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METHODS FOR DETECTION OF COMMON ADULTERANTS IN MILK AND MILK PRODUCTS

This bulletin includes technical information based on latest developments on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of dairy operations. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue is **Methods for Detection of Common Adulterants in Milk and Milk Products**. It may be understood that the information given here is by no means complete.

In this issue:

1. INTRODUCTION

Adulteration of milk and milk products is a commonly encountered problem. Deriving undue economic benefits, through artificial increase in fat and/or solids not fat (SnF) or through masking the poor quality of product, is the key motive behind adulteration. Milk is a very convenient food for adulteration because of its high water content and opacity. Further, as it is very complex mixture of several constituents, it is difficult to detect the presence of adulterants in milk and milk products.

This issue of *Technews* provides a compilation of simple methods of detection of adulterants in milk and milk products. The PFA Rule 4, Sub-rule (9) recognizes, among others, the analytical procedures provided in the Manual of Methods of Analysis of Foods brought out by the Directorate General of Health Services, Ministry of Health and Family Welfare, as the official methods of analysis. Most of the methods provided in this *Technews* are from this source. Some other methods for detection of adulterants in milk and milk products have also been included from the available literature. Additionally, a reference list of methods, which are not included above, has also been provided based on the methods provided by the Bureau of Indian Standards (BIS) for detection of adulterants in milk and milk products.

It is useful to carry out more than one test, where available, for an adulterant. It may, however, be noted that the results of the tests used for adulteration detection must be augmented through confirmatory methods, if they are to be used for legal purposes.

2. COMMON ADULTERANTS IN MILK AND MILK PRODUCTS

The Table 1 below lists common adulterants, for which detection methods have been provided in this *Technews*, and the purpose for which these adulterants are used in milk and milk products.

Table 1: Common Adulterants Used in Milk and Milk Products

Adulterant(s)	**Milk Product in Which Used**	**Purpose**
Sugar, maltodextrins, urea, ammonium sulphate, sodium chloride (common salt), skimmed milk powder, gelatin	Milks	To increase solids not fat content
Glucose	Milks and milk powders	To increase the solids not fat content in milks. To increase total solids and hence the quantity of the products.
Starch (including mashed potatoes)	Milks and milk products like *khoya*, cheese, butter, *ghee* etc.	
Cellulose	Milks and milk products like *rabri*, *khoya*, *burfi* etc.	
Non-milk fat	Milks and milk fat products	To increase the fat content in milk. To increase the quantity of milkfat products.

Adulterant(s)	Milk Product in Which Used	Purpose
Carbonates and caustic soda	Milks	To neutralize developed acidity
Hypochlorites and chloramines; quaternary ammonium compounds; formalin; hydrogen peroxide; boric acid and borates; salicylic acid; and benzoic acid	Milks	For preservation

3. METHODS FOR DETETCTION OF COMMON ADULTERANTS IN MILK AND MILK PRODUCTS

A. METHODS RECOGNIZED UNDER THE PFA RULES AND FROM LITERATURE

(i) Detection of cane sugar in milk [1]

Principle

Ketose sugars react with resorcinol to give red colour. Fructose present in the cane sugar is a ketose sugar and can, therefore, be detected with resorcinol.

Reagent(s)

- *Concentrated hydrochloric acid (specific gravity 1.16)*
- *Modified resorcinol reagent*: Dissolve 1.0 g of resorcinol in 100 ml diluted hydrochloric acid (1.5 volume of distilled water + 1 volume of concentrated hydrochloric acid).

The resorcinol flakes used should be white in colour.

Procedure

- Curdle an aliquot of the milk by adding a little concentrated hydrochloric acid. Generally, one ml of concentrated hydrochloric acid is required for 25 ml of milk.
- Let it stand for about 10 minute and filter.
- Take 5 ml of the modified resorcinol reagent in a test tube and add 1 ml of the filtered milk serum. Mix the contents.
- Place the test tube in boiling water bath for exactly 1 minute. Withdraw the tube and observe the colour.

Interpretation

Appearance of deep red colour indicates presence of sucrose, or a ketose sugar.

(ii) Detection of glucose in milk and milk powder [(1)]

Reagent(s)

- *Modified Barfoed's reagent*: Dissolve 24 g of copper acetate in 450 ml of boiling distilled water. Add 25 ml of 8.5% acetic acid, shake, cool to room temperature and make upto 500 ml with boiled and cooled distilled water. Allow to settle. Filter and store in dark coloured bottle.
- *Phosphomolybdic acid reagent*: To 150 g of pure molybdic acid in an Erlenmeyer flask, add 75 g of anhydrous sodium carbonate. Add 500 ml distilled water in small portions with shaking, heat to boiling or until all the molybdic acid has been dissolved. Filter and add 300 ml of 85% phosphoric acid to filtrate, cool and make upto 1 litre with distilled water.

