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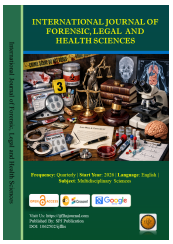

Original Research

Detection of Chemical Adulterants in Commercially Packaged Milk: An Integrated Approach Using Conventional Physicochemical Analysis and Untargeted GC-MS Screening

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ABSTRACT

Milk is among the most widely consumed food commodities and remains highly susceptible to adulteration for economic gain, necessitating continuous quality surveillance. This study evaluated the safety and authenticity of commercially packaged milk using conventional physicochemical analyses in conjunction with untargeted gas chromatography–mass spectrometry (GC–MS). Ten milk samples procured from local retail outlets were assessed for pH, ash content, and moisture, and screened for common adulterants including starch, detergent, neutralizers, hydrogen peroxide, formalin, and sodium chloride. Untargeted GC-MS analysis was additionally performed to detect chemical constituents without prior selection of specific target analytes. Conventional qualitative testing revealed detergent contamination in two samples, while starch, formalin, hydrogen peroxide, neutralizers, and sodium chloride were not detected in any sample. Physicochemical parameters remained within acceptable reference ranges across all samples. However, untargeted GC-MS analysis identified several compounds of toxicological and food-safety significance, including 2-pyrrolidone, toluene, oleic acid, glycidyl palmitate, benzyl butyl phthalate, and 2,4-di-tert-butylphenol. These findings demonstrate that untargeted GC-MS can detect chemical contaminants that evade conventional screening protocols, underscoring its value as a complementary analytical tool in food forensic investigations and milk quality assessment. The results emphasize the need to integrate advanced instrumental techniques with routine testing methods to strengthen consumer protection and food authenticity monitoring frameworks.

Keywords: Milk Adulteration, Food Forensics, Untargeted Screening, Food Authenticity

INTRODUCTION

Food is one of the most fundamental requirements for human life, providing the nutrients necessary for growth, energy production, tissue repair, and the maintenance of normal physiological functions. [1] A balanced diet supplies carbohydrates, proteins, fats, vitamins, minerals, and other essential nutrients that help maintain overall health and prevent disease. Foods may originate from plants or animals and can be consumed in raw, processed, or semi-processed forms. However, the nutritional value and safety of food depend greatly on its quality and purity. [2-4] Unfortunately, increasing commercialization of the food industry has led to a significant rise in food adulteration, making food safety a major public health concern worldwide. Food adulteration refers to the deliberate or accidental addition, substitution, or removal of substances that reduce the quality, purity, or nutritional value of food. [5-7] In many cases, adulteration is carried out to increase profits by replacing expensive ingredients with cheaper alternatives or by enhancing the appearance and shelf life of products. Such practices mislead consumers and may expose them to harmful chemicals, toxic substances, or microbial contamination. Although intentional adulteration is primarily driven by economic motives, accidental adulteration can also occur because of poor manufacturing practices, environmental contamination, improper storage, or inadequate handling during food production and distribution. Food forensics has emerged as an important scientific

discipline for identifying adulteration and ensuring food authenticity. It combines analytical chemistry, molecular biology, and forensic science to determine whether a food product is genuine and whether its composition matches the information provided on the label. [8] Food forensic investigations also help establish the geographical origin, botanical or animal source, production methods, and traceability of food products. By detecting adulterants, contaminants, and fraudulent substitutions, food forensics plays a crucial role in protecting consumers, supporting regulatory agencies, and maintaining public confidence in the food supply chain. In India, food adulteration remains a persistent challenge despite the implementation of strict food safety regulations. [9] The Prevention of Food Adulteration Act, 1954, followed by the Food Safety and Standards Act and its enforcement through the Food Safety and Standards Authority of India (FSSAI), provides legal measures to prevent the manufacture and sale of adulterated food. [10] These regulations define adulteration, establish quality standards, and prescribe penalties for violations, including imprisonment and financial fines for serious offences that endanger public health. Nevertheless, increasing consumer demand, inadequate awareness, and unethical trade practices continue to contribute to the circulation of adulterated food products.

Among commonly adulterated foods, milk is of particular concern because it is consumed daily by people of all age groups, especially infants, children, pregnant women, and older adults. Milk is a highly nutritious food containing water, proteins, fats, lactose, vitamins, and minerals in appropriate proportions. Its rich nutritional composition also makes it highly susceptible to microbial growth and spoilage. To increase volume, improve appearance, or extend shelf life, unscrupulous vendors may add substances such as water, starch, detergents, urea, formalin, hydrogen peroxide, and neutralizing agents. These adulterants not only reduce the nutritional quality of milk but may also cause gastrointestinal disorders, organ damage, toxicity, and other long-term health complications. Therefore, reliable detection methods, stringent quality control measures, consumer awareness, and effective regulatory enforcement are essential to ensure the safety, authenticity, and nutritional integrity of milk and other food products. [11-14]

MATERIALS AND METHODS

Study Design and Sample Collection: A laboratory-based analytical study was conducted to investigate the presence of adulterants in commercially available milk samples. A total of ten milk samples were collected from different confectionery shops located within the study area. The samples were purchased as regular consumer products to ensure unbiased sampling. The retail price of the collected milk samples ranged between ₹32 and ₹33 per unit. Immediately after collection, the samples were labelled (Sample 1–Sample 10) and transported to the laboratory under appropriate storage conditions for further analysis.

