Detection of Adulterants in Milk

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INTRODUCTION

- India is highest milk producer in the world.
- However, the quality of milk and milk products is far from satisfactory.
- That is why the export of dairy products from India is very small.
- Chemical quality is linked to the hidden desire of milk producers/suppliers for making more profits.
- This results in adulteration of milk.

WHAT IS MILK ADULTERATION?

"Adulteration of milk may be defined as the addition or subtraction of some of the legally prohibited substances in or from the milk with a view to increasing the quantity or reducing the quality to make extra profits."

Different types of Adulteration

- Sugar
- Starch
- Wheat flour
- Common salt
- Baking soda
- Washing soda
- Urea
- Hydrogen Peroxide
- Formalin

The adulteration of milk by unscrupulous persons is usually done for:

1. Increasing the quantity of milk
2. Extending shelf-life
3. Maintaining SNF
4. Avoiding detection of sour milk
5. Replacing milk fat with cheaper oils & fats

COMMON CATEGORY OF ADULTERANTS

- **Neutralizers**: Sodium Carbonate, Sodium bicaronate, Sodium hydroxide, calcium hydroxide etc
- **Preservatives:** Hydrogen peroxide, Formalin, Boric acid, Borate
- **Carbohydrate:** Sugar, glucose, starch, maltodextrin
- **Salts and Fertilizers:** Urea, Ammonium sulphate, Ammoniacal fertilizer, potassium sulphate, sodium chloride etc.
- **Detergent:**

Liquid detergent: Teepol, laolene, easy etc.

Solid detergents: Washing poeder like vim tide etc.

- **Oils and paints:** Vegetable oil, fat, body oil, mineral oil, white pain etc
- **Pond water:** Heavier than water

METHODS FOR DETECTION OF ADULTERATION IN MILK

NEUTRALIZERS

- Milk during transportation and storage develops acidity. Such acidic milk gets rejected at milk plant. To prevent the rejection, unscrupulous traders add neutralizers.
- In milk, NaOH, Na_2CO_3, $NaHCO_3$ etc. are added as neutralizers; however, such practice is not permissible. Their detection can be carried out by the following methods:

(a) Rosalic acid test:

Rosalic acid is an indicator which shows a change in colour on addition to alkaline milk. Rosalic acid gives rose-red colour with carbonates and bicarbonates, whereas with pure milk it gives brownish coloration.

Method:

- Take 5 ml milk in a test tube of capacity 20 ml.
- Add 5 ml ethanol (95%,v/v) and 4 drops of Rosalic acid solution (0.05%; w/v in ethanol).
- Mix the contents.
- If carbonate or bicarbonate is present, a rose red colour will appear. If NaOH is present a deep rose red colour will appear.
- In the absence of all these or in case of pure milk, only a brownish colouration will be present

- This test will work only when neutralizers are added in excess quantities and the milk is alkaline in nature. That means, this test will detect only the over-neutralized milk.
- The limitation of this test is that the under-neutralized milk will not be detected by this test.
- Under these conditions, perform the ‘alkalinity of ash content test’ of the milk sample, as follows:

(b) Alkalinity of ash test:

Principle: Neutralization of milk with lime, NaOH,Na_2CO_3, $NaHCO_3$ etc increases the ash content and also increases the alkalinity of ash.

Method:

- Take 20 ml milk in a silica crucible.
- First evaporate the water to dryness, and then burn the content to ash in a muffle furnace.
- Disperse the ash in 10 ml distilled water and titrate the ash content against N/10 HCl using phenolphthalein as indicator.
- If the volume of N/10 HCl exceeds 1.20 ml, then the milk contains the added neutralizers.

Disadvantage: It is tedious and time consuming.

Detection of Starch (Iodine solution test)

Starch is cheaply available in various forms such as wheat flour, corn flour, and commercially manufactured starch. Being cheaper, it is sometimes added in the milk by adulterators to raise the SNF. However, it can be easily detected in milk by the following method:

Principle: Iodine solution gives intense blue colour with starch due to the formation of starch-iodo-compound(Blue colour unstable complex)

Method:

- To 10 ml milk in a test tube, few drops of 1% iodine solution are added.
- Blue colour developed indicates the presence of starch.