- *Acetate buffer*: Mix 1 N sodium acetate and 1 N acetic acid in equal volume. The pH should be 4.75.

Procedure

- To 1 ml of milk sample or 1 ml of reconstituted milk powder in a test tube, add equal volume of acetate buffer and filter.
- To 0.2 ml of filtrate, add 2.8 ml distilled water and 2 ml of modified Barfoed's reagent.
- Heat the tube in boiling water for 4 minutes. Let it cool for 2 minutes.
- Add 3 ml of phosphomolybdic acid reagent and mix the contents.
- Observe for colour change.

Interpretation

Development of deep blue colour indicates the presence of glucose.

(iii) Detection of starch in milk [1]

Principle

Starch produces a blue coloured complex with iodine.

Reagent(s)

Iodine Solution: Dissolve 2.6 g of iodine and 3 g of potassium iodide in a sufficient quantity of distilled water and make-up to 200 ml.

Procedure

- Take about 5 ml of milk in a test tube.
- Bring to boil and allow it to cool to room temperature.
- Add 1-2 drops of iodine solution to the test tube and observe the colour change.

Interpretation

Development of blue colour, which disappears when sample is boiled and reappears on cooling, indicates presence of starch.

(iv) Detection of cellulose in milk [1]

Principle

Cellulose gives blue colour in presence of iodine and zinc chloride while it does not give a colour with iodine alone.

Reagent(s)

- *Iodine solution*: Dissolve 1.5 g of iodine and 3 g of potassium iodide in a sufficient quantity of distilled water and make upto 60 ml.
- *Iodine – zinc chloride reagent*: Dissolve 20 g zinc chloride in 8.5 ml distilled water and when cool, introduce the iodine solution, prepared as above, drop by drop until iodine begins to precipitate.

Procedure

- Take about 10 g of milk in a 100 ml beaker and add 50 ml of hot distilled water. Stir thoroughly for about 2 minutes.
- Pour the mixture on a nylon cloth. Wash the residue on the cloth with 50 ml of hot distilled water twice.
- Scrape the residue with a spatula and place it in a spotting plate.
- Stain a part of residue with iodine - zinc chloride reagent and another part with iodine solution.
- Observe the change in both the cases.

Interpretation

Development of blue colour in iodine - zinc chloride reagent

and absence of blue colour in iodine solution confirms presence of cellulose.

Note(s)
The method is also applicable to milk products like curd, *rabri* and evaporated milk. It is not applicable to the samples containing starch.

(v) Detection of Maltodextrins in milk [2]

Reagents
Trichloroacetic acid (TCA) solution: 10% (w/v) solution in distilled water.
Barium chloride solution: 2% (w/v) in distilled water.

Procedure
- Put 20 ml of milk in a beaker, boil and cool.
- Coagulate the milk using 10% TCA solution.
- Filter through Whatman filter paper no. 42 and collect the filtrate.
- Add 2 ml barium chloride solution to the filtrate and mix well.
- Observe the colour.

Interpretation
Appearance of blue colour indicates the presence of maltodextrins.

(vi) Detection of added urea in milk [1]

The total urea content in milk shall not exceed 700 ppm (0.07%) as per the Prevention of Food Adulteration Rules.

Two methods, based on different principles, for detection of

added urea in milk are provided below.

(a) Enzymatic method

Principle

Urea is hydrolyzed by the enzyme urease leading to generation of alkaline conditions that turn red litmus blue.

Reagent(s)

- *Urease solution*: Soak some soybeans overnight or for 12 hours in distilled water and grind to prepare a slurry. This slurry contains urease. A fresh solution of urease can also be prepared by dissolving standardized urease in distilled water and neutralizing it. Each 10 ml of neutralized solution should convert nitrogen of not more than 0.1 g pure urea. For neutralizing, first determine the alkalinity of urease solution (0.1 g commercial urease dissolved in 50 ml distilled water) by titrating with 0.1N hydrochloric acid using methyl red. Note the volume of the 0.1 N HCl solution required for titration. Add the same volume of 0.1N hydrochloric acid to each 0.1 g commercial urease used for preparing urease solution.
- *Red litmus paper*

Procedure

- Take 10 ml of milk in a test tube and add a small quantity of soybean slurry.
- Insert a strip of moistened red litmus paper into it taking care not to touch the milk and sides of the test tube.
- Cover the mouth of the tube with a cork or stopper to make it air tight. Shake the tube gently, taking care that the contents do not come in contact with the litmus paper. Keep

it aside for 5-10 minutes.
- Observe for colour change in litmus paper.

Interpretation
The test detects urea concentration greater than 0.1% while the inherent urea in milk is typically below 0.1%. Therefore, change of red litmus paper to blue colour indicates presence of added urea.

Note
The test is also applicable to formaldehyde treated milk.

(b) p–Dimethyl amino benzaldehyde method

Principle
p-Dimethyl amino benzaldehyde (DMAB) reacts with urea to give a yellow complex in acidic conditions.