Analytical Instrumentation: The study employed Gas Chromatography–Mass Spectrometry (GC–MS) as the primary analytical instrument for the identification of chemical adulterants present in milk samples. GC–MS combines the separation capability of gas chromatography with the molecular identification power of mass spectrometry, enabling both qualitative and untargeted screening of volatile and semi-volatile compounds. The technique is widely used in food quality assessment because of its high sensitivity, specificity, and reliability. The mass spectrometer consisted of an ionization chamber, accelerator plates, repeller electrode, and mass analyser. After ionization, positively charged ions were accelerated and separated according to their mass-to-charge (m/z) ratio before being detected, allowing accurate identification of chemical constituents present in the milk samples.

Parameters Evaluated: The collected milk samples were analysed using both qualitative and quantitative approaches.

Qualitative analysis included:

- Detection of starch
- Detection of sodium chloride
- Detection of detergent
- Detection of formalin
- Detection of neutralizers
- Detection of hydrogen peroxide

Quantitative analysis included:

- Ash content
- Moisture content
- pH measurement
- Untargeted GC–MS screening

Qualitative Chemical Analysis: Standard chemical tests were performed to detect common milk adulterants.

- **Starch Test:** Approximately 2 g of milk sample was mixed with distilled water, boiled, cooled, and treated with iodine solution. Development of a blue colour indicated the presence of starch.
- **Detergent Test:** Two millilitres of milk were mixed with methylene blue solution followed by chloroform extraction. Formation of a blue colour in the lower chloroform layer confirmed the presence of detergent.
- **Neutralizer Test:** Equal volumes of milk and rectified spirit were mixed, followed by the addition of Rosalic acid reagent. A deep rose-red colour indicated the presence of neutralizing agents, whereas a brownish colour indicated their absence.
- **Hydrogen Peroxide Test:** One millilitre of milk was mixed with potassium iodide-starch reagent. The appearance of a blue colour confirmed the presence of hydrogen peroxide.
- **Formalin Test:** Ten millilitres of milk were treated with ferric chloride solution, followed by careful addition of concentrated sulphuric acid. Formation of a violet or blue ring at the interface indicated the presence of formalin.

pH Determination: The pH of each milk sample was determined using a calibrated digital pH meter.

Prior to analysis, the instrument was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.0. The electrode was rinsed thoroughly with distilled water between measurements to avoid cross-contamination. The pH values obtained were compared with the standard acceptable range for fresh milk.

Ash Content Determination: Ash content was estimated to determine the total mineral residue present in the milk samples. Approximately 5 g of each sample was weighed into pre-weighed silica crucibles. The samples were initially dried at 110°C to remove moisture and subsequently charred over a burner. Complete ashing was carried out in a muffle furnace at 550°C for 2 hours. After cooling in a desiccator, the crucibles were reweighed and the percentage ash content was calculated using the standard formula:

Ash (%) = [(Final Weight − Empty Crucible Weight) / Sample Weight] × 100

Moisture Content Determination: Moisture content was determined using the oven-drying method. Approximately 5 g of each milk sample was placed in pre-weighed crucibles and partially evaporated on a hot plate before drying in a hot air oven at 100°C for 3 hours. The crucibles were cooled in a desiccator before final weighing. Moisture percentage was calculated using the standard gravimetric method.

Sample Preparation for GC–MS Analysis: Two representative milk samples were selected for GC–MS analysis. The samples were first allowed to curdle naturally, after which the liquid phase was separated. Approximately 4 mL of the extracted liquid was transferred into centrifuge tubes. A solvent mixture of n-hexane and acetone (4:1, v/v) was added, followed by vortex mixing and centrifugation at 4000 rpm for 10 minutes. The organic layer was collected and subjected to a second extraction using fresh solvent along with a small quantity of anhydrous sodium sulphate to remove residual moisture. Following vortexing and centrifugation, the final extract was filtered through a syringe filter into GC–MS vials for instrumental analysis.

Data Analysis: Qualitative observations were recorded based on colour changes observed during chemical testing. Quantitative parameters, including ash content, moisture content, and pH, were calculated using standard analytical formulas and compared with established reference values for milk quality. GC–MS data were analysed through untargeted screening to identify potential adulterants and other chemical constituents present in the selected milk samples.

