iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 14 **Issue:** I **Month of publication:** January 2026

DOI: https://doi.org/10.22214/ijraset.2026.77142

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 14 Issue I Jan 2026- Available at www.ijraset.com

Milk Adulteration in Nashik: A Comparative Study on Different Adulterants Present in Milk

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Abstract: ***Nashik, Maharashtra has seen an extensive spread of food adulteration issues, with emerging populations being especially at risk from its consequences. Milk is regarded as a complete food since it is rich in vitamins and minerals that support healthy bodily growth. According to the Department of Animal Husbandry and Dairying report 2021-22, about 878.94 tonnes of milk were produced. However, the quantity generated by producers is lower than that of consumers. Many common adulterants are used to enhance volume, simulate nutritious content, prolong shelf life and improve profit. This addition decreases the nutritive value of milk. Health risks from consuming adulterated milk include bacterial infections, chronic toxicity, cancer, gastrointestinal issues, renal damage, nutritional deficiencies and other health issues that rely on the adulterant. Thirty milk samples, comprising both packaged and dairy variants were passed through qualitative analysis to determine adulterants including formalin, salts, sugar, detergent, starch, and skimmed milk. Standard chemical tests were conducted to ascertain the presence of these substances. The results pointed out that a substantial measure of the samples contained adulterants, with ammonium salt, common salt and formalin being the contaminants detected most often. Compared to dairy milk, packaged milk samples showed elevated levels of adulteration, accompanied by traces of preservatives such as formalin detected as well. This research highlights the immediate necessity for more stringent quality control measures and regular testing to safeguard the safety of milk.***

Keywords: milk, adulteration, preservatives, Nashik, nutrition.

I. INTRODUCTION

Since the beginning of civilization, domesticated animals have been an essential part of farming systems, and millions of Indian rural households have a long-standing tradition of dairying. India is a premier agricultural nation. According to the PFA, "milk" has several different meanings. PFA (1976) defined milk as the lacteal secretion absent of colostrum that is produced by thoroughly milking one or more healthy milk cattle.

It provides enough amounts of readily absorbed proteins, fats, carbs, vitamins, and minerals. The nutritional content of milk is great when it is present in its natural state. It has more nutrients than any other food, including vitamins, minerals, lipids, carbohydrates, and high-quality proteins. [8] Numerous animals that produce milk, like goats, buffalo, cows, and others, may easily obtain it. In addition to producing 90% of the world's buffalo milk and ranking second in the world for cow's milk production (54 million metric tons), after the United States, India contributes 17% of the world's milk production. [1] On average, 87% of cow milk is made up of water, with the remaining 3.9% being fat, 4.9% being lactose, 3.5% being protein, and 0.7% being minerals and vitamins. Some of the remaining ingredients are in trace amounts.

The udder's interior, as in mastitis, its outer surface, milk handling and storage equipment, the milking and housing environment, health state, and the udder's hygienic exterior are the primary sources of bacterial contamination of milk. [8] Lactation is commonly done at the wrong time in the absence of suitable lactation guidelines and an easy-to-use lactation detector, which can lead to either milk waste (if breastfeeding starts late) or antibiotic residues in the milk (if lactation is done early). [11]

A lactometer, which gauges the change in specific gravity, is the most widely used scientific instrument for identifying water in milk. In today's world, milk adulteration is a major societal issue and a widespread food fraud. It presents health hazards in addition to the ethical and economical conundrum. Cancer, problems with the skin, kidneys, eyes, and heart are among the most prevalent conditions. For this reason, spotting milk adulteration is essential to illness prevention. Although it can occasionally be caused by insufficient detection equipment and miscommunications among dairy company employees regarding proper medication delivery protocols, adulteration is typically done on purpose to boost earnings.

India produces more than 136 million tonnes of milk annually, making it the greatest producer in the world. To increase profits, milk is tampered with by adding water or removing fat. Moreover, milk is contaminated with urea, detergent, and vegetable fat.

