Manual of
Simple methods for testing
of common adulterants in food
(Suitable for mobile food testing labs & school/college laboratories)
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I. General Laboratory Rules and Personal Safety Precautions

- Conduct yourself in a responsible manner at all times in the laboratory.
- Follow all written and verbal instructions.
- Do not smoke, eat or drink in the mobile lab. This includes chewing gum.
- Know the location and use of the fire extinguisher, Eye wash station and first aid kit in the van.
- **Wear a long-sleeved lab coat or apron** - The laboratory coat provides protection for the arms and body. It should be washed regularly and whenever they become contaminated with chemicals.
- **Suitable footwear** to be worn to protect the feet and leg from chemical spill. The wearing of open-toed shoes or sandals or slippers (chappals) is not permitted, as these make the feet extremely vulnerable to injury from broken glass, spilt corrosive substances such as acid ad alkali. Loose fitting sandals, especially those with no heel restraint, are not secure and may present a tripping hazard. **Closed shoes which cover the whole foot must be worn**
- **Loose long hair, dangling jewellery and loose baggy clothing** are a danger to personal safety. Long hair must be tied back or secured in a cap, and dangling jewelry and baggy clothing must be secured.
- **Wear Gloves** to protect the hands against one or more of a range of different hazards, which include acids, alkali, organic solvents, boiling water etc. Dispose used gloves in the disposal bin before leaving the laboratory.
- **Wear safety goggles for Eye protection** when doing laboratory tests. Wearing of contact lenses while performing laboratory experiments is not advised but is not prohibited.
- If corrosive chemicals or liquids come in contact with the skin or clothing, wash with copious amounts of water for an extended period of time.
- **Handling glassware**: Glassware must always be handled carefully. Examine glassware before each use. Never use chipped, broken, cracked, or dirty glassware.
- **Handling chemicals**: Do not taste, or smell any chemicals or food samples under testing.
- Pipetting liquids or solutions by mouth is strictly prohibited; use a pipette pump, syringe or a mechanical dispenser.
- Never deliberately taste, swallow or inhale any chemical.
• **Handling acids:** When diluting concentrated acid or base always add the concentrated acid or base to water (never the reverse), while stirring the solution. Be very careful with $\text{H}_2\text{SO}_4$, HCL and $\text{HNO}_3$ acid.

• Use a Pasteur pipette/dropper for adding acid. Avoid spilling acid on hands and skin. Skin contact with concentrated acid results in severe irritation and burns. If acid is spilled onto the body, wash the acid off with generous amounts of water for 15 min using the safety shower.

• Keep hands away from face, eyes, mouth, and body while using chemicals or lab equipment.

• Do not immerse hot glassware directly in cold water. The glassware may shatter.

• Never look/peer into a container that is being heated.

• When pouring reagents, hold the bottle such that the label points upwards facing the palm of the hand. The accumulation of reagent on bottle lip may be removed by touching the bottle lip to the rim of the receiving vessel.

• Always wash your hands with soap and water before touching other parts of your body (especially the area around the eyes), after performing the tests and before taking food.
II. Milk and Milk Products

The key motive for adulteration of milk and milk products, a very common problem, is for deriving undue economic benefits. Adulteration includes masking the poor quality of product by increasing the fat and/or solids not fat (SNF) content. The complex colloidal mixture of several constituents in milk, the high-water content and opacity, render milk as a commodity in which adulterants are not visible and therefore easy to adulterate. The fraudulent practice of adulterating milk as well as preparing synthetic milk, with some of the common practices being addition of water along with starch of skimmed milk powder, removal of fat etc. is rampant among the milk vendors. Synthetic milk, which is mixed with natural milk, contains hazardous chemicals like urea, laundry detergents, pulverized soap, boric acid, hydrogen peroxide, starch and neutralizers (caustic soda or sodium hydroxide, sodium carbonate and sodium bicarbonate. The consumption of such adulterants leads to various health hazards. There are several methods documented for detection of adulteration in milk and milk products. The methods elaborate below are simple, rapid and sensitive methods to detect adulteration that can be carried by college students in their chemistry laboratories.

1. Sample Handling of Milk and Milk Products
   i. Preparation of milk sample for analysis

   Empty the entire sample into a beaker and the temperature to 37-40°C by keeping it in a water bath maintained at 40-45°C. Stir gently and then mix sample thoroughly by pouring back into the bottle and mixing to dislodge any residual fat/cream adhering to the walls of the container. Empty the contents back in the beaker. Avoid vigorous mixing of sample. Allow the sample to come to room temperature (26-28°C) and withdraw immediately for analysis. If small clots or lumps are observed in the sample which cannot be dispersed, a few drops of liquor ammonia may be used during homogenization. If even after homogenization the sample shows lumps or clots or droplets of oil are visible suggestive of curdling/splitting of milk, the sample should be deemed unfit for analysis and rejected.

   ii. Preparation of milk products for analysis

   **Ghee and Butter:** Mix the sample in the container in which it is received until homogenous. Place the container in a water bath at a temperature not more than 50°C till completely melted. Filter through fluted filter paper.

   **Cream:** Warm the cream to 50°C Mix it thoroughly and take a representative sample
Dahi or Curd: Dahi or curd should be rendered homogenous by using a thick glass rod or a wire whisk
Khoa: homogenized by grinding to a fine powder in a mortar and pestle or grinder

2. Detection of Added Water in Milk
   a. Glass Plate Method

Safety precautions: Take care while handling the glass plates to avoid any injury

Apparatus
   i. Smooth (polished) glass plates or microscopy slides
   ii. Pasteur pipettes/Dropper

Procedure
   i. Using the dropper, pipette 1-2 ml of the sample
   ii. Place a drop on the surface of a vertically placed glass slide/plate
   iii. Observe the flow of the sample.

Inference
   i. If the drop of milk stays either on the top or moves slowly leaving a white trail behind the milk is not adulterated with water.
   ii. If the milk sample readily flows down, leaving no trail, it is adulterated with water.

Caution: This test is not suited to test the adulteration of skimmed milk (milk from which fat has been removed) with water.

   b. Lactometer Procedure

Safety precautions: Handle the lactometer and mercury thermometers with care. Mercury is very toxic and is very difficult to dispose if thermometer breaks. A broken mercury thermometer requires special cleaning procedures.

Apparatus
   i. ISI Lactometer calibrated at 27 °C
   ii. Thermometer
   iii. Measuring cylinder (250 or 500 ml)
   iv. Chemwipes

Procedure
   i. Mix the milk sample gently and pour it gently into a clean and dry measuring cylinder (250-500 ml) along the sides of the jar to avoid the incorporation of air.
ii. Clean the lactometer with water and wipe dry
iii. Lower the lactometer gently into the milk holding it by the stem with the bulb going first.
iv. Make sure that the lactometer floats freely without touching the sides of the jar.
v. Released when it is approximately in its position of equilibrium.
vi. Add milk to brim of cylinder
vii. As soon as the lactometer is at rest, read and record reading on the scale corresponding to the top of the meniscus (top surface) of the milk.
viii. The bulb of the lactometer shall not touch the sides of the measuring cylinder.
ix. Repeat the reading after depressing the lactometer about 3 mm and allowing it to come to rest
x. Note the temperature of milk with the help of the thermometer

**Inference**

i. At 27 °C the normal specific gravity of the milk ranges from 1.028 to 1.033. Below the value indicate the possible addition of water to the milk.

**Caution:** Always read the temperature of the milk first; the lactometer reading varies according to temperature. If the temperature of the milk is different from the calibration temperature of the lactometer, apply a correction factor.

### 3. Detection of Added Starch and Cereal Flours

**Safety precautions:** Be careful as you carry out the test because the iodine solution can stain skin and clothes. Prepare iodine solution in a fume hood.

**Apparatus**

i. Hard glass test tubes
ii. Bunsen Burner

**Reagents**

i. 1% iodine solution: Prepare in a fume hood. Dissolve 2 g of potassium iodide in 20 ml of distilled water; add 1 g of iodine; stir to dissolve then dilute to 100 ml. Store in a dark brown bottle with a dropper cap.

**Procedure**

i. Take about 3 ml of well mixed milk in a test tube.
ii. Boil the milk over a burner.
iii. Cool to room temperature (25±2°C)
iv. Add 2-3 drops of 1% iodine solution
v. Observe the color

**Inference**

i. Appearance of blue or bluish black color which disappears on boiling indicates the milk is adulterated with either starch or cereal flours.

ii. Iodine will remain brown in color, if starch is absent.

iii. The limit of detection is 0.02%

4. Detection of Cellulose in Milk and Milk Products

**Safety precautions**: Be careful as you carry out the test because the iodine solution can stain skin and clothes. Prepare iodine solution in a fume hood.

