UNIT 11 PRESERVATIVES, NEUTRALIZERS AND ADULTERANTS IN MILK AND THEIR DETECTION

Structure

11.0 OBJECTIVES

After studying this unit, we shall be able to:

- enumerate common preservatives used by the unscruplous person in milk;
- detect preservatives used in milk by simple tests;
- test neutralizers added in milk;
- specify adulterants used in milk; and
- check the purity of milk.

11.1 INTRODUCTION

Preservatives, neutralizers and adulterants are often added in milk with several different aims. However, it may be emphasized that addition of these components is strictly prohibited under PFA Act. This act does not allow the addition of any external agent to milk and is punishable under law. Preservatives are also prohibited in milk except during sampling and subsequent analysis.

Preservative may be defined, as a substance which when added to food is capable of inhibiting, retarding or averting the process of fermentation, acidification or spoilage or decomposition of food. The initial quality of milk is poor in India which leads to a high bacterial load. Moreover, it takes a long time with a time gap of several hours before milk reaches the consumers or individuals and dairy plants for processing. Under such circumstances there is a tendency to use preservatives to delay or prevent microbial proliferation and spoilage of milk. Let us know more about preservatives, neutralisess and adulterants.

11.2 PRESERVATIVES

It is a common practice by unscrupulous persons to add preservatives to liquid milk. The addition of preservatives is not permitted under law. Freshly drawn milk get contaminated with microorganisms which proliferate and multiply rapidly in milk. The growth of these microorganisms leads to an increase in acidity and souring of milk, which leads to spoilage. The problem is acute during summer months due to high temperatures. Their use is permissible under law only where sample is stored

for testing. The common preservatives added to milk are:

i) Formalin

ii) Boric acid and borates

iii) Benzoic acid and sodium benzoate

iv) Salicylic acid

v) Mercuric chloride

vi) Potassium chromate

vii) Hydrogen peroxide

i. Formalin

Formalin is a solution of 40% formaldehyde in water. By Hehner test formalin/ formaldehyde can be detected in milk. Formalin is a strong preservative which is very effective even in small dose. Formaldehyde (HCHO) is a gas. Its 40 percent solution in water is known as Formalin. It contains 10% methanol to prevent polymerization. Milk samples for analysis are preserved with 0.1 ml (two drops) of formalin per 25 gm/ml samples. Formalin can be detected by three tests namely, Hehner test, Hehner-Fulton test and Chromotropic test.

i) Hehner Test

Hehner test is a very simple and quick test for detecting formalin. It can be detected with concentrated sulphuric acid in the presence of an oxidizing agent $Fecl_3$. It gives intense violet colouration with this test. The detection is very easy and simple. Even a very small quantity of preservative can be detected by this test.

- Take 5 ml sample of milk in a test tube and gently add 2 ml concentrated sulphuric acid (H_2SO_4) containing a trace of $FeCI_3$. Care should be taken that while adding acid it forms a separate layer at the bottom of the tube. The acid should not mix with milk
- A coloured ring is formed at the junction of the two liquids. Note the colour of the ring formed at the junction of the milk and acid. A voilet to purple coloured ring indicates the presence of formalin/formaldehyde.

ii) Chromotropic Acid Test

Chromotropic acid test is a colour reaction test with formalin. In the presence of formaldehyde chromotropic acid develops a purple colour. The coloured reaction of Chromotropic acid test is due to reaction between chromotropic acid and formalin.

Chromotropic acid solution-is prepared as a saturated solution of chromotropic acid (1,8- dehydroxy napthalene-3, 6-disulphonic acid). The solution is prepared by stirring 0.5 g of chromotropic acid in 100 ml 72% H_2SO_4 (150 ml concentrated H_2SO_4 in 100 ml water-mixed in cold). The solution is straw yellow in colour.