RESULTS

Qualitative analysis was performed to detect the presence of common milk adulterants, including starch, sodium chloride, neutralizers, hydrogen peroxide, detergent, and formalin, using standard chemical methods. The findings demonstrated that starch, sodium chloride, neutralizers, hydrogen peroxide, and formalin were absent in all ten milk samples. However, detergent was detected in Samples 1 and 2, while the remaining samples tested negative for this adulterant (Table 1). The presence of detergent indicates possible intentional adulteration aimed at improving the appearance and consistency of diluted milk.

Table 1. Various test for Qualitative detection are been performed to check the adulteration in milk sample

Test Performed	Result
Detection of Starch	Absent
Detection of Sodium Chloride	Absent
Detection of Neutralizer	Absent
Detection of Hydrogen Peroxide	Absent
Detection of Detergent	Present in Sample and 2
Detection of Formalin	Absent

Quantitative Analysis

Ash Content: Ash analysis was carried out to determine the total mineral content of the milk samples. The obtained ash values ranged from 0.1% to 2.0% (Table 2).

Table 2. Result of Ash testing

SAMPLE NAME	READING
SAMPLE 1	.9%
SAMPLE 2	.9%
SAMPLE 3	.7%
SAMPLE 4	.1%
SAMPLE 5	.8%
SAMPLE 6	2%
SAMPLE 7	2%
SAMPLE 8	.6%
SAMPLE 9	.9%
SAMPLE 10	.7%

Samples 1, 2, and 9 showed ash values of 0.9%, while Samples 6 and 7 exhibited the highest ash content (2.0%). Samples 4 (0.1%), 8 (0.6%), and 3 and 10 (0.7%) showed comparatively lower values. Although several samples demonstrated ash values within the expected range, the elevated ash content observed in Samples 6 and 7 may indicate the presence of additional inorganic substances or adulterants, warranting further investigation.

Moisture Content: The moisture content of all ten milk samples was found to be 85%, which corresponds to the standard moisture content reported for fresh cow's milk (Table 3).

Table 3. Standard Physicochemical Values of Milk Used for Quantitative Analysis

Parameter	Standard Value
Ash (%)	0.1%
Moisture (%)	85%
pH	6.9

The uniform moisture values suggest that no significant variation in water content was observed among the collected samples.

pH Analysis: The pH values of the milk samples ranged between 6.91 and 6.95, which fall within the normal physiological pH range of fresh milk (Table 4).

Table 4. pH Values of the Analysed Milk Samples

Sample Name	pH Value
Sample 1	6.95
Sample 2	6.94
Sample 3	6.91
Sample 4	6.94
Sample 5	6.94
Sample 6	6.95
Sample 7	6.94
Sample 8	6.92
Sample 9	6.92
Sample 10	6.95

Sample 3 recorded the lowest pH (6.91), whereas Samples 1, 6, and 10 showed the highest value (6.95). These findings indicate that the collected samples had not undergone significant acidification or spoilage at the time of analysis.

GC–MS Analysis: In Sample 1, GC–MS analysis identified 2-Pyrrolidione, Toluene, Glycidyl Palmitate, Oleic Acid, and Benzyl Butyl Phthalate, with 2-Pyrrolidione showing the highest peak intensity (100%) (Table 5).

Table 5. GC–MS Detected Compounds in Sample 2

Retention Time (min)	Detected Compound	Peak Area (%)
1.6940	2-Hexanone, 3,3-dimethyl	100.00
2.8127	Toluene	0.89
16.5235	2,4-Di-tert-butylphenol	0.90
27.4300	Oleic Acid	0.84

Trace amounts of Toluene (0.66%), Glycidyl Palmitate (0.21%), Oleic Acid (1.85%), and Benzyl Butyl Phthalate (0.36%) were also detected. Similarly, Sample 2 showed the presence of 2-Hexanone, 3,3-dimethyl, Toluene, 2,4-Di-tert-butylphenol, and Oleic Acid, with 2-Hexanone, 3,3-dimethyl exhibiting the dominant peak (100%) (Table 9). Minor peaks corresponding to Toluene (0.89%), 2,4-Di-tert-butylphenol (0.90%), and Oleic Acid (0.84%) were also observed. The detection of compounds such as Toluene, Benzyl Butyl Phthalate, and 2,4-Di-tert-butylphenol suggests possible contamination arising from packaging materials, environmental exposure, or processing conditions. Their presence highlights the usefulness of GC–MS as a sensitive analytical technique for the detection of trace contaminants that cannot be identified through routine chemical tests.

