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Qualitative Detection of milk Adulterants A Qualitative study on milk Adulterants in Capital of Jharkhand, Ranchi India

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Abstract

Introduction: Food is essential for sustenance of life. Adulteration of food cheats the consumer and can pose serious risk to health in some cases. major food adulteration and contamination events seems to occur with some regularities, such as the widely publicized adulteration of milk products with melamine and the recent microbial contamination of vegetables across Europe for example. With globalization and rapid distribution systems, these can have international impacts with far–reaching and sometimes lethal consequences. These events, through potentially global in the modern era, are in fact far from contemporary and deliberate adulteration of food products is probably as old as the food processing and production system Themselves. Food forensic is a sub field of forensic chemistry which is used to detect when it is contaminated or adulterant, it helps to check the purity of the food item. Food adulteration is the most leading fraud which is growing rapidly.

Methodology: The study was conducted is several stages to assess the presence of adulterants in the milk samples collected from different local areas of Ranchi. the methodology involved the following steps such as sample collection, presumptive testing, documentation and data recording, Analysis, interpretation using forensic chemistry principles.

Results: The qualitative analysis of 11 different brands of milk samples collected from different regions of Ranchi revealed a significant presence of hydrogen peroxide, starch, sodium chloride etc. the presumptive tests detected multiple adulterants that pose considerable health risk to consumers.

Conclusion: The study demonstrate that milk adulteration is a prevalent and alarming issue in the city of Ranchi. It also emphasizes the urgent need for regulatory bodies, public health authorities, and forensic science professionals to work collaboratively to ensure the availability of safe and unadulterated milk for all consumers.

Keywords: Adulteration of food, globalization, and rapid distribution system, adulterants, forensic chemistry presumptive

Introduction

Forensic chemistry is a branch of forensic science which deals with the principle of chemical techniques to aid in investigation agencies and law Enforcement, in one such application food forensic is a field of study under the forensic chemistry division and deals with the detection and identification of illegalities in relation to food products or items of local consumptions. Forensic studies with regards to food have not been widely popular especially in India, were most of the cases of adulteration and testing are carried out by the food and drug Administration. The study is carried to analyse the purity of milk available in market both branded and local[1].

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Milk

Milk is nutrient rich, white liquid food produced by the mammary glands of mammals. Milk is considered as nearly perfect which is readily digested and absorbed.

Milk is very important due to its special nutritive value and important role for human and animal health. It is chemically very complex system containing more than one lakh substances. physically milk is heterogenous mixture of various components, present either in solution, suspension or emulsion in water. The major constituents of milk include fat, casein and proteins the major proteins of milk, lactose- milk sugar and ash – minerals matter of milk[2].

Main constituent	Range	Mean (%)
Water	85.5-89.5	87.0
Total Solids	10.5-14.5	13.0
Fat	2.5-6.0	4.0
Protein	2.9-5.0	3.4
Lactose	3.6-5.5	4.8
Minerals	06-0.9	0.8

Adulteration

In general adulteration may be defined as any addition of the legally prohibited substances into or from a more valuable genuine product. whereas adulteration of milk is defined as" any change caused in the normal levels of milk constituents is known as adulteration of milk. The change may be brought about by addition of some foreign matter to milk or by removing some more valuable ingredients out of it." Some of the cause ofadulteration in milk are demand and supply gap, physical nature of milk. degraded moral of the society, spoiled economic structure, perishable nature of milk, this unorganized condition of dairy industry, lack of strict and effective regulatory system and lack of suitable, rapid and sure tests[3].

One of the important reasons for wide spread adulteration of milk sits physical and chemical nature, due to which it can hide many things when added to it. Thus, milk can be easily and many ways that affect the quality of dairy products manufactured out of it. In the milk mainly two groups of substances are added adulterating agents, whose purpose is to increase the economical yield and preservatives, which delay or inhibit the spoilage of the milk. addition of cheaper material like water and skimmed milk powder is one of the most common ways of adulteration. water is added for increasing volume of milk while theskim milk powder is added to increase the SNF content of milk. starch, glucose, gelatine, glucose, malt dextrin and cellulose are added in milk as thickening agent as well as to increase the SNF content of milk. some miscellaneous adulterants are also added in milk and milk product such as vegetable oil, soybean protein, antibiotics detergents etc[4].

Adulterants is one of the major problems that stands against the progress of dairying in India and has detrimental effect on export of our dairy products. A worrying 79% of branded or loose milk available in the market is adulterated, the latest annual report by the consumer guidance society of India (CGSI) has found. The report said of all the milk samples 73 were branded packets and only 11of them (15%) adhered to the standard mark. The remaining 85% of the branded milk currently sold in the market was adulterated[5].

Milk standards as per FSSAI (current as of 2025)[2]

S. no	Parameters	Standards limit	
		FSSR 2011	FSSR2011, AMENDED 2018
1	**fat**	0.5-6.0%	0.5-6.0%
2	**SNF**	8.5-9.0%	6.0-9.0%
3	**Vegetable oil/fat**	Negative	negative

Continue....