Adulterants include water, flour, salt, powdered soap, detergents, urea, skim milk powder, and preservatives like formalin and hydrogen peroxide will all degrade the quality of milk. The so-called artificial milk is made from these ingredients. There are numerous foods and beverages at the market that can be used in place of milk, including soy milk, vegan yoghurt, rice milk, oat milk, almond milk, hazelnut milk, coconut milk, quinoa milk, and potato milk. Local milk is more contaminated than the pasteurized milk produced in factories. The chemical substance which are used as adulterant in milk are mentioned in below:

Properties	Adulterants
Preservative	Formaldehyde, Salicylic acid, Antibiotics, Benzoic acid, Boric acid and Hydrogen peroxide
Density enhancer	Sodium carbonate, Sodium Bicarbonate, Sodium Hydroxide, and Calcium hydroxide
Insecticide and Pesticide	Organochlorine, Organophosphate, Synthetic pyrethroid and Triazine
Fats	Vegetable oil and Palm Oil
Emulsifier	Detergent
Others	Water and another animal's milk

Table No. 1: Milk adulterants and their properties

A. Analysis of Adulterants

1) Detergent: The main purpose of adding detergent is to cover up the presence of tainted foreign lipids in the milk. Vanaspati oil or other inferior fats are added to change the fat content after the pricey milk fat has been removed and detergents have been added to help emulsify it. In addition to cancer, the detergent's dioxane causes neurotoxicity, endocrine disruption, nausea, skin irritation, and heart issues.

2) Ammonium Sulphate: One ingredient used in milk for its preservation properties is ammonium sulphate. The density of milk is artificially increased by its presence. The WHO does not classify it as an adulterant because the amount in milk might vary depending on how the animals are fed. However, when added to raise the apparent protein content, it performs better than urea and melamine.

3) Formalin: Illegally, trace amounts of formalin are used as a preservative to prolong the shelf life of milk. Formaldehyde combines with aldehyde dehydrogenase in the body to make formic acid, which is fatal to the individual. In spite of temperatures as high as 40 to 50 °C, it is commonly used to keep milk from deteriorating.

4) Starch: Diabetics are seriously harmed by it. Since diluted milk is not commercially desirable, starch is employed to retain density and solid components. To raise the SNF level of milk, wheat, and rice flour, starches and compounds like arrowroot can be added.

5) Cane Sugar: Sometimes, cane sugar is added to watered milk to improve its flavour and mask the presence of extra water. It is true that adding sugar to milk causes the lactometer reading to rise and, as a result, the specific gravity of watered milk to rise. This makes it challenging to use the lactometer test to identify too much water.

6) Sodium Chloride: It thickens artificial milk by increasing its specific gravity. Adding salt can also improve the lactometer results. When salt is added to milk, it greatly increases the freezing point depression number but has no influence on the total SNF content, making it difficult to determine the amount of additional water in the milk.

7) Skimmed Milk Powder: Skim milk powder can be used to change the milk solids in toned, double-toned, and recombined milk. It can raise the milk's protein level, giving the impression that it is healthier. Since milk powder is a dried product that keeps better than fresh milk, it can also be used to extend the milk's shelf life.

Yadav, A. K., Gattupalli: The primary milk adulterants in India were examined by Ajay Kr. Yadav et. al., who concentrated on how to identify them and why they were added. They talked about the benefits of milk as a stand-alone diet for both babies and adults, but the idea that milk is high in nutrients has resulted in dishonest adulteration to increase profits. The percentage of milk and milk products that do not satisfy acceptable quality requirements in India is 68.7%.

Azad, T., & Ahmed, S. (2016).: Tanzina Azad and Shoeb Ahmed studied frequent milk adulteration and how to detect it using both qualitative and quantitative methods. They also talked about the usage of foreign proteins including soy, rice, wheat, and almonds, as well as the drawbacks of qualitative approaches.

Nascimento, C. F: Carina F. Nascimento and her team looked at the most recent advancements in milk adulterant detection, with a particular emphasis on the identification of contaminants and adulterants in milk using mid-infrared spectroscopy (MIRS). A diluted sample is needed for analysis using MIRS technology, which is non-destructive. A rapid method for detecting food adulterants or defects in the quality of fresh milk or milk powder is FT-MIR. In addition to polarimetric, isoelectric precipitation, SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis), HPLC (High Performance Liquid Chromatography), and immunodiffusion techniques, polyclonal antibodies are employed to identify foreign proteins and other adulterants.

