

TECHNOLOGICAL FRAUDS AND MILK ADULTERATIONS: A REVIEW

Alina-Daiana IONESCU, Alexandru Ionuț CÎRÎC*, Mihaela BEGEA

University Politehnica of Bucharest, 313 Splaiul Independenței, 060042, Bucharest/Romania

Corresponding author emails: mihaela.begea@gmail.com, alexcrc@yahoo.com

Abstract

The milk consumption has increased constantly, with milk being part of the diet of a large proportion of the global population. As a result of this growing demand, the increased competition in the dairy market and the increasing complexity of the supply chain, the producers in the sector of milk and dairy products resort to technological fraud, which is considered to be a predominant problem in countries without a specific legislation. Therefore, this paper aims to review the main adulterants, the counterfeiting techniques and various methods of detecting counterfeiting.

Key words: adulteration, detection techniques, fraud, milk.

INTRODUCTION

Milk, according to the *Codex Alimentarius*, is the normal mammary secretion of milking animals, without any addition or extraction thereof, intended for consumption as liquid milk or for further processing (Morin & Lees, 2018).

Adulteration of milk and dairy products has become a worldwide concern, immediately after the discovery of melamine contamination of infant products in China in 2008, but the history of counterfeiting of milk is very old. In the old German Empire, milk was diluted and then its consistency was restored by adding sugar, flour or calcium carbonate. In addition, this process dates back to 1850, when 8,000 babies died in New York from milk produced by the Swill factories because the milk came from animals fed on by-products from distilleries and was then adulterated by dilution with water, bleached by adding plaster and thickened with starch. Until the early 1900s, the milk was often adulterated with foreign substances, obtained from sick cows or mishandled during milking and storage. As a result, the milk was often the host of tuberculosis, cholera, typhoid fever and other life-threatening diseases. It was not until the end of the 19th century, when scientists began to fully understand theories about microorganisms, that they realized that diseases

are transferred through milk and that they can intervene to eliminate this risk (Handford et al., 2015).

Milk is the best source of protein, fat, carbohydrates, vitamins and minerals, but unfortunately, it can be easily counterfeited worldwide. The reasons behind this fraud are mainly the perishable nature of milk, the shortage of supply and demand to meet urban demand and the lack of adequate detection methods (Tanzina & Shoeb, 2016). According to studies, milk is the second most prone to counterfeiting, after olive oil. Thus, it is adulterated with harmful substances that increase its quantity, but considerably reduce its quality (Gawali, 2021).

In addition to its microbiological quality and safety, the quality of milk is usually defined on the basis of the nutrient levels (mainly protein and fat) (Marin et al., 2019). These parameters were used to calculate payment to the supplier. In general, the parameters usually used to assess the milk quality are fat, protein, solids-non-fat and freezing point. The adulterants added to milk improve the value of these parameters, thus increasing milk quality in a dishonest way (Kedjia, 2018).

Since it is equally important to know about common adulterants and their effects on health, appropriate consumer awareness has been taken into account as a solution to prevent counterfeiting (Gawali, 2021).

MATERIALS AND METHODS

The current review was restricted to articles with English full-text availability. MDPI, Google Scholar, MedCrave, Springer Link, Springer Nature, Elsevier, and Juniper Publishers were among the databases used. The most common search terms were: milk quality, milk adulteration, potential milk adulterants, and detection methods. Additionally, searches were conducted using each adulterant in turn. We also looked for additional references in the bibliographies of the included papers. In our review of the literature, we discovered a sizable number of studies that mostly discussed the chemical makeup of milk and how the most crucial elements of milk change when adulterants are added. The results of the thorough search are sorted into categories and listed according to the best techniques discovered. We only included the more than 120 research articles and review papers that were discovered after 2009 because that is when the majority of adulterant detection techniques were created, which was necessary following the significant finding of the falsification of powdered milk from China in 2008.

RESULTS AND DISCUSSIONS

I. The most common milk adulterations

The different types of adulterants found in milk can be categorized into intentionally or accidentally added adulterants. However, only adulterants that have been added intentionally will be presented below. Also, water, vegetable proteins, whey and milk of different species (cow, buffalo, goat, sheep, camel, etc.) form the main constituents of economically motivated adulterants and do not pose a serious health risk. However, adulterants such as urea (to increase the non-protein nitrogen content and make milk white), formalin and boric / benzoic acid (to increase the shelf life of milk), detergents (to emulsify the oil in diluted milk), chlorine (to compensate for the density of diluted milk after adulteration) and ammonium sulphate (to maintain milk density) pose serious health risks (Kumar & Dash, 2021).