4	**sugar**	negative	negative
5	**glucose**	negative	Negative
6	**urea**	700mg/kg	Negative
7	**starch**	negative	Negative
8	**detergent**	negative	negative
9	**Hydrogen peroxide**	negative	negative
10	**Maltodextrin**	negative	negative

Adulteration of Milk in India

In 2019, the national survey on adulteration confirmed that about 68% of milk did not match the standards of food safety and FSSAI different surveys are carried out to detect the adulteration of milk in different cities of India .as a result about 80 to 90 of milk is found to be adulterated. So, there is a need for strict analytical control for detection of adulteration in milk to safeguards the citizens health. There are number of instrumental methods, test kits, physical and chemical method for detecting the adulterants present in milk. For an example ammonium salt can be detected by various tests likeNessler's reagent test, phenol test and turmeric test. For the detection of detergent methylene blue test is commonly done[6]. Detection of sulphate can be done by barium chloride test. Some of the quantitative tests for detecting the adulterants present in milk are described in methodology section.

Key Recent FSSAI Updates (2023–2025)

Initiative	Highlights
"Food Safety on Wheels" (March 2023)	FSSAI deployed 168 mobile testing vans equipped with Milk-o-Screen systems to conduct **on-the-spot testing** of milk samples for constituents like fats, SNF, protein, and adulterants such as water, urea, sucrose, maltodextrin, ammonium sulphate.
Festive Season Surveillance Intensified	During major festivals, FSSAI has repeatedly directed States/UTs to ramp up surveillance of dairy products—including sweets, milk, paneer, ghee, etc.
SOPs for Dairy Producers (Oct 2024)	New standard operating procedures for cleanliness, animal health, milking hygiene, and milk handling/storage (e.g., transportation within 3–4 hours or refrigerated at 4–6 °C) were introduced to reduce contamination risk.
Clarifications on Additives (Oct 2023)	FSSAI clarified that **protein binders are not permitted** in milk and milk products—only additives listed in Appendix A of FSS (Food Products Standards and Food Additives)
Labelling and Testing Protocols	Drafts for enhanced labelling (e.g., mandatory milk logos, prominent nutritional info), faster lab timelines (within 14 days), and adoption of international methods (AOAC, ISO, Codex) have been issued.
Ongoing Surveys	A large-scale survey across 766 districts (over 10,000 samples) was launched by FSSAI in 2023 to assess compliance of milk and dairy products, with findings expected in reports to the Health Ministry.

Methodology

The study is planned to determine the qualitative test used for the detection of adulterants in milk. The materials and methodology used in this detection technique are mentioned as follows:

List of Chemicals

Phenol, Phenolphthalein, Sodiumbicarbonate, Sodiumhydroxide, sulphuricacid, Chloroform, Diethylether, Iodine solution Nessler'sreagent, Nitric acid methylene blue etc.

Apparatus and Glassware

Beaker, Conicalflask, Measuringcylinder, Testtube, Glassrod, Funnel, Pipettes, Spiritlamp, Waterbath, Centrifuge Refrigerators etc.

Analytical Techniques for Detection of Adulteration in Milk

Various measures are taken to contain the menace of adulteration; including regulatory monitoring, enhancing awareness in the communities, extensive testing/analysis of raw milk, etc. Analysis is one of the essential parts of overall quality assurance system operated by dairy plants. Various analytical methods are used for the purpose of checking adulteration of milk including physical methods, instrumental methods and chemical methods.

Physical Methods

Methods based on physical properties of milk are density (lactometer reading), freezing point, refractive index, etc, which are easy to perform, but can be very easily manipulated due to natural variations in milk composition. Physical methods are simple, fast, easy, cheap and convenient. However, sensitivity of these tests is less in comparison to chemical and instrumental methods[7].

Freezing point can be significantly affected by seasonal and regional factors. Thus, considering geographical vastness of India and consequent seasonal and regional variation it cannot be a reliable means of adulteration detection.

The density (or specific gravity) depends on composition, temperature and temperature history of milk. As Indian dairy sector is still predominantly unorganized in nature; it is difficult to control most parameters affecting density. Therefore, density measurement cannot be a useful tool for adulteration detection. Thus, physical methods suffer from some of the general limitations due to large natural variations, lower sensitivity, poor specificity, proneness to manipulation[8].

Instrumental methods are one of the good options for quality control of milk and milk products. Though, it possesses several advantages like higher sensitivity, high specificity and reliability, it also suffers from several limitations as described below.

- Very limited adoptability for practical applications.
- Requires high initiOal investment, operational cost and expensive maintenance.
- Most methods are time consuming as it necessitates isolation, purification, concentration &/or derivatization of the target analyte.
- Impractical for routine analysis and field applications.

As these methods require skilled manpower it is difficult to use as routine methods. Indian dairy industry is still characterised by small scale farming and small cooperative societies which may not be able to afford capital requirements for sophisticated instruments as well as its maintenance.

