Tool Kit For Identification of Adulteration in Milk

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ABSTRACT

Milk adulteration remains a critical food safety issue worldwide, with the problem being more acute in developing countries due to weak regulatory enforcement and lack of consumer-level detection methods. Adulterants such as starch, detergents, urea, formalin, hydrogen peroxide, and melamine are frequently added to milk to enhance profit margins but pose significant health risks, ranging from gastrointestinal disorders to kidney damage and cancer. The present study introduces a novel Toolkit for the Detection of Milk Adulterants, designed to provide rapid, reliable, and cost-effective identification of common adulterants. The toolkit integrates qualitative colorimetric tests for on-site screening with guidelines for advanced confirmatory methods such as chromatography, spectroscopy, biosensors, and chemometric analysis. Its portability, low cost, and simplicity make it suitable for field applications, small laboratories, dairy industries, and even consumer-level use, thereby filling a crucial gap between laboratory-based techniques and real-world needs. The toolkit is expected to improve milk quality monitoring, raise consumer awareness, and support regulatory authorities in enforcing food safety standards.

Keywords: Milk adulteration, Tool kit, TLC, Harmful substances.

INTRODUCTION

Adulteration refers to the deliberate lowering of food quality by mixing in inferior substances, substituting key ingredients, or removing valuable components. It encompasses not only intentional tampering that compromises the food's nature and quality, but also accidental contamination that may occur during cultivation, harvesting, storage, processing, transportation, or distribution. An adulterant is any substance that renders food unsafe, substandard, falsely labelled, or contaminated with foreign matter.[1]

TYPES OF ADULTERATION

1. Intentional adulteration

It refers to the deliberate act by unscrupulous manufacturers or vendors to compromise the quality of food items. This is often done to falsely enhance the nutritional content or increase the quantity of the product, thereby maximizing profit. Common tactics include the addition of harmful chemicals like urea and melamine, or bulk-increasing substances such as starch, flour, cane sugar, vegetable oils, water, skim milk, and even hazardous materials like sand, chalk powder, molasses, brick powder, stone particles, ergot, chicory, roasted barley powder, and ground papaya seeds. These practices pose serious health risks and undermine food safety standards.[2]

2. Incidental adulteration

It refers to the unintentional contamination of food, often stemming from a lack of awareness or inadequate resources to preserve its quality. Such adulteration may occur due to chemical residues from pesticides and fertilizers, or through leakage and exposure during various stages of production. Improper handling, storage, and packaging practices can also contribute to this type of contamination. As the term suggests, accidental adulteration happens unknowingly and without deliberate intent.[3]

3. Metallic contaminants

It can infiltrate the food supply either through environmental exposure or during various stages of food processing. These substances often appear in trace quantities and may originate from industrial pollution, agricultural inputs, or equipment used in manufacturing and packaging.[4]

MILK AND MILK PRODUCTS ADULTERATION

In many developing nations, industrial growth though beneficial in numerous ways has also introduced significant challenges. One such issue is the large-scale collection and distribution of milk from multiple sources, which has become a potential pathway for the spread of diseases.[5] Milk adulteration typically includes diluting milk with water and extracting its nutritious fat content, which significantly reduces its overall quality and health benefits. Manufacturers intentionally tamper with milk and dairy products by reducing their natural nutrient content and then artificially boosting it to create a false impression of quality.[6] This deceptive practice is aimed at misleading consumers and maximizing profits. To achieve this, they often add substances such as urea, starch, flour, cane sugar, vegetable oils, and detergents compromising both safety and nutritional integrity. Milk is often treated with preservatives such as formalin and certain antibiotics to extend its shelf life and delay spoilage. These additives help maintain the product's appearance and usability over time, though they may pose health concerns if used improperly.[7]

Milk is widely considered as a complete food, rich in essential nutrients like proteins, vitamins, minerals, and healthy fats that support growth and development. Yet, its purity has come under threat in recent years due to increasing cases of adulteration. Whether driven by profit motives or resulting from careless practices, food adulteration presents serious risks to public health and consumer safety[8].Common contaminants such as water, detergents, starch, formalin, urea, and harmful chemicals diminish milk's nutritional value. These substances can lead to dangerous health issues, including digestive problems, damage to internal organs, and chronic illnesses. In many developing countries, weak regulatory frameworks and poor enforcement make it difficult to curb adulteration. Misleading marketing and the complexity of today's supply chains further complicate consumers' ability to identify safe products. Strengthening detection technologies and increasing public awareness are essential steps in addressing this widespread issue. The project was carried out in three distinct stages to address the issue of milk adulteration[9].

In **Stage I**, essential raw materials and chemical agents were obtained from multiple suppliers and systematically packed into clearly labelled containers to ensure proper handling and identification.