1. Adulteration that generally aimed at increasing the volume of milk

This type of adulteration can be done by adding water or skimmed milk to whole milk, as well as double forging using both adding water and reducing the fat content of the milk (Kedjia, 2018).

1.1. *Addition of liquid whey (a by-product of cheese making)*

This is a well-known practice to increase the volume of milk, especially in areas where a huge amount of cottage cheese is produced. The advantage of this addition is that it does not change the lactose content of the milk. In addition, the whey added to the milk does not change the overall milk density, but reduces the solids and fat content, which decreases in proportion to the whey added. In this particular case, it is the fat that reflects the adulteration. Moreover, the addition of whey cheese to milk is very difficult to detect by formal analytical procedures that make as necessary to implement new experimental procedures/tests (Aquino et al., 2014).

1.2. *Addition of reconstituted milk or synthetic milk*

Synthetic milk is an excellent imitation of natural milk containing vegetable oil, urea and emulsifiers. It is characterized by the fat, nitrogen, glucose and foam content, and a specific gravity similar to natural milk. When the synthetic milk is mixed with normal milk in different proportions, becomes identical to it including flavour. It is reported that synthetic milk is used to alter milk by 5-10% (Morin & Lees, 2018).

1.3. *Partial skimming and addition of skimmed milk*

This practice is most often used when milk with a high fat content, milked in the evening, is skimmed and added on top of whole milk. Milk fat is one of the most valuable components and therefore can be subject to fraud, hence removing a quantity of fat leads to changes in the characteristics of the milk: the density increases, and the fat content decreases in proportion to the degree of skimming. The dry matter in the milk may increase, or its value may remain unchanged (Hanganu & Chira, 2021).

1.4. *Diluting milk with water*

This is the most common, simplest, and oldest method of adulteration, based on the percentage reduction of all milk components and the nutritional value and other quality control parameters of the milk (such as density, fat, SNF, protein and freezing point) will decrease. Therefore, dilution of milk with water can lead

addition of chemicals to bring the density and colour to normal parameters. The milk will increase considerably in volume, hence the producers will make a considerable profit, but to changes in nutritional, hygienic and technological quality in addition to changes in chemical composition (Kedjia, 2018).

Table 1. Variations of milk quality control parameters according to the degree of dilution with water (Vujadinovic et al., 2017)

Models	Density [g/cm ³]	Viscosity [mPas]	Freezing point [°C]	pH	Fat of milk [%]
Undiluted milk	1.0571	33.83	-0.501	6.70	3.20
5% H ₂ O	1.0531	32.83	-0.484	6.72	3.00
10% H ₂ O	1.0517	32.63	-0.457	6.73	2.90
15% H ₂ O	1.0505	31.63	-0.433	6.75	2.80
20% H ₂ O	1.0478	31.33	-0.405	6.76	2.55
25% H ₂ O	1.0469	30.90	-0.378	6.77	2.45
30% H ₂ O	1.0447	29.27	-0.352	6.78	2.25
35% H ₂ O	1.0436	28.90	-0.327	6.78	2.00
40% H ₂ O	1.0430	28.73	-0.301	6.80	1.80
45% H ₂ O	1.0412	27.90	-0.275	6.80	1.73
50% H ₂ O	1.0397	27.13	-0.250	6.81	1.48

2. Adulterations involving the increased protein content

Nitrogen-rich adulterants are also a well-known issue in recent years precisely because of food safety incidents. This type of adulteration is very common because non-protein nitrogen cannot be distinguished by the Kjeldahl and Dumas methods that are commonly used to determine the total protein content of milk. Melamine, urea and whey are the main adulterants for this purpose due to their high nitrogen content and low cost (Morin & Lees, 2018).