Reagent(s)
DMAB reagent: Dissolve 1.6 g of p-Dimethyl amino benzaldehyde in 100 ml ethyl alcohol and add 10 ml concentrated hydrochloric acid.

Procedure
- Mix 5 ml of milk sample with 5 ml of DMAB reagent.
- Mix 5 ml of control milk (known to be free from added urea) with 5 ml of DMAB reagent.
- Observe for colour change in both the cases.

Interpretation
Development of a distinct yellow colour indicates presence of added urea in milk. The control milk shows a slight yellow colour due to presence of natural urea.

(vii) Detection of ammonium sulphate in milk [1, 3]

Principle

Phenol reacts with ammonia ions and forms a green-blue coloured complex, whose intensity is proportional to the ammonia concentration in sample.

Reagent(s)

- *Sodium hydroxide solution*: 2% (w/v) solution in distilled water.
- *Sodium hypochlorite solution*: 2% (v/v) solution with distilled *water.*
- *Phenol solution*: 5% (w/v) solution in distilled water.

Procedure

- Take 1.0 ml of milk in a test tube and add 0.5 ml of sodium hydroxide solution, 0.5 ml of sodium hypochlorite solution and 0.5 ml of phenol solution.
- Heat for 20 seconds in boiling water bath.
- Observe for colour change.

Interpretation

A bluish colour immediately forms, which turns deep blue in case of a sample containing ammonium sulphate. The colour remains stable for 12 hours. The development of pink colour shows that the sample is free from ammonium sulphate. Detection limit is 0.1% added ammonium sulphate.

(viii) Detection of sodium chloride (common salt) in milk [1]

Principle

Silver chloride, produced on reaction between silver nitrate and sodium chloride, gives yellow colour while silver nitrate gives brick red colour with potassium chromate.

Reagent(s)

- *Silver nitrate solution*: 0.1 N.
- *Potassium chromate solution*: 5% (w/v) solution in distilled water.

Procedure

- Take 2.0 ml of milk and add 1.0 ml of potassium chromate solution followed by 2.0 ml of silver nitrate solution.
- Observe for colour change.

Interpretation

Appearance of yellow colour indicates presence of dissolved chloride and appearance of red precipitate indicates the absence of dissolved chloride in milk.

(ix) Detection of skimmed milk powder in milk [1]

Principle

The reducing groups present in the proteins of milk powder reduce molybdenum resulting in formation of blue colour.

Reagent(s)

- *Diluted acetic acid*: 4% (v/v).
- *Phosphomolybdic acid solution*: 1% (w/v) solution in distilled water.

Procedure

- Take 50 ml of milk in a 60 ml centrifuge tube.
- Place the tube in the centrifuge and balance it properly. Centrifuge at 3000 rpm for 15 minutes.
- Decant the supernatant creamy layer carefully.
- Add 0.5 ml of diluted acetic acid for coagulation and then add 2 ml of phosphomolybdic acid solution. Mix the contents thoroughly.

- Heat in a water bath at boiling temperature for 15 minutes and then cool.
- Observe the colour of the curd obtained.

Interpretation

The curd obtained from pure milk shall be greenish in colour whereas the curd of sample containing skimmed milk powder shall be bluish in colour. The intensity of bluish colour depends on the amount of the skim milk powder present in the sample.

(x) Detection of gelatin in milk [1]

Principle

The type and colour of the precipitate formed by picric acid and mercuric nitrate in presence of gelatin differ from the precipitate formed in absence of gelatin in milk.

Reagent(s)

- *Mercuric nitrate solution*: Dissolved mercury in twice its weight of nitric acid and dilute to 25 times its volume with distilled water.
- *Picric acid solution*: Saturated solution of picric acid in distilled water.

Procedure

- Take 10 ml of sample, add 10 ml mercuric nitrate solution and shake the mixture.
- Add 20 ml distilled water, shake again and let it stand for 5 minutes and filter. If much gelatin is present, filtrate will be opalescent and a clear filtrate cannot be obtained.
- To a portion of filtrate in a test tube, add equal volume of

saturated aqueous picric acid solution.
- Observe the colour and type of the precipitate formed.

Interpretation

Yellow precipitate is produced in the presence of considerable amount of gelatin. Smaller amounts of gelatin are indicated by cloudiness.