Stage II focused on performing a range of chemical analyses to detect prevalent adulterants in milk. These tests included identifying the presence of water using a lactometer, as well as detecting formalin, benzoic acid, salicylic acid, starch, and detergents. Advanced methods such as thin layer chromatography and litmus paper testing were utilized to improve precision and reliability[10]

In **Stage III**, following the confirmation of adulterants, a comprehensive detection toolkit was prepared and packaged for practical application. This initiative aims to enhance consumer protection by facilitating the accurate detection of contaminated milk and raising awareness about maintaining food quality standards.

METHODS

a) Detection of water content

To determine the water content present in the sample of milk gently place the lactometer into the milk, ensuring it floats vertically at a controlled temperature (around 15.5°C or 60°F) and wait about 30 seconds, then read the level at the top of the milk's curved surface (the meniscus) where it meets lactometer's scale[11]

b) Detection of starch

Place a small quantity of amount of milk into a test tube and add a few drops of iodine solution to the milk observe any colour change in milk[12].

c) Detection of formalin

Take about 10 ml of the milk sample & add about 5 ml of concentrated sulfuric acid to the test tube along the inner sides, allowing the acid to form a distinct layer without mixing with the milk[13].

d) Detergent test

Take 5 to 10 ml of the milk sample add an equal amount of water to the milk sample in test tube. Shake the mixture vigorously for at least 30-40 seconds[14].

e) Detection of benzoic and salicylic acid

Take 5 mL of the milk sample in a test tube and acidify it by adding concentrated sulphuric acid. Then, add 0.5% ferric chloride solution drop wise while mixing the contents thoroughly. The appearance of a buff colour indicates the presence of benzoic acid, whereas a violet colour signifies the presence of salicylic acid.[15]

f) Litmus test

Take a small piece of red litmus paper and dip it into the milk solution and observe any colour change[16].

g) Detection of salt

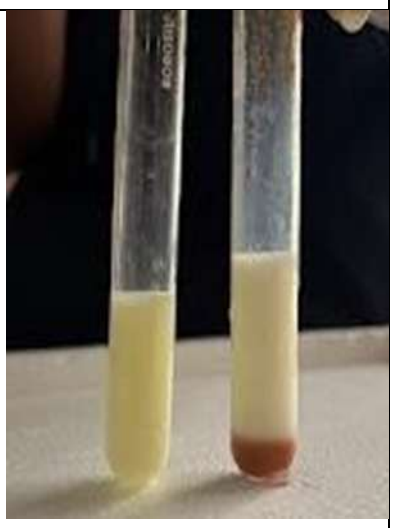
To the 2ml of milk add silver nitrate and observe the colour change in the test tube.[17].

h. Thin layer chromatography

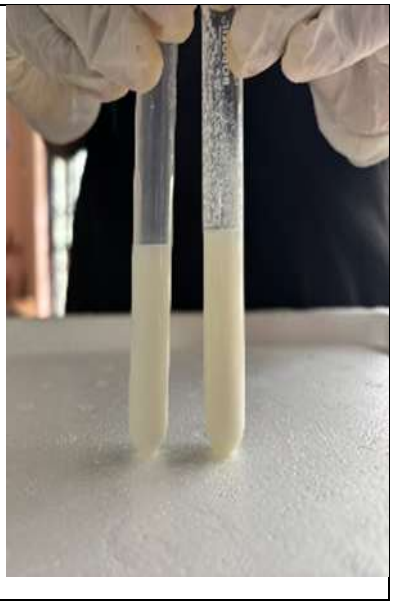
To perform TLC of milk, prepare a TLC plate with a silica gel stationary phase, spot the milk sample and a standard into the baseline, place it in a development chamber with a mobile phase which is a mixture of ethyl acetate: acetic acid:ethanol 10:6:4, allow capillary action to develop the chromatogram and mark the solvent front, visualize the separated component by spraying iodine solution and calculate the Retention Factor (Rf) value[18].

RESULTS

ADULTERANT	REASON	HEALTH RISKS	OBSERVATIONS
Water	To increase volume	Reduces nutritional value	

Starch	To thicken the milk	Harmful to diabetics	
Formalin	Preservative	Carcinogenic and highly toxic	
Detergent	Milk appears frothy	Damages internal organs	

Benzoic and salicylic acid	Preservatives	Toxic	
Litmus test	Alkali in milk	Kidney damages and renal failure	
Thin layer chromatography	Identify compounds	Can Prevent all harmful effects	

Detection of salt	Increases density	Affects blood pressure	

Packing of the kit:

After performing the chemical test to determine the adulteration in milk, tool kit packing was done.