2.1. *Adulteration of milk with melamine*

According to the World Health Organization, melamine is a nitrogen-rich substance, which is used to increase the apparent protein content and therefore the economic value of milk. Until melamine contamination was reported in China in 2008 the limit of melamine in EU food legislation was not established. Both the European Commission and the United States Food and Drug Administration imposed the maximum allowable limit, which was later followed by the Codex Alimentarius Commission in a new ruling in 2010. According to the Regulation 1881/2006, the maximum level of melamine in food, except

infant formula, is 2.5 mg/kg and in powdered infant formula and follow-on formula the maximum level allowed is 1 mg/kg. Melamine is described as harmful if swallowed, inhaled or absorbed through the skin. However, it is not a carcinogenic compound and has low oral toxicity; but it does cause kidney and urinary problems and even infant death when it reacts with cyanuric acid inside the body. The FDA has reported that when melamine and cyanuric acid are absorbed into the bloodstream, they concentrate and form a large number of crystals, which block and destroy kidney cells. The toxic dose of melamine is on par with common table salt with an average lethal dose (LD50) greater than 3 grams per kilogram of body weight (Jalili, 2017).

2.2. *Adulteration of milk with urea*

Adding water to milk leads to a reduction in whiteness and density, and to maintain these properties, urea is generally used as a adulterating agent. Urea acts as preservative, increases SNF and non-protein nitrogen, but decreases the titratable acidity and suppresses the milk fermentation. At the same time, it makes the milk viscous, giving the impression of thicker milk. Urea, being a natural component of milk, accounts for most of the

non-protein nitrogen in milk, i.e. 55%. According to Food Safety and Standards Authority of India (FSSAI), the maximum permissible limit for urea in milk is 70 mg/100 ml. Milk can be adulterated with urea in two ways: by intentionally adding urea and by adding synthetic milk to natural milk (Kumar & Dash, 2021).

2.3. Adulteration of milk with proteins resulting from the activity of genetically modified yeasts

The technology for obtaining these types of protein allows being applied anywhere, even in urbanised areas. This technology is still being studied and the aim is to make these caseins allergen-free. Since these proteins are based on GMO technology, no milk protein produced in this way has so far received regulatory approval in Europe. In the US, the products containing proteins identical to those of natural milk, produced by a controlled fermentation process, are already on the market. In the future, it is likely that these proteins will also be the main ingredient in synthetic milk, as a response to the huge demand for the product, the drawbacks of factory farming and concerns about lactose allergies, hormones and antibiotics (Slane, 2019).

3. Counterfeiting involving the addition of unusual substances

This practice is applied in order to hide another type of falsification and involves the addition of the following compounds:

3.1. Detergents or soap

This type of compounds are added to achieve the natural characteristics of milk, especially to make the milk thicker and to emulsify and dissolve the vegetable oil previously added to replace the extracted fat, forming a solution that resembles the froth of freshly milked milk. Detergent is added mainly to synthetic milk, which is similar to natural milk, being white in colour, and is produced by mixing urea, detergent, vegetable oil, neutralisers, sugar and water. Detergents have been shown to be the essential components of such a milk-like preparation. The anionic detergents are widely used in such practices precisely because of their low cost and easy availability (Barui et al., 2013).

3.2. Vegetable oil

Milk fat is an important component of food and plays a significant role in the economics, nutrition and physical and chemical properties of milk. However, the incorporation of vegetable oils alters the content, the type and distribution of fat droplets in the protein network, causing changes in the microstructure and textural behaviour of dairy products. Olive oil in particular is used to replace milk fat and it is the added element after the fat content is removed. Since milk fatty acids are short-chain fatty acids (caprylic, capric, butyric) and vegetable fats are long-chain fatty acids, only a simple analysis of the fatty acid profile by chromatographic method can show that milk has been adulterated with vegetable oils (Ntakatsane et al., 2013).

3.3. Calcium carbonate or calcium chloride

It is added to correct the density and to mask the dilution of the milk with water. When the calcium value rises above the normal range, the milk can be said to be falsified (Kedjia, 2018).

3.4. Maltodextrin

Maltodextrins are polysaccharides containing dextrose and are obtained either chemically or by enzymatic hydrolysis of starch. Maltodextrins are classified on the basis of the amount of reducing sugar in relation to total carbohydrates, ranging from 3 to 20%. Maltodextrin is highly soluble in water with a solubility of about 1.2 kg/l; it is mainly used in foods and beverages as a thickener, sweetener and/or stabiliser. The maltodextrin imparts important functional properties such as bulking, gelling, binding, prevention of crystallisation, promotion of dispersibility, freezing control. It has been reported that maltodextrin is added as an adulterant to milk mainly to increase the density and also to increase the yield of the product prepared from it (Aparnathi et al., 2020).