Note(s)

- The test is applicable to milk products also.
- In applying this test to sour, fermented, cultured, or very old samples of milk, cream or butter milk, sterilized cream or evaporated milk or cottage cheese, care should be exercised to recognize precipitate produced by picric acid when added to the mercuric nitrate filtrates from these materials in absence of gelatin. Such samples with or without rennet and entirely free from gelatin, give on standing distinct precipitate when treated as above. In every case, however these precipitates differ in character than those produced by picric acid with gelatin. Gelatin picric acid precipitate is finely divided, more apt to remain in suspension, settles only slowly and adheres tenaciously to the bottom of the container, from which it is rinsed with difficulty. Precipitates produced by picric acid in the absence of gelatin are flocculent, separate readily (leaving serum practically clear) do not adhere to walls of container and are easily removed by rinsing with distilled water. When gelatin is present in sample gelatin picric acid precipitate will remain in suspension long after flocculent precipitate has settled, but on standing overnight the characteristic sticky deposit will be found adhering tenaciously to bottom and sides of the test vessel. If gelatin is present in relatively high concentration

(1%), picric acid precipitate will be voluminous and will settle rather quickly.

(xi) Detection of foreign fat in milk by butyro-refractometer reading [4]

Principle

Fat is extracted from milk and its refractive index is determined and compared with that of the pure milk fat. This test can be done with the fat extracted during determination of fat in milk with the Rose-Gottlieb method.

Procedure

- Stabilize the temperature of the refractometer to within 40ºC ± 0.1ºC by using a thermostatically controlled water bath with a provision to circulate water through the refractometer.
- Calibrate the refractometer with a glass plate of known refractive index by placing it on the prism with a drop of alpha bromonapthalene as the contact liquid. (In the absence of butyro refractometer, use Abbe refractometer which can be standardised with distilled water. The refractive index of distilled water at 20ºC is 1.3330 and at 40ºC is 1.3306).
- Take the Butyro-refractometer reading (BRR) at 40ºC of the fat extracted during determination of fat in milk sample by Rose-Gottlieb method.

Interpretation

If the BRR differs from the prescribed limit of variability, presence of foreign fat in the milk may be suspected. Butyro-refractometer reading of 1-100 corresponds to the refractive index between 1.4220-1.895 and the refractive index can be read to the fourth decimal place.

Note

The tests based on determination of BRR of milk fat cannot detect presence of hydrogenated fat, palm kernel oils and coconut oil as their BRR are close to those of milk fat.

(xii) Detection of *vanaspati* in *ghee* by critical temperature of dissolution test (4)

Principle

Critical temperature of dissolution (CTD) reflects the temperature at which the turbidity appears on gradual cooling of fats dissolved in a warm solvent. The CTD is specific for a type of fat.

Reagent(s)

- *Solvent mixture*: Mix two volumes of ethyl alcohol (95%, v/v) and one volume of iso-amyl alcohol (boiling point 128-132°C).

Procedure

- Take 2 ml of melted ghee sample in a test tube (15 cm length, 150 mm outer diameter).
- Add 2 ml of solvent mixture.
- Heat in a liquid paraffin bath using thermometer as stirring rod.
- When two layers of ghee sample and the solvent mixture become homogeneous and clear, remove the tube from the water bath and continue stirring until a definite turbidity appears.
- Record the temperature, when the definite turbidity appears, as the CTD.

Interpretation

A CTD value of 55°C is taken as a cut-off limit beyond which the sample may be suspected to be adulterated.

Note

The test cannot detect adulteration of *ghee* with *vanaspati* at a level lower than 15%.

(xiii) Detection of *vanaspati* in *ghee* by apparent solidification time test [3]

Principle

The apparent solidification time (AST) of the fat sample is defined as the time taken by the melted fat sample to get solidified apparently at a particular temperature. The AST is specific for a type of fat.

Procedure

- Take 3 g of melted ghee sample in a test tube (10 x 1 cm) and maintain it at 60°C for 5 minutes.
- Transfer the test tube in refrigerated water bath maintained at 18 $\pm$ 0.2°C and start the stop watch simultaneously.
- Observe the test tube constantly till the apparent solidification of fat sample takes place. Confirm by tilting the test tube – the fat sample should not show any movement.
- Stop the stop-watch and record the time taken for apparent solidification as the AST.

Interpretation

An AST value of 2.31-3.25 minutes corresponds to pure cow/buffalo *ghee*. Any deviation from these values gives an

indication of adulteration of milk fat.

(xiv) Test for detection of cotton seed oil in *ghee* by methylene blue reduction test [4]

Principle

Cotton seed oil contains cyclopropenoic acid which instantaneously reduces the methylene blue dye leading to the decolourization of the dye.

Reagent(s)

Methylene blue dye solution: Dissolve 0.1 g methylene blue dye in 100 ml methanol - chloroform mixture (1 volume of methanol + 1 volume of chloroform)

Procedure

- Take about 5 g melted *ghee* sample in a test tube.
- Add 0.1 ml of the methylene blue dye solution. Shake the content taking care that the contents do not solidify.
- Observe the change in colour.

Interpretation

Decolourization of the solution in the test tube indicates presence of cotton seed oil. Normal ghee would not reduce the dye and hence the solution in the test tube will remain blue.

Note

The test cannot differentiate between the *ghee* adulterated with cotton seed oil and the *ghee* from the cotton seed tract area (area where cotton seed is extensively fed to the cattle).