Chemical Methods

The chemical methods are simple, fast, easy, cheap, convenient and have better specificity for adulterant/ chemical compounds being tested. However numerous qualitative tests are reported for detection of adulterants in milk with wide variation in procedure for a given test. There is lack of information regarding sensitivity between various reported qualitative tests. To overcome these limitations various reported tests and procedural variations were evaluated[9].

Qualitative Tests for Detection of Adulterants in Milk

Upon review and evaluation of qualitative tests available for detection of common adulterants reported in milk, it was observed that there is wide variation related to several test performance parameters like sensitivity, convenience, cost etc. This variation in performance was mainly attributable to variations in the procedures of the test. Further, it also appeared from the survey of literature that scant attention has been paid on systematic work for improving performance of the qualitative tests for detection of adulterants in the milk. Thus, it was envisaged to undertake work for improving some qualitative tests suggested for detection of common adulterants encountered in milk. The qualitative tests were optimized considering three different aspects of the test procedure.

1. To select suitable medium for performing the tests in detection of adulterants.
2. To standardize various chemicals/reagents used in the tests.
3. To optimize different conditions involved in performing the tests.

Considering the requirements for improving the performance of existing qualitative tests reported for common adulterants, including detergent, urea, ammonium salts, glucose, sucrose, maltodextrin, starch, hydrogen peroxide, salt, nitrate, sulphate, formaldehyde and neutralizers were modified[10].

Detection of Detergents by Methylene Blue Test

Methylene blue is cationic dye which forms complex with anionic detergents. It is normally water-soluble compound; however, it shows affinity for anionic detergents, if they are present. In this method, detergent is first extracted in chloroform and then methylene blue solution is added. In presence of detergent blue colour is developed in chloroform layer of the sample, whereas blue colour is observed in milk layer in control (pure milk). Chloroform is heavier settles at the bottom. This implies that observation of blue colour in the bottom layer indicates presence of detergents[11].

Reagents

1. Methanol (AR)
2. Methylene blue solution: 12.5 mg methylene blue (AR) is dissolved in 100 ml of distilled water. Protect thesolution from direct sunlight.
3. Chloroform (AR):

Precaution: Inflammable and toxic on inhalation. Mouth pipetting is not recommended

Procedure

1. Take 2.5 ml of suspected milk sample in a test tube and add 7.5 methanol.
2. Filter the content through Whatman No. 1 filter paper.
3. Take 2 ml filtrate in a test tube.
4. Add 2 ml of methylene blue solution and shake well.
5. Subsequently add 4 ml chloroform and shake well again.
6. Allow the chloroform layer to separate.
7. Compare the colour extracted in the chloroform layer in suspected milk with that for pure milk.

Interpretation: If the methylene blue colour extracted from a suspected sample into the chloroform layer is darker than that extracted from pure milk sample, it indicates the presence of detergent in milk.

Limit of detection: 0.02 g/ 100 ml milk

(**Note**: The method reported by Varadkar*et al*. (2000b) is modified. Methanol was used in place of ethanol. Methylene blue concentration was reduced from 25 mg/100 ml to 12.5 mg/100 ml.) **Benefits of modified method over reported method**

- Better differentiation between adulterated and pure sample
- Eliminates use of ethanol (a regulated chemical)

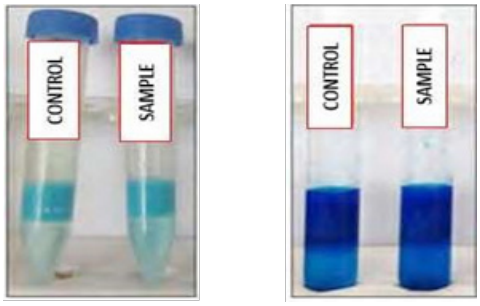


Figure 1: Test of Detergents

Detection of Urea By Dmab Test

A yellow-coloured complex is formed between urea and *p*-dimethyl amino benzaldehyde (DMAB) reagent in low acidic alcoholic solution at room temperature. The intensity of colour can be measured at 440 nm (Leafier, 1996). The colour developed is in proportion to urea content in the sample

Reagents

Dimethylaminbenzaldehyde reagent (DMAB): The reagent is prepared by dissolving 1.6 g of *p*-dimethylamine benzaldehyde (AR) in methanol (AR) subsequently adding 10 ml of concentrated HCl (AR) and making volume to 100 ml with methanol.

Procedure

1. Take 5 ml milk in a test tube.
2. Add 5 ml DMAB reagent.
3. Mix the content and observe the colour.

Interpretation: Formation of distinct yellow colour indicates the presence of added urea in milk sample. Pure milk shows light yellow colour due to natural urea.

Limit of detection: 0.2 g/ 100 ml milk

Benefits of modified method over reported method

- Better differentiation between adulterated and pure sample.
- Eliminates use of ethanol (a regulated chemical).

