TO THE POINT CLASS LECTURES

MILK AND MILK PRODUCTS INSPECTION

(MICRO 404)

MID COMPLETE

PART TWO

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Milk Fat

Milk fat present in the milk in the forms of small globules which are rounded. And have diameter 10-100 μm. These fat globules are present in the form of fine suspension (so milk also called "oil in water" suspension).

Formation of such type of fine suspension is due to some emulsifying agents or repoilded substances present in the milk.

Fat Globule of Cow and Camel Milk

The fat globules possess negative charge, due to which remain suspended in the milk.

Take the milk of cow and camel in the separate cylinders and stabilize them at 45 C for 3-4 hours. After this period, we can observe that in cow's milk there is a clear fat layer on the top of the milk while fat globules are equally distributed throughout the body of the milk in case of camel's milk.

It happens: as fat globules of camel milk fat are very small; 10-15 μm in diameter and that of cow's milk are quite bigger in size; 70-80 μm in diameter, so they make a layer on the top of the milk and fat layer is easily seperatable.

- Due to this reason, yogurt may not be prepared from camel's milk but remember that curdling will occur upon formation but a true texture of yogurt, we may not get.

Physical Properties of Milk Fat

Melting point: 32-36 C (average: 34 C) for all kinds of fat of all species.
Specific gravity: 0.930 at 21 C.

Composition of Milk Fat

Basically milk fat is triglycerides in nature; One mole of glycerol with three fatty acids.
Difference in fat (in terms of nature and type) is made by fatty acids.
About > 64 types of fatty acids (F.A.) are discovered to be present in the milk fat with various combinations. Variations in the characteristics of milk fat are only brought about by dominant fatty acids.

Fatty Acids in Milk Fat

Milk fat generally consists of two types of fatty acids as described below:

a) **Volatile Fatty Acids (VFAs):**
   Major fatty acids which are present in all milk fats are as given below:
   Butyric acid
   Capric acid
   Caproic acid
   Caprylic acid
   Laureic acid

   These fatty acids belongs a specific category of fatty acids named as Volatile Fatty Acids. These fatty acids adds taste and flavour in the raw milk and these are diffusible on heat treatment of milk.

   Note: Cow milk fat is rich in butyric acid contents so it is also called "Butyrate Fat". Fat name is given after the name of most abundant fatty acid present in the milk fat.

b) **Non-Volatile Fatty Acids (NVFAs):**
   Palmitic acid

Myristic acid
Stearic acid
Oleic acid
Linoleic acid
These fatty acids give flavour and taste to the milk after boiling/pasteurization.

Free Fatty Acids in Milk Fat
In certain circumstances, there are free fatty acids in milk fat due to which milk becomes off-flavour- which is not acceptable by the consumers.
For example: when lactating animals are fed on cotton seed meal or concentrates, then butyric acid contents increased in the milk fat – gives a peculiar aroma.

Solubility of Fatty Acids
Among volatile fatty acids; butyric acid, capric acid, caproic acid and caprylic acid are soluble in nature but while other volatile and non-volatile fatty acids are insoluble in nature; but soluble in chloroform, ethyl ether, benzene and carbon tetrachloride (CCl_4).. If these fatty acids get solublized, then removal is very difficult.

Saturation Nature of Fatty Acids
Except oleic and linoleic acid, all fatty acids are saturated. Oleic and linoleic acids are unsaturated in nature (Milk fat must contain unsaturated fatty acid contents 35-40% of total)

Soft Fat
Milk fat with more than 35-40% unsaturated fatty acids (Oleic and linoleic acids) of total contents, may be regarded as very good (health wise) and known as Soft Fat.

Oleic and linoliec acids are rich in Omega-3 fatty acid which helps to reduce the blood cholesterol level. It is also known as essential fatty acid as it is required by the body. Researchers are now working with poultry eggs to get increased amount of omega-3 F.A.

Hard Fat
Milk fat with increased proportion of saturated fatty acids is known as Hard Fat.

