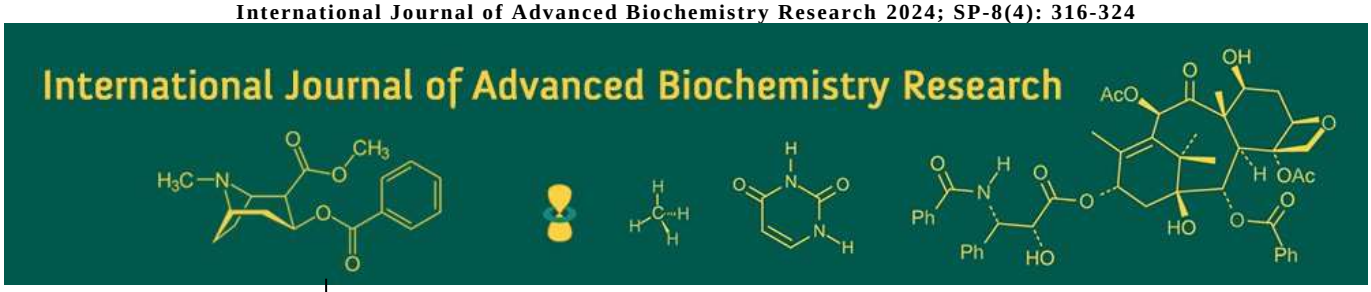

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A comprehensive review on adulteration of milk

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Abstract
Milk is a wholesome nutritious dairy product and is consumed by a majority of the population worldwide for drinking as such, as well as via dairy products. However, the practice of adulteration of milk invariably reduces its quality and may introduce hazardous substances into the dairy supply chain jeopardizing consumers' health. Various instances of adulteration of milk have been reported globally, wherein substances such as extraneous water, foreign proteins, whey proteins, melamine and urea, vegetable or animal fats, plus many minor constituents of milk fat have been added as potential adulterants in milk and milk products. This review focused on some common and some advanced methods for detection of these adulterants. This review includes some laboratory methods that regularly followed in dairy industry and some advanced technique like HPLC, Mid-Infrared Spectroscopy, DNA based methods like PCR, Immunological procedures, Gas chromatography, Biosensor etc. This review intends to contribute towards the common as well as advanced knowledge base regarding possible milk adulterants and their detection techniques.

Keywords: Milk, adulteration, HPCL, chromatography, PCR

Introduction
Milk has a great nutritional value because it is a wonderful source of high-quality lipids, proteins, carbohydrates, vitamins, and minerals. (Neumann *et al*., 2002) [47]. In ancient science, Acharya Sushruta explained properties of milk such as milk is Madhura rasatmaka, Sheeta viryatmaka, it gives strength to body, increase life span also rejuvenating property (Ambikadatta, 2005) [3].

It is the first food that mammals eat and offers all the energy and nutrients required for healthy growth and development. It is also very important for the production of bone mass (Paula, 2014) [48].

Milk is an integral part of a developing newborn's and expecting mother's daily nourishment. It includes all the materials needed by the species for both young and old, even in its most fragile condition (Reddy *et al*., 2017) [53].

Adulteration is defined as "the process by which the quality or the nature of a given substance is reduced through (Kamthania *et al.*, 2014) [29]. The purpose of adulteration can be twofold: either it is done intentionally to increase profits by adding extraneous water, non-dairy proteins, urea, melamine, animal fat, reconstituted milk, or synthetic milk; or it happens accidentally due to natural processes such as the mastitis-treated cattle's milk naturally containing antibiotics, or dust particles or other foreign objects that may have gotten into the milk during processing (Poonia *et al.*, 2016) [51].

The rates of food adulteration are rising daily, and when it comes to milk, India leads the United States and Russia in terms of adulteration rates. The Food Safety and Standard Authority of India (FSSAI) has set requirements for milk and milk products, yet about 68.7% of those sold in the country do not meet those norms. Compared to southern states, it is more common in northern states.

Xin & Stone, in 2008 [69] reported that history of milk adulteration is very old. Swill milk scandal has been reported in 1850 which killed 8000 infants in New York alone. However, (Kamthania, 2014) [29] claimed that in 2008, melamine-contaminated newborn milk powder poisoned over 2,90,000 people (the most of them were young children) and killed at least six of them. The melamine incident resulted in severe sanctions for both individuals and companies involved, according to the Chinese authorities.