3.5. Starch, wheat flour or rice flour

These are added precisely to increase the density of the milk but also to increase the milk solids content. Functional maize starch is also specially designed for introduction into the milk industry. It successfully replaces modified starches with much better stability and is suitable for use in food processes that undergo heating or shearing. It has no impact on product, colour and taste (Kedjia, 2018).

3.6. *Salt*

It is added mainly to get a correct lactometer reading by increasing the milk density. It is the most commonly used because its properties lend themselves very well to such adulteration. It is relatively difficult to detect, as there is quite a large amount of chlorides in milk, and in some particular situations (milk from cows with mastitis) the chlorides exceed the normal maximum limit. However, the natural content of chlorides in mixed milk (expressed as sodium chloride) varies between 120-170 mg/100 ml of milk, with an average of 140 mg/100 ml, and up to 200 mg/100 ml in colostrum milk or milk from cows with mastitis (Aparnathi et al., 2020).

4. Adulterations associated with increasing the shelf life of milk

4.1. *Neutralizers*

These are added to cover the acidity and sour taste of the milk. The most popular neutralisers are sodium bicarbonate, hydrated lime, sodium hydroxide and sodium carbonate. Although the addition of such alkaline substances is not allowed by law, the producers tend to neutralise milk to avoid rejection in milk collection centres and factories (Kedjia, 2018).

4.2. *Formaldehyde*

This is an antiseptic, disinfectant and a good preservative. It is used to preserve milk for a long period of time (1 ml per 10 L of milk preserves it for about 10 days), especially during transport and storage to avoid refrigeration costs. It is a toxic and very dangerous substance, considered carcinogenic, and a high dose of formaldehyde can affect the liver and cause kidney damage (Mabood et al., 2017).

4.3. *Hydrogen peroxide*

It has a similar role to formaldehyde and helps to increase shelf life by acting as a preservative. It decreases the souring of milk when hygiene and low temperature storage rules are not followed by stopping bacterial growth. Hydrogen peroxide (H_2O_2) has a long history of use as a preservative in milk around the world. The use of H_2O_2 to activate the inherent lactoperoxidase enzyme system has improved the quality of milk and dairy products in areas where refrigeration is not widely available. Even though, due to chemical processes within raw milk, it may contain small amounts of hydrogen peroxide (1-2 mg/L), the

concentration necessary to inactivate pathogens is 10 times higher. In addition, the presence of H_2O_2 in high concentrations can lead to modifications in the milk chemical composition. At the same time, the addition of hydrogen peroxide for the purpose of preserving milk is prohibited for the following reasons: it masks to some extent negligence in the observance of hygienic conditions; it has a germicidal action which is not selective, acting more strongly on lactic bacteria than on the spoilage microflora; used in larger quantities to ensure preservation for 1-2 days, it can impart undesirable sensory properties to milk (bitter, irritating taste); added even in small quantities, hydrogen peroxide, through the active oxygen released, causes incipient oxidation of milk fat. The US Food and Drug Administration (FDA) only allows hydrogen peroxide in milk that is used for cheese production (Ivanova et al., 2019).

4.4. *Acids*

These are added to milk as preservatives. The most common are salicylic acid and benzoic acid, which are responsible for increasing shelf life. Salicylic acid added to milk at a rate of 0.04-0.05% preserves the product for several days. Salicylic acid and its salts are prohibited for use in the preservation of milk because of their harmful effects on the body and other negative implications. Benzoic acid is widely used in foodstuffs as a preservative. Although benzoic acid is generally recognised as safe (GRAS) under the food regulations, benzoic acid is not a permitted preservative in milk and milk beverages in EU and China. WHO has assessed and established an acceptable daily intake (ADI) for benzoic acid of 0-5 mg/kg body weight (Qi et al., 2009).

5. Adulterations involving species substitutions

Of the many possible adulterations in milk and dairy products, one of the most common concerns the origin of the species, i.e. replacing milk with higher nutritional value (such as sheep, goat or buffalo) with cheaper cow's milk to reduce production costs and increase profits. This is explained by seasonal fluctuations and lower yields of sheep, goat and buffalo milk (or more exotic species such as camel or donkey), which raises the economic values of these types of milk and derived products. Replacing milk from these species, in addition to having a