Milk Fat Tests

Iodine Test
Saturation of fatty acids can be determined by this test.
It can be stated that: it is the number of grams of iodine used for 100 grams of milk fat.
Iodine value = No. of grams of iodine used / 100 grams of milk fat

Reference Iodine Values:
Butter fat: 30-34 Tallow: 35-40 Lard: 48-60
Tallow: is the milk fat of sheep/goat.
Lard: is the milk fat of pig.
Conclusion: Increased iodine value ------ Increased unsaturated fatty acids. Hence, Lard is rich in unsaturated fatty acids. Lard is used for nutritive and medicinal purpose commercially as it is rich in unsaturated fatty acids which are good for health.

Sponification Test

Fatty acids in the milk fat contain carbon atoms from 4 to 21 (C4-C21). Mostly fatty acids are branched in nature. These fatty acids may be:

a) Long chain fatty acids
b) Short chain fatty acids

Increased number of chains of fatty acids in the milk fat shows increased Sponification value. What is Sponification? It is the formation of soap when water is added in the fat.

Sponification Value:
It is the measure of no. of mg of KOH (potassium hydroxide) used to form soap from one gram of milk fat.

Reference Sponification Values:
Butter fat : 231 Tallow : 203 Lard : 196
As butter fat possess high sponification value, so It is obvious that it is widely used in soap industry for soap formation.

MILK AND MILK PRODUCTS FLAVOUR

Acid Degree Value

Flavour of milk and milk products can be assessed by:

a) Physical method
b) Independent chemical test(s) i.e. acid degree value.

a) Physical method:
This method is adopted by a panel of international judges. They can approve the taste and flavour of a certain product meet required international standards. This type of evaluation of a product is known as "organo-leptic evaluation".

b) Independent chemical tests:
These tests are performed on the basis of availability of free fatty acids in the milk fat.
↑ free fatty acids ------ ↑ off flavored product

Acid Degree Value
It is the measure of no. of mg of KOH required for neutralizing free fatty acids in 100 grams of fat of milk/product.
It can be stated as:
Acid degree value = No. of mg of KOH / 100 gram of fat of milk/product

Reference values:
Acid degree value for fresh milk : 0.4-0.56
Acid degree value for off-flavoured milk : 0.9-1.0
It is said that sensory evaluation is only valid when acid degree value is almost 3 times to declare the product as off-flavour. It means:
If you are not performing acid degree value test, then if a product declared as off-flavoured via sensory evaluation will have acid detergent value: 3.0-4.0

Note: Acid degree value is more efficient and reasonable measure to declare the commercial product as off-flavour.

Energy Value

Energy value of milk depends not only on the fat contents of the milk but also on the total solids of milk.

Formula:

E = 12 (F+2) = total calories / 100 grams of milk

Here: 12 = T.S. F = Fat%

Milk Protein

Milk protein is very exclusive in nature and it is the natural source of amino acids. More than 95 amino acids of total declared amino acids are available in the milk protein.

Milk protein is regarded as high resource and matchless resource of amino acids particularly it contains many essentials amino acids which are not synthesized in our body and required for normal metabolism.

Fractions of Milk Protein

1) **Casein**
   It is the 1st major milk protein, which is always present in the form of calcium caseinate.
   Molecular weight = 125-375,000 Daltons Diameter = 0.8-1.0 μm.
   It is present in the suspicion / colloidal form in the milk.
2) Albumin:
   It is less in concentration and always present in the soluble form in the milk.
   Molecular weight = 1000-25,000 Daltons Diameter = 0.-0.4 μm
3) Globulin:
   It is not more than 0.4% of the total protein in the milk.
4) Enzymes:
   These are protein in nature.
5) Vitamins / Flavoproteins:
   Vitamin B_{12} is abundantly present in the milk.

No. 4 and 5 are found in traces in the milk but these are essentially required.

Casein

Casein is a very versatile component of milk – having high energy values and also many elements such as H, S, P, N, Ca, O. It is used commercially in textile, paint and furniture industry.

Chemical Properties

- Milk casein is easily digestible protein.
- It stays in the stomach for about 6-7 hours; no other stay so long in the stomach. Due to this property, it gives "Stomach-fill effect"
- Casein products are advised by many dieticians as fat-free diet in obese patients.
- Casein molecule contains both +ve and –ve groups in its structure – so it is also called amphoteric molecule.

Fresh Milk pH = 6.6

Iso-electric pH = 4.7 (at this point molecules becomes inert, it means there is equally distribution of +ve and –ve charge). This property of pH of casein molecules helpful for producing a lot of milk products such as: cheese.