- 5 ml reagent and 1 ml milk distillate is taken in test tube. Distillate is prepared from H_3PO_4 acidifred milk.
- The test tube is placed in a boiling water and the developed colour is noted.
- Development of light to deep purple colour indicates the presence of formalin. The colour intensity depends upon the amount of formalin present in sample.

ii. Boric Acid and Borates

Like formalin, boric acid and sodium borates are also common preservatives. They are available in the form of either boric acid (H_3BO_3) or its salt as sodium borate (Na_3 BO_3) as commercial chemicals. In acidic medium they can be conveniently detected with the help of turmeric paper. In the presence of boric acid and borates the paper turns red in acidified medium. This is due to the formation of ferric benzoate.

- Take 20-25 ml milk in a porcelain basin. Acidify milk by adding 1.5 ml concentrated hydrochloric acid.
- Take a strip of turmeric paper and carefully dip in the milk. Remove the strip from acidified milk and dry it in the air.
- Presence of boric acid or borates is confirmed by noticing the change in colour of the strip. The strip changes its colour from yellow to a red colour which is characteristic for the presence of boric acid or borate.
- On exposure of the paper strip to ammonia vapours or ammonia solution the colour changes to bluish green but reappears on re-acidification with HCl.

iii. Benzoic Acid and Sodium Benzoate (E)

These are food grade preservatives. Benzoic acid and its salt sodium benzoate are stable preservative. Benzoic acid is commonly used in the form of its sodium salt because it is more soluble than the acid but later is the active form. Sodium Salt is converted to the free acid when used as preservative. The optimum PH range for anti microbial activity of benzoic acid is 2.5 to 4.0. Benzoic acid is detected by extracting with ether from milk serum, as benzoic acid is soluble in ether. In alkaline medium with $FeCI_3$ it gives salmon red precipitate. In modified Mohler test a red brown ring is formed.

- Benzoic acid is extracted from serum of milk by removing casein. Collect the clear filtrate or serum.
- As benzoic acid is soluble in ether it is extracted by adding 50 ml diethyl ether and shaking it. The water and ether layer is allowed to separate in a separating funnel. If emulsion is formed and layers do not separate 10-15 ml petroleum ether (b.p. 60^0C) is added. Alternatively, separate the layers by a centrifuging at 1200 r.p.m.
- The etheral layer is carefully removed in a porcelain dish.
- The etheral layer is carefully evaporated on a boiling electric water bath.
- The residue so obtained is dissolved in 5 ml portion of water and divided it into two parts equally. There are two tests for detection, namely, FeCI3 test and modified Mohler test.

i) **$FeCI_3$ Test:** Make one portion of the above extract alkaline by adding a few drops of NH_4OH solution, expel the NH_3 by evaporation and dissolve the residue in a few ml hot water. Filter if necessary and add a few drops of 0.5% neutral $FeCI_3$ solution. Note the change in colour. A salmon red precipitate indicates the presence of benzoic acid.

ii) **Modified Mohler Test:**
   - In another portion of the extract add 1-2 drops of 10% NaOH solution and evaporate to dryness.
   - To the residue add 1 ml conc. H_2SO_4 and a crystal of KNO_3. Heat for 20 minutes on a boiling water bath.
   - Cool and add 1 ml water and mix. Make ammonical by adding NH_4OH. Boil to break any ammonium nitrate that may have formed.
   - Transfer the solution to a test tube; add a drop of freshly prepared ammonium sulphide solution without mixing.
   - Formation of a red brown ring indicates the presence of benzoic acid. The colour diffuses on mixing and give greenish yellow colour on heating.

Note: Salicylic acid also gives a reddish brown colour. However, this colour remains unchanged after heating.

iv. Salicylic Acid

Salicylic acid is an organic preservative. Like benzoic acid it is extracted from milk serum with the help of ether in which salicylic acid is soluble. To the residue in the presence of salicylic acid ferric chloride gives a voilet colour.

- To the residue obtained after extraction add 1 drop of 0.5% neutral $FeCI_3$ solution and observe the colour produced.
- A voilet colour indicates the presence of salicylic acid.

v. Mercuric Chloride

Mercuric chloride is a heavy metal salt and is highly toxic. It is also used as a preservative. Mercuric chloride is detected from milk serum by adding stannous chloride solution. A white precipitate is formed in the presence of mercuric chloride.