negative economic impact, is also a problem for many consumer groups for other reasons such as religious, ethical or cultural objections. In several EU countries, especially those in the Mediterranean and other areas such as the Eastern Europe and the Middle East, a variety of cheeses are prized as traditional products made from goat's milk, sheep's milk, their milk in a mixture or buffalo milk. Traditionally produced cheeses are regarded as specialities and generally fetch higher market prices and are therefore more prone to adulteration of the raw material from which they are made. There is now a growing market for non-cow's milk in some countries, particularly goat's milk, due to its superior nutritional characteristics, but also to other aspects such as its attractive smell and taste and superior digestibility. In addition, according to studies, goat's milk may be a possible alternative to cow's milk as it is considered to be hypoallergenic. In this case, if cow's milk is not listed on the label it could pose a health risk to consumers who are allergic. However, because the proteins are very similar, people allergic to cow's milk proteins can be affected by milk from any species, demonstrating the importance of correct labelling (Morin, 2018). The composition of milk of different types of farm animals differs significantly in physico-chemical indicators such as the mass fraction of proteins and fats, minerals, vitamins, enzymes, etc. An important identification criterion for the type of milk is also the polymorphism of caseins, the constituent technological components of raw milk that determine its possibility of industrial processing. In order to avoid possible fraudulent substitution of goat and sheep milk by cow's milk, it is necessary to develop analytical procedures capable of detecting such frauds and protecting consumers against misleading mislabelling (Gilmanov et al., 2020).

II. Methods for detecting adulterants in milk

The control of milk quality is very important for the safety reasons. Therefore, the adulteration of milk decreases its quality and can even affect its safety. The methods for detecting adulteration in milk are generally classified into qualitative and quantitative detection methods. Although the quantitative detection methods include complex biotechnological and electrical methods, the qualitative

detection methods based on colour chemical reactions (biochemical, physical-chemical) are advantageous because they are simple, fast and very easy to perform, even if not very accurate (Kedjia, 2018).

1. Physical methods (qualitative)

Methods based on the physical properties of milk are density (reading by lactometer), freezing point, refractive index, etc., which are easy to perform but may be inaccurate due to natural variations in milk composition. Physical methods are simple, quick, easy, cheap and convenient. However, the sensitivity of these tests is lower compared to chemical and instrumental methods. The freezing point can be significantly affected by seasonal changes and regional factors. Density (or specific gravity) depends on the composition and temperature history of the milk. Therefore, density measurement may not be a useful tool for detecting adulteration. Thus, physical methods have a number of general limitations due to natural variations, lower sensitivity, poor specificity, susceptibility to manipulation, etc. (Aparnathi et al., 2020)

2. Chemical methods (qualitative)

Chemical methods for detecting milk adulteration are based on observable physico-chemical changes. They can be performed in any biosafety level 1 laboratory with the availability of chemical reagents and the necessary precautions. Chemical changes may occur as a result of chemical reaction between the adulterant in the milk and a specific chemical reagent, resulting in the appropriate colorimetric detection (Rupak et al., 2021). When reviewing and evaluating available qualitative tests for the detection of common adulterants reported in milk, it was observed that there is a wide variation related to the performance of several tests in terms of aspects such as sensitivity, convenience, cost. In view of performance improvement requirements the existing qualitative tests for the determination of common adulterants including detergent, urea, ammonium salts, glucose, sucrose, maltodextrin, starch, hydrogen peroxide, salt, nitrate, sulphate, formaldehyde and neutralisers have undergone a number of changes (Aparnathi et al., 2020).

Table 2. List of qualitative tests for finding common milk adulterants that have been reported (Aparnathi et al., 2020)

Sr. No.	Adulterants	Details of tests selected for optimization	
		Test	Reference
1.	Detergent	Methylene blue	Paradkar et al. (2000)
2.	Urea	p-dimethylaminobenzaldehyde test (DMAB)	FSSAI (2016)
3.	Ammonium salts	Nessler	Sharma et al. (2012)
4.	Sucrose	Seliwanoff	Srivastava (2010)
5.	Glucose	Barfoed	Barfoed (1873)
6.	Maltodextrin	Iodine	Sharma et al. (2012)
7.	Starch	Iodine	BIS (1960)
8.	Salt	Silver nitrate test	FSSAI (2016)
9.	Nitrate	Diphenylamine	FAO (1986)
10.	Sulphate	Barium chloride	FSSAI (2016)
11.	Hydrogen peroxide	Iodometric test	FSSAI (2016)
12.	Formaldehyde	Hehner	Draaiyer et al. (2009)
13.	Neutralizers	Rosolic acid	DGHS (2005)