Figure 2: Test of Urea

Detection of Ammonium Salts By Nessler's Test

Nessler's test is one of the classical methods for qualitative and quantitative analysis of ammonia and ammonium ions. Nessler's reagent is an alkaline solution of potassium mercuric iodide (K2HgI4). On reaction with ammonium ion Nessler's reagent produces a yellowish-brown colour. The intensity of the colour is directly proportional to the amount of ammonia/ammonium ion present[12].

Reagents

1) Nessler's reagent: Dissolve the following chemicals separately. a)8.0 g of mercuric chloride (AR) in 150 ml distilled water.

b) 60.0 g of sodium hydroxide (AR) in 150 ml distilled water.

c) 16.0 g of potassium iodide (AR) in 150 ml distilled water.

Add reagent „a ''to reagent „b'' and mix well. To this mixture, add reagent „c'', mix and dilute the contents to 500 ml with distilled water. Leave this solution undisturbed and decant the clear upper layer of the solution. Store in a stoppered amber glass bottle.

Note: Alternatively, commercially available (readymade) Nessler's reagent can also be used.

2) Citric acid solution (5%): Dissolve 5 g citric acid monohydrate (AR) in distilled water and make up the volume to 100 ml with distilled water.

Procedure

1) Take 20 ml milk in a conical flask.
2) Warm the milk to 70-80 °C either on direct flame or water bath.
3) Add 5% citric acid solution drop wise in the milk with gentle stirring till visible coagulation occurs. Flask if stirred vigorously will result in fine curd particles and which may impact colour observation.
4) Filter the content using Whatman No. 1 filter paper.
5) Take 5 ml of filtrate into a test tube.
6) Add 0.4 ml of Nessler's reagent.
7) Observe the colour without shaking the test tube.

Interpretation: Carefully observe instant development of orange colour in milk adulterated with ammonium salts. Whereas pale yellow colour indicates unadulterated milk.

Limit of detection: 0.02 g/ 100 ml milk

Benefits of modified method over reported method.

- Improved sensitivity over the reported method.
- Better differentiation between adulterated and pure sample.

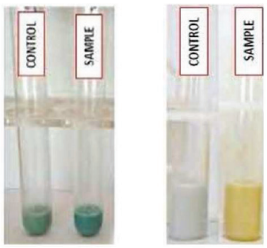


Figure 3: Test for Ammonium Salts

Detection of Sucrose by Seliwanoff's Test

Sucrose is a disaccharide containing glucose and fructose (a ketose sugar). Seminoff test is used for detection of ketoses. The dilute hydrochloric acid used in Seminoff reagent along with heat leads to hydrolysis of sucrose and subsequent dehydration of fructose. Further keto group more actively attacks resorcinol in comparison to aldehyde group. The dehydration product 5-hydroxymethylfurfural condenses with resorcinol forming cherry red colour. Ketoses react rapidly in comparison to aldoses because dehydration of aldoses to 5-hydroxymethylfurfural proceeds in a much slower way than the reaction of ketoses[13].

Reagents

Resorcinol solution (0.05%): The reagent is prepared by dissolving 0.05 g of resorcinol (AR) in 100 ml hydrochloric acid (The acid is prepared by taking 30 ml conc. HCl and diluting to 100 ml with distilled water.)

Procedure (using milk as medium)

1) Take 3 ml milk and 5 ml resorcinol solution in a test tube.
2) Keep the content in boiling water bath for 6 min.
3) Cool the tubes immediately after heating under tap water to retard the rate of reaction, which if not done would narrow the colour difference between negative and positive samples.
4) Observe for colour development.

Procedure (using whey as medium):

1) Take 3 ml milk and 5 ml resorcinol solution in a test tube. (Quantity of both milk and reagent can be doubled proportionately to get sufficient filtrate.)
2) Mix and filter the content using Whatman No. 1 filter paper.
3) Keep the filtrate in boiling water bath for 4 min.
4) Cool the tubes immediately after heating under tap water to retard the progress of reaction, which if not done would narrow the colour difference between negative and positive samples.
5) Observe for colour development.

Interpretation: Development of red colour indicates adulteration of sucrose in milk. The intensity of red colour increases with increase in the sucrose content in the milk. Pure milk remains light in colour.

Limit of detection:

0.1g/ 100 ml milk (When test is performed in milk)

0.06 g/100 ml milk (When test is performed in whey)

Benefits of modified method over reported method

- Better differentiation between adulterated and pure sample.

Different concentration of hydrochloric acid (30 ml conc. hydrochloric acid diluted to 100 ml) was used instead of reported concentration (1-part HCl: 2 parts distilled water). The test was also modified using whey as medium.

Figure 4: Test for Sucrose

Detection of Glucose by Barfoed Test

Barford's test is routinely used for detection of extraneous glucose in milk. By means of the Barford's reaction it is possible to differentiate reducing monosaccharaides from reducing disaccharides as monosaccharaides can reduce copper fast enough in comparison to disaccharides. The formation of green, red, or yellow precipitate is a positive test for reducing monosaccharides[14].