Limit of detection (LOD): 0.01% starch

Detection of Sugar (Resorcinol test):

Sugar is generally added in order to raise the lactometer reading of milk which has been diluted with water. Added sugar can be easily detected by following method.

Principle: Resorcinol produces red coloured solution with sucrose in acid medium.

Procedure:

- To 10 ml milk, add 1 ml of conc. HCl and 0.1 g of resorcinol.
- Stopper the test tube and mix the contents.
- Remove the stopper and place the test tube in a boiling water bath for 5 min.
- If cane sugar is present in milk, the red colour will be produced.
- In pure milk, only pale yellowish or brownish colour will develop.

LOD: 0.2% sucrose

Detection of Glucose (Modified Barfoed's reagent method):

Glucose being a reducing sugar poses many problems in its detection. Moreover, it is easily available in commercial form as concentrated syrup. These days adulteration of milk with glucose is increasing.

Reagents:

- **Modified Barford's Reagent:** Dissolve 24 gm of cupric acetate in 450 ml boiling distilled water and immediately add 25 ml of 8.5% lactic acid to the hot solution. Cool and dilute to 500 ml.
- **Phosphomolybdic acid reagent:** Take 35 gm of Ammonium molybdate and 5 gm of sodium tungstate in a one litre beaker. Add 200 ml of 10% (w/v) NaOH and 200 ml of distilled water. Boil vigorously for 20 min. cool, dilute to about 350 ml and add 125 ml of concentrated (85%) phosphoric acid. Dilute to 500 ml.

Principle: Glucose gives deep blue colour with phosphomolybdic acid in presence of modified Barfoed's reagent which contains cupric acetate and lactic acid.

Procedure:

- Take 1 ml adulterated milk sample in a test tube and add 1 ml of Barfoed's reagent.
- Heat the mixture for 3 min. in a boiling water bath and cool for 3 min. under tap water.
- Add 1 ml of phosphomolybdic acid reagent and mix.
- **Immediate** formation of deep blue colour indicates the presence of glucose.
- In case of control, only faint bluish colour appears due to slower reaction of disaccharides as compared to monosaccharides.

LOD: 0.1% glucose

Detection of Maltodextrin:

Maltodextrin is hydrolysed starch and soluble in water, and does not give characteristic blue colour with iodine solution unlike starches.

Procedure:

- To 5 ml milk sample in a test tube
- Add 2 ml of dilute iodine solution (0.05 N).
- Appearance of chocolate red brown colour developed indicates the presence of maltodextrin.

LOD: 0.3% maltodextrin.

Detection of Hydrogen peroxide:

Hydrogen Peroxide is a preservative, but as per rules, it is not permitted to be added to milk. Two methods are given for its detection.

Method 1 (Using p-phenylene-diamine reagent):

Principle: *p*-phenylenediamine hydrochloride gives intense blue colour with H_2O_2

Procedure:

- Take 5 ml milk in a test tube and few drops of *p*-phenylenediamine hydrochloride solution (2%, w/v, aq.).
- The content will be blue in colour if hydrogen peroxide is present in milk.
- Pure milk does not give blue colour and remains white in colour.

LOD: 0.025% H_2O_2

Method 2 (using KI and starch reagent):

Principle: H_2O_2 gives blue colour with potassium iodide-starch reagent. Iodine released from potassium iodide by H_2O_2 gives blue colour starch-iodo-compound(Unstable blue colour complex)

Procedure:

- Take 1 ml milk sample in a test tube.
- Add 1 ml of potassium iodide-starch reagent (mix equal volumes of 20% KI solution and 1% starch solution) to the test tube.
- Appearance of blue colour indicates the presence of hydrogen peroxide in the milk sample; whereas control samples remain colorless.

LOD: 0.004% H_2O_2

Detection of Formaldehyde

Formalin (40% water solution of formaldehyde) is generally used to preserve the milk samples for chemical analysis. It should never be added to milk meant for human consumption due to its poisonous property. If milk kept at room temperature (25 to 35°C) for longer time, did not sour, then that milk must be tested for formaldehyde by the following simple methods:

1. Hehner test
2. Leach test
3. Chromotropic acid test

Principle: In the Hehner and Leach test added formaldehyde gives characteristic violet colour with ferric salts and other oxidizing agents.