Cheese

It is a protein-rich diet easily digestible. It has high nutritive value. Shelf-life is longer than ordinary milk.

Processing
Milk is processed via curdling process. Water is removed and milk casein (rich in Ca^{+}) is separated to use for making the cheese.

Major processing procedures:
i) Acidic precipitation
ii) Alcoholic precipitation
iii) Rennet precipitation

i) **Acidic precipitation:**
Lactic acid/ acetic acid is used to precipitate the milk protein. Milk pH is decreased from 6.6 to 4.7 at 35 C to make the curdling of milk by making it acidic. After precipitation, water is easily removed and Ca+ caseinate is separated.
ii) **Alcoholic precipitation:**
Add 10% ethyl alcohol in the milk. It will dehydrate the milk protein and ultimately precipitation occurs. Remove the water to get the cheese.
iii) **Rennet precipitation:**
Rennet is the stomach lime of cow calves or pepsin of the piglets, which make the curdling of milk immediately. 0.01% rennet is used for cheese preparation.

Acidic and alcoholic precipitations are reversible type of processing procedures while rennet precipitation is an irreversible process which results in splitting of alpha and beta caseinate.

Effect of processing procedure on the quality of cheese
>> Cheese obtained via rennet precipitation is regarded as of high nutritive value because there is minimum loss of essential amino acids in the rennet precipitation.
>> Cheese obtained via acidic and alcoholic precipitation is of low energy value as it is deficient in many essential amino acids.

Casein: pH = 4.7
It can be separated by alcoholic, acidic and rennet precipitation.

Albumin: pH = 4.5
It can be separated by acidic precipitation. Albumin is easily digestible and has high nutritive value. Albumin cheese is also available but very costly.

Globulin: It is 0.3-22% of the total protein in the colostrum.
It provides immunity to the newborn. It is present in the form of immunoglobulins i.e. IgA, IgG, IgM.

Enzymes:
Major enzymes present in raw milk are;
a) lactoperoxidase b) lysozyme
Their concentration in the raw milk is much high and these are natural source of microorganism inhibitors – provides protection for 12 hours when milk is drawn aseptically.
These are heat labile – easily destroyed when milk is heated at 62 C for 30 minutes as it is pasteurization temperature.

Due to the destruction of such precious enzymes, now-a-days people prefer raw milk on pasteurized milk. In addition to these enzymes, a lot of milk minerals are also destroyed during pasteurization.

BURNING ISSUES IN THE MILK INDUSTRY

1) Added Water:

Purpose: water is added in the milk to increase the volume of milk. Addition of water dilutes the components of milk leads to decrease in energy value.
Addition of water in the milk is an unlawful practice and it is regarded as adulteration as it makes the milk unwholesome. It changes the whole composition of the milk and enzymatic activity of the milk also goes down.

Detection of added water in the milk
Certain techniques and methods are used to detect this adulteration as follows:

1. Lactometer method
This is an old method and it tells us about the specific gravity of the milk specific gravity value decrease when we add water into the milk but the disadvantage of this method is that it only give the result when we add water more then 10%.

2. Refractive index method:
Rays of specific wavelength are passes via the body of the milk and absorbed on the other side. Zeiss immersion rafractrometer is used made by a Germen company; it is a handy instrument and has digital display.

- Refractive index is measured in term of optical density OD.
- Pure Milk serum has OD value = 36
- 36 OD value is also equal to 7.26% of copper sulphate solution.

So we make a graph and take the OD on the Y axis and percentage of water on the X axis.

There is a standard curve and we can calculate added water % by comparing our curve with standard curve and got result. This method can tells about the presence of water even if it is less then 10%.

2) Added Vegetable Fat

Milk fat is the natural source of variable variety of fatty acids diversified in nature. It is separated to make the cream and sold at high prices.
People separate the cream from the milk and add vegetable fat into the milk and then sell it after homogenization. Gerber's method tells us only about the percentage of the fat not about the nature and source of the fat.

- We can add vegetable fat but for some special purpose. For example: a person is ill and he can not digest then vegetable fat can be used in the milk otherwise we can't add vegetable fat. Vegetable fat can be added in the milk for research purpose.