According to Spink and Moyer (2011) [66], Food fraud is a food danger that is becoming well recognized and concerning since it poses a significant global hazard to public health, particularly through the more specific subcategory of economically motivated adulteration (EMA). As a means of raising the product's apparent worth or lowering its production costs for financial advantage, the Food and Drug Administration has developed a working definition of EMA, which is defined as the "fraudulent, intentional substitution or addition of a substance in a product."

In order to determine the quality of milk and pinpoint various sources of adulteration in liquid milk across the nation, the Food Safety and Standards Authority of India (FSSAI) carried out a recent surveillance study titled The National Survey on Milk Adulteration (Executive Summary on National Survey on Milk Adulteration, India 2011) [18]. According to the survey, water was the most often used adulterant added to milk. In addition to lowering milk's nutritious content, this puts customers' health at danger. In a different investigation, (Singuluri and Sukumaran, 2014) [64] measured the amounts of various adulterants in milk that were conducted in Hyderabad, India. The examinations made it clear that several samples did not meet the legal requirements set forth by the FSSAI.

History of adulteration

Adulteration use was first investigated in 1820 by the German chemist Frederick Accum, who identified many toxic metal colonings in ford and drink. The physician author Hill Hossal conducted extensive studies in the early 1850 which were published in the lancet and led Typical adulterants in milk to the 1860 food Adulteration Act and rather legislation (Ghimire, 2016) [21].

Types of adulterants

Economically motivated adulteration of milk refers to the inclusion of vegetable protein, milk from various species, whey, and watering (Fischer, Schilter, Tritscher, & Stadler, 2011; Singh & Gandhi, 2015) [20, 62]. There is no serious health risk associated with these adulterations. Urea, formalin, detergents, ammonium sulfate, boric acid, caustic soda, benzoic acid, salicylic acid, hydrogen peroxide, sugars, and melamine are a few of the main adulterants in milk that have been shown to have substantial negative health effects. The heart, liver, and kidneys are among the important organs that are negatively impacted by contaminated milk (Lahankar *et al*., 2019).

To raise the non-protein nitrogen content in milk, commercial urea is added (Sharma *et al.*, 2012) [59]. Melamine is also added in an attempt to artificially raise the protein level (Liu *et al*., 2012) [37]. In order to retain the density of diluted milk while increasing the lactometer value, ammonium sulphate is added. As preservatives, hydrogen peroxide, salicylic acid, and benzoic acid lengthen the milk's shelf life (Singh & Gandhi, 2015) [62]. Due to the high cost of milk fat, some producers of dairy products and milk remove it in order to increase their profits; in order to make up for this, they add non-milk fat, like vegetable oil. The oil is emulsified and dissolved in water with the addition of detergents, creating a frothy solution that resembles milk (Singuluri & Sukumaran, 2014) [64].

Impact of adulteration on health

Some kinds of adulterant and their impact on health of human body are discussed below.

1. Water: The simplest and least expensive method of adulterating milk has been water since prehistoric times. Human diseases such diarrhea, amoebiasis, shigellosis, cholera, giardiasis, and others can be transmitted through contaminated water.

2. Urea: It is the natural constituent of raw milk and its maximum value declared by Food Safety Standards Authority of India (FSSAI), Act 2006 and Prevention of Food Adulteration (PFA) rules 1955 is 70mg/100ml (Sharma *et al.*, 2012) [59]. It is added to milk to increase the protein content of the milk. It may also rise as a result of cows being fed unevenly. Singh *et al*., 2008) [61]. It is also used to improve the consistency of milk, level the content of solid not fat (SNF), give whiteness, and maintain heat stability. A higher urea load in milk can be detrimental to the kidneys, liver, and heart. It causes symptoms such as cancer, ulcers, indigestion, and acidity. The kidneys are particularly affected because they have to work harder to remove urea from urine.

3. Hydrogen peroxide (H_2O_2): It prolongs the shelf life of milk by acting as a preservative, as reduce the development of micro-organisms in milk. It may result in digestive issues, which may then exacerbate into gastritis and intestinal inflammation. Additionally, it interferes with the body's antioxidant system, disrupting natural immunity and speeding up aging. (Sahil and Yang 2017) [55].