Kandpal, S. D., Srivastava: Using a milk adulteration testing equipment, Kandpal SD and their group focused on estimating the quality of raw milk. Only 12 out of 60 milk samples that were analysed had a specific gravity of 26, with the other samples having lower values. In order to ensure milk purity, enhance consumer safety, and promote confidence, the plan incorporated machine learning techniques. Milk adulterants such as formalin, sugar, starch, gelatine, urea, detergent, hydrogen peroxide, ammonium sulphate, benzoic acid, salicylic acid, nitrate borax, boric acid, Vanaspati, sodium chloride, buffalo milk in cow milk, neutralizers and other substances can significantly jeopardize human health. Such substances are analyzed by means of qualitative testing techniques like colour tests.

Aqeel, M., Sohaib: Researchers have investigated cutting-edge technologies like potentiometric electronic tongue and near-infrared spectroscopy for the detection and classification of milk adulteration, with Muhammad Aqeel's study on hyperspectral imaging and machine learning focusing on both destructive and non-destructive methods.

Kamthania, M., Saxena: The Milk Adulteration: Methods of Detection & Corrective Measures project by Mohit Kamthania, Jyoti Saxena, and their team discovered a number of adulterants in milk using simple and quick methods. The government has taken steps to stop fraudulent practices by passing a number of laws, and the study indicates that low-income consumers are the least educated and unaware of food adulteration, underscoring the need for better education and training on the issue. In conclusion, those research offers important new information about the techniques employed in India to identify milk adulterants. To solve the intricate problems pertaining to milk adulteration and guarantee the security and caliber of milk products, extra investigation is necessary.

II. MATERIALS AND METHODS

For preparation of reagents all the salts namely Ferric Chloride, Mercuric Chloride, Sodium Hydroxide, Potassium Iodied, Methylene blue solution, Chloroform, Silver Nitrate, Potassium Chromate, Resorcinol, Hydrochloric Acid, Phosphomolybdic Acid, Acetic Acid, Iodine Solution, were purchase from Sigma Aldrich Inc, Thermo fisher scientific India Pvt. Ltd, Modern Industries-74, EMPLURA, EMPATRA and Addendum etc. are given below:

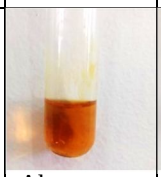
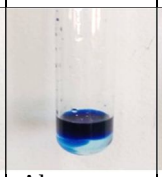
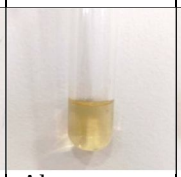
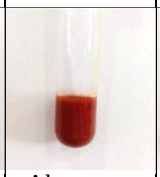
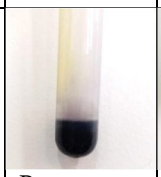
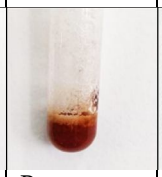
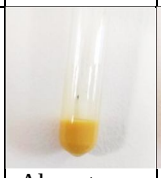
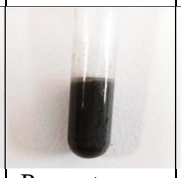
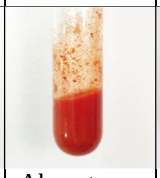
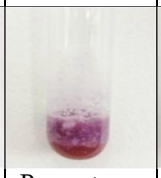
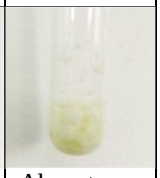
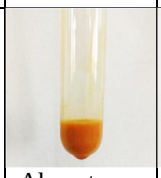
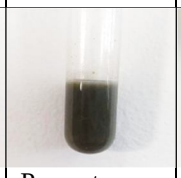
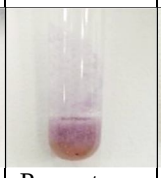
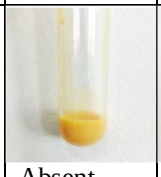
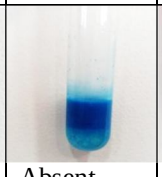
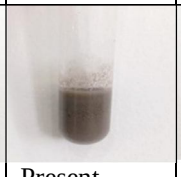
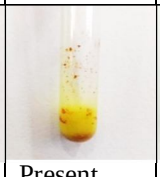
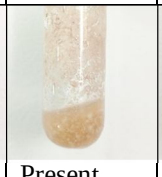
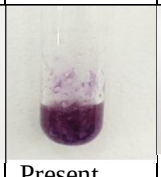
The standard reagent solutions were prepared in distilled water. All the borosilicate glassware were thoroughly washed with aqua regia and rinsed with water prior to use.