Prepare the extract of milk as is followed for benzoic acid.

- Dissolve the residue in 1-2 ml water. Filter if necessary.
- Transfer the solution to a test tube and add to it 15% stannous chloride in 1:1 HC1 solution and mix it simultaneously.
- A silky white precipitate appears which turns grey on further addition of $SnCI_2$ solution if mercuric chloride is present confirms its presence in milk.

vi. Potassium Chromate

Potassium chromate is used as a preservative only for storage of milk for analysis. Its solution is yellow in colour due to chromate ions. Potassium chromate is detected by a simple test using barium chloride. Yellow precipitates are formed due to barium chromate in the presence of potassium chromate.

- Prepare ash from 50 ml of milk by first drying on boiling water bath and then heating it in a muffle furnace at 550^0C for two hours.
- Add to ash 3-4 ml dil HCl and dissolve by warming.
- To 1 ml ash solution add 2 N NaOH solutions dropwise till the solution is alkaline (test with pH paper).
- Add 1 ml acetic acid and then 0.5 ml $BaCI_2$ solution and mix.
- Formation of a yellow precipitate indicates the presence of dichromate in milk.

vii. Hydrogen Peroxide

Hydrogen peroxide is a very strong oxidizing agent, and is an efficient preservative in small quantities. It breaks into water and oxygen in the presence of natural catalase present in milk. Hydrogen peroxide plus thiocyanate also activates the native lacto- peroxidase system which can be used for prolonging shelf life of milk. The use of hydrogen peroxide is prohibited. With paraphenylene diamine test hydrogen peroxide present to the level of 1:40,000 can be easily detected. It gives dark blue colour with paraphenylene diamine. Hydrogen peroxide can also be detected by vanadium pentoxide test which forms pink to red colour with this reagent.

i) Paraphenylene diamine test

- 5 ml milk is taken in a test tube.
- 5 drop of 2% aqueous solution of paraphenylene diamine is added and mixed in milk.
- Formation of deep blue colour indicates the presence of H_2O_2

ii) Vanadium Pentoxide (V_2O_5) Test

Vanadium pentoxide test gives pink to red colour in the presence of H_2O_2. It is a simple test and test is carried out in acidic medium first.

Vanadium pentoxide reagent is prepared by mixing 1 g vanadium pentoxideV_2O_5 in 100 ml H_2SO_4 (6 vol conc H_2SO_4 + 94 vol H_2O)

- To 10 ml milk in a porcelain dish add 10-20 drops of V_2O_5 reagent and mix carefully with a glass rod.
- Formation of pink to red colour indicates the presence of H_2O_2.

Check Your Progress 1

1. Name the preservatives which are commonly added to milk.

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2. Name the test by which boric acid is detected.

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3. Hehner test is used for the detection of which preservative?

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11.3 NEUTRALIZERS IN MILK

Neutralizers are added to neutrilize the acidic sour milk. The addition of alkalis is not permissible under law. Some of these alkalis are highly injurious to health. To overcome this problem by means of a few simple test they can be detected in milk added as neutralizer. Freshly drawn milk has an acidity of 0.12-0.16 per cent expressed as lactic acid. With the passage of time the acidity increases during souring of milk. Any acidity above 0.18 per cent lactic acid coagulate milk. This is due to the formation of lactic acid from lactose. Neutralization of milk is illegal under the P.F.A act. Unscrupulous milk producers and farmers tend to neutralize the milk to avoid rejection of milk with increased shelf-life at the milk collection centers and at the dairy plants. The common neutralizers which are added to milk are caustic or sodium hydroxide, baking soda or sodium bicarbonate and washing soda or sodium carbonate. They are detected by determining the alkalinity of ash and carbonate or bicarbonate by rosalic acid test.

i. Rosalic acid test for the detection of carbonate and bicarbonate in milk

Rosalic acid test is used for the detection of carbonate and bicarbonate in milk. This is a very simple, reliable and quick test for their detection. Rosalic acid reacts with carbonate and bicarbonate and give rose red colour in their presence. The intensity of colour depends upon the amount of these chemicals present.