4. Detergents: It is added to milk to change the oil's color and dissolve it in the water, creating a frothy solution that gives the milk its distinctive white hue. Gastrointestinal issues may result from it.

5. Starch: The presence of undigested starch in the colon caused by a high starch addition to milk might cause diarrhea. Because it accumulates in the body, it proves lethal in patients with diabetes. (Mohammad *et al.*, 2007) [44].

6. Carbonates and bicarbonates: These products are added as an adulterant in milk which may leads to disruption of hormone which can helps us to regulate and development of reproduction. (mohfw.nic.in)

7. Chlorine: Chlorine is added after the water to adjust for the diluted milk's density. The amount of chlorine in milk is also increased by mastitis in cows (Lima *et al.*, 2004) [35]. It may have impacted the heart by blocking arteries, leading to the development of heart-related issues. Due to its ease of use and simplicity, potentiometric detection and conductometric sequential injection analysis can be used to titrate milk in order to detect it (Silva *et al.*, 1999) [60].

8. Antibiotics: Adulteration with antibiotic can result in tissue damage, allergic reactions, an increase in antibiotic-resistant bacteria, disruption of the intestinal flora, and some antibiotics, such as those with sulfamethazine residues, may even have carcinogenic qualities. In 2016 [14], Das and Goswami. It disrupts the process of bacterial fermentation, leading to significant losses in fermented products (Tan *et al.*, 2009) [67]. Urticaria can be brought on by traces of penicillin.

9. Whey / liquid whey: When used in little amounts, it is safe. Increased bowel motions, nausea, thirst, bloating, cramps, anorexia, exhaustion, and headaches are among the adverse effects that high dosages might bring on.

10. Chloride and Ammonia: Milk's chlorides change the pH of blood and throw off the body's acid-base equilibrium. Milk containing ammonia can cause sensory issues, renal problems, regression, and loss of acquired speech.

11. Formalin: It prolongs the shelf life of milk by acting as a preservative. It is carcinogenic in addition to losing some of its nutrients. It has no discernible effect on milk's freezing point or specific gravity. However, it lowers the milk pH.

12. Melamine: To artificially increase the protein content of milk, melamine is added. It is added to a variety of foods, including processed meals, wheat gluten, and poultry feed, in addition to milk. It is not carcinogenic, but in severe situations, it can cause neonatal mortality and renal failure.

Methods for detection of Common milk adulteration

1. Detection of different edible adulterants in milk

Following table presents methods for detecting common edible adulterants in milk, with a focus on ensuring the purity and quality of this essential dairy product. The highlighted techniques offer quick and effective means of identifying the presence of common adulterants, providing valuable insights for quality control and regulatory compliance in the dairy industry.

Table 1: Detection of different edible adulterants in milk

Adulterant	Procedure	Observation	Limit of detection (R. Sharma, Rajput, Barui, & N., 2012) [6]	References
Sugar	Take 5 mL milk sample in a test tube. Add 1 mL conc. HCl and 0.1 g resorcinol solution. Place the test tube in water bath for 5 min.	Appearance of red color indicates presence of added sugar.	0.2% (w/v)	Sharma *et al*., 2012) [59]; (Singh *et al*., 2012)
Starch	Take 3 mL sample in a test tube. After boiling it thoroughly, cool it to room temperature. Add 1 drop of 1% iodine solution.	Appearance of blue color indicates the presence of starch.	0.02% (w/v)	(Sharma *et al*., 2012) [59]; (Singh *et al*., 2012), (Kumar *et al*., 1998) [32]
Glucose	Take 1 ml of milk sample in a test tube. Add 1 ml of modified Barfoed's reagent. Heat the mixture for exact 3 min in a boiling water bath. Rapidly cool under tap water.	Immediate appearance of deep blue color indicates presence of glucose.	0.1% (w/v)	(Sharma *et al*., 2011) [58]
Common salt	Take 5 ml of milk sample into a test tube. Add 1 ml of 0.1 N silver nitrate solution. Mix the content thoroughly and add 0.5 ml of 10% potassium chromate solution.	Appearance of yellow color indicates presence of added salts, whereas, brick red color indicates the milk free from added salt.	0.02% (w/v)	(Sharma *et al*., 2012) [59]
Buffalo milk	Dilute the milk 1/10. Put a drop of diluted milk on the centre of a glass slide. Now place a drops of Hansa test serum (duly preserved) on the drop of milk and mix together with a glass rod or clean tooth pick.	Curdy particles develop within half a minute in milk containing buffalo milk.	----	(Kamthania *et al*., 2014) [29]; (Singh *et al*., 2012)

