Detection of Adulterants in Milk Products of a Well-Known Brand: An Investigation into Quality Assurance and Consumer Safety

Investigation

Milk is a strategic product in any country as it is a basic food for children and adults. The demand for milk and dairy products has continuously increased, but the offer is not sufficient to cover the needs of the world population. Milk supply is in a continuous decline due to small number of specialized farms market oriented, the low production performance, and milk quality which does not compile with the EU requirements. So, the imports of milk and dairy products were required to cover the population and industry needs. The areas with the highest contribution to Romania's milk production are the Central region and North East region, fresh dairy products are mainly carried out in the Central and South Muntenia and Bucharest and Ilfov County and cheese production is especially achieved in the North-West, Central and North Eastern Romania.

Food adulteration is a global concern and developing countries are at higher risk associated with it due to lack of monitoring and policies. However, this is one of the most common phenomena that has been overlooked in many countries. Unfortunately, in contrast to common belief, milk adulterants can pose serious health hazards leading to fatal diseases. Qualitative detection of adulterants in milk are simple color based chemical reactions.¹

Aim

The main objective of this investigative endeavor is to thoroughly identify and analyze potential additives or contaminants within milk products originating from a widely recognized brands. Through meticulous scrutiny and rigorous testing methodologies, the goal is to ensure the milk products uphold stringent quality standards and comply with regulatory mandates. By pinpointing any adulterants, whether deliberate or inadvertent, this inquiry aims to uphold consumer well-being and confidence by advocating for transparency, accountability, and adherence to industry standards.

Objective/World link

Food adulteration is a global concern and developing countries are at higher risk associated with it due to lack of monitoring and policies. However, this is one of the most common phenomena that has been overlooked in many countries. Unfortunately, in contrast to common belief, milk adulterants can pose serious health hazards leading to fatal diseases. Qualitative detection of adulterants in milk are simple color based chemical reactions.

Research Question

How can we effectively identify and quantify adulterants in milk products from reputable brands, and what are the implications for consumer safety and regulatory compliance in the dairy industry?

Background research

Under the Food Safety Regulation (2011), milk is defined as the normal mammary secretion derived from complete milking of healthy milch animal without either addition thereto or extraction therefrom.

Thus, no extraneous addition of any substance in milk is permitted under legal provisions. Further as per FSS regulations an adulterant is defined as "Any material which is or which could be employed for making food unsafe or substandard or misbranded or containing extraneous matter".

Reasons for adulteration of milk and milk products

The important reason for wide spread adulteration of milk is its physical and chemical nature, due to which it can hide many things when added to it. Thus milk can be adulterated easily and in many ways that affect its quality and safety of dairy products manufactured.

Some reasons for prevalence of milk and milk products adulteration are discussed hereunder:

- To make more profi
- Degraded moral of the society
- Gap between demand and supply in some pockets of the country
- Competition to capture more market
- Difficulties in enforcement of the available regulatory standards

Types of adulterants:

- (1) Addition of cheaper ingredients like water, skimmed milk, synthetic milk, vegetable oils, etc.
- (2) Separation of costly ingredient *i.e.* fat.
- (3) To improve keeping quality, addition of preservatives and neutralizers.
- (4) To improve physical characteristics
- Addition of thickening agents to increase viscosity and specific gravity.
- Colouring agents to mimic natural colour of milk.
- (5) Interspecies adulteration: Addition of buffalo milk in cow milk followed by addition of some masking agent.

Adulterants in milk can be generally classified into two major groups of substances. The first group comprises of those substances whose purpose is to increase the economical yield and the second group comprises of those substances whose purpose is to increase/extend the storage of milk by delaying its spoilage. In economic adulteration of milk when water is mixed, other

additional substances are also necessitated in order to conceal the watering. The selection of these substances is carried out in such a way that visual appearance, some of the common physical properties (e.g. density and viscosity) and gross chemical composition are simulated to that of the genuine milk. To make up the fat content, cheaper vegetable oils often of dubious quality are added. To raise the SNF and mask dilution mixture of carbohydrates, non-protein-nitrogenous compounds and some selected salts are added. From carbohydrate glucose, sucrose, maltodextrin, starch and/or cellulose may find their way in to milk. For simulating the protein in terms of nitrogen content, use of ammonium sulphate, urea or such other compounds are encountered. In addition other miscellaneous adulterants like salt, detergent, gelatin, colouring matter, neutralizers, etc. are also mixed.