**Apparatus**

i. Nylon cloth

ii. Spotting plate

**Reagents**

i. Iodine solution: Weigh 1.5 g of iodine and 3 g of potassium iodide. Dissolve in a sufficient quantity of distilled water and make up the volume to 60 ml.

ii. Iodine – zinc chloride reagent: Dissolve 20 g zinc chloride in 8.5 ml distilled water and cool. Add the prepared iodine solution drop by drop until iodine begins to precipitate.

**Procedure**

i. To 10 g of milk or milk product add 50 ml of hot distilled water and stir thoroughly for about 2 minutes.

ii. Pour the mixture on a nylon cloth and allow liquid to flow through.

iii. Wash the residue on the cloth with 50 ml of hot distilled water two times.

iv. Scrape the residue with a spatula and place on a spotting plate at two different places.

v. Stain one with iodine - zinc chloride reagent and the other with iodine solution.

vi. Observe the color change in both the parts.

**Inference**

i. Development of blue color with iodine - zinc chloride reagent and absence of blue color in iodine solution, confirms presence of cellulose.

ii. Absence of blue color with iodine-zinc chloride reagent indicates absence of cellulose.

*Note*: Test can be applied to curd, rabri, evaporated milk etc. Test is not applicable if sample tests positive for added starch with 1% iodine
5. Detection of Added Cane Sugar (Sucrose)

Safety precautions: Take care while handling Concentrated HCl. It can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

Apparatus
i. Hard glass test tubes
ii. Water bath

Reagents
i. Resorcinol solution (0.5%): Weigh 0.5 g of resorcinol (should be white in g) and dissolve in 40 ml of distilled water. Add 35 ml of concentrated HCl (11.6 M) carefully along the sides and make up the volume to 100 ml using distilled water.

Procedure
i. Take 1 ml of milk in a test tube.
ii. Add 1 ml of Resorcinol solution and mix gently.
iii. Place tube in a boiling water bath for 5 min.
iv. Observe the color of the milk

Inference:
   i. The appearance of a deep cherry red color indicates the presence of sucrose, or a ketose sugar.
   ii. No change in the color of milk indicates no sucrose (table sugar) added
   iii. The limit of detection for this method is 0.1% (w/v)

6. Detection of Added Glucose

Safety precautions: Take care while handling Phosphoric acid and NaOH pellets. NaOH is extremely corrosive and can cause severe skin burns and eye damage.

Apparatus:
   i. Hard glass test tubes
   ii. Large beaker or Erlenmeyer flasks
   iii. Boiling water bath

Reagents
i. 10% NaOH: Carefully weigh 10g of NaOH pellets and dissolve in 100 ml distilled water. Cool the solution and store only in polypropylene bottle.
**Note:** Glass containers should be completely avoided in the preparation and storage of NaOH solutions.

ii. 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 25±2 °C and make up to 500 ml. After sedimentation filter the reagent and store in dark brown glass bottle.

iii. Phosphomolybdic acid: Weigh 35 g ammonium molybdate and 5 g sodium tungstate in a large beaker or Erlenmeyer flask; add 200 ml of 10% NaOH solution and 200 ml water. Boil vigorously (20-60 min) so as to remove ammonia. Check removal of ammonia with the help of red litmus paper. Cool, dilute with water to about 350 ml. Add 125 ml concentrated Phosphoric acid (H₃PO₄) (85%) and dilute to 500 ml.

**Procedure:**

i. Setup the boiling water bath

ii. To 1 ml of milk sample in a test tube add 1 ml of modified Barfoed’s reagent.

iii. Heat the mixture for exactly 3 min in a boiling water bath.

iv. Rapidly cool under tap water.

v. Add 1 ml of phosphomolybdic acid reagent to the turbid solution.

vi. Observe the color

**Inference:**

i. Formation of deep blue color immediately after adding phosphomolybdic acid reagent indicates the presence of added glucose in the sample.

ii. A faint bluish color is observed due to the dilution of Barfoed’s reagent in a sample of pure milk.

7. Detection of Maltodextrin/Dextrin

**Safety precautions:** Handle Trichloroacetic Acid (TCA) with caution as it is a corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage

**Apparatus**

i. Whatman No 42 filter paper

**Reagents**

i. Trichloroacetic acid solution: 10% (w/v): Dissolve 10 g of TCA in 100 ml of distilled water. Note TCA is very hygroscopic

ii. Barium chloride solution: 2% (w/v): Weigh and dissolve 2 g of Barium chloride in 100 ml of distilled water
Procedure
i. Boil 20 ml of milk in a beaker, and cool.
ii. Coagulate the milk using 10% TCA solution.
iii. Filter through Whatman filter paper no. 42 and collect the filtrate.
iv. Add 2 ml barium chloride solution to the filtrate and mix.
v. Observe the color.

Inference
i. Appearance of blue color indicates the presence of maltodextrins.
ii. Absence of blue color indicates absence of maltodextrins

Detection of Added Urea

Safety precautions: Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

Reagents
i. DMAB reagent (1.6%, w/v): Dissolve 1.6 g para-dimethylamino benzaldehyde (DMAB) in 100 ml of 5% ethyl alcohol and add 10 ml concentrate HCl. The reagent is stable for one month.

Procedure
i. In a test tube mix 1 ml of the milk sample with 1 ml of DMAB reagent.
ii. Mix 1 ml of pure milk (known to be free from added urea) with 1 ml of DMAB reagent.
iii. Observe the color change in both the tubes

Inference
i. A distinct yellow color is observed in milk containing added urea.
ii. The control (pure milk) shows a slight yellow color due to presence of natural urea.
iii. Limit of detection for this method is 0.2% (w/v)

Detection of Ammonium Salts in Milk

Safety precautions: Take care while handling NaOH, which is extremely corrosive and can cause severe skin burns and eye damage. Avoid inhaling Sodium hypochlorite, as it can irritate nose, throat and eyes.
**Reagents**

- **i.** 2% NaOH (w/v): Weigh 2 g of NaOH pellets and dissolve it in 100 ml of distilled water. Cool and store in a plastic bottle.
- **ii.** 2% Sodium hypochlorite (v/v): Weigh 2 g of Sodium hypochlorite p and dissolve in 100 ml of distilled water.
- **iii.** Phenol 5% (w/v): Weigh 5 g of phenol and dissolve in 100 ml of distilled water. Store in a dark brown bottle.

**Procedure**

- **i.** Take 1.0 ml of milk in a test tube
- **ii.** Add 0.5 ml of 2% NaOH solution, 0.5 ml of 2% sodium hypochlorite solution and 0.5 ml of 5% phenol solution.
- **iii.** Heat for 20 seconds in boiling water bath.
- **iv.** Observe for color change.

**Inference**

- **i.** A bluish color, which turns deep blue, indicates the presence of ammonium compounds like ammonium sulphate. The color is stable for 12 hours
- **ii.** The development of pink color shows the absence of ammonium compounds.

**10. Detection of Added Sulphates in Milk**

**Safety precautions:** Handle Trichloroacetic Acid (TCA) with caution as it is a corrosive chemical and contact can severely irritate and burn the skin and eyes with possible eye damage

**Apparatus**

- **i.** Glass stoppered test tubes
- **ii.** Whatman No 42 Filter paper

**Reagents**

- **i.** 5% Barium chloride (w/v): Dissolve 5.0 g barium chloride (BaCl$_2$.2H$_2$O) in distilled water and make the final volume to 100 ml.
- **ii.** 24% Trichloroacetic acid (w/v): Weigh 24 g of TCA add distilled water to dissolve and make the final volume to 100 ml,

**Procedure**

- **i.** Take 10 ml of milk in a 50-ml stoppered test tube.
- **ii.** Add 10 ml of 24% TCA solution and mix.
- **iii.** Filter the coagulated milk using Whatman filter paper No 42
iv. Take 5 ml of clear filtrate in a clean test tube.

v. Add a few drops of 10% barium chloride solution.

vi. Observe for any visible precipitate/cloudiness in the tube

**Inference**

i. Formation of milky-white precipitate or cloudiness indicates the presence of added sulfates like ammonium sulfate, sodium sulfate, zinc sulfate and magnesium sulfate.

ii. A clear solution with no visible change indicates the absence of sulfates.

iii. The Limit of detection is 0.05%

11. Detection of Sodium Chloride in Milk

**Safety precautions:** Silver nitrate causes discoloration of skin

**Reagents**

i. Silver nitrate (AgNO₃) 0.1 N solutions: Dissolve 1.7g of AgNO₃ in distilled water and make up to 100 ml.

ii. Potassium chromate (K₂CrO₄) 10% (w/v): Dissolve 10.0 g of potassium chromate in water, and dilute with water to 100 ml.