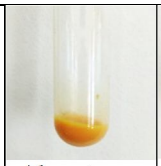
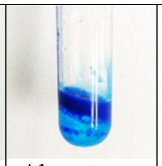
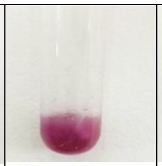
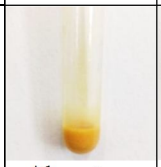
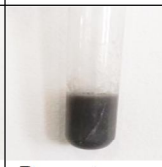
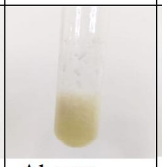
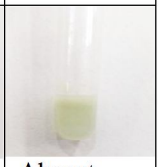
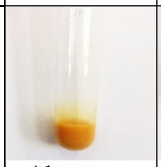
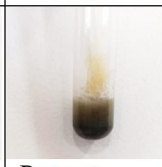
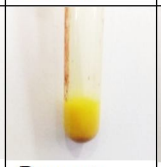
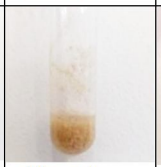
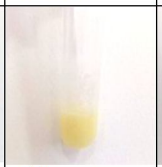
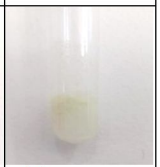
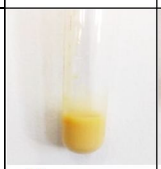
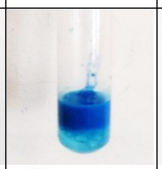
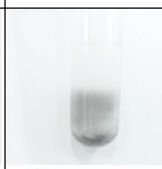
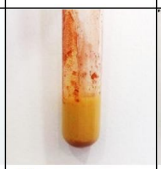
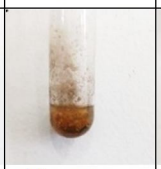
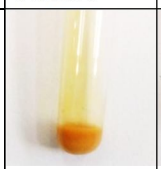
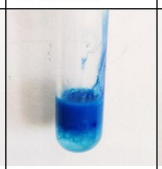
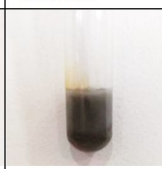
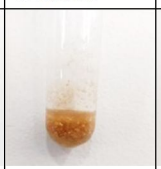
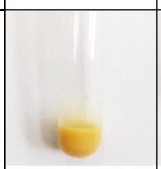
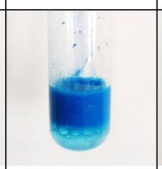
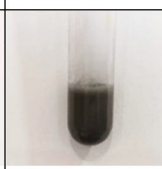
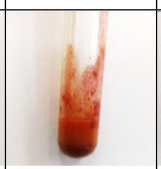
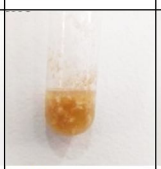
Sr. No.	Adulterant	Test for detection	Observation
1	Starch	Take 1 ml milk in test tube, add 2 drops of iodine solution.	Appearance of blue colour indicates the presence of starch in the milk
2	Detergent	Take 1 ml of milk in test tube, add 1 ml of Conc. HCl in it. Take 0.1 gm resorcinol and mixed well in test tube. Place the test tube in boiling water bath for 5 min.	More intense blue colour in lower layer indicates presence of detergent in milk
3	Ammonium Salt	Take 2 ml of milk sample into test tube. Add 1ml of Nessler's reagent and mix the content thoroughly	Appearance of dark brown colour confirms the presence of added ammonium salts in milk.
4	Common Salt	Take 1 ml of milk sample into test tube. Add 1ml of 0.1 N silver nitrate solution mix it thoroughly. Add 0.5 ml of 10% potassium chromate solution in test tube.	Appearance of yellow colour indicates the presence of added salts.
5	Cane Sugar	Take 1 ml of milk in test tube, add 1 ml of Conc.	In the presence of cane sugar