DISCUSSION

Milk is one of the most widely consumed foods because of its high nutritional value and its rich composition of proteins, carbohydrates, fats, vitamins, and minerals. However, its complex composition and high demand make it highly susceptible to adulteration and contamination at different stages of production, processing, transportation, and marketing. Adulteration not only reduces the nutritional quality of milk but also poses significant health risks to consumers. Therefore, the present study combined conventional chemical methods with Gas Chromatography–Mass Spectrometry (GC–MS) to comprehensively evaluate the quality and safety of commercially available milk samples. The qualitative analysis demonstrated that starch, sodium chloride, neutralizers, hydrogen peroxide, and formalin were absent in all analysed milk samples, whereas detergent was detected in Samples 1 and 2 (Table 1). The absence of most commonly encountered adulterants indicates that the majority of the collected milk samples complied with basic quality requirements. However, the presence of detergent in two samples is a matter of concern because detergents are intentionally added to diluted milk to restore its foamy appearance and improve its texture. Consumption of detergent-adulterated milk has been associated with gastrointestinal irritation, nausea, vomiting, and long-term digestive complications. These findings suggest that although widespread adulteration was not observed, isolated cases of intentional adulteration continue to exist in the local milk supply. [15-17]

Quantitative evaluation further supported the overall quality assessment of the collected samples. Ash analysis revealed values ranging from 0.1% to 2.0% (Table 2). Most samples showed ash values close to the expected range; however, Samples 6 and 7 exhibited comparatively higher ash content (2.0%). Elevated ash values may indicate increased concentrations of inorganic minerals or the possible addition of mineral-containing substances during milk processing. Although ash content alone cannot confirm adulteration, abnormal values warrant further analytical investigation using advanced instrumental techniques. The moisture content of all analysed samples remained constant at 85%, which is consistent with the normal moisture content of fresh cow's milk (Table 3). This observation indicates that no substantial differences in water content existed among the collected samples. Similarly, the measured pH values ranged from 6.91 to 6.95 (Table 4), which falls within the normal physiological pH range of fresh milk. These findings suggest that the analysed samples had not undergone significant microbial spoilage or acidification before testing and retained acceptable physicochemical quality. GC–MS analysis provided a more detailed chemical profile of the selected milk samples than routine chemical tests. [18-20]

In Sample 1, compounds including 2-Pyrrolidione, Toluene, Glycidyl Palmitate, Oleic Acid, and Benzyl Butyl Phthalate were identified, with 2-Pyrrolidione representing the dominant chromatographic peak. In Sample 2, 2-Hexanone, 3,3-dimethyl was identified as the major component, while Toluene, 2,4-Di-tert-butylphenol, and Oleic Acid were detected in relatively smaller quantities (Table 5). Oleic acid is a naturally occurring fatty acid and is considered a normal constituent of milk fat. In contrast, compounds such as Toluene, Benzyl Butyl Phthalate, and 2,4-Di-tert-butylphenol are not natural components of milk and may originate from environmental contamination, packaging materials, plasticizers, laboratory handling, or industrial processing. Their detection at low concentrations does not necessarily confirm deliberate adulteration but indicates the possibility of external contamination during production or storage. The results of the present investigation demonstrate the complementary roles of conventional chemical testing and instrumental analysis. While routine qualitative tests effectively detected common adulterants such as detergent, GC–MS enabled the identification of trace organic compounds that are not detectable using conventional methods alone. This highlights the importance of incorporating advanced analytical techniques into routine food quality surveillance programmes to ensure comprehensive assessment of milk safety.

CONCLUSION

The present study demonstrated that the majority of the analysed milk samples were of acceptable physicochemical quality, with normal moisture content and pH values. Qualitative analysis detected detergent only in Samples 1 and 2, while starch, sodium chloride, neutralizers, hydrogen peroxide, and formalin were absent. Although most samples showed acceptable ash values, higher ash content in two samples suggested the need for further investigation. GC–MS analysis successfully identified several compounds, including 2-Pyrrolidione, 2-Hexanone (3,3-dimethyl), Toluene, Oleic Acid, Benzyl Butyl Phthalate, and 2,4-Di-tert-butylphenol, demonstrating its effectiveness in detecting trace contaminants that cannot be identified by conventional chemical tests. Overall, the findings highlight the importance of combining routine chemical analysis with advanced instrumental techniques such as GC–MS to ensure milk quality, detect adulteration, and protect consumer health.

DECLARATIONS

Ethics Approval and Consent to Participate: The present study involved the analysis of commercially available milk samples collected from local confectionery shops. No human participants, animals, or clinical specimens were involved in this research. Therefore, ethical approval and informed consent were not required.
Consent for Publication: Not applicable.
Availability of Data and Materials: The data generated and analysed during this study are included within this manuscript. Additional information may be made available by the corresponding author upon reasonable request.
Conflict of Interest: The author(s) declare that there are no conflicts of interest regarding the publication of this research.
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