**Procedure**

i. To 5.0 ml of milk add 1.0 ml of 0.1 N AgNO₃ solution

ii. Mix the content thoroughly

iii. Add 0.5 ml of 10% potassium chromate solution

iv. Observe the color of reaction

**Inference**

i. Appearance of yellow color indicates presence of dissolved chloride

ii. Appearance of brick red/chocolate brown precipitate indicates the absence of dissolved chloride in milk

iii. The limit of detection of this method is 0.02%.

12. Detection of Vanaspati (Hydrogenated Fat) in Milk

**Safety precautions:** Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

**Reagents**

i. 1% sucrose in Concentrated HCl: Dissolve one g of sucrose in 100 ml Concentrated HCl
Procedure

i. Extract fat from the milk sample by Rose-Gottlieb method as described below

ii. Add 1ml sucrose in HCl to extracted fat, shake and allow the mixture to stand for 5 min.

iii. The development of a pink color indicates the presence of sesame oil/vanaspati.

Rose Gottlieb Method

Apparatus:

1. Mojonnier fat extraction flask or any other suitable extraction tube (as per IS specification).
2. Cork or stopper of synthetic rubber unaffected by usual fat solvents.
3. Butyro Refractometer

Reagents

1. Ammonia solution, containing approximately 25% (m/m) of NH₃,
2. Ethyl alcohol (95%).
3. Diethyl ether, peroxide-free.
4. Petroleum ether, boiling range 40-60°C.

Procedure

Extract fat from the milk sample by Rose-Gottlieb method

1. Weigh accurately about 10 g of sample (liquid milk), transfer to extraction tube.
2. Add 1.25 ml of ammonia sp. gr. 0.91 (or an equivalent volume of a more concentrated ammonia solution may be used), mix and shake thoroughly.
3. Add 10 ml ethyl alcohol and mix again.
4. Add 25 ml of diethyl ether (peroxide free) stopper and shake vigorously for about a minute.
5. Then add 25 ml petroleum ether (boiling range 40– 60°C and shake again vigorously for about half a minute.
6. Let it stand until the upper ethereal layer has separated completely and is clear.
7. If there is a tendency to form emulsion, a little alcohol may be added to help separation of the layers.
8. Decant off the clear the real layer into a suitable vessel (flask, glass bowl, aluminium dish, etc.).
9. Dry the flask in an air oven at 102 ± 2°C for two hours, cool in a desiccator.
10. Take the B.R. reading at 40°C of the extracted fat and interpret. A correction of 0.55 is added to the observed B.R. reading for each degree above 40°C or subtracted for each degree below 40°C to get corrected B.R. reading of the sample

**Calculation**

Calculate the Corrected B.R. reading of isolated fat as follows:

\[
\text{Corrected B.R.} = \text{Observed B.R.} \times 1.08
\]

**Interpretation**

If the BR reading differs from the prescribed limit of variability (not more than 42 in case of non-cotton tract area and not more than 45 in case of cotton tract area), presence of foreign fat in the milk may be suspected.

**13. Detection of Nitrates (Pond Water) in Milk**

**Safety precautions:** Take care while handling Concentrated\(\text{H}_2\text{SO}_4\). Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

**Apparatus**

i. Test tubes  
ii. Pasteur pipettes/ Dropper

**Reagents**

i. Diphenylamine (2%, w/v, in \(\text{H}_2\text{SO}_4\) acid): Weigh 2 g of diphenylamine and dissolve it in concentrated \(\text{H}_2\text{SO}_4\) acid to obtain final volume of 100 ml.

**Procedure**

i. Take 2 ml of milk in a test tube.  
ii. Rinse the tube with the milk and drain the milk from the test tube.  
iii. Add two-three drops of the reagent along the side of the test tube.  
iv. Note the developed color.

**Inference**

i. A deep blue color on the walls of the tubes as well at bottom portion indicates the presence of nitrate in the milk sample.  
ii. Pure milk sample will not develop any color.  
iii. The limit of detection for this is 0.2% (w/v) of KNO\(_3\)
14. Detection of Neutralizers in Milk: Rosalic Acid Test

**Apparatus**
i. Test tubes

**Reagents**
i. Rosalic acid solution (1% w/v): Weigh 1g of Rosalic acid powder and dissolve it in 30 ml ethyl alcohol and make up the volume with distilled water to a final volume of 100 ml.

ii. Ethyl alcohol (95%): Take 95 ml of ethyl alcohol in a 100 ml volumetric flask and make the volume up to the mark with distilled water and mix well.

**Procedure**
i. Take 5 ml milk in a test tube.

ii. Add 5 ml 95% ethyl alcohol and mix well.

iii. Add of 2-3 drops of Rosalic acid solution.

iv. Note the developed color

**Inference**
i. The appearance of a rose-red color indicates the presence of neutralizer

ii. A brown color develops for pure milk

iii. The limit of detection of method is 0.1% for sodium hydroxide, 0.1% for Sodium carbonate and 0.2% for sodium bicarbonate.

15. Detection of Detergents in Milk

**Apparatus**
i. Pasteur pipettes/ Dropper

**Reagents**
i. Bromocresol purple (0.5%): Dissolve 0.5 g of Bromocresol purple in distilled water and make up to 100ml

**Procedure**
i. Take 5 ml of the milk sample test tube.

ii. Add 0.1 ml (1 to 2 drops) of Bromocresol purple solution.

iii. Observe color of the milk

**Inference**
i. A violet to purple color indicates presence of detergent in milk.

ii. A faint violet color indicates absence of detergent in milk.
16. Detection of Pulverised Soap in Milk

**Reagents**

i. Phenolphthalein indicator solution (1%): Dissolve 1 g of phenolphthalein in 100 ml of rectified spirit.

**Procedure**

i. Take 10 ml of milk in a test tube
ii. Add 10 ml of hot water followed by 2-3 drops of phenolphthalein indicator.
iii. Observe the color change of milk

**Inference**

i. Development of red/pink color denotes the presence of soap in milk
ii. No color change observed in pure milk.

17. Test for Skimmed Milk Powder in Natural Milk

**Safety precautions:** Take care while handling Concentrated HNO₃. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

**Apparatus**

i. Pasteur pipettes/ Dropper

**Reagents**

i. Concentrated HNO₃

**Procedure**

i. Take 5 ml of milk in a test tube
ii. Add concentrated HNO₃ drop by drop using a Pasteur pipette or an eye dropper.
iii. Look for the development of yellow or orange color.

**Inference**

i. An orange color indicates the presence of skimmed milk powder
ii. A yellow color indicates the absence of skimmed milk powder

18. Detection of Gelatin in Milk

**Safety precautions:** Take care while handling Concentrated HNO₃. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents. **Dry picric acid is very, very dangerous**
Apparatus
i. Whatman Filter paper

Reagents
i. Stokes reagent (Acid Mercuric nitrate (Hg (NO₃)₂)). Mercury dissolved in two times its weight of concentrated HNO₃. This solution is diluted 25 times to its volume with water
ii. Saturated aqueous picric acid solution: Add 1.5 g wet picric acid to 100 ml distilled water and stirs it overnight. This is an excess of ~0.1 g.

Note: Picric acid is shipped wet and absolutely must stay wet. Dry picric acid is very, very dangerous! Check the hydration of picric acid as part of regular laboratory inspection and add distilled water if needed to maintain a wet paste (minimum 10% water by volume).

Procedure
i. Take 10 ml of sample in a large test tube or conical flask
ii. Add 10 ml Stokes reagent
iii. Shake mixture, add 20 ml water, shake again, let stand 5 minutes and filter.
iv. If much gelatin is present, filtrate will be of pale scent and a clear filtrate cannot be obtained
v. To 5 ml of filtrate in test tube add an equal volume of saturated aqueous picric acid solution.
vi. Observe the color and type of the precipitate formed

Inference
i. Finely divided yellow crystalline precipitate in suspension is produced in the presence of considerable amount of gelatin. Smaller amount of gelatin is indicated by cloudiness.
ii. Absence of yellow precipitate or cloudiness indicates the absence of gelatin

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

19. Detection of Preservatives added to Milk
a. Formalin in Milk
   - Hehner’s Procedure

Safety precautions: Take care while handling Concentrated H₂SO₄. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

Reagents
   - Gerber H₂SO₄ acid: Specific gravity 1.807-1.812 at 27°C, colorless corresponding to a concentration of H₂SO₄ acid from 90 to 91 percent by mass. Take 10 ml of distilled water in a Pyrex flask kept in a basin of ice cold water. Carefully add the commercial H₂SO₄ acid in small quantities at a time keeping the container sufficiently cold. Mix gently.
   - Ferric chloride solution: 10% (w/v) solution in distilled water.