		HCl in it. Take 0.1 gm resorcinol and mixed well in test tube. Place the test tube in boiling water bath for 5 min.	in the milk sample, red colour is produced.
6	Formalin	(From 10 % of ferric chloride add 2-3 drops in 5ml HCl). Take equal amount of milk with ferric chloride. Let the test tube sit in a boiling water bath for 3-4 min	Appearance of brownish violet colour confirms the presence of formalin in the sample
7	Skimmed milk powder	Take milk which centrifuge at 5000 rpm for 15 min. Remove cream layer and take out 5 ml of milk. Add 2ml of acetic acid for coagulation and wash curd with distilled water. To the curd add 2ml of 1% phosphomolybdic acid. Mix the content and heat in water bath for 5 minutes.	Curd indicate bluish colour in the presence of skimmed milk.

Table no.2 Tests for adulterants with observation

III. RESULTS

Sr. No	Test product	Starch (Iodine solution)	Detergent (methylene blue sol)	Ammonium salt (Nessler reagent)	Salt, NaCl (silver nitrate)	Cane sugar (Resorcinol)	Formalin (Leach test)	Milk powder (Phosphomolybdic acid)
	control	 Absent	 Absent	 Absent	 Absent	 Absent	 Absent	 Absent
	standard	 Present	 Present	 Present	 Present	 Present	 Present	 Present
1	sample 1	 Absent	 Absent	 Present	 Absent	 Present	 Present	 Absent
2	sample 2	 Absent	 Absent	 Present	 Absent	 Present	 Present	 Absent
3	sample 3	 Absent	 Absent	 Present	 Present	 Present	 Present	 Present