White coloured milk-like fluid (so called "synthetic milk") is produced by blending a welldesigned assortment of vegetable oil, detergent, urea, sugar, neutralizers and water for adulteration of milk. This fluid is partially blended with pure milk to earn profit.

Since, it is made with several chemicals of dubious nature it poses serious health implications to consumers.

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.²



Hypothesis

Our hypothesis posits that in the detection of adulterants in well-known brands milk, the cheapest sample will exhibit the highest prevalence and concentration of adulterants. We anticipate that cost-cutting measures may lead to compromises in quality control and sourcing practices, thereby increasing the likelihood of adulteration in lower-priced milk products. Through our investigation, we expect to observe a positive correlation between the price point of milk samples and the presence of adulterants, with cheaper variants displaying a greater extent of adulteration compared to higher-priced counterparts.

Variables

Independent Variable		Manipulating the type of adulterant added to
1.	Type of adulterants	the milk (e.g., water, starch, urea, detergent)
2.	Concentration of adulterants	to observe its effects on the results of
3.	Testing methods	qualitative tests.
4.	Temperature and pH conditions	Testing milk samples from different sources
5.	Duration of testing	(e.g., different brands, regions, or types of
6.	Source of milk	milk)
Dependent Variable		Observation of color changes or the
1.	Presence/Absence of adulterants	formation of precipitates.
2.	Color change or precipitation	The intensisty of color changes.
	formation	The accuracy of detecting can be determined
3.	Intensity of reaction	by comparing the test results with known
4.	Accuracy of detection	standard sample.
Controlled Variable		Use the same volume of milk for each sample
1.	Volume of milk samples	tested.
2.	Type of water used-distilled water	Use the same qualitative analysis methods
3.	Concentration of chemical solutions	equipment (test-tubes, pipettes) and reagents
4.	Same test solutions for the samples	(chemical solutions, indicators).
5.	Same test-tubes	
Uncontrolled Variable		Milk samples stored under different
	Storage history of milk samples	conditions.
2.	Presence of contaminants	Contamination of milk samples with
3.	Variability in Laboratory Conditions	unintended substances during collection,
		storage.
		Differences in laboratory conditions
		(temperature, humidity) across experimental
		sessions.

Materials & Equipment used

- 4 samples of different brands milk
- Test-tubes
- Test-tube rack
- Pipette
- Bunsen burner
- Distilled water
- Phenolphtalein
- Nitric acid
- Sulphuric acid
- Iron chloride
- Hydrochloric acid
- Glucose
- Iodine
- Silver nitrate

- Potassium chromate
- Beakers

Week 1: The team of budding scientists convened to select a suitable title for their research project, aligning it with their defined objectives and scope.

Week 2: Research activities commenced as the team delved into relevant literature, consolidating their understanding of the subject matter and identifying areas requiring further investigation.

Week 3: Samples of cow milk were procured from local supermarkets, with meticulous attention given to ensuring uniformity in parameters across selected specimens.

Week 4: Preparation of necessary reagents commenced alongside the optimization of their concentrations, ensuring the precision and reliability of subsequent experimental procedures.

Week 5: Rigorous experimentation ensued, with each test meticulously repeated thrice to ensure the consistency and accuracy of the obtained results, adhering to robust scientific methodologies.

Week 6: Preparations for dissemination began, involving the development of a comprehensive display board and the creation of a video presentation showcasing the project. The team presented their work at the school's Science Fair, earning the notable achievement of second place.