Reagents

Barfoed reagent: Dissolve 13.3 g of copper acetate (AR) in distilled water, subsequently add 2.0 ml of lactic acid (AR) and make up the total volume to 200 ml.

Procedure (using milk as medium)

1) Take 1 ml milk in a test tube.
2) Add 2 ml Barfoed reagent.
3) Keep the test tube in boiling water bath for 6 min.
4) Cool the test tube to room temperature under tap water.
5) Observe for colour development.

Procedure (using whey as medium)

1) Take 1 ml milk and 2 ml Barfoed reagent in a test tube (quantity of both milk and reagent can be doubled proportionately to get sufficient filtrate).
2) Mix the content and filter using Whatman No. 1 filter paper.
3) Keep test tube containing filtrate in water bath for 4 min.
4) Cool the test tube to room temperature using water.
5) Observe for colour development.

Interpretation: Development of green colour indicates presence of glucose in milk.

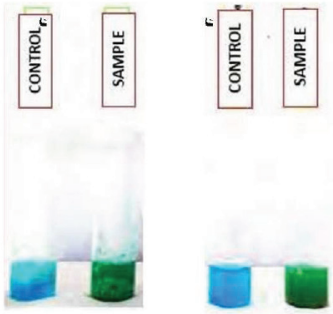
Limit of detection:

0.1 g/100 ml of milk (milk as medium)

0.15 g /100 ml of milk (whey as medium)

Benefits of modified method over reported method -better differentiation between adulterated and pure sample.

- Better clarity in whey as compared to milk.
- Eliminates large number of costly chemicals.
- One step procedure, improved convenience.



Barfoed milk Barfoed whey

Figure 5: Test for Glucose

Detection of Maltodextrin by Iodine Test

Similar to starch maltodextrin also forms complex with iodine. However, instead of blue colour, red to brown colour is observed. This is because the complex formed is dependent on chain length of dextrin's. The chain length more than 45 DP (degree of polymerization) gives blue colour, whereas lesser DP gives red to brown colour complex, as in the case of maltodextrin[15].

Reagents: Iodine solution (1%): Dissolve 2.5 g potassium iodide (AR) in 100 ml distilled water. Then, add 1 g pure iodine crystals (AR). Prepare iodine solution at least a day before as iodine dissolves slowly.

Procedure

1) Take 20 ml milk in a conical flask.
2) Warm the milk to 70-80
3) Add 5% citric acid solution drop wise in the milk with gentle stirring until clear coagulation occurs (Approximate consumption of citric acid required would be 1.5-2 ml.). Flask, if stirred vigorously will result in fine curd particles and which may impact colour observation.
4) Filter the content using Whatman No. 1 filter paper.
5) Take 5 ml filtrate in a test tube.
6) Add 0.25 ml of 1% iodine solution.
7) Mix the content and observe the colour.

Interpretation: Development of red-brown colour indicates adulteration of milk with maltodextrin. Pure milk remains yellow in colour. **Limit of detection:**0.1 g /100 ml milk.

Benefits of modified method over reported method

- Better differentiation between adulterated and pure sample.
- Significant improvement in sensitivity.

Iodine Test (Whey)

Figure 6: Test for Maltodextrin

Detection of Starch by Iodine Test

The development of blue colour on addition of iodine solution in starch containing milk is due to complex formation between iodine and amylose component of starch. The other component, amylopectin, gives a red-purple colour which is much less intense than the amylose. The acidic condition in the reagent mixture accentuates the blue colour, whereas alkali reduces its intensity, the blue colour disappears above a pH of about 9.5. Heating the solution containing starch-iodine complex also destroys the colour although reversibly.

Reagents

1) Iodine solution: Dissolve 2.5 g potassium iodide (AR) and 1 g of pure iodine crystals (AR) in 100 ml distilled water. Prepare iodine solution at least a day before as iodine dissolves slowly.

2) Acetic acid (10%): Dissolve 10 ml glacial acetic acid (AR) in distilled water and make up the volume to 100 ml.
3) Citric acid solution (5%): Dissolve 5 g citric acid monohydrate (AR) in distilled water and make up the volume to 100 ml with distilled water.

Procedure (using milk as medium)

1) Take 3 ml milk in test tube.
2) Bring the milk to boil on a direct flame or on a boiling water bath.
3) Cool the test tube to room temperature under tap water.
4) Add a drop of 10% acetic acid in the test tube.
5) Add 0.2 ml of iodine solution.
6) Mix the content and observe colour.

Procedure (using whey as medium)

1) Take 20 ml milk in a conical flask.
2) Bring the milk to boil on a direct flame or on a boiling waterbath.
3) Add 5% citric drop wise till visible coagulation. (Approximate consumption of citric acid would be 1.5-2 ml.)
4) Filter the content using Whatman No. 1 filter paper. Let the filtrate cool down to room temperature.
5) Take 3 ml filtrate in another test tube and add 0.1 ml of iodine solution.
6) Mix the content and observe colour.