2. Detection of different hazardous chemicals in milk

Below table outlines methods for the detection of hazardous chemical adulterants in milk, emphasizing the critical need to ensure the safety and integrity of this essential food product. The presented techniques offer valuable insights into identifying and quantifying specific harmful substances, contributing to stringent quality control measures and safeguarding public health within the dairy industry.

Table 2: Detection of different hazardous chemicals in milk

Adulterant	Procedure	Observation	Limit of detection	References
Hydrogen peroxide	2 ml of milk sample were added and 2 ml of HCL (1%) were added, thoroughly mixed, then 2 ml of potassium iodide (10%) were added. The tube was immersed in hot water (80-90°C) for 1 min after which the tube was quickly cooled in running water. 2 ml of starch solution (1%) were added as an indicator solution of paraphenyl-enediamine.	A blue colour is developed in presence of hydrogen peroxide.	0.025%	(Pien *et al*., 1953) [49]
formalin	Two milliliters of milk sample were mixed with 2 ml of distilled water in a test tube, and then sulphuric acid (90% containing a trace of ferric chloride) was poured down the side of the tube.	Development of violet ring at the junction between the two layers indicates the presence of formalin.	0.1%	(Ling, 1963) [36]
Ammonium sulphate	Take 2 ml. milk in a test tube and add 0.5 ml NaOH (2%) 0.5 ml sodium hypochlorite (2%) and 0.5 ml phenol (5%) Heat in boiling water bath for 20 sec	A bluish colour forms immediately, which turns deep blue afterward.	0.05%	(Kumar *et al*., 2002) [33]
Urea	Take 5 mL milk sample in a test tube. Add equal volume of 24% TCA to precipitate fat and proteins of milk. Take 1 mL filtrate and add 0.5 mL 2% sodium hypochlorite, 0.5 mL 2% sodium hydroxide and add 0.5 mL 5% phenol solution, then mix.	A characteristic blue or bluish green colour develops in presence of added urea whereas pure milk remains colourless.	0.2%	(Meisel, 1995) [42]

3. Detection of different mixed adulterants in milk

Beneath the table provides an overview of various methods for detecting mixed adulterants in milk, highlighting the importance of comprehensive screening for a range of potential contaminants.

Table 3: Detection of different mixed adulterants in milk

Adulterant	Procedure	Observation	Reference
Detergent	Take 5 ml in a test tube and add 0.1 ml 0.5% Bromocresol Purple (BCP) solution.	Appearance of violet colour indicates the presence of detergent. Unadulterated milk shows faint violet color.	(Singhal, 1980) [63]; (Singh *et al*., 2012)
Pulverized soap	Take 10 ml milk sample in a test tube. Add equal quantity of hot water to it, then add 1-2 drops of phenolphthalein indicator.	Appearance of pink color indicates presence of soap.	(Ghodekar, 1974) [22]
Coloring matter	1. Take 10 mL milk sample in attest tube. Add 10 ml diethyl ether. After shaking, allow it to stand.	Appearance of yellow color in ethereal layer indicates the presence of added color.	(Batis *et al*., 1981) [7]
	2. Make the milk sample alkaline with sodium bicarbonate. Dip a strip of filter paper for 2 hours.	Appearance of red color on filter paper indicates the presence of annatto. Treatment of this paper with stannous chloride gives pink color.	(Lechner and Klostermeyer, 1981) [34]
	3. Add a few drops of hydrochloric acid to milk sample.	Appearance of pink color indicates azo dyes.	(DE Souza *et al*., 2000) [17]

4. Advanced methods for detection of milk adulterants

Under the provided table presents an overview of advanced methods employed for the detection of adulterants in milk, showcasing cutting-edge techniques that go beyond traditional approaches. These sophisticated methods, including chromatography, spectroscopy, and molecular analysis etc.