- Vegetable fat is unsaturated and it get oxidized and becomes rancid when exposed to air so become hepato-toxic and cause liver cirrhoses in the person who is using the milk with vegetable fat since long time.
- We use the vegetable oils in cooking but the container or the bottle of oil should not expose to open air.
- If one litter bottle of oil remains exposed to open air for one week it become rancid.
- Small milk globules become rancid when oil and vegetable fat is being added and most important is that colour and consistency of the milk remains normal if the rancidity even has occurred.
- There is another bad practice in some countries where vegetable fat is added and it labeled as vegetable fat so no one can see easily.

Note: any thing added in the milk that is not mentioned in the label is comes in the category of adulteration.

How we can check vegetable fat in milk sample?

1) Alcohol Test
Take 1 ml milk and 9 ml absolute alcohol
Mix and shake well and then stand it for 5 minutes.
Conclusion: If the fat globules start floating on the top this is natural fat but if they sunk in the bottom then it indicates the addition of vegetable fat in the milk sample.

Natural fat floats because its globule has pits is it and air entrapped in that pits that makes the globule lighter and these float on the surface. There are no such pits in the vegetable fat globule.
Vegetable fat can also be detected with other tests such as:
2) Butyric acid content (present in the milk fat)
3) Iso-valeric acid contents (only present in milk fat)

SPOILAGE OF MILK

Spoilage makes the milk unwholesome. It occurs in different ways and in a variety of shapes. Microorganisms cause deterioration of milk and these may come from the environment or may come from the person(s) involved in the milking process.

Spoilage may be prevented in the raw milk as it contains;
- Lactoperoxidase
- Agglutinins
- Lyzozymes

These are also called inhibitory substances of spoilage.

Types of Spoilage

There are six (6) different types of spoilage as follows;
(i) Gas production
(ii) Proteolysis

(iii) Sliminess / Ropiness
(iv) Changes in milk fat
(v) Changes in milk flavor
(vi) Changes in milk color

Note: Once the source of spoilage is known to us, then its prevention is easy to do.
Spoilage may be regarded as: mild, moderate or sever (Degrees of Spoilage).

(1) Gas production

- Milk contains lactose which produces acids (such as propionic, lactic, and butyric) and CO_2 on fermentation.
- When this fermentation occurs at faster rate, then raw milk present a foamy layer on the upper surface. If it continues then air bubbles becomes entrapped and gas becomes saturated throughout the body of the milk. It is also termed as "Stormy Fermentation". Such milk is not fit for human consumption.
- Gas production may be due to gas producing bacteria such as:
- Colliforms, clostridium and bacillus species.
- This condition is due to fermentation phenomenon.

How it becomes sever?
Only the reason is holding temperature which is 30-35 C – very suitable for it. A small number of bacteria coming from the environment in the milk will result into stormy fermentation within 2 hours. Milk enzymes and defense mechanism can prevent it to a certain limit.

(2) Proteolysis

It is the destruction of milk proteins especially of casein. It reflected in a variety of shape, apparently look in different ways; shrunken coagulated molecules and whey is easily separated. Proteolysis may causes:

i) Acid proteolysis
Milk whey separated and pH >5.2. Milk taste will be soured.

ii) Alkaline proteolysis
Milk whey separated and pH >6.9 (towards neutral and alkaline)
Milk taste will be bitter.

iii) Sweet curdling
It is due to bacterial contamination i.e. *bacillus cerus* – it release enzymes "proteasis" which targeting casein. Bacteria use lactose and convert into acids and aldehyde components. Milk is sweet in taste

iv) Slow proteolysis:
If raw milk is heated to certain pasteurized temperature or boiling temperature, it will cause destruction of bacteria which results into release of endogenous proteases in the milk. These proteases cause slow proteolysis of the milk.

v) Residual proteolysis:
It is proteolysis in the residual component of bacteria i.e. *Pseudomonas flourescense* (found in plants and soils) - release protease enzyme which is heat stable. If this enzyme is found in the milk, it causes proteolysis.

Difference between slow proteolysis and residual proteolysis:
In case of residual proteolysis, there is no bacteria in the medium but only residues (i.e. enzymes). In slow proteolysis, bacteria also present.

Proteolysis due to anaerobe bacteria

Bacillus and *clostridial* species are heat resistance. Their presence not eliminated easily at any stage. That's why result into specific kind of smell names as "butyric acid fermentation (butyrine smell)". *Clostridium* species are mostly involved in the alkaline proteolysis of milk.