Interpretation: Blue/Dark blue colour formation indicates adulteration of milk with starch. Whereas pure milk remains yellow due to colour of iodine.

Limit of detection:

0.02 g/ 100 ml milk (When test is performed in milk)

0.01 g /100 ml milk (When test is performed in whey)

Benefits of modified method over reported method

- Better differentiation between adulterated and pure sample.
- Better sensitivity
- Reduces the chances of interference of neutralizers on the detection of starch.

Detection of Sodium Chloride by Silver Nitrate Test

The chloride ion (Cl) from sodium chloride reacts with silver ion of silver nitrate forming white precipitates of silver chloride. Simultaneously water-soluble sodium nitrate is also formed. After the Ag from silver nitrate has complexed with all the available chloride in the sample, the Ag reacts with chromate from silver chromate added in the reaction mixture; forming an orange-coloured precipitates of silver chromate.

Reagents

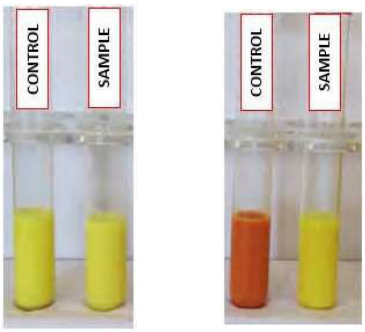
1. Silver nitrate solution (0.1N): The reagent is prepared by dissolving 16.987 g silver nitrate (AR) in 1000 ml distilled water.
2. Potassium chromate solution (5%): The reagent is prepared bydissolving 5 g potassium chromate (AR) in 100 ml distilled water. Procedure:
3. Take 5 ml milk in test tube.
4. Add 0.5 ml of 5% potassium chromate solution.
5. Add 2 ml 0.1 N silver nitrate and mix the contents.
6. Observe for colour change.

Interpretation: Yellow colour indicates adulteration of milk with common salt (sodium chloride). Unadulterated milk gives chocolate or reddish-brown colour.

Limit of detection: 0.04g /100 ml of milk

Benefits of modified method over reported method

Better differentiation between adulterated and pure sample.



Chromate Test (Milk)

Figure 7: Test for Salt (Sodium Chloride)

Detection of Nitrate by Diphenylamine Test

The test consists of adding a solution of diphenylamine in sulphuric acid to milk. Nitrates are considered as oxidising agent. Under the conditions of test, diphenylamine is oxidized by nitrate to the intensely blue quinone-ammonium salt *by*diphenyl benzidine.

Reagents

Diphenylamine solution: The reagent is prepared by dissolving 0.085 g diphenylamine in 50 ml distilled water and gradually 450 ml of concentrated sulphuric acid is added with constant stirring. During preparation of reagent the content is kept cool by dipping in cold water. Diphenylamine solution should be prepared freshly and shall be colourless.

Acetic acid (10%): Take 10 ml glacial acetic acid (AR) in 100 ml volumetric flask and make up the volume with distilled water.

Procedure

1) Take 20 ml milk in a conical flask.
2) Warm the milk to 70-80 C either on direct flame or boiling water bath.
3) Add 10% acetic acid drop wise in the milk with gentle stirring till visiblecoagulation.
4) Filter the content using Whatman No. 1 filter paper.
5) Take 2 ml diphenylamine solution in a test tube.
6) Add 1 ml filtrate in test tube containing diphenylamine solution.
7) Observe for ring formation at the junction of two solutions.

Interpretation: Formation of blue ring at the junction of two solutions indicates adulteration of milk with nitrate or surface water.

Limit of detection: 0.002g /100 ml milk

Benefits of modified method over reported method

- Better differentiation between adulterated and pure sample.
- Elimination of toxic chemicals from precipitating reagent.

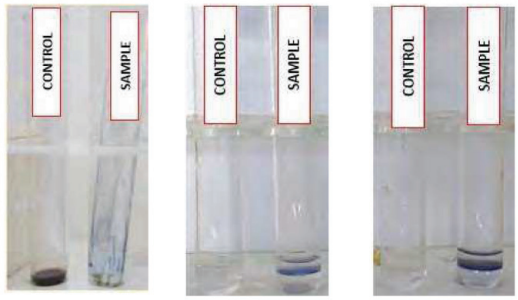


Figure 8: Test for Nitrate

Detection of Sulphate by Barium Chloride Test

Barium chloride test is one of the commonly used methods for detection of sulphate adulteration in milk. The barium ion (Ba^{2+}) reacts with sulphate ion (SO^{2-}) to[4]give white precipitates of barium sulphate (BaSO4). Using this test, presence of added sulphate like ammonium sulphate, sodium sulphate, zinc sulphate, magnesium sulphate, etc. to milk can be confirmed by observing milky-white precipitates.

Reagents

1) Barium chloride solution (5%): Dissolve 5.0 g barium chloride (AR) in distilled water and make up the final volume to 100 ml.
2) Lactic acid solution (5%): Take 5 ml lactic acid (AR) in 100 ml volumetric flask and make up the volume with distilled water.