4	sample 4	 Absent	 Absent	 Absent	 Absent	 Present	 Present	 Absent
5	sample 5	 Absent	 Absent	 Present	 Absent	 Absent	 Absent	 Absent
6	sample 6	 Absent	 Absent	 Present	 Present	 Absent	 Absent	 Absent
7	sample 7	 Absent	 Absent	 Absent	 Present	 Absent	 Absent	 Absent
8	sample 8	 Absent	 Absent	 Present	 Absent	 Absent	 Absent	 Absent
9	sample 9	 Absent	 Absent	 Present	 Absent	 Absent	Absent	Absent
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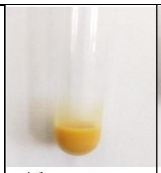
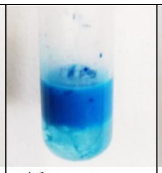
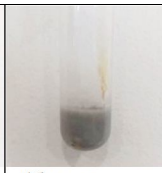
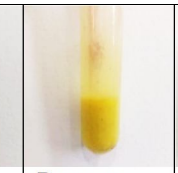
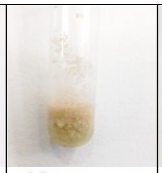
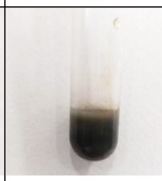
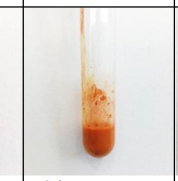
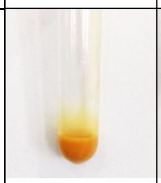
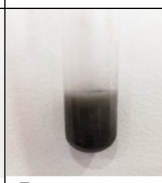
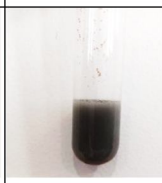
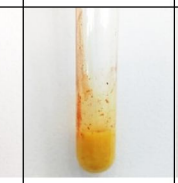
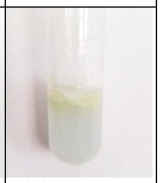
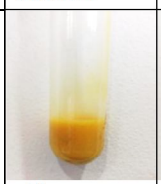
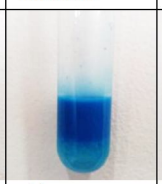
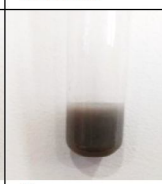
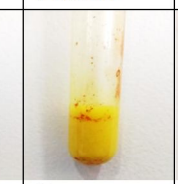
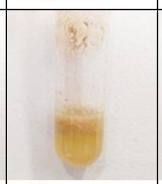
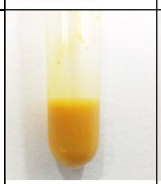
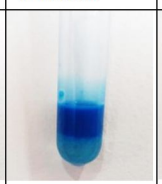
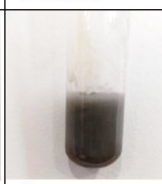
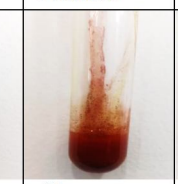
13	sample 13	 Absent	 Absent	 Absent	 Present	 Absent	 Absent	 Absent
14	sample 14	 Absent	 Absent	 Present	 Absent	 Absent	 Absent	 Absent
15	sample 15	 Absent	 Absent	 Present	 Absent	 Absent	 Absent	 Absent
16	sample 16	 Absent	 Absent	 Present	 Present	 Absent	 Absent	 Absent
17	sample 17	 Absent	 Absent	 Present	 Present	 Absent	 Present	 Present
18	sample 18	 Absent	 Absent	 Present	 Absent	 Absent	Absent	Absent
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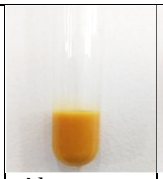
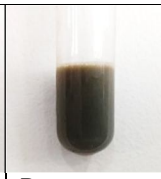
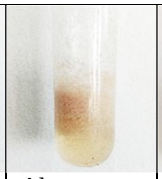
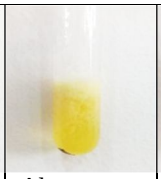
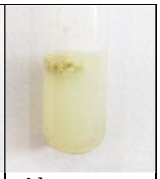
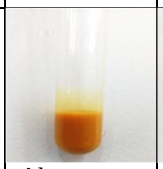
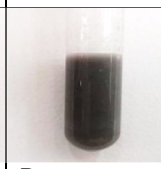
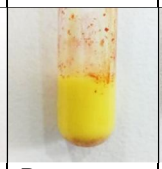
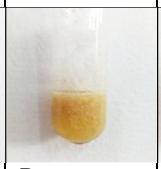
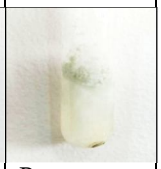
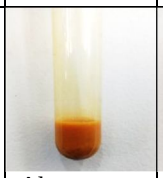
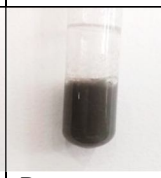
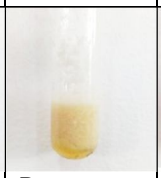
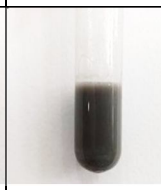
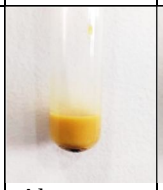
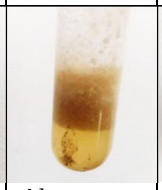
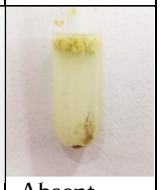
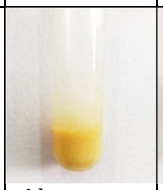
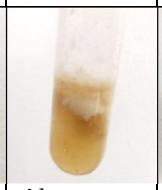
21	sample 21	 Absent	 Absent	 Present	 Absent	 Absent	 Absent	 Absent
22	sample 22	 Absent	 Absent	 Present	 Present	 Absent	 Present	 Present
23	sample 23	 Absent	 Absent	 Present	 Present	 Absent	 Present	 Absent
24	sample 24	 Absent	 Absent	 Present	 Present	 Present	 Present	 Present
25	sample 25	 Absent	 Absent	 Present	 Present	 Absent	 Present	 Absent
26	sample 26	 Absent	 Absent	 Present	 Present	 Absent	Present	Absent
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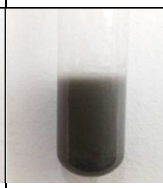
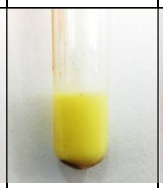
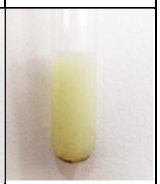
30	sample 30	 Absent	 Absent	 Present	 Present	 Absent	 Absent	 Absent