Table 4: Advanced methods for detection of milk adulterants

Name of adulterant	Technique	Advantage	Disadvantage	Reference
Extraneous water	Mobile phone (as spectroscopic analysis tool)	Affordable and can detect 3% of Extraneous water	Considerable additional work with regard to sampling, data treatment is necessary.	(Iqbal and Bjorklund, 2011) [27]
Tap water, Urea, Liquid whey and synthetic milk	Impendance sensor (constant phase angle based))	Cheap, biocompatible and will not contaminate the test milk. Performance of sensor is not affected by change of temperature or humidity	In some cases, sensitivity is less and precise instrumentation system is required to see the change	(Das *et al*., 2011) [15]
Vegetable proteins	ELISA (PAB)	Large sample throughput High sensitivity. It permits the detection of wheat proteins and adulteration of high heat milk powders	Selection of suitable antigens still remains the major problem Semi-quantitative	(Sanchez *et al*., 2002) [56]
Soya, pea and soluble wheat proteins	Optical biosensor	Good speed, sensitivity and stability	High cost	(Abdulhalim *et al*., 2007) [1]
Vegetable proteins	CE	Rapid and automated analysis High resolution	It is not independent of milk-processing conditions Wheat proteins are not detected Low reproducibility	(Manso *et al*., 2002) [39]
Rennet whey	Immuno-chromatographic lateral-flow test dipstick test	It can detect rennet whey content above 4%	Milk with a poor bacteriological quality, the presence of pseudo-c-GMP arising from the action of proteinases from psychrotrophic bacteria can also give rise to positive results	(Martin-Hernandez *et al*., 2009) [40]
Acid whey	UV spectroscopy	Suitable for routine analysis Cheap Easy sample Preparation	It requires tedious calibration studies with different types of milk samples	(Miralles *et al*., 2000) [43]
Cheese whey	GMP SDS-PAGE	Useful tool for routine detection of fraudulent manipulation of milk and dairy products with whey	Resolving power is low to separate the peptides, give false-positive results Tedious, time-consuming and have problems of sensitivity and accuracy at low concentrations	(Chavez *et al*., 2008) [11]
Nondairy (vegetable and animal) fats in milk fat	GC long capillary columns	Well standardised It is adopted by the International Dairy Federation as the official method	Limited by the natural variability of fatty acids High detection limits (>15%) Large data sets for statistic are required	(Molkentin and Precht, 2001) [46]
Nondairy fats in milk fat cows' milk fat in fat from goats or ewes	GC packed and short capillary columns	Cheap, fast and well standardised Applicability confirmed (method adopted by the EU) Limit of detection below 5%	Accuracy is affected in lipolysed samples It requires long previous studies to determine the standard triglyceride composition	(Goudjil *et al*., 2003) [23]
Vegetable fats in milk fat	GC	Very selective and Sensitive	Tedious and time-consuming. High variability depending mainly on several steps required	(Alonso *et al*., 1997) [2]
Sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$),	Ultrasonic method	Less expensive, less time consuming than spectroscopic, chromatographic methods	Required expert technician Validation is required	(Mohanan *et al*., 2002) [45]

formalin (HCHO)				
Urea, tetracycline, sugar and salt	FTIR with 2D spectroscopy	Convenient, rapid, automated and simplify sample handling	High cost	(He *et al*., 2010) [25]
Synthetic milk (based on detergent detection)	Paper chromatography	Convenient	---	(Barui *et al*., 2013) [6]

(CE- Capillary Electrophoresis, FTIR- Fourier Transform Infrared, GC- Gas Chromatography, GMP- Glycomacropeptide, SDS-PAGE - Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis).

1. Mid-Infrared Spectroscopy

Two of the most important technologies that are traditionally used in the dairy industry are mid-infrared spectroscopy and Fourier-transform infrared. These technologies are used to detect and quantify adulterants, milk dilution, the presence of pathogenic bacteria, veterinary drugs, and hazardous substances in milk (Ceniti *et al.*, 2023) [10].