(xv) Test for detection of vegetable oils in *ghee* through detection of antioxidants [4]

Principle

Tannins present in oils give prussian blue colour with potassium ferricyanide and ferric chloride reagent.

Reagent(s)

- *Acetonitrile*
- *Ferric chloride solution*: 0.008 M
- *Potassium ferricyanide solution*: 0.003 M

Procedure

- Take 2 ml of clear melted fat sample in a test tube.
- Add 5 ml of acetonitrile into the test tube.
- Shake the contents and centrifuge.
- Take the acetonitrile layer (lower layer) in a test tube and add 1ml of ferric chloride solution and 0.3 ml of potassium ferricyanide solution.
- Observe the colour of the solution immediately.

Interpretation

Immediate appearance of the bluish tinge after addition of the reagents indicates presence of vegetable oils while appearance of bluish tinge after 5 minutes shows that the milk fat sample is pure.

Note

The test is not useful if a vegetable oil, which does not contain tannins (such as refined vegetable oils), is used for adulteration of ghee. Also, the test may be falsely positive if the ghee contains permitted added antioxidant Butylated Hydroxy Anisole (BHA).

(xvi) Detection of carbonates in milk by rosalic acid test [1]

Principle

Rosalic acid gives rose red colour in alkaline conditions due to presence of carbonates.

Reagent(s)

- *Rosalic acid solution*: 1% (w/v) in ethyl alcohol
- *Ethyl alcohol* - 95% (v/v)

Procedure

- To 10 ml of milk add equal volume of ethyl alcohol in a test tube.
- Add a few drops of rosalic acid solution.
- Observe the colour of the solution.

Interpretation

If alkali is present a rose red colour appears whereas pure milk shows only a brownish colour.

Note

This test does not indicate presence of carbonates in milk if the developed acidity in milk has neutralized the added carbonates.

(xvii) Detection of carbonates in milk through difference in acidity before and after boiling [5]

Principle

Addition of carbonate neutralizers increases the carbon dioxide content and its contribution to the acidity in milk. As carbon dioxide can be expelled from the milk by heating, difference in the acidity before and after boiling of milk can be used to detect presence of carbonate neutralizers in milk.

Procedure

- Divide the milk sample into two portions.
- Determine acidity of one portion of the milk sample.
- Boil the other portion of the milk sample, cool and determine its acidity.
- Calculate the difference between the two acidity values.

Interpretation

Carbon dioxide, naturally present in milk, contributes to its acidity by upto 0.01% or 0.02%. A difference of more than 0.02% in the two acidity values obtained indicates presence of carbonate neutralizers.

Note

This test does not apply to heated/pasteurized milk.

(xviii) Detection of carbonates/caustic soda in milk [5]

Principle

Milk has some natural acidity. However, on improper storage and handling, it also develops acidity through bacterial action, which is due to the lactic acid produced by the bacteria from lactose. Presence of lactic acid beyond a level in milk, which is negative for clot-on-boiling test, gives indication that it has been neutralized by using carbonates and/or caustic soda.

Reagent(s)

- *Barium chloride solution*: Dissolve 19.75 g of barium chloride in distilled water and make up the volume to 100 ml using distilled water.
- *Sodium hydroxide solution*: 1.32 N
- *Zinc sulphate solution*: Dissolve 22.5 g of zinc sulphate in distilled water and make up the volume to 100 ml using

distilled water.

- *Ferric chloride solution*: Dissolve 5 g of ferric chloride in 100 ml of 1/8 N hydrochloric acid. This should be diluted to 1% solution before use by mixing its 1 volume with four volumes of distilled water.

Procedure

- Take 225 ml of milk in a conical flask.
- Add 5 ml of barium chloride, 5 ml of sodium hydroxide and 5 ml of zinc sulphate solution. Add in the order mentioned.
- Shake the contents of the flask thoroughly and allow them to stand for 30 seconds.
- Filter through fluted Whatman no. 4 filter paper and collect the filtrate into a clean conical flask.
- Take 0.5 ml of 1% ferric chloride solution in a Lovibond comparator cuvette marked at 10ml. Mix the contents and place the cuvette in the right hand compartment of the Lovibond comparator.
- Prepare a blank in a similar way by taking distilled water instead of the filtrate. Place this cuvette in the left hand compartment of the Lovibond comparator.
- Insert the standard tintometer disc number 6 (covering a range of 0-0.05 % lactic acid in five steps of 0.01%, with first labeled '0' to represent the colour of the ferric chloride in the blank) into the comparator.
- Read the lactic acid content.

Interpretation

A reading of 0.03% is suspicious while a reading above 0.03% confirms presence of neutralizer, if the milk is negative for clot-on-boiling test. Normal milk has a developed lactic acid content of 0.01% to 0.02%.

Note

This test will not confirm presence of neutralizers in milk which is positive for clot–on-boiling test. However, such milk should be rejected anyway.