Pasteurization Protocol:

Temperature: 62 C Time: 30 min
Now replaced with:
Temperature: 71 C Time 15 sec

It kills also infectious bacteria i.e. Mycobacterium tuberculosis. But this much temperature destroy 70% of the milk Ca+ (present in the form of Ca+ phosphate).

Ultrahigh temperature (UHT) Protocol:

Temperature: 170 C Time: 2-3 sec

(3) Roppiness or Sliminess

It developed in the milk; thick consistency. Milk viscosity increased – if you touch the surface of milk, a cord/rope like structure formed.

Roppiness or sliminess is may be bacterial or non-bacterial in origin;

i) **Non-bacterial:**
   a) It may be due to normal thickness of cream itself. Such milk is not regarded as spoiled milk.
   b) It may be due thin film of casein and lactalbumin. These when mix together at refrigeration temperature, sliminess may be seen in the bottom part of the milk
   c) If there is non-infectious mastitis to the animal, then leukocytes and fibrin contents enhanced. These two make the milk thicker and produce roppiness or sliminess in the milk.

   Depending upon the type of changes in the milk – milk may be considered as unwholesome and should not be used for human consumption.

ii) Bacterial:

Conditions are irreversible. Certain microorganisms have properties to produce sliminess at the top and other produce throughout the body of the milk.

Bacteria produces surface roppiness; at the top of the milk:

a) *Alcaligen viscolactis*

Bacteria produces roppiness throughout the body of the milk:

a) *Enterobacter*
b) *Klebsiella*
c) E.*coli*
d) *Streptococcus*

Mostly capsulated bacteria produce sliminess because it comes from the capsule.

All these roppiness or sliminess (surface or throughout the body) in the milk is controlled through pasteurization.

(4) Change in Milk Fat

There are three particular conditions which cause changes in the milk fat.

a) Oxidation of unsaturated fatty acids:
Upon oxidation, milk fat yields a lot of aldehydes, ketones and variety of acids. Oxidation imparts tellowy odour.

b) Hydrolysis of overall milk fat:

Hydrolysis occur due to the acidity of enzyme "lipase" which separates the molecules of glycerols from fatty acids and results into release of free fatty acids in the milk which brought obnoxious change in the milk. Hydrolysis gives rise to putrefied odour (rotten egg like smell).

c) Combined condition of above:

Change in milk fat may be due to certain bacteria which gives a combination of both odours discussed before:

Pseudomonas
Staphlococcus
Proteus
Brucella

(5) Change in the Color of Milk

Normal color of milk ---------- Light or off-white (cow) and shining white (buffalo)
Variety of bacteria responsible for bringing change in the color of the milk:
Blue color ---------- *Pseudomonas synciani*
Red color ---------- *Brubibacterium erythrogenes, Sarcinia marcense*
Yellow color ---------- *Pseudomonas synxantha*
Brown color ---------- *Pseudomonas putrificians*
Green color ---------- *Pseudomonas aurogenosa*

- Blood mixed milk must be differentiated which may be due to any internal bleeding.
- These above are all bacteria except *brubibacterium* which is yeast.
- Most of these bacteria give rise the color pigmentation on milk supplementation in addition to the media because milk is also a good bacterial medium.
- Milk with any abnormal discoloration must be declared s unwholesome and spoiled.

↑ bacterial load ----- ↑ intensity of the color

6) Change in the Flavour of Milk

The change in the flavour of the milk is discussed on two aspects:

a) whether it is sour
b) whether it is bitter

Sour flavour:

It is due to acidic changes in the milk.

Sourness in the milk is described in 3 different forms:

i) **Clean**: Low contents of acids due to environment of *Streptococcus lactis*. There will be sour acidic flavour.

ii) **Aromatic**: It is due to *leuknostae* species (bacteria) which are responsible for moderated type of acidic components.

iii) **Sharp**: It is due to the environment of clostridial species; high acidic contents.

Bitter flavour: It is due to alkaline changes in the milk.

Potato-like Flavour:

Pseudomonas mucidolense (a bacteria) produce potato like flavour in the milk.

Fishiness:

A typical flavour of fishiness is produced by; *Acromian hydrophila*. It is due to formation of tri-methyl amine when lactose converts into it. Trimethyl amine gives fishiness to the milk.

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