Procedure

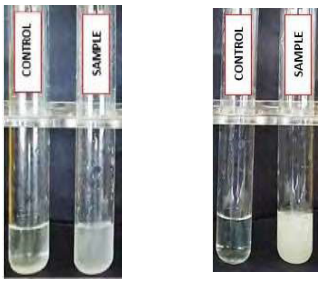
1) Take 20 ml milk in a conical flask/test tube bring it to boil on direct flame.
2) Add 2 ml of 5% lactic acid in hot milk and filter the content using Whatman No. 42 filter paper.
3) Take 2 ml filtrate in a separate test tube.
4) Add 0.2 ml of 5% barium chloride and observe for turbidity development.

Interpretation: Formation of turbidity after barium chloride solution addition indicates adulteration of milk with sulphate.

Limit of detection: 0.015 g /100 ml milk

Benefits of modified method over reported method -better differentiation between adulterated and pure sample.

- Improved sensitivity.
- Elimination of TCA (hazardous chemical).



Barium Chloride Test **Barium Chloride Test**

Figure 9: Test for Sulphate

Detection of Hydrogen Peroxide by Iodine Test

Hydrogen peroxide presence can be detected iodometric test. Hydrogen peroxide oxidises iodide to iodine. Starch forms a deep, dark blue complex with minute amounts of triiodide ions that are formed only in the presence of both iodine and iodide in solution. Thus, formation of blue colour indicates presence of hydrogen peroxide.

Reagents

1. Potassium iodide solution (20%): Weigh 20 g of potassium iodide (AR) and dissolve it in distilled water to obtain 100 ml solution. The solution should be prepared fresh before every use.
2. Starch solution (1%): Take 1 g of soluble starch and make paste using cold water. Transfer the paste to 100 ml volumetric flask and make the volume to 100 ml using boiling distilled water. Cool and decant the clear solution. The solution should be prepared fresh before every use.
3. Starch-potassium iodide reagent: The reagent is prepared by mixing equal volumes of 20 per cent potassium iodide solution and 1 per cent starch solution. The solution should be prepared fresh before every use.
4. Acetic acid (10%): Dissolve 10 ml glacial acetic acid in water and make up the volume to 100 ml.

Procedure

1. Take 20 ml milk in a conical flask.
2. Add 2 ml 10% acetic acid.
3. Mix the content and filter using Whatman No. 1 filter paper.
4. Take 1 ml filtrate and add 1 ml starch-potassium iodine reagent.
5. Observe for colour development.

Interpretation: Appearance of bluish black colour indicates the presence of hydrogen peroxide in the milk sample whereas control milk sample remains colourless.

Limit of detection: Modified test: 0.015 g /100 ml of milk

Benefits of modified method over reported method ·Better differentiation between adulterated and pure sample.

- Improved sensitivity.
- Method uses relatively benign chemicals in place of potentially harmful chemical paraphenylenediamine.

phenylenediamine Iodometric Iodometric Test

Figure 10: Test for Hydrogen Peroxide

Detection of Formaldehyde by Hehner Test

In the Hehner test for detection of formaldehyde in milk, concentrated sulphuric acid and ferric chloride is used. The test is an aldehyde- oxidation reaction of an aromatic amine. Ferric chloride acts as oxidising agent for formaldehyde. The formaldehyde reaction depends on the presence of the tryptophan in the protein molecule. The violet colour develops as a result of the reaction of oxidised formaldehyde with tryptophan. The intensity of the reaction with different proteins varies in direct proportion to the amount of tryptophan present in the protein molecule.

Reagents

- Ferric chloride (10%): Take 10 g of ferric chloride (AR) in 100 ml volumetric flask and mark up the volume with distilled water.
- Sulphuric acid (80%): Add 80 ml concentrated sulphuric acid (AR) into 20 ml distilled water.

Procedure

1) Take 5 milk sample in a test tube.
2) Add 5 ml distilled water and 0.1 ml of 10% FeCl3 solution.
3) Mix the content and add 10 ml H2SO4 (80%) from the side of the test tube.

Interpretation: Violet ring at the junction of two layers indicates presence of formaldehyde.

Limit of detection: 0.0005 ml formalin/ 100 ml milk

Benefits of modified method over reported method - better differentiation between adulterated and pure sample.

Improved sensitivity.

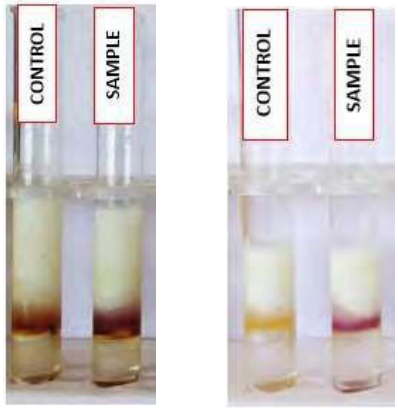


Figure 11: Test for Formaldehyde

Detection of Neutralizers by Phenol Red Test

Phenol red is used as a pH indicator and shows colour transition from yellow to red over the pH range 6.8 to 8.2. However, at pH value greater than 8.2 phenol red turns bright pink. As neutralization increases the pH of milk (beyond 6.8), it can be detected using phenol red indicator.