(xix) Detection of hypochlorite and chloramine (1)

Principle

Iodine released from potassium iodide in presence of hypochlorites/chloramines under acidic conditions gives blue colour with starch.

Reagent(s)

- *Potassium iodide solution*: 7% (w/v) in distilled water. Prepare fresh.
- *Diluted hydrochloric acid*: Mix 2 volumes of distilled water with 1 volume of concentrated hydrochloric acid.
- *Starch solution*: Boil 1 g starch in 100 ml distilled water. Cool before using.

Procedure and interpretation

- To 5 ml of sample in a test tube add 1.5 ml of potassium iodide solution, mix thoroughly and observe colour. A yellowish brown to deep yellow colour may be formed. If unaltered, add 4 ml of diluted hydrochloric acid, mix thoroughly with a glass rod flattened at one end and note colour of curd. A yellowish brown to deep yellow colour may be formed.
- Place the test tube in a large water bath previously heated to 85ºC and allow it to remain for 10 minutes. The curd will rise to the surface. The liquid and the curd will have yellowish brown to deep blue colour.

- Next add 0.5 to 1.0 ml of starch solution to the liquid below curd. A blue purple colour will be formed if hypochlorites and/or chloramines are present.

(xx) Detection of hypochlorite [2]

Reagent(s)

Stannous chloride solution: 0.025% (w/v) in 73.5% sulphuric acid (1 volume of distilled water + 3 volumes of concentrated sulphuric acid).

Procedure

- Cool 3 ml of milk in a test tube to 2-5ºC.
- In another tube, take an equal volume of the stannous chloride solution, similarly cool, and add to milk.
- Gently shake the tube while in the freezing mixture for 3 minutes.
- Pour in to a 12.5 ml centrifuge tube and centrifuge for 3 minutes at 2500 rpm.

Interpretation

A yellow-green colour indicates the presence of hypochlorite.

(xxi) Detection of quarternary ammonium compounds (QAC, detergent) in milk [1]

Reagent(s)

- *Indicator solution*: Prepare a stock solution by dissolving 0.05 g eosin in 100 ml acetone. Shake 10 ml of stock solution with 90 ml of tetrachloroethane and 1 g citric acid and filter before use.
- *Buffer*: Dissolve 25 g citric acid in 100 ml distilled water and adjust to pH 3.5 with 50% sodium hydroxide solution

(approximately 15 ml of sodium hydroxide solution is required).

Procedure

- To a centrifuge tube add 1 ml milk, 5 ml distilled water, 1 ml indicator solution and 0.2 ml buffer and shake vigorously for 10 seconds.
- Centrifuge for 5 minutes at 3200 rpm.
- Observe the colour of the bottom layer.

Interpretation

If QAC is present, the bottom layer assumes a red or pink colour.

Note

The method detects presence of about 5 mg/kg of the QAC in milk. Samples containing about 1 mg QAC/kg of sample show a faint pink colour, which may not be clear and conclusive.

(xxii) <u>Detection of formalin in milk</u> [(2)]

Principle

Formaldehyde produces purple colour with sulphuric acid in presence of traces of ferric chloride.

Reagent(s)

Gerber Sulphuric acid: Specific gravity 1.807-1.812 at 27ºC, colourless.

Ferric chloride solution: 10% (w/v) solution in distilled water.

Procedure

- Take 5 ml milk sample in a graduated test tube and add 5 ml

of distilled water.

- Add 1 drop of ferric chloride solution to 10 ml Gerber sulphuric acid in another test tube.
- Gently pour the acid carefully down the side of the test tube with milk-water mixture so that it forms a layer at the bottom without mixing with milk.
- Observe the colour of the junction between the two layers formed.

Interpretation

Formation of violet or blue ring at the junction indicates formaldehyde is present in milk. The test detects about 1 ml of 40% formaldehyde solution in 100 litres of milk, that is, about 10 ppm of 40% formaldehyde can be detected.

A green or brown colour indicates formaldehyde is not present.

(xxiii)<u>Detection of hydrogen peroxide in milk</u>

(a) ***Vanadium pentoxide test*** [1]

Reagent(s)

Vanadium pentoxide reagent: Dissolve 1 g vanadium pentoxide in 100 ml of diluted sulphuric acid (94 ml distilled water + 6 ml sulphuric acid).

Procedure

- To 10 ml of sample in a test tube add 10-20 drops of the reagent and mix.
- Observe the colour of the solution.

Interpretation

The development of pink or red colour indicates presence of

hydrogen peroxide.

Note
Hydrogen peroxide is removed from milk when it is heated/pasteurized or stored for a long period. The test may not detect hydrogen peroxide in such milks.

(b) Paraphenylenediamine test [2]

Reagent(s)
Paraphenylenediamine reagent: 2% (w/v) solution in distilled water, freshly prepared.