Procedure
   i. Take 5 ml milk sample in a graduated test tube and add 5 ml of distilled water.
   ii. Add 1 drop of ferric chloride solution to 10 ml Gerber H₂SO₄ acid in another test tube.
   iii. Gently add the acid carefully along the sides of the test tube to diluted milk- so that a layer is formed at the bottom without mixing with milk.
   iv. Observe the color formed at the junction between the two layers.

Inference
   i. Formation of violet or blue ring at the junction indicates formalin is present in milk.
   ii. A green or brown color indicates absence of formalin.
   iii. Limit of detection is 0.05% of formalin

Note: If H₂SO₄ is added from the top and not along the side of the test tube, it may burn the milk solids and affect the end result.
**Leach Test**

**Safety precautions:** Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

**Apparatus**

i. Water bath

**Reagents**

i. Ferric Chloride (10%, w/v): Weigh 10 g of ferric chloride and dissolve it in distilled water to obtain 100 ml.

ii. Concentrated HCl containing Ferric Chloride: Take 1 ml of 10% ferric chloride solution in a 500 ml volumetric flask and make up the volume using Concentrated HCl.

**Procedure:**

i. Take 5.0 ml of milk in a test tube.

ii. Add an equal volume of concentrated HCl containing ferric chloride solution.

iii. Place in a boiling water bath five minutes.

iv. Observe the color change of the solution

**Inference:**

i. Appearance of brownish pink color indicates the presence of formalin in the sample

ii. If the sample remain white indicates the absence of formalin

**b. Hydrogen Peroxide in Milk**

**Reagents**

i. Dissolve 2.0 g of p-phenylenediamine distilled water to obtain 100 ml solution i.e. 2% aqueous solution. Dissolution of p-phenylenediamine in water is difficult and requires thorough mixing. The solution will be pale (straw) yellow and should be freshly prepared.

**Procedure**

i. Take 2 ml of milk sample in a test tube.

ii. Add an equal volume of raw milk

iii. Add 5 drops of the reagent and mix.

iv. Observe the color of the solution.

**Inference**

i. The development of a deep blue color indicates presence of hydrogen peroxide.
ii. No color change indicates the absence of hydrogen peroxide.

iii. The limit of detection for this method is 0.025% (v/v) H₂O₂ in milk

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

High amount of H₂O₂ are known to inactivate peroxidase, it is always advisable to add to the sample an equal volume of raw unpreserved milk and to follow with addition of a few drops of a 0.2% solution of p-phenylenediamine. Under these circumstances a blue color will develop immediately if H₂O₂ has been added to the milk sample.

c. Boric Acid and Borate in Milk

**Safety precautions:** Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents. DO not inhale Ammonium hydroxide directly.

**Apparatus:**

i. Turmeric powder

ii. Ethanol

iii. Whatman Filter paper, No 2

iv. Concentrated HCl

v. Ammonium hydroxide (sp. gr. 0.88)

vi. Lime water or caustic soda

**Reagents**

i. Turmeric paper: Weigh 1.5 to 2.0 g of turmeric powder in 250 ml an Erlenmeyer flask and add 100 ml of 80% (v/v) ethanol. Shake for 5 min and filter. Collect the filtrate in a flat bottom dish. Dip Whatman filter paper Grade 2 in the clear filtrate. Using tweezers remove the paper and hang to dry in air. After 1 h, cut the paper into 6 × 1 cm strips and store in tightly stoppered dark brown bottle protected from light. Alternately these strips are available commercially.

**Procedure:**

i. Take 20 ml of milk in a porcelain dish and add 1.4 ml of Conc. HCl and mix it thoroughly.

ii. Dip a strip of turmeric paper in the acidified milk.

iii. Observe the color change in the turmeric paper
Inference

i. Appearance of a characteristic red color on the turmeric paper indicates the presence of boric acid or borax. The red color changes to dark blue green on adding ammonium hydroxide, but reappears on re-acidification with HCl.

ii. No change in the yellow color indicates the absence of boric acid/borates.

d. Benzoic and Sodium Benzoate in Milk

Safety precautions: Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents. DO not inhale Ammonium hydroxide directly.

Reagents

i. Dilute HCl-1:3 by volume. Add 25 ml of Concentrated HCl slowly to 75 ml of water cooled in an ice bath.

ii. Neutral Ferric chloride solution - 0·5 percent (w/v): Dissolve 0.5 g of Ferric chloride in distilled water

iii. Ethyl ether

iv. Ammonium hydroxide (sp gr 0·88).

Procedure

i. Acidify 100 ml of milk with 5 ml of dilute HCl. Shake until curdled.

ii. Filter and extract the filtrate with 50 to 100 ml of ether.

iii. Wash the ether extract layer with two 5-ml portions of water.

iv. Evaporate the greater portion of ether in a porcelain dish on a water-bath

v. Allow the remainder to evaporate spontaneously.

vi. Make alkaline by adding a few drops of ammonium hydroxide,

vii. Expel the excess of ammonia by evaporation,

viii. Dissolve the residue in a few ml of hot water.

ix. Add a few drops of the neutral ferric chloride solution.

x. Observe the color of the precipitate

Inference

i. A salmon pink colored precipitate indicates the presence of benzoic acid.

ii. No color change is observed in pure milk sample.
e. Detection of Salicylic Acid in Milk

Safety precautions: Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents. DO not inhale Ammonium hydroxide directly.

Apparatus
i. Separating funnel

Reagents
i. Dilute HCl-1:3 by volume. Add 25 ml of Concentrated HCl slowly to 75 ml of water cooled in an ice bath.
ii. Neutral Ferric chloride solution - 0-5 percent (w/v): Dissolve 0.5 g of Ferric chloride in distilled water
iii. Ethyl ether
iv. Petroleum ether (boiling below 60 °C)

Procedure
i. Acidify 100 ml of milk with 5 ml of dilute HCl in a separating funnel. Shake until curdled.
ii. Filter and extract the filtrate with 50 to 100 ml of ethyl ether.
iii. 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.
iv. Drain the aqueous layer and shake vigorously and again let separate and drain the aqueous layer.
v. Wash the ether extract layer with two 5-ml portions of water.
vi. Evaporate the greater portion of ether in a porcelain dish on a steam bath
vii. Allow the remainder to evaporate spontaneously.
viii. Add one drop of the neutral ferric chloride solution.
ix. Observe the color

Inference
i. A violet (amethyst) color indicates the presence of salicylic acid.
ii. No color change is observed in pure milk sample.
f. Detection of Mercuric Chloride

Safety precautions: Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents. DO not inhale Ammonium hydroxide directly.

Reagents

i. Dilute HCl: Dilute concentrated HCl 1:1 with distilled water.

ii. Stannous Chloride (15%) in dilute HCl: Weigh 15 g of Stannous chloride and dissolve in dilute HCl

Procedure

i. Acidify 100 ml of milk with 5 ml of dilute HCl. Shake until curdled.

ii. Filter and extract the filtrate with 50 to 100 ml of ether.

iii. Wash the ether extract layer with two 5-ml portions of water.

iv. Evaporate the greater portion of ether in a porcelain dish on a water-bath

v. Allow the remainder to evaporate spontaneously.

vi. Make alkaline by adding a few drops of ammonium hydroxide,

vii. Expel the excess of ammonia by evaporation,

viii. Dissolve the residue in 1-2 ml water. Filter if necessary.

ix. Transfer the solution to a test tube and add Stannous chloride solution and mix it simultaneously.

Inference

i. A silky white precipitate appears which turns grey on further addition of SnCl₂ solution confirms the presence of mercuric chloride in milk.

ii. No precipitate is visible in pure milk

20. Alkaline Phosphatase Test for Checking Efficiency of Pasteurization in Liquid Milk

Apparatus

i. UV-Visible spectrophotometer or colorimeter

ii. Timer

iii. Water bath set at 37 °C
Reagents

i. Carbonate Buffer solution: Dissolve 1.5 g of Sodium bi carbonate (NaHCO₃) and 3.5 g of Anhydrous Sodium Carbonate (Na₂CO₃) in water and make up to one litre. Store in a refrigerator and discard after 1 month.

ii. Buffered substrate solution: Weigh accurately 150 mg of disodium p—nitrophenyl phosphate (PNP) into a 100 ml measuring cylinder, add 5-10 ml carbonate buffer and stir to dissolve all PNP and make up to 100 ml with carbonate. The solution should be stored in refrigerator and protected from light. The solution should be gless. The solution must be discarded after one week.