The technique known as infrared spectroscopy measures the wavelength and intensity of light absorbed by a substance in the infrared spectrum (Putzig, 2011) [52]. To put it briefly, the vibrational frequency of each functional group in a molecule can be used to identify which functional groups are present in a given sample. The radio frequency (1 cm - 1m), the microwave region (100 μm - 1 cm), the X-ray region (0.5 - 10 nm), the mid-infrared region (MIR) (2500 - 25,000 nm), the near-infrared region (NIR) (800 - 2500 nm), the visible region (350 - 800 nm), and the UV region (10 - 350 nm) are among the regions, depending on the wavelength. Electromagnetic radiation travels through materials in the mid-infrared range (2500 - 25,000 nm), causing molecular movements (such as rotation and vibration) through chemical bonds and various degrees of energy absorption. The chemical makeup of the sample under examination can be ascertained by examining the energy given and the amount absorbed by the sample.

Fourier-transform infrared (FTIR) spectrometry, a type of MIRS, facilitates the rapid scanning of a complete spectrum of electromagnetic waves, ranging from 4000 cm^{-1} to 400 cm^{-1} (Soyeurt, *et al*., 2023 and Karoui *et al*., 2010) [65, 30]. It is extensively used to forecast the fat, protein, lactose, and casein contents of milk samples, which are routinely collected in accordance with various countries' milk-recording systems. The International Committee for Animal Recording (ICAR, 2012) has approved FTIR spectrometry as the standard operating procedure for assessing the components of milk.

2. Biosensors

Molecularly imprinted polymers and surface-enhanced Raman spectroscopy (MIPs-SERS) is a new biosensor that combines the two techniques to detect melamine in whole milk, as proven by (Hu *et al.*, 2015) [26]. With limits of detection (LOD) and quantification (LOQ) of 0.012 and 0.039 mmol/L, respectively, it was evident that this biosensor had a high sensitivity for precisely measuring melamine in whole milk. Some benefits of this technique are shorter run times and easy sample pretreatment. In a another study, a piezoelectric sensor-which gauges the pressure of the gas released from the sample-was used to build an enzyme-based sensor for the detection of urea in milk. When the samples' urea concentration varied, the sensor responded linearly. After analyzing the sensor's temporal response, it was discovered that a liquid to gas ratio of 1:2.5 produced output that was suitable for the sensor. According to the findings, urea levels in milk may be found using this method (Renny *et al.*, 2005) [54]. The biosensors provided simple experimentation analysis, convenience of handling, high specificity and accuracy, and comparatively reduced cost.

3. Immunological procedures

Milk and other food adulterants are also detected by a number of immunological techniques. When it comes to identifying foreign proteins in milk and other foods, the most popular immunological method is the enzyme linked immunosorbent test (ELISA). The ELISA method for identifying several analyte types is shown in Fig. 2. Both qualitative and quantitative methods of detecting milk adulterants can be carried out with the ELISA. ELISA-inspired detection techniques have been used to identify vegetable-derived proteins in milk. Such immunological techniques have been used to identify the phony whey addition to milk. Whey content in tampered milk has been determined both qualitatively and quantitatively utilizing the sandwich-ELISA method using polyclonal antibodies. Melamine was discovered in one of the most common dairy prostitutes, using one of the variants of ELISA called indirect-ELISA. Additionally, immuno-chromatographic analysis and fluorescence polarization immunoassay (FPIA) were used to identify whey and melamine in milk, respectively. Specific detection was demonstrated by both techniques with little to no cross-reactivity. Various milk and food adulterants are detected in different capacities using immunological techniques. However, some of the limiting aspects that raise questions about the efficacy of these detection techniques include the possibility of cross reactivity, the formation of antibodies, and the need for lengthy washing operations. These assays are not appropriate for regular analysis since they require a large investment of time, money, and expensive equipment and materials (Nagraik *et al.*, 2021).