Reagents

Phenol red solution: 0.05 g of phenol red is dissolved in 20 ml ethanol and volume is made up to 100 ml using distilled water.

Procedure

1) Take 4 ml milk in a test tube.
2) Add 1 ml phenol red solution.
3) Mix the content and observe the colour.

Interpretation: Development of pink colour indicates presence of sodium hydroxide (NaOH), whereas orange colour indicates sodium carbonate (Na2CO3) or sodium bicarbonate (NaHCO3) in milk. Milk without added neutralizers shows yellow colour.

Limit of Detection:

NaOH: 0.04 g/ 100 ml milk

Na2CO3: 0.08 g/ 100 ml milk

NaHCO3: 0.2 g/ 100 ml milk

Benefits of developed method over reported method -better differentiation between adulterated and pure sample.

- Improved sensitivity.
- Elimination of ethanol (a regulated chemical)

Figure 12: Test for Neutralizers

Result

S. NO	MILK BRAND SAMPLE	ADULTERANTS					
		detergent	urea	Sodium chloride	Hydrogen peroxide	starch	sucrose
Positive spot result		Blue	Intense yellow	yellow	Bluish black	Dark blue	red
1.	MILKY KOOL	X	X	X	✓	X	X
2	OSEM DIARY	X	X	X	X	X	X
3	BERRY DIARY	X	X	✓	X	X	X
4	SUDHA MILK	X	X	X	X	X	X
5	MEDHA	X	X	X	X	✓	X
6	AMUL	X	X	X	X	X	X
7	W-ORGANIC	X	X	X	✓	X	X
8	MOTHER DAIRY	X	X	X	X	X	X
9	ORGANIC	X	X	X	X	✓	X
10	DAIRYBEST	✓	X	X	X	X	X
11	OMFED	X	X	X	X	X	X

Discussion

There are numerous variations are observed in particular qualitative tests used for detection of specific adulterants. therefore, it is important from the public health view point to evaluate the detection of adulterants in milk.moreover, the presence of adulterant in milk is a major challenge for the country. The supplies of milk appear to have found three ways to increase their margin or profit

from the sale of milk ;(a) dilution (b) extraction of valuable components i.e. milk fat removed as cream (c) a combination of cheaper bulking additives and some chemicals, adulterants and malpractices results in public health concern and malnutrition.

Basedon research, it was found that many of the milk samples shows the presence ofadulterants. which has been shown on the above. For the confirmatory conclusive we can proceed for quantitative techniques like SDS-PAGE, HPLC, IMMUNODIFFUSION METHOD, NIR SPECTROSCOPY.

DAIRY BEST milk sample indicates the presence of detergent, which can lead to gastrointestinal issues, tissue damages, &food poisoning.

BERRY DIARY indicates the presence of sodium chloride (NACL), excessive amount of NACL may cause vomiting,hypernatremia, respiratory distress, physio-chemical changes like PH Disbalance.

MILKY KOOL & W-ORGANIC shows the presence of hydrogen peroxide(H2O2), it is milk preservative used to inhibit microbial growth and spoilage. Higher exposures may cause a build-up of fluid in the lungs (pulmonary oedema) with severe shortness of breath, nausea and vomiting.

MEDHA & ORGANIC milk sample shows the presence of starch. A higher amount of starch in milk can cause Diarrhoea and other gastrointestinal problems such as gastric and bloating.

After surveying and testing the milk sample, the result was quite shocking. People expected water to be present as an adulterant in their consumed milk but for some cases test indicated the presence of other adulterants that are harmful for health. Therefore, this contradictory information shows that consumers are unaware of these adulteration practices. For tackling this problem government should periodically collect samples and do some test to make sure quality of milk supplied and consumed by people.

Detection of adulteration is complicated as the indicators of adulterants in milk can vary in quality due to various biological, climatic and agronomic factor. Moreover, processing can also dramatically change the composition of minor constituents, which can be problematic in setting the specification ranges for various inspection methods.thus, the challenge is to develop simple and cost-effective techniques for detecting adulteration in milk, which could be used with high degree of responsibility.

Conclusion

The findings of this study underscore the widespread and alarming issue of milk adulteration in Ranchi, with multiple samples showing the presence of harmful substances such as hydrogen peroxide, starch, and sodium chloride. These adulterants pose significant health risks to consumers, highlighting the urgent need for effective monitoring and strict regulatory measures. The application of forensic chemistry has proven to be a valuable tool in identifying food adulterants and ensuring food safety. Collaborative efforts between public health authorities, regulatory bodies, and forensic experts are crucial to curb the growing threat of food fraud and to guarantee the distribution of safe and unadulterated milk to the public**.**

Ethical Clearance : NA

Conflict of Interest : NA

Source of Funding : NA

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