Procedure

i. Pipette 5 ml of buffered substrate solution into two separate test tubes, stopper and bring the temperature to 37°C by incubating for 5 mins.

ii. Label one as test and the other as blank.

iii. Add 1 ml of test milk to tube labeled test and 1 ml of boiled and cooled test sample to the Blank.

iv. Shake and replace stopper, incubate at 37°C for 2 hrs.

v. Remove the tubes after 2 hrs and the content should be well mixed. Take the reading of test vs blank at 405 in a UV-Vis spectrophotometer.

vi. Create a standard curve (0-50 μg) at 405 nm using p-nitro phenol standard solution diluted with buffer. Compare the reading of the test sample with the curve and calculate the p-nitrophenol released.

Inference:-

i. The test is considered satisfactory if it gives a reading of 10 μg or less of p-nitrophenol per ml of milk. Properly pasteurized milk gives no discernible color.

ii. Reading greater than 10 μg released per ml of milk indicates pasteurization is not satisfactory.

Note: The sample of milk should be examined as soon as possible after arrival at the laboratory. If not examined immediately, store in refrigerator (3-5°C). The sample must be brought to room temperature (25 ±2 °C) before being tested.

21. Turbidity Test for Checking Efficiency of Sterilization in Liquid Milk

Apparatus

i. Conical flask, 50 ml.
ii. Graduated cylinder, 25ml.
iii. Whatman No. 12 or equivalent, 12.5 cm folded filter paper
iv. Water bath

**Reagents**

i. Ammonium sulphate AR ((NH₄)₂SO₄)

**Procedure**

i. Weigh 4g of (NH₄)₂SO₄ in a 50-ml Erlenmeyer and add 20 ± 0.5 ml of the sample at 25±2°C.
ii. Agitate the flask for about one minute until the (NH₄)₂SO₄ dissolves.
iii. Allow the solution to stand for at least 5 minutes.
iv. Filter through the pleated filter paper.
v. Collect 5 ml of the filtrate in the glass test tube
vi. Place the tube in a boiling water-bath.
vii. Examine the tube for turbidity by moving it before a source of light.

**Inference**

i. The milk is considered sterilized when the filtrate shows no turbidity.
ii. Turbidity indicates inadequate sterilization

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**22. Detection of Coloring Matter in Milk and Milk Products**

**a. Detection of Metanil Yellow**

**Safety precautions:** Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents. Diethyl ether is inflammable. Do not open or use near open flame.

**Reagents**

i. Diethyl ether
ii. Concentrated HCl

**Procedure**

i. To 10 ml milk in a test tube, add 10 ml diethyl ether and shake vigorously.
ii. Allow to stand.
iii. Presence of any color is indicated by yellow color of the ethereal layer
iv. Add a few drops of Concentrated HCl
v. Observe the color change in ether layer
Inference
i. No change in color of ether layer indicates the absence of Metanil yellow
ii. Pink to dark red color indicates the presence of Metanil yellow

b. Detection of Annato in Milk

Reagents
i. Sodium bicarbonate
ii. 40% Stannous chloride: Weigh 4 g of Stannous chloride and dissolve in 100 ml distilled water

Procedure
i. Add sodium bicarbonate to milk to make it alkaline.
ii. Immerse a strip of filter paper for 2 hours in the milk.
iii. Yellowish color observed on filter paper indicates the presence of annatto.
iv. Dry the paper carefully
v. Add a few drops of 40% stannous chloride
vi. Observe color of paper

Inference
i. Yellow color turning pinkish orange on adding Stannous chloride indicates presence of annatto
ii. No color change is observed for pure milk

c. Detection of Azo (Coal Tar Dyes) Dyes in Milk

Safety precautions: Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents. Diethyl ether is inflammable. Do not open or use near open flame.

Reagents
i. Concentrated HCl
ii. Diethyl ether

Procedure
i. Take 2 ml of milk in a test tube and add 3 ml of ether.
ii. Shake it thoroughly
iii. Add 1 ml of concentrated HCl.
iv. Shake well and allow it to stand.

Inference
i. Appearance of pink –to crimson red color indicates the presence of coal tar dyes

d. Coal Tar Dyes in Ghee, Butter, Khoa, Cheese, Condensed Milk, Milk Powder

Safety precautions: Take care while handling Concentrated HCl and H₂SO₄. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

Reagents
i. Concentrated HCl
ii. Dilute H₂SO₄

Procedure
i. Take one full teaspoon (2 ml) of melted sample in a test tube
ii. Add 5 ml of concentrated HCl or dilute H₂SO₄.
iii. Shake well and allow it to stand.
iv. Observe the color

Inference
i. Appearance of pink color with Dilute H₂SO₄ or crimson red color with concentrated HCl indicates the presence of coal tar dyes

23. Detection of Thickeners in Milk Products (Cream, Dahi, Khoa, Butter, Ghee and Cheese Etc)
a. Starch, Mashed Potato and Cereal Flours

Starch, mashed potato and cereals flours all contain Starch. Detection of starch using iodine solution infers adulteration.

Apparatus
i. Hard glass test tubes
ii. Bunsen Burner

Reagents
i. 1% iodine solution: Prepare in a fume hood. Dissolve 2 g of potassium iodide in 20 ml of distilled water; add 1 g of iodine; stir to dissolve then dilute to 100 ml. Store in a dark brown bottle with a dropper cap.
Procedure
i. Take about 2 g of milk product in a test-tube
ii. Add about 5 ml of water and boil for a few minutes
iii. In case of butter and ghee melt the sample.
iv. Cool to room temperature (25±2°C)
v. Add 2-3 drops of 1% iodine solution
vi. Observe the color
vii. The appearance of a blue color indicates the presence of starch

b. Gelatin

Apparatus
i. Whatman Filter paper

Reagents
i. Stokes reagent (Acid Mercuric nitrate (Hg (NO₃)₂)). Mercury dissolved in two times its weight of concentrated HNO₃. This solution is diluted 25 times to its volume with water
ii. Saturated aqueous picric acid solution: Add 1.5 g wet picric acid to 100 ml distilled water and stirs it overnight. This is an excess of ~0.1 g.

*Note*: Picric acid is shipped wet and absolutely must stay wet. Dry picric acid is very, very dangerous! Check the hydration of picric acid as part of regular laboratory inspection and add distilled water if needed to maintain a wet paste (minimum 10% water by volume).

Procedure
i. The presence of gelatin is detected by Stokes Test.
ii. Mix together 10 ml cream, 20 ml water and 20 ml of Stokes reagent.
iii. Filter the mixture
iv. To the 10 ml of filtrate add an equal volume of saturated picric acid solution.
v. A characteristic yellow precipitate is produced in presence of considerable amount of gelatin; smaller amounts are indicated by cloudiness as described in 18.

c. Blotting Paper in Rabdi

Safety precautions: Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.
Reagent
i. Concentrated HCl

Procedure
i. Take a teaspoon of rabdi (1g) in a test tube.
ii. Add 3 ml of Concentrated HCl and 3 ml of distilled water.
iii. Stir the content with a glass rod.
iv. Remove and examine the glass rod.

Inference
i. Presence of fine fibers to the glass rod will indicate the presence of blotting paper in rabdi.
ii. A clean glass rod indicates the absence of blotting paper

24. Detection of Vanaspati/Hydrogenated Edible Fat in Ghee

Safety precautions: Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents.

Reagent
i. Fuming Concentrated HCl
ii. Sugar (Sucrose)

Procedure
i. To 5 ml melted ghee in a test tube, add 5 ml conc. HCl
ii. Add half tea spoon table (cane) sugar.
iii. Stopper and shake vigorously for 2 minutes and allow the mixture to separate.
iv. The development of red (crimson) or pink color in acid layer indicates the presence of vanaspati/ sesame oil.
v. This is confirmed by adding 5 ml water and shaking again.
vi. If color in acid layer persists, sesame oil/vanaspati is added.

Inference
i. Persistent pink (crimson) color in acid layer indicates that vanaspati/sesame oil added
ii. Absence of pink color indicates absence of vanaspati

Note: The test does not differentiate between sesame oil and vanaspati. Some Coal Tar dyes also give the pink color with dilute HCl. If the test is positive ie the crimson g develops with adding Conc. HCl and without adding sugar, then the sample is adulterated with coal tar dyes.
References

- Methods for detection of common adulterants in milk and milk Products (2009),Technews, 83, 1-30
III. Edible Oils & Fats

Oils and fats are used as either cooking or frying oils, salad oils or in the formulation of processed foods such as infant foods, ready-to-cook and ready-to eat foods. Edible oils are fats are highly priced, which causes them to be adulterated with less expensive oils and fats for economic gain. Adulterations have become more sophisticated; therefore, it is imperative to advanced and suitable methods to detect the adulterants. Each oil and fat generally has a unique component. Their presence is used as a detection tool.