Week 7: The focus shifted towards crafting a scholarly article for submission to the Cambridge Science Competition, with meticulous attention given to validating and scrutinizing all aspects of the research findings and interpretations.

Method

1. Detetion of sodium chloride by silver nitrate

The chloride ion (Cl^{-}) from sodium chloride reacts with silver ion (Ag^{+}) 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 by dissolving 5 g potassium chromate (AR) in 100 ml distilled water.

Procedure :

1. Take 5 ml milk in test tube.

- 2. Add 0.5 ml of 5% potassium chromate solution.
- 3. Add 2 ml 0.1 N silver nitrate and mix the contents.
- 4. Observe for colour change.

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

2. Test for detection of starch by iodine test

Reagents :

1. Iodine solution: Dissolve 2.6 g of iodine and 3 g of potassium iodide in a sufficient quantity of water and make up to 200 ml.

Procedure :

Take about 5 ml of milk in a test tube. Bring to boiling condition and allow the test tube to cool to room temperature. Add 1-2 drops of iodine solution to the test tube. Development of blue colour indicates presence of starch which disappears when sample is boiled and reappears on cooling.

3. Pulverized soap test

Soap is added to milk to increase the foaming of milk and thus to have thick milk. Addition of such chemicals will cause health problem especially related to stomach and kidneys.

Reagents:

- 1. 10 mL hot water
- 2. 2-3 drops phenolphtalein

Procedure:

Soap can be detected by adding phenolphthalein indicator to the adulterated milk. A pink color will be observed if soap is present as the alkali will be neutralized by the acidity of the milk when phenolphthalein indicator is added.

4. Skim milk powder test

Skim milk powder is add in milk to increase the protein and solids content of milk. So i if milk has a low concentration of protein it will be add it.

Reagents:

1. few drops of nitric acid

Procedure:

If the addition of nitric acid drop by drop in to the test milk sample results in the development of orange colour, it indicates the milk is adulterated with skim milk powder. Samples with out skim milk powder shows yellow colour.

5. Salicylic acid test

Salicylic acid is used as a preservative in food industry. It is added to milk to preserve and thus increase the shelf life of milk.

Reagents:

- 1. few drops of sulphuric acid
- 2. iron chloride

Procedure:

Presence of these acids can be detected by adding concentrated sulfuric acid and iron chloride, which when reacts with salicylic acid to give buff colored and violet colored reaction products.

6. Vanaspati test

Manufacturers tend to include artificial trans fats to increase the density of the milk and mimic the properties of natural fats. Some manufacturers also tend to add trans fats containing hydrogenated vegetable oils in milk to increase its fat contents.

Reagents:

- 1. 10 mL of hydrochloric acid
- 2. 1 tablepoon of sugar

Procedure:

Add 5 mL milk, 10 drops of hydrochloric acid and 1 tablespoon of sugar inside a test tube. Next, they have to rest the mixture for 5 minutes and examine the change of colouration. If the mixture turns red, it means the milk contains vanaspati.

7. Synthetic milk test

Synthetic milk has a bitter aftertaste, gives a soapy feeling on rubbing between the fingers, and turns yellowish on heating. Synthetic milk is made by adding white colour water paint, oils, alkali, urea, detergent.

Reagents:

1. 5 mL of milk

Procedure:

Boil some milk on a slow heat while moving it with a spoon till it becomes solid (khoya). Take it off the heat and wait for 2-3 hours. If the produced solid is oily, the milk is of good quality if it's not, it means the milk is synthetic.^{2,3,4,5,6}

Our work





















Safety Procedure

1. Utilize Proper Protective Gear (PPE):

- When dealing with the specified chemicals, especially potent acids and corrosive substances like sulfuric acid and nitric acid, ensure proper PPE is worn and accessible.
- Wear goggles to shield against splashes, a chemical-resistant apron, gloves, and consider a face shield for added protection from vapors, especially when handling highly hazardous substances like hydrochloric acid and silver nitrate.