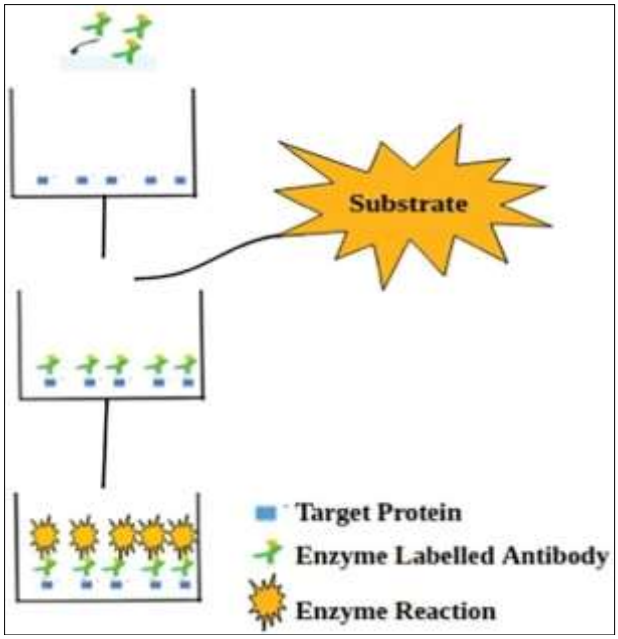

Fig 1: Representation analyte detection by ELISA

4. Polymerase chain reaction

It is possible to distinguish between the milks of several species in a blend using the polymerase chain reaction (PCR), which is based on the amplification of species-specific DNA sequences. By using sequence-specific retardation, which is accomplished by an intercalating agent during agarose gel electrophoresis, Plath *et al.,* (1997) [50] were able to effectively separate the b-casein PCR products from bovine or buffalo milks from those of caprine or ovine milk. Additionally, other research describe using PCR to detect bovine milk in buffalo milk (Darwish *et al.*, 2009) [13], caprine milk (Cheng *et al.*, 2006), ovine milk (Lopez-Calleja *et al*., 2004) [38], and caprine milk (Maudet and Taberlet, 2001) [41]. A PCR algorithm was developed by (Diaz *et al*., 2007) to identify the adulteration of ovine cheeses with caprine milk. (Darwish *et al*., 2009) [13] used primers based on cow mitochondrial 12S rRNA genes to report the presence of bovine milk in buffalo milk down to a level as low as 0.5%. Additionally, up to 1% of bovine milk has been effectively identified in caprine milk using duplex PCR, a more sophisticated PCR technique that targets the mitochondrial D-loop area with two sets of primers (Kotowich *et al.,* 2007) [31].

Similarly, for the purpose of simultaneously detecting the milks of two or more species present in dairy products, (Bottero *et al.,* 2002-03) [8-9] created duplex and multiplex PCR formats. A few more publications on the use of PCR techniques to identify different kinds of animal milks.

5. High-performance liquid chromatography (HPLC) and other chromatographic techniques

For the purpose of detecting different milk adulterants, chromatographic techniques of various kinds have been routinely employed in conjunction with mass spectrometry (MS) and other detection technologies. Milk containing soy and extraneous whey proteins can be separated and detected using reverse phase high performance liquid chromatography (RP-HPLC) (Jablonski *et al*., 2014) [28]. Fig.2 shows the general HPLC instrument and it's working. The general HPLC equipment is seen operating in a different kind of chromatographic methods, is used to extract various foreign proteins from skim milk powder (Filazi *et al.,* 2012) [19].

Melamine in tainted milk is routinely detected using tandem mass spectrometry coupled with high performance liquid chromatography (HPLC-MS/MS) (Chilbule *et al*., 2019) [12]. Other detection methods, like fluorescence and UV-Vis, have also been coupled with HPLC to detect milk adulterants (Scano *et al.,* 2014) [57].

Analyte instability, matrix effects, and contamination can all lead to issues during analysis when using any of these methods.

These issues have different effects based on the analyte being studied, the food matrices involved, and the methodology employed. While melamine can be quantified at the ppm level with the HPLC methodology, qualitative and trace-level analysis cannot be adequately accomplished with this method (Tittlemier, *et al.,* 2010) [68].

While there are several chromatographic techniques available for detecting milk adulteration, they are all beset by expensive instrument costs and laborious sample pre-treatment procedures.