Procedure
- Take about 2 ml of milk sample in a test tube.
- Add 5 drops of the reagent and mix.
- Observe the colour of the solution.

Interpretation
The development of deep blue colour indicates presence of hydrogen peroxide.

Note
Hydrogen peroxide is removed from milk when it is heated/pasteurized or stored for a long period. The test may not detect hydrogen peroxide in such milks.

(xxiv) <u>Detection of boric acid and borates in milk</u> [1, 3]

Principle
Boric acid and its salt produce red colour on reaction with turmeric.

Reagent(s)
- *Turmeric paper (dried)*: Take 1.5-2.0 g turmeric powder in a

250 ml conical flask and add 100 ml 80% (v/v) ethyl alcohol. Shake for 5 minutes and filter. Collect the filtrate in a flat bottom dish. Dip sheets of Whatman no. 2 filter papers in the clear filtrate. Remove the papers and hang on dry in air. After 1 hour, cut the papers into 6 x 1 cm strips (or other convenient size) and store in tightly stoppered bottle protected from light.

- *Concentrated hydrochloric acid (specific gravity 1.16)*
- *Ammonium hydroxide (specific gravity 0.88)*
- *Caustic soda or lime water*

Procedure

- Make about 25 ml milk sample strongly alkaline with lime water or caustic soda and evaporate to dryness on a water bath.
- Ignite the residue on a low red heat to destroy organic matter. Let it cool.
- Mix about 25 ml distilled water, add concentrated hydrochloric acid drop by drop until the ignited residue is dissolved. Add approx 1 ml in excess.
- Saturate a piece of turmeric paper with this solution and allow it to dry.
- Observe the colour change in paper.

Interpretation

The colour of the paper turning red indicates the presence of boric acid or borate.

(xxv) Detection of salicylic acid [1]

Reagent(s)

- *Diluted hydrochloric acid*: Mix 3 volumes of distilled water

+ 1 volume of concentrated hydrochloric acid.
- *Ethyl ether*
- *Petroleum ether (boiling below 60ºC)*
- *Ferric chloride solution*: 0.5% (w/v), neutral

Procedure
- Place 50 ml of sample in a separating funnel. Add 5 ml of diluted hydrochloric acid and extract with 50 ml ethyl ether. If mixture emulsifies, add 10-15 ml petroleum ether and shake. If this treatment fails to break emulsion, centrifuge or let stand until considerable portion of aqueous layer separates.
- Drain the aqueous layer and shake vigorously and again let separate and drain the aqueous layer.
- Wash ether layer with two 5 ml portions of distilled water.
- Evaporate ether in a porcelain dish.
- Add 1 drop of neutral ferric chloride solution.
- Observe the colour of the solution.

Interpretation
A violet colour indicates presence of salicylic acid.

B. METHODS PROVIDED BY THE BUREAU OF INDIAN STANDARDS

The BIS has provided methods for detection of adulterants in milk, which are largely same as provided by the PFA Rules, details of which are provided in the previous section. There are, however, some additional methods provided by the BIS for this purpose, which are listed in the Table below.

Table 2: Additional Methods for Detection of Adulterants in Milk and Milk Products Provided by the Bureau of Indian Standards (BIS) [6, 7, 8]

S. No.	Method	Reference
1.	Detection of vegetable oils/foreign fat in milk using modified Gerber method and butryo-refractometer reading.	IS 1479 (Part I): 1960 [6]
2.	Test for benzoic acid in milk.	
3.	Detection of neutralizers in milk – Test for total alkalinity of ash.	
4.	Detection of boric acid and borates in milk – Glycerol test	IS 1479 (Part II): 1961 [7]
5.	Detection of formaldehyde in milk – Leach test, chromotropic acid test, and phenylhydrazine hydrochloride/ferric chloride test	
6.	Detection of salicylic acid in milk – Jorissen test	
7.	Detection of nitrates in milk	
8.	Detection of starch in milk products like *khoya*, butter and cheese	IS 15642 (Part 2): 2006 [8]
9.	Detection of vanaspati in ghee and butter through detection of presence of sesame oil (Baudouin test)	
10.	Detection of mashed potatoes and other starches in ghee/butter	

REFERENCES

1. DGHS (2005). Manual of Methods of Analysis of Foods – Milk and Milk Products. Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India.
2. FAO (2009). Milk Testing and Payment Systems Resource Book – A Practical Guide to Assist Milk Producer Groups. FAO, Rome. Pp. 38-43.
3. Roy, N.K. and Sen, D.C. (1994). Rapid Analysis of Milk. In Textbook of Practical Dairy Chemistry. Kalyani Publishers. New Delhi. Pp. 85-118.
4. Darshan Lal, Raman Seth, Rajan Sharma and Arun Kumar (2005). Approaches for detection of Adulteration in Milk Fat – An Overview. Indian Dairyman, 57, 10. Pp. 31-43.
5. Gunnery, K.S. (1979). Additives in Milk and Their Detection. Indian Dairyman, 31, 9. Pp. 665-669.
6. BIS (1960). Indian Standard – Methods of Test for Dairy Industry. Part I Rapid Examination of Milk (IS 1479 (Part I): 1960)
7. BIS (1961). Indian Standard – Methods of Test for Dairy Industry. Part II Chemical Analysis of Milk (IS 1479 (Part II): 1961)
8. BIS (2006). Indian Standard: Quick Methods for Detection of Adulterants/Contaminants in Common Food Products – Chemical Methods (IS 15642 (Part 2): 2006)