IV. DISCUSSION

When combined with anionic detergents, the cationic dye methylene blue creates a complex. Usually water soluble, it exhibits affinity for anionic detergents when they are present. Chloroform is one example of an organic solvent that is used to extract an anionic surfactant. When detergent is present, the sample's chloroform layer develops a blue hue. Since chloroform has a lower density than milk, it sinks to the bottom. [39,42]

When iodine solution is added to milk that contains starch, a compound between the iodine and the starch's amylose component causes the milk to turn blue. Compared to amylose, amylopectin is less potent. Above roughly 9.5 pH, the blue colour vanishes. [39]

The complex that forms between Hg2- and NH3 in the presence of OH- (alkaline) medium may be the cause of the first yellow colour production in the ammonium sulphate and Nessler reagent test that gradually turns brown and its following transaction. Furthermore, the temperature may have an impact on the stability of the complex that is created during the reaction. [40]

Using potassium chromate as the indicator and silver nitrate as the titrant, the sodium chloride test first produces insoluble white silver chloride by a selective reaction with the sample's chloride. Following the precipitation of all the chloride, the potassium chromate and silver nitrate combine to generate a reddish-orange precipitate of silver chromate. [16]

$$AgNO_3 + K_2CrO_4 + Cl^- \rightarrow AgCl + NO_3^- + K_2CrO_4$$

$$2AgNO_3 + K_2CrO_4 \rightarrow Ag_2CrO_4 + 2KNO_3$$

(Orange)

Fig. Reaction mechanism in silver nitrate test in Sodium Chloride

Red is produced when the fructose in cane sugar combines with the resorcinol in HCl. [41] Boiling in aqueous hydrochloric acid (HCl) containing resorcinol causes this well-known colour response for ketoses. It is predicated on the observation that ketoses yield a furfural derivative more quickly than aldoses. The process involves triple dehydration of the monosaccharide to either furfural (ketopentoses) or HMF (ketohexoses). Lastly, resorcinol reacts with furfural and HMF to produce bluish-green and red hues, respectively. [43]

Specifically, tryptophan, an amino acid, reacts with milk proteins when formalin is present, forming unstable aldehyde-protein complexes. The unstable chemicals react with the acid/ferric salt mixture to form a violet-coloured material. This colour change is a sign that formalin is present. [44]

The technique is predicated on the observation that the coagulum produced from reconstituted skim milk powder by adding acetic acid exhibits a strong blue hue when boiled with phosphomolybdic acid. This is because the milk powder's proteins contain specific reducing groups that can reduce molybdenum blue, forming a blue hue. [41]

V. CONCLUSION

One of the main motivations for milk adulteration is financial gain. The majority of the milk samples had adulterants introduced either purposefully or during manufacture and processing in accordance with one's own decision to make money. Because milk can be tainted with subpar, less expensive components or toxic substances including pond water, milk powder, cane sugar, urea, melamine, glucose, and detergents, tainted milk and milk products pose a threat to all of humanity. A number of health issues arise when people consume such contaminated and fake milk. High quality protein, important amino acids, and other vitamins and minerals, including as calcium, are all abundant in milk and are critical for healthy growth and development. Milk contributes to energy needs by providing fats and carbs.

There are compounds in milk that help strengthen the immune system.

Milk can help with vitamin absorption and digestion. However, there are serious health hazards associated with milk adulteration, which involves the addition of dangerous compounds, such as nutritional deficits, digestive issues, and even possibly toxic effects. Therefore, in order to guarantee the best possible health and wellbeing, it is essential to recognize and stay away from contaminated milk. Respondents from the low-income category were the least educated, less aware of their rights and obligations, and less aware of food adulteration. milk is a staple and complete diet in India, this analysis is done to educate the public about the health risks associated with milk production malpractices. To prevent milk adulteration in our nation, strict regulations, public education, and a well-organized dairy business are needed. Each and every departing human being must be aware of food adulteration.