CONCLUSION

The study successfully developed a comprehensive, multipurpose toolkit for detecting adulterants in milk and milk products, addressing the urgent need for accessible and reliable detection methods. By combining simple chemical spot tests with validated instrumental techniques, the toolkit ensures both rapid screening and confirmatory analysis. It also incorporated thin layer chromatography for the purpose of quantification of the adulterants. Its affordability and user-friendliness make it suitable for deployment in rural areas, small-scale dairy sectors, and developing regions, where milk adulteration is most prevalent. Importantly, this initiative not only supports regulatory enforcement but also empowers consumers and producers to identify adulteration at the earliest stage, thereby reducing health risks and economic losses. Future directions include integration of biosensors, portable digital devices, and mobile-app-based data recording to enhance precision, real-time monitoring, and traceability. Overall, this toolkit represents a significant innovation in food safety, with the potential to serve as a model framework for developing similar detection kits for other food commodities.

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REFERENCES:

[1] M.N.L. Aishwarya, Dr. Mohammad Badrud Duza. A REVIEW ON ADULTERATION OF MILK. Indo American journal of Pharmaceutical Research. 2017 Sep 5;7(8):630–7.

[2] Ayza A, Belete E. Food adulteration: its challenges and impacts. Food Sci Qual Manag. 2015;41(2015):50-6.

[3] Mansuri, Z. (2011). Study of adulterants in foods. Delhi, India.

[4] Deepti Narayan, 2014, Food Adulteration-Types, worldwide lows and future Category: Healthcare Views: 20277

[5] Chanda T, Debnath GK, Hossain ME, Islam MA, Begum MK. Adulteration of raw milk in the rural areas of Barisal district of Bangladesh. Bangladesh Journal of Animal Sciences. 2012; 41(2):112-115

[6] Choudhary A, Gupta N, Hameed F, Choton S. An overview of food adulteration: Concept, sources, impact, challenges and detection. International Journal of Chemical Studies. 2020 Jan 1;8(1):2564–73.

[7] Awan Adeela, Naseer Misbah, Aasfa Ali, Muhammad Rehana, Furhan. A study on chemical composition and detection of chemical adulteration in tetra pack milk samples commercially available in Multan. Pak. J Pharma. Scie. 2014; 27(1):183

[8] VB Khomane, Kamble D, AT Lokhande, Patil B, SV Lashkare, KB Kubade, et al. A comprehensive review on adulteration of milk. International journal of advanced biochemistry research. 2024 Jan 1;8(4S):316–24.

[9] Azad T, Ahmed S. Common milk adulteration and their detection techniques. International Journal of Food Contamination. 2016 Dec;3(1).

[10] Geisler JF. SIGNIFICATION OF LACTOMETER TESTS. Journal of the American Chemical Society. 1891 Feb 1;13(2):93–8.

[11] Ystgaard, O.M., et al. “Detection of Adulteration of Milk by Lactometric and Freezing Point Methods.” *Journal of Dairy Science*, vol. 34, no. 7, July 1951, pp. 680–688, https://doi.org/10.3168/jds.s0022-0302(51)91765-1. Accessed 26 Dec. 2019.

[12] Luther, Jamie L., et al. “Paper Test Card for Detection of Adulterated Milk.” Analytical Methods, vol. 9, no. 38, 2017, pp. 5674–5683, https://doi.org/10.1039/c7ay01321c. Accessed 6 Apr. 2022.

[13] Hehner, Otto. “The Detection of Formalin.” The Analyst, vol. 21, no. April, 1896, p. 94, https://doi.org/10.1039/an8962100094. Accessed 16 Sept. 2025.

[14]Ho, Thao M., et al. “Foaming Properties and Foam Structure of Milk during Storage.” Food Research International (Ottawa, Ont.), vol. 116, 1 Feb. 2019, pp. 379–386,pubmed.ncbi.nlm.nih.gov/30716960/ https://doi.org/10.1016/j.foodres.2018.08.051. Accessed 5 Oct. 2021.

[15] https://foodsafetyandrisk.biomedcentral.com/articles/10.1186/s40550-016-0045-3#ref-CR4

[16] McInerney, T.J. "A Note on the Acidity of Fresh Milk." *Journal of Dairy Science*, vol. 3, no. 3, May 1920, pp. 227–229, https://doi.org/10.3168/jds.s0022-0302(20)94267-4. Accessed 29 Dec. 2021.

[17] Yadav, Ajay Kr., et al. "Key Milk Adulterants in India and Their Detection Techniques: A Review." Food Analytical Methods, 8 Dec. 2022, https://doi.org/10.1007/s12161-022-02427-8. Accessed 8 Dec. 2022.

[18] Kumar, Ranjith N., et al. "Development of a Thin Layer Chromatography-Based Method to Detect Sorbitol Presence in Milk and Its Applicability in Formalin Preserved Milk Samples." Journal of Food Science and Technology, vol. 61, no. 5, 1 May 2024, pp. 870–878, pubmed.ncbi.nlm.nih.gov/38487282/, https://doi.org/10.1007/s13197-023-05880-3. Accessed 17 Mar. 2024.