1. Detection of Rancidity in Edible Oil and Ghee

Safety precautions: Concentrated HCl is corrosive and can cause burns. Handle it with care. Use a dropper or Pasteur pipette to add acid.

Reagents

i. 0.1% phloroglucinol in diethyl ether solution: Weigh 100 mg of phloroglucinol and dissolve in 100 ml diethyl ether.

ii. Concentrated HCl.

Procedure

i. Take 3 ml of oil or melted fat in a test tube.

ii. Add 3 ml of concentrated HCl.

iii. Shake the tube carefully to mix up the contents thoroughly.

iv. Add 3 ml of 0.1% phloroglucinol solution in ether.

v. Shake the tube vigorously for 2 minutes and keep it aside.

vi. Examine the tube after half an hour.

Inference

i. A red or pink coloration in acid layer indicates that the oil/fat sample in rancid

ii. The absence of color development indicates the oil is not rancid

2. Detection of Argemone Oil

Safety precautions: Concentrated HNO₃ and its vapors are corrosive to the eyes, skin, and mucous membranes. Concentrated H₂SO₄ is highly corrosive. Contact with these acids can cause severe burns and permanent damage. Handle with care. Use a Pasteur pipette or automated pipetting device.
Reagents
i. 2% Salicylic acid in methanol: Weigh two grams of Salicylic acid and dissolved in methanol.
ii. Concentrated HNO₃
iii. Concentrated H₂SO₄

Procedure
i. Take five drops of the oil (or melted fat) in a dry test tube
ii. Add successively 1) 0.5 ml of 2% Salicylic acid solution, 2) 2 ml of Conc. HNO₃ and 3) Finally 2-4 drops of Conc. H₂SO₄.
iii. Shake well
iv. Observe the development of color (20-30 Sec)

Inference
i. A crimson red color within 20-30 sec indicates the presence of argemone oil
ii. No color change is observed in pure oil.

3. Detection of Cottonseed Oil

Safety precautions: Use safety goggles and hand protection when handling tubes in oil bath/brine bath.

Apparatus
i. Stoppered glass test tubes
ii. Water bath

Reagents
iii. Amyl alcohol
iv. Precipitated sulphur 1% w/v sol: Dissolve one gram of sulphur in 100 ml of carbon disulphide (CS₂)
v. Halphen’s solution: Mix equal volumes of amyl alcohol and Sulphur solution

Procedure
i. Take five ml of oil or melted fat in a glass stoppered test tube
ii. Add an equal volume of Halphen’s solution
iii. Mix thoroughly on a vortex.
iv. Heat gently in a water bath (70-80 °C) with occasional shaking till the CS2 has boiled off and foaming ceases.
v. Place in an oil-bath or brine bath maintained at 110-115 C
vi. Hold in bath for 2.5 h.

vii. Observe the color developed

Inference
i. A red color seen at the end of this period indicates the presence of cotton seed oil.

ii. No color development indicates the absence of cottonseed oil.

Note: The test is sensitive up to 0.5 % cottonseed oil. The test is positive for hempseed oil, kapok oil and oils containing cyclopropenoi d acids.

4. Detection of Mineral Oil

Safety precautions: Use safety goggles and hand gloves when handling boiling water to avoid burns.

Apparatus
i. Boiling water bath

ii. Test tubes

Reagent
i. Alcoholic potash: Dissolve 8.6g of KOH pellets in two ml of water. Then make up to 100 ml with aldehyde free alcohol

Procedure
i. Take 2 ml of oil sample and add an equal quality of Alcoholic Potash.

ii. Heat in boiling water bath (dip in boiling water) for about 15 minutes

iii. Add 10 ml of hot water.

iv. Look for turbidity

Inference
i. Any turbidity shows the presence of Mineral oil greater than 1%.

ii. The depth of turbidity depends on the percentage of mineral oil present

iii. A clear solution indicates the absence of mineral oil

Note: This method is not applicable on high content un-saponifiable value and below 1% content of Mineral oil.

5. Detection of Castor Oil in Edible Oil

Safety precautions: Concentrated H₂SO₄ is corrosive and can cause burns. Handle it with care. Use a dropper or Pasteur pipette to add acid. Petroleum ether is highly inflammable. Do not open or use near open flame.
Reagents
   i. Petroleum ether
   ii. Ammonium molybdate reagent: Dissolve 1.25g ammonium molybdate in 100 ml of Conc. H$_2$SO$_4$

Procedure
   i. Take 1ml oil in a dry test tube.
   ii. Add 10 ml of petroleum ether.
   iii. Shake vigorously for 2 minutes.
   iv. Add 1-2 drops of ammonium molybdate reagent

Inference
   i. Instantaneous development of turbidity indicates presence of castor oil.
   ii. Transparent solution indicates absence of castor oil.

6. Detection of Karanjia Oil in Edible Oil

Safety precautions: Chloroform is a carcinogen. Avid prolonged exposure to vapors of the solvent.

Reagents
   i. Antimony trichloride solution 20 % (m/v) in chloroform. Weigh 20 g antimony trichloride crystals and add to 100 ml chloroform. Shake for a few minutes till the crystals dissolve.
   ii. Chloroform

Procedure
   i. Take 1-2 drops of the Oil in a test tube.
   ii. Add 1-2 ml of antimony trichloride solution in chloroform, mix well.
   iii. Look for yellow or orange color.

Inference
   i. Appearance of a canary yellow or orange color indicates presence of Karanja oil
   ii. No color change indicates absence of Karanjia oil

7. Detection of Cyanide in Edible Oil

Safety precautions: Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume
hood. Always add acid to water when diluting or preparing reagents. Take a little amount (3 ml) of the sample in a test tube.

**Reagents**

i. Potassium Hydroxide (2 N): Dissolve 11.2 g of KOH in 100 ml of distilled water.

ii. Ferrous sulphate solution (2 %): Dissolve two g of Ferrous sulphate in distilled water.

iii. Ferric chloride solution (20 %): Dissolve 20 g of Ferric Chloride in water to which sufficient HCl has been added to prevent hydrolysis.

iv. Concentrated HCl

**Procedure**

i. Take 3 ml of oil

ii. Add 10 drops of 2N KOH and heat the tube on the flame.

iii. Add a few drops of ferrous sulphate solution and shake well

iv. Acidify with a few drops of HCl and warm gently

v. Filter if necessary

vi. Add a few drops of Ferric Chloride

vii. Observe the development of a blue color

**Inference**

i. A blue coloration indicates the presence of hydrocyanic acid which is produced due to presence of cyanide in edible oil.

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8. **Detection of Mobile (Lube) Oil in Edible Oil**

**Safety precautions:** Potassium hydroxide is corrosive and can cause burns

**Reagents**

i. Alcoholic Potassium Hydroxide Solution: Dissolve 70 to 80 g of potassium hydroxide pellets in an equal quantity of distilled water and add two litres of ethyl alcohol or aldehyde-free rectified spirit Allow to stand overnight decant the clear liquid and keep in a bottle closed tightly with a cork or rubber stopper.

ii. Dichloroquinol chlorimide Solution (Gibbs reagent): Dissolve one gram of 2, 6-dichloroquinone chlorimide in 200 ml of absolute ethyl alcohol. Store at 10 °C and use within 5 days

**Procedure**

i. Take 20 drops of edible oil in a test tube.

ii. Add 10 drops of Alcoholic potash.
iii. Heat the tube on the flame.
iv. The mixture will de-colourise.
v. Now add 10 drops of dichloroquinol chloride.
vi. Warm the tube.
vii. Look for the development of a blue color

Inference
i. The appearance of the blue color indicates the presence of a compound of triorthocrysyl phosphate. Traces of this compound in edible oil, point to adulteration of edible oil, with lube/mobile/engine oil.

9. Detection of Adulteration in Coconut Oil

Procedure
i. Place a small bottle of oil in a refrigerator (5-10 °C)
ii. Check after 60-90 minutes.

Inference
i. Pure Coconut oil solidifies completely with no separated layer
ii. If adulterated a separate layer of adulterant oil is visible
References

- IS: 548 (Part II ) 1976 Reaffirmed 2010 “Indian Standard Methods Of Sampling And Test For Oilsand Fats Part II Purity Test ( Third Revision)
- IS 15642 (Parts 1 and 2) :2006 (Reaffirmed 2011) Quick Methods for Detection of Adulterants/Contaminants in CommonFood Products
IV. Spices and Condiments

1. Detection of Lead Salts in Turmeric Powder

**Safety precautions:** Concentrated HCl is corrosive and can cause burns. Handle it with care. Use a dropper or Pasteur pipette to add acid.