1. **Hehner test :**

Reagents: $FeCl_3$ 1%, Conc. H_2SO_4

Procedure:

- Take 5 ml of milk sample in a test tube and add a trace of ferric chloride.
- Add 5 ml of conc. H_2SO_4 carefully by the side of the test tube so that it should make a separate layer under the milk.
- Formation of purple colored ring at the junction indicates the presence of formaldehyde in milk.

2. **Leach Test:**

Reagents: $FeCl_3$ 10%, Conc. HCl

Procedure:

- Take 5 ml of milk in a test tube.
- Add 5 ml of leach reagent (Conc. HCl containing 1 ml of 10% ferric chloride solution for each 500 ml of acid).
- Sample containing HCHO will turn violet to brown black.
- Pure milk will give yellowish colour.

LOD for leach test: 0.1% HCHO.

3. Chromotropic acid test:

Procedure:

- Take 1 ml of milk in a test tube.
- Add 1 ml of chromotropic acid reagent (saturated solution of chromotropic acid in about 72% sulphuric acid).
- Appearance of yellow colour confirms the presence of formalin in sample.
- Control sample remains white.

LOD: 0.05% formalin

DETECTION OF BORIC ACID AND BORATES

Principle: Boric acid and its salts give characteristic red colour with turmeric paper under acidic conditions. This red colour turns to dark green on addition of NH_4OH.

Procedure:

- Take 5 ml milk in a test tube.
- Add 1 ml conc. HCl and mix.
- Dip turmeric paper in it and dry the paper.
- Red colour development indicates the presence of Boric acid and Borates.
- Put NH_4OH drop over the paper.
- Development of dark green colour confirms the presence of Boric acid and Borates.

Detection of common salt (NaCl):

Silver nitrate test:

Procedure:

- Take 5 ml of milk and 1 ml of silver nitrate solution (0.1 N).
- Mix well and add two drops of a solution of 10% potassium chromate.
- Yellow color indicates the presence of added salt.
- Otherwise, red color will appear.

LOD: 0.02% NaCl

Detection of Urea (DMAB method)

- Urea is a natural constituent of milk and it forms a major part of the non-protein nitrogen of milk.
- Urea is one of the ingredients of synthetic milk along with caustic soda, detergent, sugar and foreign fats.
- Adulteration of natural milk with synthetic milk increases the level of urea to such an extent that on consumption of this adulterated milk causes toxicological hazards.
- Estimation of urea concentration in milk may serve as a tool for checking the menace of adulteration of natural milk with synthetic milk.
- The average urea content in milk of cows and buffaloes ranges between 25 to 40 mg/100 ml.

DMAB method (Procedure):

Principle:

This method is based on the principle that urea forms a yellow complex with DMAB in a low acidic solution at room temperature.

Procedure:

- Take 5 ml of milk in a test tube and add 5 ml of 1.6% DMAB reagent
- Appearance of distinct yellow colour indicates the presence of added urea whereas that of slightly yellow colour is due to natural urea in milk.

LOD: 0.25% Urea.

Detection of Ammonium salts

The added ammonium salts e.g ammonium chloride, ammonium sulfate, ammonium nitrate and ammonium dihydrogen orthophosphate can be detected in milk by two methods:

1. Nessler's reagent method
2. Turmeric paper method

Method I: Nessler's reagent method :

It is based on the principle that Nessler's reagent gives yellow or grey color with ammonium salts.

- Nessler's reagent: Dissolve the following chemicals separately.
  a) 8.0 g of mercuric chloride in 150 ml distilled water.
  b) 60.0 g of sodium hydroxide in 150 ml distilled water.
  c) 6.0 g of potassium iodide in 150 ml distilled water.

Preparation of Nessler's reagent:

Add reagent ***a*** to reagent ***b*** and mix well. To this mixture, add reagent ***c,*** mix and dilute the contents to 500 ml. Leave this solution undisturbed and decant the clear upper layer of the solution and store in a stoppered glass bottle.