2. Emergency Equipment Availability:

- Guarantee that eyewash stations and safety showers are easily reachable and operational.
- In case of accidental exposure, immediate access to these facilities is crucial to minimize injury risks.

3. Guidelines for Initial Aid:

- Inhalation: If exposed to harmful vapors or gases, relocate to a well-ventilated area immediately. If breathing becomes challenging, administer oxygen if trained to do so. In case of respiratory failure, begin artificial respiration and seek immediate medical attention.
- Eye Contact: Should chemicals contact the eyes, remove any contact lenses and promptly flush with copious amounts of water for a minimum of 15 minutes, ensuring the eyelids are held open. Prompt medical attention is essential for further evaluation and treatment.

4. Skin Contact Response:

- Upon skin contact, promptly remove contaminated clothing and thoroughly rinse the affected area with water.
- Seek medical attention if signs of irritation, redness, or chemical burns manifest.

5. Managing Spills:

- Adhere to established spill response protocols if a spill occurs.
- If necessary, evacuate the area, contain the spill, and utilize suitable absorbent materials and protective gear during cleanup.

6. Education and Awareness:

- Provide comprehensive training to all individuals likely to handle or encounter the specified chemicals.
- Regularly review safety protocols, emergency procedures, and first aid measures to ensure readiness for potential incidents.

7. Medical Care:

- In the event of exposure, irrespective of severity, promptly seek medical attention.
- Furnish healthcare providers with pertinent information, including Safety Data Sheets (SDS), to facilitate proper evaluation and treatment.⁷

Data



Further implements

Literature Review: Conduct a comprehensive literature review to synthesize existing knowledge on the detection of adulterants in milk. Identify gaps and opportunities for improvement in current methodologies.

Adulterant Selection: Employ criteria such as prevalence, potential health hazards, and regulatory significance to select target adulterants for detection in milk samples.

Standardized Sample Handling: Establish standardized protocols for sample collection and preparation to ensure consistency and minimize variability in subsequent analyses.

Methodological Optimization: Optimize analytical techniques, including chromatography, spectroscopy, immunoassays, and molecular methods, to maximize sensitivity in detecting adulterants.

Enhanced Sensitivity Strategies: Implement signal amplification techniques, such as nanoparticle probes or catalytic amplification, to enhance the sensitivity of detection methods.

Validation and Quality Assurance: Validate developed methods according to established standards, utilizing certified reference materials and proficiency testing to assess method performance and reliability.

Advanced Data Analysis: Employ chemometric methods, pattern recognition techniques, and machine learning algorithms to interpret complex datasets and differentiate authentic milk from adulterated samples.

Interdisciplinary Collaboration: Foster collaboration among researchers from diverse disciplines, including chemistry, biology, food science, and regulatory affairs, to leverage expertise and ensure comprehensive method development.

Continuous Improvement: Emphasize ongoing evaluation and refinement of experimental protocols based on emerging research, technological advancements, and feedback from stakeholders to continually enhance sensitivity and robustness in adulterant detection.

Conclusion

In conclusion, our research emphasizes the crucial necessity of effective methods for detecting adulterants in milk to ensure its safety and authenticity. Through rigorous analysis utilizing advanced techniques, we have demonstrated the effectiveness of our approach in identifying adulterants present in milk samples. Our results indicate a notable prevalence of common salt adulteration in all tested samples, revealing a significant vulnerability in the milk supply chain. The widespread occurrence of added salt underscores the urgent requirement for improved regulatory measures and vigilant monitoring practices to protect consumer well-being and maintain industry standards. Additionally, the absence of other detected adulterants suggests potential limitations in current detection methods or variations in adulteration practices. Future studies should focus on refining detection techniques and broadening the scope of analysis to encompass a wider range of adulterants. By addressing these issues, we can mitigate the risks associated with consuming adulterated milk, enhance transparency within the dairy industry, and ensure the integrity of milk products for consumers globally.

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