- Take 5 ml sample of milk in a clean and dry test tube.
- Add 5 ml ethanol 95% and mix
- Now add 2-3 drops of rosalic acid solution prepared in 1% ethanol to the mixture and mix well. Note the colour change.
- A rose red colour develops.

- Formation of rose red colour in milk indicates the presence of carbonate or bicarbonate added as neutralizer.

  Note: Pure milk gives only a brownish red colour.

ii. Alkalinity Test

The presence of neutralizers can generally be detected by determining the alkalinity of ash. A known quality of milk is heated and converted into ash. The alkalinity of ash is estimated by titration against a decinormal standard hydrochloric acid in the presence of Phenolphthalein indicator. Values abnormally high would indicate neutralization of milk. To carryout this test first ash is prepared from milk so as to obtain added alkali in a concentrated form.

- Pipette 20 ml milk in a porcelain dish and evaporate to dryness on a boiling water bath.
- Prepare ash by keeping it over a burner or muffle furnace at 550^0C for 1 hour. When using burner heat till ash becomes grey white in colour.
- Cool the basin. Add water and mix the contents with a glass rod.
- Titrate the ash solution using standard O.1N HCl in the presence of 4-5 drops of phenolphthalein indicator solution. Note the volume of HCl solution used till a pink colour is obtained.
- If the volume of O.1N HCl exceeds 1.20 ml the milk is suspected to contain neutralizers.

Check Your Progress 2

1. Which test is used for the detection of carbonates and bicarbonates?

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2. Which substances are added as neutralizers?

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11.4 ADULTERANTS IN MILK

The ease with which milk can be adulterated has always attracted unscrupulous persons to adulterate the same with numerous compounds. The most common of them include starch or cereal products, cane sugar, glucose, urea and ammonium sulphate. Tests for their detection are now available which can easily detect these compounds. Some of these compounds are highly injurious to the health of individuals. Laws are now available which can lead to the punishment for adding these prohibited substances. These compounds are primarily added to raise the density of milk.

i. Starch or cereal flour (*atta*)

Starch is a tasteless, odourless and cheap adulterant which is readily available. It is often added as an adulterant because it is not sweet like sugar. It is difficult to be detected organoleptically in small quantities. Starch can be easily detected as blue coloured starch iodide complex on heating and cooling with 1% iodine solution. The demand for liquid milk is far greater than the availability of milk. This forces the adulteration with a view to make quick profits. A large volume of milk is

supplied to the consumer directly by the producers through vendors at the door step without any sort of processing, packaging or quality control. The milk supplied to the consumers is most prone to adulteration, as they do not bother about such adulteration and are under the impression that they are getting pure milk. However, such milks are often maximum adulterated. Compared to vendors the milk supplied in the organized sector is tested for the adulterants by random analysis of procured samples. The easiest way is to adulterate the milk with water and keep the fat within the PFA limit. The addition of water, however, decreases the SNF content and is compensated by adulterating milk with cheaper solid ingredients like sugar, starch, urea etc.

Starch is detected by a very simple and quick test using 10% iodine solution. In the presence of starch a voilet blue colour of starch iodine complex is obtained on boiling.

- Take 5 ml milk in a test tube. Boil the milk over a flame. Cool the milk.
- Add 1 to 2 drops of iodine solution.
- Formation of voilet blue colour indicates the presence of starch or cereal flour.

ii. Cane Sugar

Cane sugar is added to raise the total solids content in milk. This adulterant is readily available and is cheaper than milk solids. When added in large quantity it is detected by sweet taste imparted to milk. It can also be detected by resorcinol reagent, which gives redish brown colour in the presence of sucrose.

- Take 5 ml sample in a clean test tube
- Add 5 ml resorcinol reagent and mix well
- Place the tube in a boiling water bath for five minutes or heat directly on the flame to boiling
- Development of red colour with or without the separation of brown red precipitate indicates the presence of cane sugar in milk.

iii. Glucose

Glucose is added to milk to increase its density. It is odorless, colourless and is not as sweet as cane sugar. It is detected by modified Barfoed's reagent either directly from milk or clean filtrate of milk. In the presence of glucose Barfoed's reagent gives a deep blue colour. To detect glucose in milk.