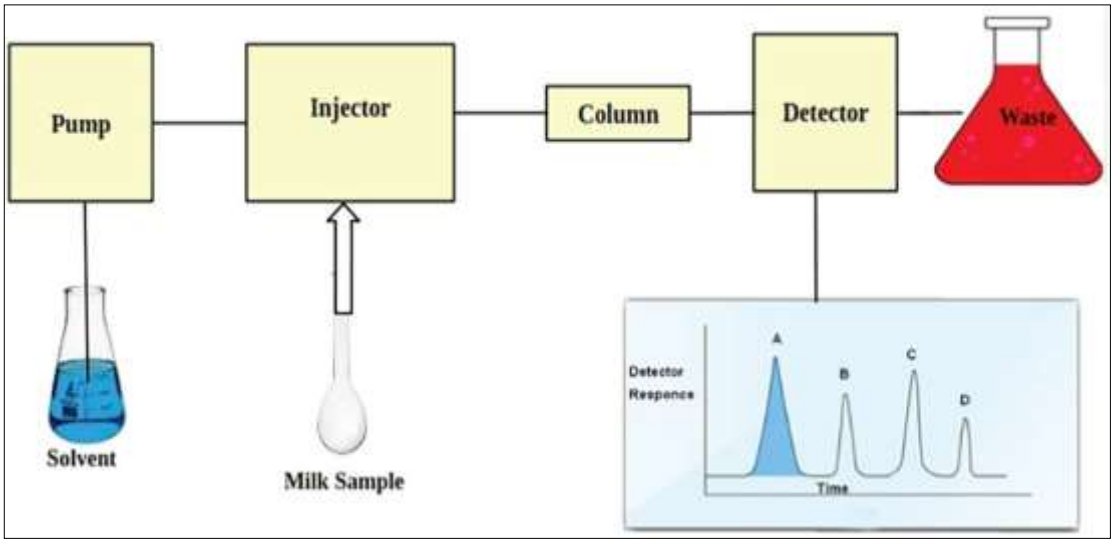

Fig 2: Schematic presentation of HPLC.

6. Gas chromatography

Gas chromatography was utilized to determine triacylglycerol profiles in milk and non-milk fat. The values of triacylglycerol were subjected to linear discriminant analysis to detect and quantify non-milk fat in milk fat by following procedure;

Weigh a known volume of milk sample to a clean, dry vial and add an appropriate volume of a suitable solvent (e.g., hexane) in the vial then Shake the mixture vigorously to extract lipids from the milk into the solvent. Centrifuge the mixture to separate the lipid-solvent layer from the aqueous layer. Transfer the upper layer (containing lipids) to a new vial. Evaporate the solvent using a gentle stream of nitrogen or under a gentle flow of nitrogen in a fume hood. If necessary, derivatize the lipid extract to improve volatility and detectability in the gas chromatograph then Inject the derivatized or non-derivatized lipid extract into the gas chromatograph using an appropriate injector system. It Separate the components on the capillary column using the gas chromatograph. Finally Detect and quantify the separated components using the flame ionization detector (FID) or other suitable detectors.

Calibration Curve: Prepare a calibration curve using standard solutions of pure milk and known concentrations of vegetable oil. Inject the standard solutions into the gas chromatograph under the same conditions as the sample.
Quantification: Compare the chromatogram of the sample with the calibration curve to determine the concentration of vegetable oil in the milk sample.
Quality Control: Include quality control samples to ensure the accuracy and precision of the analysis. Monitor the performance of the gas chromatograph regularly (Gutierrez *et al.*, 2009) [24].

Conclusions

The detection and prevention of milk adulteration are crucial for ensuring consumer safety and maintaining the integrity of the dairy industry. Through this review, we have explored a variety of common and advanced techniques employed for identifying milk adulteration. Traditional methods such as chemical tests and physical examinations remain valuable tools, providing cost-effective and accessible means for initial screening. However, the limitations of these methods in terms of accuracy and sensitivity have led to the development and adoption of more sophisticated techniques. Advanced technologies including chromatography, spectroscopy, immunoassays, and molecular techniques offer enhanced sensitivity, specificity and reliability in detecting adulterants at trace levels. Additionally, the cost and complexity associated with some advanced techniques may limit their widespread adoption, particularly in resource-constrained settings. Overall, advancements in analytical technologies continue to drive progress in milk adulteration detection, offering greater precision and efficiency in safeguarding consumer health and upholding the quality and authenticity of dairy products. By leveraging the strengths of both traditional and cutting-edge approaches, we can mitigate the risks posed by adulteration practices and ensure the integrity of the milk supply chain for years to come.

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