EXPERIMENT NO: 9

EVALUATION AND TOXICOLOGICAL ANALYSIS OF MILK AND DAIRY PRODUCTS

Milk analysis begins with checking the appearance, color, smell and taste of milk. Normally, the color of milk is yellowish white due to calcium caseinate, and if water is added, it becomes bluish white. Significant changes in the color of milk can be caused by some microbial diseases, or by feeding the animal with different plants. The physical and chemical analyzes are made after having a statement about the quality of the milk with the organoleptic properties such as color, taste and smell.

1. Determination of Illegal Additives in Milk (such as formaldehyde, salicylic acid):

Some chemicals are added to milk in order to prevent bacterial growth. These are formaldehyde, salicylic acid, carbonates, boric acid and borates.

a. Determination of Formaldehyde

1. 10 ml of milk + 5 mL of HCl and 1-2 drops of 10% $FeCl_3$ ---------- mix and heat
Blue color formation indicates the presence of formaldehyde.

2. 3-5 ml of milk + 1-2 drops of Nessler reagent
Dark brown color formation indicates the presence of formaldehyde.

b. Determination of Salicylic Acid

2 ml of milk + 2 drops of 1% $FeCl_3$ solution
Purple violet color formation indicates the presence of salicylic acid.

c. Determination of Carbonates

5 ml of milk + 5 ml of ethanol ---- mix---- add 1 - 2 drops of a 0.5% solution of rozalic acid
Rose-red color formation indicates the presence of salicylic acid. The color gets darker in pure milk.

d. Determination of Starch

2 ml of milk + 2-3 drops of lugol solution --- mix
Blue color formation indicates the presence of starch. Yellow color formation indicates the absence of starch.

2. Determination of the Changes in *Milk Composition:*

a. Reductase test: Milk has reducing capacity. This property increases with the number of microbes in the milk. It is possible to make a statement about the color of the milk by reducing the methylene blue, the microbes in the removal time. reductase enzyme. Reductase enzyme converts methylene blue into leuco derivative and causes the color to disappear.

Reagent: Methylene blue (5 mL of saturated solution in water is taken, and diluted to 200 mL.)

Experimental Procedure: 1 ml of methylene blue solution is added to 20 ml of milk. The tube is inverted twice and put in 40ºC water bath or incubator. The disappearance time of the color of the solution is determined If the color dissappears in less than 3 hours, the milk is not healty.

Milk status	Disappearance time	Microbe in 1 ml milk
I Very good	6 h	< 500.000
II Good	5-6 h	<1.000.000 den
III Middle	4-5 h	<1.500.000 den
IV Bad	2-4 h	<1.5-2.000.000
V. Very bad	< 3 h	> 2.000.000

b. Milk Acidity Tests:

The determination of acidity of milk is important in investigating whether milk is suitable for processing. The milk which shows acidity reaciton is cut as soon as they are heated. Various tests are carried out to determine whether or not the milk will be cut.

In terms of defining the acidity of milk, there are three concepts: pH, titratable acidity and Sokshlet-Henkel degree.

***pH*:** The pH of the fresh milk is 6.4-6.9. When the pH is 4.7, the milk clots. In mastitis milk, pH is around 7.6.

The titratable acidity: It is carried out with the amount of alkaline required to neutralize 100 mL of milk and is indicated in% lactic acid.

Soxhlet-Henkel (SH) degree: It is based on the titrated acidity determination principle. The assessment is indicated in terms of the amount of ¼ N NaOH required to neutralize 100 mL of milk.

When the Sokshlet-Henkel degree in milk is 12; pH is 4.7.
When it is 7.5, pH is 6.4,
When it is 6.5, pH is 6,9.

If pH is lower than 6.4, milk clots.

a. Determination of pH in Milk

10 mL of milk is mixed with methyl alcohol. Then filtered. The filtered serum is treated with the necessary indicators to confirm the pH.

i) 1-2 drops of 4% bromtimol blue is added to 1 mL milk serum. The color turns from blue to yellow if the pH is between 6 and 7.6.
ii). 1-2 drops of 4% methyl red is added to 1 mL milk serum. The color changes from red to yellow if the pH is between 4.4 and 6.8.

b. Determination of Acidity in Milk

i. Empirical method:

a. Boiling test: 5 mL milk is heated in a tube. No change in healthy milk. High acidic milk is cut. The acidity degree (SH degree) of the clotted milk is 12 and the pH is 5.

b. Alcohol test: 5 mL of milk is shaken in a tube with 5 mL of 68% ethyl alcohol. If the SH is 8.5-9, milk precipitates. Moderate sour milk can be detected with these methods. According to the regulation the degree of SH should not be more than 8. Even if the goat milk is not soured, this experiment gives positive results.

ii) Determination of the degree of acidity in milk by titrimetric methods:

a) SH titration: 25 mL of milk is taken to a clean erlene and 1 mL of phenolphthalein is added. With ¼ N NaOH, the mixture is titrated to a light pink color. If the amount of base spent is multiplied by 4, the degree of acidity of the milk is found. If the acidity of milk is more than 8, this means the milk is harmful to health and sale of it is prohibited.

b) Alizarin assay: Add 1mL of saturated alizarin (in ethanol) solution to 2mL of milk and leave to water bath. According to the degree of SH, the following results are obtained in the waiting period in the water bath.

SH degree	The Color of Alizarin	Sediment	Time
7	Lilak	None	>7 hours
8	Pale red	Very thin	5-7 h
9	Bluish red	Thin	3-5 h
10	Bluish red	Thick	1-3 h
11	Orange	Very thick	0-30 min
12	Yellowish orange	Very thick	Immediately
16	Yellow	Very thick	Immediately

3. Determination of the Pasteurization in Milk:

Determination of Peroxidase: Peroxidase is an enzyme found in milk. The persistence of lacto-peroxidase activity in pasteurized milk provides a good indication of the quality of a product. Only a raw milk of good microbiological quality can be put through a mild pasteurization process in order to not inactivate this enzyme.
The absence of peroxidase in a milk sample indicates that the milk is pasteurized above 80ºC.

Benzidine assay: 2ml of benzidine solution (in 4% ethanol), 2-3 drops of acetic acid, 2 mL of 3% H_2O_2 are added to 10 ml. Blue color formation indicates the presence of peroxidase. The peroxidase enzyme allows the oxygen of H_2O_2 to be used by oxidizing substances. Thus causing the colorless substances to turn into colored ones.