- On the basis of this test milk can be graded as follows:

Excellent: Very low bacterial count, decolorizaition time is about 8 hours

Good: Low bacterial count, decolorization time is 6-8 hrs **Fair:** High bacterial count, decolorization time is below 2 hours

Poor: Very high bacterial count, decolorization time is below 2 hours

B. Resazurin test(10 minute)

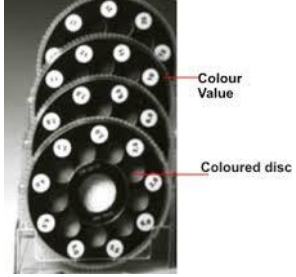
- Resazurin test is the most widely used test for hygiene and the potential keeping quality of raw milk and later microbial activity
- Resazurin is a dye indicator and under specified conditions and dissolved in distilled boiled water
- The solution of Resazurin as prepared by adding one tablet to 50 ml of distilled sterile water

NB. Rasazurin solution must not be exposed to sunlight, and it should not be used for more than eight hours because it losses strength

Procedure

- Mix the milk and with a sanitized dipper put 10 ml milk into a sterile test tube
- Add one ml of Resazurin solution, stopper with a sterile stopper, mix gently the dye into the milk
- Mark the tube before the incubation in a water bath at 37 oC and record the time for 10 minute
- Transfer and place the test tube in a Lovibond comparator with Resazurin disc
- Revolve the disc until the color of the sample matches with one of the color of the disc

Interpretation



Disc No.	Colour	Grade	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

Butter fat determination(Gerber method)

Purpose:

- To determine the price based on fat composition
- To calculate the correct amount of feed ration for high yielding cow
- For breeding programs
- To make accurate adjustment of the fat percentage in standardized milk and milk products

Procedure

- Mix the milk sample (temperature about 20°C) thoroughly, taking care to minimize incorporation of air
- Allow the sample to stand for a few minutes to discharge any air bubbles
- Mix gently again before pipetting
- Pipette or dispense 10 ml of sulphuric acid into the butyrometer
- Pipette the required volume of milk into the butyrometer
- Care must be taken to avoid charring of the milk, by ensuring that the milk flows gently down the inside of the butyrometer; It then rests on top of the acid

- Pipette or dispense 1 ml of amyl alcohol
- Clean the neck of the butyrometer with a tissue or dry cloth
- Stopper the butyrometer tightly using a clean, dry stopper. Shake and invert the butyrometer several times until all the milk has been absorbed by the acid
- Then place the butyrometer in a water bath at 65°C for 5 minutes
- Centrifuge for 4 minutes at 1100 rpm

- Return the butyrometer to the water bath for 5 minutes at 65° , ensuring that the water level is high enough to heat the fat column
- Read the fat percentage. If necessary, the fat column can be adjusted by regulating the position of the stopper

NB. Average fat percentage of cow milk is 4.92

Quality control on heat treated milk

- Raw milk contains enzymes which could be destroyed by heat treatment

- Pasteurization destroy pathogenic bacteria rendering milk safe

Peroxidase test

- Used to ascertain whether the milk has been subjected to sever heat treatment
- Native lactoperoxidase **survives pasteurization** of milk, but it is inactivated at high temperature(85oc) and ultra high temperature

Method:

- Pour 5 ml milk and 0.5 ml of the reagent phenol into test tube
- Mix thoroughly and put aside for awhile and observe for color change

Interpretation

- Red brown color suggests positive i.e. the milk has been heated at 85
- If the milk retains it white color it indicates that the milk has not been heated at 85 oc

Phosphatase test

- Acidic phosphatase is heat stable and can only be inactivated at 90 oc where as alkaline phosphatase is heat sensitive and does not survive pasteurization temperature
- Alkaline phosphatase test is used to determine efficiency of pasteurization using ***lovibond comparator***
- A negative alkaline phosphatase result indicate that the enzymes and any pathogenic bacteria have been destroyed during pasteurization

Interpretation

- ✓0-10-properly pasteurized
- ✓10-18 slightly under pasteurized
- ✓18-42 under pasteurized
- ✓>42 not pasteurized

Turbidity test(Aschaffenburg test)

Purpose : To distinguish between pasteurized and sterilized milk

Method

- Put 4g of ammonium sulphate into 50ml of flask
- Pour 20 ml of milk into it
- Shake and set aside for 5 minutes
- Filter the solution and retain it in a test tube
- Put 5ml of the filtrate in another test tube and heat in water bath at 100 oc for 5 minute
- Cool and examine for the presence of turbidity

Interpretation:

Presence of turbidity- milk is sterile

Absence of turbidity- milk is not sterile

Milk microbiology

- Milk is, an efficient carrier for a variety of disease producing microbial agents.

- A variety of pathogens may gain access in to milk from different sources and cause different types of milk borne illness.

Source of milk borne zoonotic disease

- **Animals**
- **Handlers**
- **Environment**

Common milk borne zoonotic diseases

- ✓ Tuberculosis
- ✓ Brucellosis
- ✓ diphtheria
- ✓ Q – Fever
- ✓ Viral infections
- ✓ Enteroviruses
- ✓ Poliomyelitis
- ✓ Infectious hepatitis

End of session!!!!