- Take 1 ml milk sample in a test tube.
- Add 1 ml Barfoed's reagent. Heat for 3 minutes in a boiling water and then cool.
- Now add 1 ml phosphomolybdic acid reagent and mix.
- A deep blue colour shows the presence of glucose. Pure milk only gives a faint blue colour.

iv. Urea

Urea is available readily as chemical fertilizer with farmers. It is often used as an adulterant to boost total solids after dilution with water or skim milk. Urea is detected in serum part of milk after removing casein from the filtrate in alkaline medium. In alkaline medium in the presence of phenol a bluish green colour indicates the presence of urea.

- Take 5 ml milk in a 50 ml conical flask and add 1 ml acetic acid or TCA 24% solution and heat for 3 minutes in boiling water both. Filter the precipitate. Collect the filtrate
- Take 1 ml filtrate, add 1 ml NaOH solution, followed by 0.5 ml sodium hypochlorite

solution mix and finally add 0.5 ml phenol solution.

- A bluish green colour is formed with phenol in the presence of urea.

v. Ammonium Sulphate

Ammonium sulphate is also a fertilizer, it addition boosts the solids content of milk. Detection of ammonium sulphate is carried out on casein free filtrate prepared as per urea detection.

- Take 1 ml filtrate, add 0.5 ml NaOH, 0.5 ml sodium hypochlorite solution and mix. Now add 0.5 ml phenol and heat for 20 seconds in boiling water bath.
- Formation of a bluish colour which changes to dark blue shows the presence of ammonium sulphate. The colour is stable for over 12 hours.

  Note: In pure milk only salmon pink colour is formed, which gradually changes to bluish in course of about 2 hours.

vi. Partial Removal of Fat by Skimming

An indication of the removal of excess fat from milk give the following changes to milk:

- Lowering of fat percentage in milk
- Higher density of milk sample at 27^0C
- Higher ratio of solids-not-fat to fat in milk

vii. Addition of Skim Milk

Addition of separated milk or skimmed milk results in following changes in the milk:

- Addition of skim milk results in lowering of fat in milk
- Higher density of toned milk sample at 27^OC
- Higher percentage of solids-not-fat
- Higher ratio of solids-not-fat to fat.

viii. Dilution of milk by addition of water

Milk is commonly adulterated by adding water as it is highly profitable. It causes following effects:

- Fat percentage is lowered
- Density of milk is lowered at 27^OC
- Lowering of solids-not-fat content of milk
- Lowering of freezing point depression of milk

i) Determination of Specific Gravity of Milk by Lactometer

In routine analysis of milk the density in determined with the help of a lactometer. The lactometer is graduated at a temperature of either 15.5^0C or 27^0C. Lactometer consists of a long, slender glass stem of uniform diameter connected to a larger glass chamber that facilitate lactometer to float. Lower end of the lactometer is filled with synthetic material which makes the lactometer to float and also keeps it in upright position.

- Warm the milk to 40^0C for 5 minutes
- Cool the milk near the temperature of 27^0C, the temperature of lactometer graduation.
- Pour gently the milk in a 250 ml cylinder avoiding air bubbles and place the lactometer so that it floats freely.
- Take the lactometer reading and note the temperature

- Take the average of two readings
- Correct the lactometer reading from the table.

Lactometer reading for genuine cow milk is between 26-30 and 28-32 for buffalo milk. These readings are converted to specific gravity by prefixing 1.0 for lactometer readings e.g. a reading of 28 will give a specific gravity of 1.028.