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NEWS SECTION

Indian Food Laws

- **Notification No.1-54/08-MMPO/FSSAI. ADVT-III/4/Exty./187-O/09 of 4 December 2009 of the Ministry of Health and Family Welfare (Food Safety and standards Authority of India):** It provides the regulations 'Food Safety and Standards Authority of India (Milk and Milk Products) Amendment Regulations, 2009'. These replace the Milk and Milk Products Order (MMPO), 1992. The major changes are:
  - The fee (non-refundable) for registration of new plant or expansion/modification of the existing plant is Rs.5,000/- (Rupees Five Thousand Only). The demand draft to be drawn in favour of the senior Accounts Officer, Food Safety and Standards Authority of India payable at New Delhi; and,
  - The Chief Executive Officer of the Food Safety and Standards Authority of India is the Controller under the 'Food Safety and Standards Authority of India (Milk and Milk Products) Regulations'.

Codex Alimentarius Commission (CAC)

Meetings of the following Codex Committees are scheduled during March-April 2010:

→ Codex Committee on Food Import and Export Inspection and Certification Systems, 1-5 March 2010, Queensland, Australia

→ Codex Committee on Methods of Analysis and Sampling, 8-13 March 2010, Budapest, Hungary
→ Codex Committee on Food Additives, 15-19 March 2010, Beijing, China
→ Codex Committee on General Principles, 12-16 April 2010, Paris, France
→ Codex Committee on Pesticide Residues, 19-24 April 2010, Xian, China
→ Codex Committee on Contaminants in Foods, 26-30 April 2010, Izmir, Turkey

International Dairy Federation (IDF)

IDF has published the following Bulletin/Standards recently:

Bulletins

- IDF Bulletin No.440/2009: Interlaboratory Collaborative Study on the Kjeldahl Reference Method for Nitrogen Determination in Sheep and Goat Milk According to ISO 8968-1/2 | IDF 20-1/2
- IDF Bulletin No.441/2009: Monitoring success of paratuberculosis programs Proceedings of 2nd Paratuberculosis Forum, Minneapolis, August 2009.

Standards

- IDF/RM 216 - ISO/TS 27105: Milk and milk products - Determination of hen's egg white lysozyme by HPLC
- IDF/RM 217 - ISO/TS 27106: Cheese - Determination of nisin A content by LC-MS and LC-MS-MS

For purchasing the IDF publications, the following may be contacted:

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The following ISO/IDF standards will be withdrawn from 1st March 2010:

- ISO NWIP N729/IDF 27:64 - Determination of the ash content of processed cheese products
- IDF 51B:91 - Processed cheese products – Calculation of content of added phosphate expressed as phosphorus
- IDF 87:79 - Instant dried milk – Determination of the dispersibility and wettability
- IDF 102:89 - Dried milk – Guidelines for the detection of neutralizers
- ISO 1546:81 - Procedure for milk recording for cows
- ISO 2449:74 - Milk & liquid milk products – Density hydrometers for use in products with a surface tension of approximately 45 mN/m

- ISO 3594:76/IDF 54:70 - Milk fat – Detection of vegetable fat by gas-liquid chromatography of sterols
- ISO 5542:84/IDF 98A:85 - Milk – Determination of protein content – Amido black dye-binding method (Routine method)
- ISO 6092:80/IDF 81:81 - Dried milk – Determination of titratable acidity (Routine method)
- ISO 6735:85/IDF114:82 - Dried milk – Assessment of heat class – Heat number reference method
- ISO 7586:85/IDF 112A:89 - Butter – Determination of water dispersion value

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Issues of *Technews* during 2009

Issue	Month	Theme
78	Jan-Feb	Somatic Cells in Milk
79	Mar-Apr	*Trans*-Fatty Acids and Cholesterol in Milk and Dairy Products
80	May-Jun	New Codex Standards Relevant to Dairy Industry
81	Jul-Aug	Codex, WTO and IDF
82	Sep-Oct	Greenhouse Gas Emissions
83	Nov-Dec	Methods for Detection of Common Adulterants in Milk and Milk Products

METHODS FOR DETECTION OF COMMON ADULTERANTS IN MILK AND MILK PRODUCTS

I find this bulletin:

Useful	☐	Informative	☐
Only entertaining	☐	Boring	☐

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I would like information in any subsequent issue on _____

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