Procedure:

- Pipette 5 ml of suspected milk sample into a test tube
- Add 1 ml of Nessler's reagent.
- Mix the contents of the tube thoroughly.
- Appearance of yellowish or grey colour confirms the presence of added ammonium salts in milk.

LOD: 0.15% ammonium salts

Method 2. Turmeric paper method

Principle:

In this method ammonium salts on addition of strong alkali liberate ammonia and the liberated ammonia turns turmeric paper to pinkish red.

Reagents:

1. **Turmeric paper:** Dissolve 10 g of pure turmeric powder in 100 ml distilled water and dip Whatman filter paper Grade 1 strips into it for 2 min. Dry the paper at room temperature. The dried filter paper is wetted with distilled water before use.
2. **Sodium hydroxide solution:** 10% (aq.)

Procedure:

- Pipette 5 ml of suspected milk sample in a test tube
- Add 1 ml of 10% NaOH solution in such a manner that it should not touch the rim of the test tube while adding.
- Mix the contents of the tube.
- Place a piece of wet turmeric paper on the rim of the test tube and keep the test tube undisturbed.
- Observe the change in the colour of the turmeric paper.
- Appearance of pinkish red colour confirms the presence of ammonium salt in milk.

Detection of Sulfate salts (Barium chloride test):

Presence of sulfates in milk can be detected by using barium chloride.

Reagents:

1. Barium chloride ($BaCl_2.2H_2O$) solution: 5% (w/v, aq.)
2. Trichloroacetic acid (TCA): 24% (w/v, aq.)

Principle:

Sulphates give white precipitates with barium chloride solution.

Procedure:

- Take 10 ml of milk in a 50 ml stoppered test tube and add 10 ml of TCA solution.
- Filter the coagulated milk through Whatman filter paper Grade 42.
- Take 5 ml of clear filtrate and add few drops of barium chloride solution.
- Formation of milky-white precipitates indicates the presence of added sulfates like ammonium sulfate, sodium sulfate, zinc sulfate and magnesium sulfate etc. to milk.

LOD: 0.05% sulphate salts.

Detection of Detergents in milk (Methylene blue test):

Detergents are not present in natural milk. However, they gain entry into natural milk when synthetic milk is added to it, as they are used in the preparation of synthetic milk. Detergents can be detected in milk by using indicators like methylene blue.

Principle:

- Detergents can be anionic, cationic and non-ionic, but in general, anionic detergents are most commonly used commercially.
- This method is based on the principle that methylene blue, which is soluble in an aqueous phase, but forms a complex with anionic detergents, which is again blue in color and that complex becomes soluble in chloroform.
- Therefore, if the color of the chloroform added to suspected milk sample becomes blue after the addition of methylene blue, it indicates the presence of anionic detergent in milk.

Reagents

1. **Methylene blue solution:** Dissolve 12.5 mg of methylene blue in water and make volume to 100 ml.
2. **Chloroform**: AR Grade quality.

Procedure

1. Take 1 ml of suspected milk sample. Add 1 ml of dye solution followed by addition of 2 ml chloroform.
2. Vortex the contents for about 15 seconds and centrifuge at about 1100 rpm for 3 min.
3. Relatively more intense blue color in lower layer indicates presence of detergent in milk.
4. Relatively more intense blue color in upper layer indicates absence of detergent in milk.

LOD: 0.0125 % detergents

Detection of Pond water (Diphenylamine test for nitrates)

Nitrate test or Nitrate reduction test

Principle:

Pond water is heavier than the tap water due to the presence of certain constituents soluble in water; some unscrupulous persons usually prefer it for adulteration in milk. This method actually detects nitrates present in the pond water. In the pond water, nitrates may come from fertilizers used in the fields or from the nitrifying bacteria. Nitrates give intense blue colour with diphenylamine whereas pure milk does not give this test.

Procedure:

- Rinse a test tube with the suspected milk sample.
- Along the side of the test tube, add about 1 or 2 drops of 2% solution of diphenylamine.
- The sides of the test tube will turn blue if the milk sample contains pond water.

LOD: 0.2% KNO_3.

Thank You