**Apparatus**
- i. Pasteur pipettes/ Dropper

**Reagent**
- i. Concentrated HCl

**Procedure**
- i. Take one g of turmeric/chilli powder in a tube
- ii. Add 1 ml concentrated HCl.
- iii. Observe the change in color

**Inference**
- i. The appearance of magenta color shows the presence of yellow oxides of lead in turmeric powder

2. Detection of Lead Chromate in Turmeric Powder

**Safety precautions:** Concentrated H₂SO₄ is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid.

**Apparatus**
- i. Pasteur pipettes/ Dropper

**Reagents**
- i. 1:7 H₂SO₄: Add 10 ml of concentrated H₂SO₄ carefully to 70 ml of distilled water.
- ii. Diphenylcarbazide (0.2%): Weigh 200 mg of Diphenylcarbazide and dissolve in 100 ml of 95% alcohol.

**Procedure**
- i. Ash about 2 grams the sample.
- ii. Dissolve it in 4-5 ml of 1:7 sulphuric acid (H₂SO₄) and filter.
- iii. Add 1ml of 0.2% diphenylcarbazide.
- iv. Observe any change in color

**Inference**
- i. A pink color indicates presence of lead chromate.
3. Detection of Metanil Yellow in Turmeric Powder

**Safety precautions:** Concentrated HCl is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid.

**Apparatus**
- Pasteur pipettes/Dropper

**Reagent**
- Concentrated HCl

**Procedure**
- Take one gram turmeric powder in a test tube.
- Add 5 ml of water.
- Add a few drops of concentrated HCl.
- Observe any change in color.

**Inference**
- Appearance of pink/violet color which disappears on dilution with water shows the presence of unadulterated turmeric.
- If the color persists, indicates the presence of metanil yellow.

4. Detection of Aniline Dyes in Turmeric Powder

**Safety precaution:** Rectified spirit is flammable. Store appropriately.

**Reagent**
- Rectified spirit

**Procedure**
- Take one-gram turmeric powder in a test tube and add water to make a suspension. Add 1-2 ml of rectified spirit.
- Observe the color of the rectified spirit layer.

**Inference**
- Immediate separation of yellow color in the rectified spirit layer indicates the presence of dyes.

5. Detection of Chalk Powder in Turmeric Powder

**Reagent**
- Concentrated HCl
Procedure
i. Take about 1 g of turmeric powder in a test tube containing 2-3 ml of water.
ii. Add a few drops of concentrated Hydrochloric acid.

Inference
i. Effervescence indicates the presence of chalk powder.

6. Detection of Added Starch in Powdered Spices other than Turmeric Powder

Reagents
i. Iodine solution (1%): Prepare in a fume hood. Dissolve 2 g of potassium iodide in 20 ml of distilled water; add 1 g of iodine; stir to dissolve then dilute to 100 ml. Store in a dark brown bottle with a dropper cap.

Procedure
i. Add a few drops of Iodine solution to the spice powder in a petri dish.
ii. Observe the development of blue color if any

Inference
i. A blue color shows the presence of starch.

7. Detection of Brick Powder in Chilli Powder

Safety precaution: Chloroform and Carbon tetrachloride are suspect carcinogens. Avoid inhaling vapor and contact with eyes, skin and clothing. Use only with adequate ventilation.

Reagents
i. Chloroform-Carbon tetrachloride mixture: Mix equal volumes of the two solvents

Procedure
i. Take chloroform/Carbon tetra chloride mixture in a beaker
ii. Pour the sample in a beaker containing a mixture of chloroform and carbon tetrachloride.
iii. Observe the bottom of the beaker

Inference
i. Brick powder and dirt will settle at the bottom.

8. Detection of Added Color in Chilli, Turmeric and Other Curry Powders

Safety precaution: Concentrated H₂SO₄ is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid. Petroleum ether is highly flammable. Store away from open flame.
Reagent
i. 13N H$_2$SO$_4$: 88 ml of concentrated H$_2$SO$_4$ diluted to 250 ml with distilled water
ii. Petroleum ether

Procedure
i. Extract one gram of the sample with 3-4 ml petroleum ether
ii. Add 13N H$_2$SO$_4$ to the extract.
iii. Observe color of lower acid layer

Inference
i. Appearance of red color (which persists even upon adding little distilled water) indicates the presence of added color.
ii. If the color disappears upon adding distilled water, the sample is not adulterated.

9. Detection of Oil Soluble Color in Chilli Powder

Safety precaution: Concentrated HCl is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid. Petroleum ether is highly flammable. Store away from open flame.

Reagents
i. 50% HCl: Add 50 ml of concentrated HCl to 50 ml of distilled water.
ii. Petroleum ether

Procedure
i. Take 2 g of the sample in a test tube, add few ml of ether and shake.
ii. Decant ether layer into a test tube containing 2 ml of dilute HCl (50%) and shake.
iii. Observe color of the lower acid layer

Inference
i. Red color of the lower acid layer indicates the presence of oil soluble color.

10. Detection of Sudan Dye III in Chilli Powder

Safety precautions: Hexane is inflammable. Keep away from open flame.

Reagents
i. Acetonitrile reagent: Add 70 ml of acetonitrile to 30 ml of water and allow it to come to room temperature.
ii. Hexane

Procedure
i. Take one g of chilli powder in a test-tube
ii. Add 2 ml of hexane to it, and shake well.
iii. Allow it to settle.
iv. Decant the clear solution into another test tube.
v. Add 2 ml of acetonitrile reagent and shake well.
vi. Observe color of lower acetonitrile layer

**Inference**

i. The appearance of a red color in the lower acetonitrile layer indicates the presence of Sudan red III.

11. Detection of Rhodamine B in Chilli Powder

**Safety precautions**: Acetone is highly flammable solvent. Keep away from open flame. Store in appropriate cabinet

**Reagent**

i. Acetone

**Procedure**

i. Take 2 grams sample in a test tube
ii. Add 5 ml of acetone.
iii. Observe color of acetone layer

**Inference**

i. Immediate appearance of red color indicates presence of Rhodamine-B.

12. Detection of Papaya Seeds in Black Pepper

**Reagents**

i. Ethyl alcohol (97.2% (v/v))
ii. Iodine reagent: Dissolve 2 g of potassium iodide in 20 ml of distilled water; add 0.2 g of iodine; stir to dissolve then dilute to 100 ml with alcohol. Store in a dark brown bottle with a dropper cap.

**Procedure**

i. Float 15g seeds in a beaker containing 150 ml alcohol
ii. Stir the seeds with a glass rod.
iii. Mature black pepper berries will sink
iv. Spoon of all the floating berries and examine
v. Cut the seeds into two halves and examine visually
vi. Add a drop or two of iodine reagent
vii. Observe the developed color

Inference
i. A blue color indicates it is pepper due to the presence of starch
ii. A pale color indicates it is papaya, due to the presence of dextrin
iii. Papaya is a dicot; a thin line partition shows two cotyledons.
iv. Pepper is a monocot, the seed halves will show a central hole

13. Detection of Common Salt in Coriander Powder

Safety precaution: Silver nitrate solution causes skin to discolor

Reagent
i. Silver nitrate reagent (0.1 M): Weigh 1.7 g of Silver nitrate and dissolve in 100 ml of distilled water. Store in an amber colored bottle.

Procedure
i. Suspend one gram of the sample in water
ii. Add a few drops of silver nitrate reagent.
iii. Look for a white precipitate

Inference
i. A white precipitate indicates the presence of sodium chloride.

Note: Added water should be free from chloride

14. Detection of Chalk in Asafoetida

Safety precaution: Concentrated HCl is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid. Petroleum ether is highly flammable. Store away from open flame

Reagent
i. Dilute HCl: Dilute concentrated HCl, 1:1 with distilled water
ii. Carbon Tetrachloride

Procedure
i. Shake sample with CCl4
ii. Allow to settled down
iii. Decant the top layer
iv. Add dil. HCl to the residue
v. Effervescence indicates the presence of chalk in sample.
15. Detection of Colophon Residue in Asafoetida

Safety precautions: Concentrated $\text{H}_2\text{SO}_4$ is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid. Acetic Anhydride is a highly corrosive chemical and contact can severely irritate and burn the skin and eyes.

**Reagents**

i. Acetic anhydride
ii. Concentrated $\text{H}_2\text{SO}_4$

**Procedure**

i. Dissolve about 100 mg of asafoetida (or 0.5 g of compounded asafoetida) in 10 ml of acetic anhydride.
ii. Heat gently and then cool
iii. Add one drop of Concentrated $\text{H}_2\text{SO}_4$.
iv. Observe the color

**Inference**

i. A bright purplish-red color, rapidly changing to violet, indicates the presence of colophon is present.