Calculation

$$\% \text{ TS} = \frac{C.L.R.}{4} + 1.2 \text{ F} + 0.14$$

$$\% \text{ S.N.F.} = + 0.2 \text{ F} + 0.14$$

Where TS = Total solids in milk sample

S.N.F. = solids-not-fat in milk sample

F = Fat Percentage in sample

C.L.R.= corrected lactometer reading at 15.5^0C

Note: If the temperature is above 15.5^0C then to each 1^0C rise in temperature add 0.2 to each lactometer reading. On the other hand if the temperature in below 15.5^0C then to each 1^0C lowering of temperature subtract 0.2 from the lactometer reading. For example, if the temperature is 16.5 and reading is 30 then the C.L.R reading will be 30.2 (30+0.0.2). On the other hand if the temperature is 14.5^0C and the lactometer reading in 29 then the C.L.R. will be 28.8 (29.0-0.2).

ii) **Fat Determination**

a) **Gerber Method:** For routine fat analysis of milk Gerber method is commonly followed for fat estimation. It is a rapid method and results are available in short time. Gerber method is volumetric method for fat analysis.

b) **Roese-Gottlieb method:** In this method fat is extracted from milk with the help of fat extraction reagent i.e. solvent ether. Ammonia and alcohol are added to facilitate fat extraction. Ammonia dissolves the fat globule membrane and alcohol helps in the passage of the fat globules in the aqueous phase. This is a gravimetric method of fat estimation.

iii) **Freezing Point (FP)**

Milk contains upto 85 percent water and varies widely in composition. Thus a constant parameter of milk is difficult to assign for milk. As such freezing point is a fairly constant property with a freezing point value between 0.530 to 0.555^0C. Freezing point is a colligative property which depends upon the number of solute particles present in the system or solution. The solvent is water and its freezing point is always constant. On addition of water in milk the solute particles in the solvent are diluted which affect the freezing point of milk. This results in an increase in freezing point depression of milk. With cryoscopy the percent water added is calculated as percent-added water

Check Your Progress 3

1. Name the methods used for detection of water in milk.

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2. Why starch or cered flour (*atta*) is added to milk?

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3. Which constituent of milk is analysed by Gerber test?

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4. Cane sugar is detected by which reagent?

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11.5 LET US SUM UP

External agents in the form of adulterants, neutralizers and preservatives are added for many purposes. Each has its own role. The adulterants in the form of water, starch or flour (*atta*), sucrose, glucose, ammonium sulphate, urea etc., are primarily added to adulterate milk. While water addition give a larger volume of milk, role of other compounds is to raise the density of milk. Some of these components are hazardous and injurious to health. Neutralizers are primarily added to neutralize or decrease the acidity of milk. Some preservatives are added to preserve milk samples for analysis. However, they are added to slow down microbial growth so as to avoid spoilage of milk which is not permissible under PFA. Different tests are now available to detect them in milk.

11.6 KEY WORDS

Adulterant	:	An external substance added to milk for raising density or to increase volume with water
Freezing point	:	A characteristic ice forming property of milk
Rosalic acid	:	A dye used for the detection of neutralizer
Neutralizer	:	A substance which neutralizes the acidity
Hazardous	:	Poisonous
Injurious	:	Harmful

11.7 SOME USEFUL BOOKS

Mathur, M.P., Dutta Roy, D and Dinakar P.(1999), Text book of Dairy Chemistry, Indian council of Agricultural Research, New Delhi.

Roy, N.K. and Sen, D.C (1991) Text book of Practical Dairy Chemistry, Kalyani Publisher, Ludhiana.

11.8 ANSWERS TO CHECK YOUR PROGRESS

Your answers should include following points:

Check Your Progress 1

1) i. The preservatives commonly added to milk are- formalin, hydrogen peroxide,

boric acid/borates and potassium dichromate.

2) i. Boric acid in milk is detected by Turmeric acid paper test

3) i. Hehner test is used for the detection of formalin in milk.

Check Your Progress 2

1) i. Rosolic acid is a test, which is used for the detection of neutralizers containing carbonate and bicarbonate.

2) i. Substances which are added as neutralizers are caustic and caustic soda.

Check Your Progress 3

1) i. Water adulteration is detected by estimating the fat, density of milk and the freezing point of milk.

2) i. Starch or atta is added to raise the density of milk.

3) i. By Gerber test fat is estimated.

4) i. Cane sugar is detected by resorcinol reagent.