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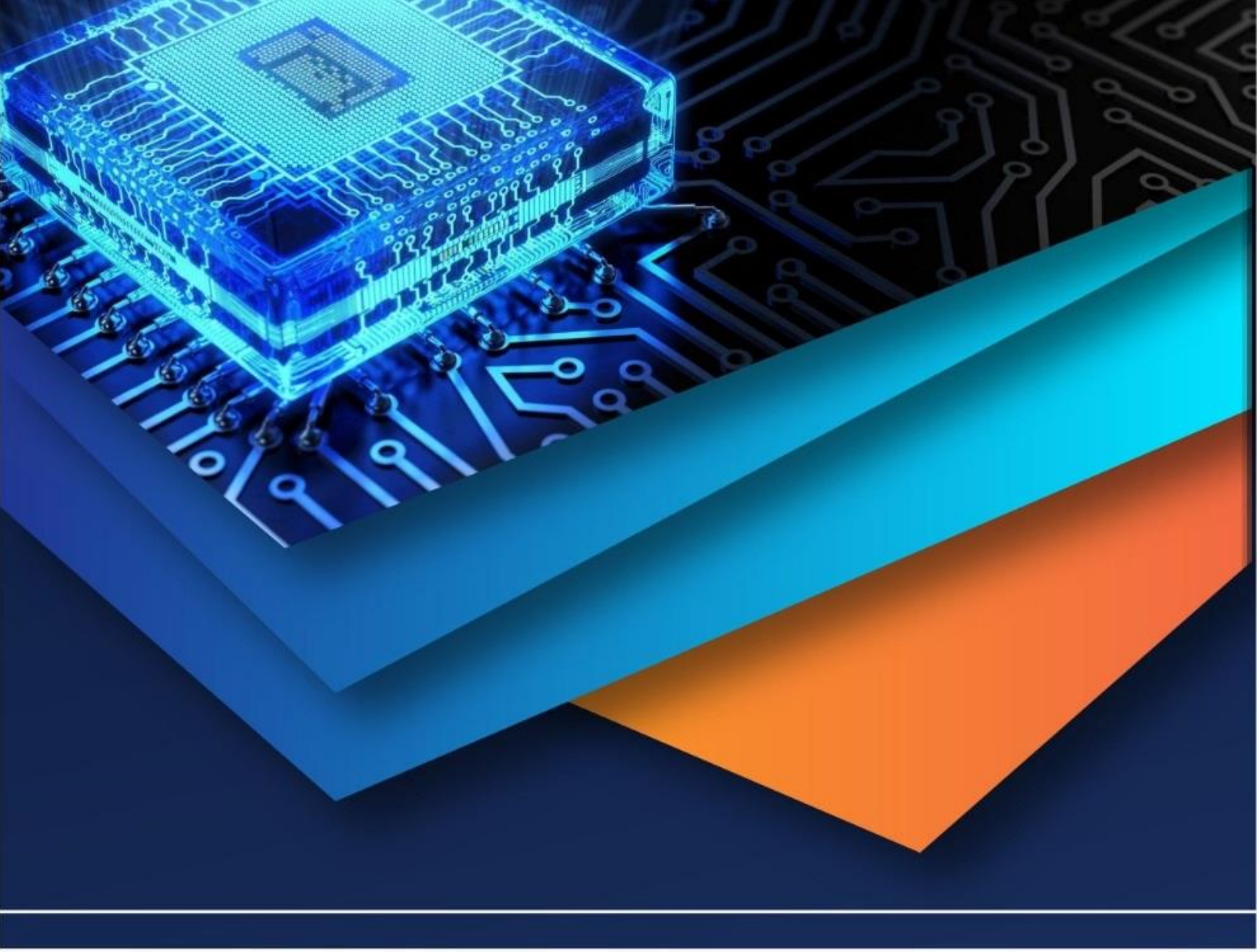

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