16. Detection of Foreign Resins in Asafoedita

**Reagents**

i. Ferric chloride (6%): Dissolve 6 g of Ferric chloride in 100 ml of distilled water
ii. Rectified spirit

**Procedure**

i. Take 1 g of asafoetida, powder it thoroughly, and take it in a test-tube.
ii. Add some rectified spirit
iii. Filter/ decant the solution.
iv. To 5 ml of filtrate add few drops of ferric chloride (6%) solution.
v. Observe any color change

**Inference**

i. Olive green color shows the presence of adulteration with other resins.

17. Detection of Dried Tendrils of Maize Cob in Saffron

**Procedure**

i. Take a few strands of saffron
ii. Add hot water 70-80 °C

iii. Observe the diffusion of color

**Inference**

i. Pure saffron when allowed to dissolve in water will continue to give its saffron color so long as it lasts. Genuine saffron will not break easily like artificial saffron.
References

- Pearsons’ Composition and Analysis of Food 9th Edition,
- IS 15642 (Parts 1 and 2) :2006 (Reaffirmed 2011) Quick Methods for Detection of Adulterants/Contaminants in Common Food Products
V. Detection of Adulterants in Other Foods

1. Detection of Chalk Powder And Washing Soda in Sugar, Bura Sugar/Wheat Flour/Ice Cream

Safety precaution: Concentrated HCl is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid.

Apparatus
i. Pasteur pipette / Dropper

Reagent
i. Dilute HCl: Dilute concentrated HCl, 1: 1 with distilled water
ii. Lime water

Procedure
i. Take small amount of sugar/ wheat flour/ice cream in a test tube
ii. Add few drops of dil. HCl.
iii. Brisk effervescence or bubbles indicates the presence of chalk powder or washing soda.
iv. Confirm the test by bubbling the gas into lime water
v. Observe the color of the lime water

Inference
i. Effervescence appears, and lime water becomes cloudy or milky, indicates the presence of chalk/washing soda in the sample

2. Detection of Metanil Yellow in Pulses/ Parboiled (Sella) Rice/Sweetmeats/Ice Cream

Safety precaution: Concentrated HCl is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid.

Apparatus
i. Pasteur pipette / Dropper

Reagent
i. Concentrated HCl

Procedure
i. Take one g of the pulse/food in a test tube and add luke warm water to bring in solution
ii. Vortex the contents and let stand
iii. Add a few drops of concentrated HCl
iv. Observe the change in color
Inference

i. The immediate development of pink color indicates the presence of metanil yellow dye.

3. Detection of Lead Chromate in Pulses/Other Foods

Safety precaution: Concentrated HCl is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid.

Apparatus

i. Pasteur pipette / Dropper

Reagent

i. Concentrated HCl

Procedure

i. Shake 5g of pulse/food with 5ml of water
ii. Add a few drops of concentrated HCl
iii. Observe any change in color

Inference

i. A pink color obtained indicates the presence of lead chromate in the sample.

4. Detection of Sand/Dirt in Wheat and Other Flours

Safety precautions: Carbon tetrachloride is a carcinogen. Avoid spilling and inhalation of solvent vapors

Apparatus

i. Measuring Cylinder

Reagent

i. Carbon tetrachloride

Procedure

i. Weigh 5 gms of flour into a beaker
ii. Add 20 ml of Carbon tetrachloride
iii. Allow it to stand
iv. Observe the debris at the bottom of the beaker

Inference

i. Sand and filth will collect at the bottom of the beaker
5. Detection of Iron Filing in Tea Leaves/Wheat Flour

Apparatus
i. Powerful bar magnet

Procedure
i. Spread 50-100g of the tea leaves/sooji/maida/flour evenly on a clean paper
ii. Run a bar magnet repeatedly over the spread sample
iii. Iron filings if present will cling to the magnet

Inference
i. Iron filing if present will cling to the bar magnet

6. Detection of Artificially Coloured Tea Dust Mixed with Genuine Tea or Used Tea Leaves

Apparatus
i. Filter paper

Procedure
i. Take about 5 g of tea leaves/dust and place it in the center of a filter paper.
ii. Using the dropper, add water drop by drop at the heap of the tea leaves/dust.
iii. If the genuine tea is adulterated with a colored tea, water will dissolve the added color
iv. Streaks of visible color on the filter paper.

Inference
i. Pink or red color diffused on filter paper indicates colored used tea leaves
ii. Pure tea leaves leave no color

7. Detection of Malachite Green in Green Vegetables

Apparatus
i. Blotting paper

Reagent
i. Liquid paraffin

Procedure
i. Take moist blotting paper and spread the greens/vegetables on moist white blotting paper
ii. Look for color on filter paper
iii. Rub the outer green surface of the vegetable with liquid paraffin soaked cotton.
iv. Soak green peas in a beaker of water
**Inference**

i. Green color impressions on paper indicates the presence of Malachite green

ii. Cotton ball shows green color indicates the presence of Malachite green

iii. Water in beaker is green indicates peas are artificially colored.

8. **Detection of Artificial Invert Sugar Syrup in Honey (Fieh’s Test)**

**Safety precautions:** Take care while handling Concentrated HCl. Concentrated acids can cause severe skin burns and damage. Concentrated acids should be opened and used only in a fume hood. Always add acid to water when diluting or preparing reagents. Diethyl ether is inflammable. Do not open or use near open flame.

**Reagents**

i. Resorcinol reagent freshly prepared: Weigh one g of resorcinol and dissolve in 100 ml concentrated HCl

ii. Diethyl ether

**Procedure**

i. Take 5 ml of honey in a small beaker.

ii. Add 5 ml of cold water and mix well.

iii. Extract with 10 ml of ether

iv. Decant the ether layer in a Petri dish and allow the ether to evaporate.

v. Dissolve the residue in 5 ml ether

vi. To 2 ml of above add 2 ml of freshly prepared resorcinol reagent

vii. Look for color change

**Inference**

i. A pink to cherry red color indicates presence of invert sugar

*Note: This test should not be taken as conclusive. Perform Aniline chloride test for confirmation.*

9. **Detection of Artificial Invert Sugar Syrup in Honey if Fieh’s Test Is Positive**

**Safety precaution:** Concentrated HCl is corrosive and can cause burns. Handle it with care. Use Pasteur pipette to add acid. Ether is highly flammable. Store away from open flame

**Reagents**

i. Aniline chloride solution: To 100 ml of aniline add 30 ml of 25% concentrated HCl

ii. Diethyl ether
Procedure
i. Take 5 g of honey in a porcelain dish.
ii. Add 2.5 ml of aniline chloride solution and stir well.
iii. Observe any changes in color

Inference
i. Orange red color indicates presence of invert sugar.

10. Detection of Mineral Acid in Vinegar/Carbonated Beverages

Reagent
i. Metanil yellow indicator paper: Metanil yellow indicator paper can be prepared by dipping a strip of filter paper in metanil yellow solution (1%) in water.

Procedure
i. Take 5-10 ml of vinegar or beverage in a test tube
ii. Dip the Metanil yellow indicator paper,
iii. Observe color change of indicator paper

Inference
i. If the color changes from yellow to pink, mineral acid is present.

11. Determination of Boric Acid in Maida/Rice Flour

Reagents
i. Turmeric indicator paper: Turmeric indicator paper can be prepared by dipping a strip of filter paper in turmeric solution (1 gm turmeric in 4 ml ethanol, let it stand for some time and filter) in ethanol.
ii. Concentrated HCl

Procedure
i. Take one g sample in a test tube,
ii. Add 5 ml water and shake.
iii. Add a few drops of concentrated HCl.
iv. Dip a turmeric paper strip
v. Observe color change of turmeric paper

Inference
i. If the turmeric indicator paper turns red, boric acid is present
Reference

- Pearsons’ Composition and Analysis of Food 9th Edition,
- IS 15642 (Parts 1 and 2) :2006 (Reaffirmed 2011) Quick Methods for Detection of Adulterants/Contaminants in CommonFood Products
- Wood, R; Foster, L;Damant, A and Key, P in Analytical Methods for Food Additives, Woodhead Publishing Ltd and CRC Press LLC, 2004
ABOUT THE MANUAL

The current manual provides a comprehensive view of test methods for determination of adulterants in various food products. This manual not only meets the need of any analyst working in the MFTL, but can also be used to educate the school/college students about simple food testing methods. FSSAI gratefully acknowledges the help and assistance of Dr. Lalitha R. Gowda, member of the Empowered Committee for her precious time in developing and compiling the manual related to safety procedures and test methods.