3. Instrumental methods (quantitative)

Modern biotechnology offers a range of rapid, sensitive and accurate methods for the detection and analysis of adulterants in food products. However, recent incidents of adulterated milk contamination have shown that standards in milk quality control are insufficient in identifying poor quality milk. Therefore, different methods have been studied and applied precisely to combat these problems. However, such detection methods require large investments. Another important aspect is that the type of quantitative detection techniques depends on the nature of the adulterants in milk. Although there are fairly well known techniques based on portable equipment, those designed and developed recently require experimental set-up and much more expensive equipment, as well as a number of operational procedures (Kedjia, 2018). A situation often arises when the indicators of the device used, calibrated according to the

manufacturer's data, differ from the indicators obtained by reference methods, e.g. the Gerber method for fat determination. The difference can be as significant as 0.3% fat. With the apparent simplicity of the method, overlooking its complexity can lead to systematic errors in the analysis. For example, the temperature regime is not maintained, the isomeric composition of the isoamyl alcohol used is not monitored. This leads to discrepancies between the milk analyser readings and the results obtained from the chemical analysis data precisely because the Gerber method was performed incorrectly. In addition, thanks to new technologies, quality analysis is carried out much faster and is less dependent on the qualifications of employees. Therefore, more and more priority is given to analysers using indirect methods of data collection. The analytical methods by which milk authenticity can be demonstrated are presented below (Smirnova et al., 2020).

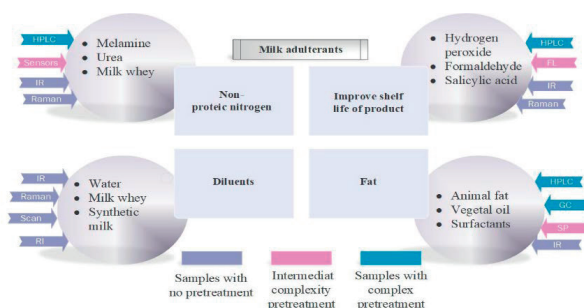


Figure 1. A description of the main milk adulterants, along with information on sample pretreatment complexity and the analytical techniques used for determining the most commonly (Nascimento et al., 2017)

FL: molecular fluorescence; GC: gas chromatography; HPLC: high performance liquid chromatography; IR: infrared spectrometry; RI: measurement of refractive index; Scan: scanometry; SP: UV-vis spectrophotometry

3.1. Identification of milk components and quantification of added adulterants by spectroscopy (NIR, Raman)

Fingerprinting methods are ideal candidates to replace analytical procedures. The term 'fingerprinting' can be defined as a variety of techniques that can measure food composition in a non-selective way. Among these methods, infrared-based vibrational spectroscopy methods and Raman spectroscopic techniques use information from the major compounds present in milk. Organic compounds absorb radiation at specific wavelengths or frequencies, giving rise to spectral signatures that are characteristic of the food composition and can be considered as 'fingerprints' of the product. These signatures also include interference due to variation arising from natural events (e.g. weather, climate, disease, etc.). Vibrational spectroscopy is suitable for implementation in factories and dairy laboratories as it allows on-line control and screening of a large number of samples per unit time. Fingerprinting methods are also of interest to regulatory bodies as they allow for rapid preventive action (Morin, 2018).

3.2. Evaluation of proteins by mass spectrometry (MS) or liquid chromatography (LC) coupled with MS

Evaluation of proteins and/or peptide sequence by mass spectrometry (MS) or liquid chromatography (LC) coupled with MS is increasingly used to prove the authenticity of milk. This has been made possible by several technological advances that allow for accurate protein and peptide analysis using ionization techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) provides informative fingerprints of milk proteins for the authentication of dairy products and is also a simple, fast, sensitive and highly reproducible technique. LC-MS techniques are advantageous in terms of high selectivity and sensitivity, which makes them useful as confirmatory techniques (Morin, 2018).

3.3. Different methods of species identification

In recent years, analytical methods based on DNA analysis have progressed rapidly, going

beyond protein analysis and have been successfully applied in testing the authenticity of milk (Morin & Lees, 2018). DNA-based methods have several advantages, in particular the ubiquity of nucleic acids in each cell type and their superior stability compared to proteins. Most DNA tests are based on the polymerase chain reaction (PCR) technique, precisely because of its high specificity, sensitivity, simplicity and speed, allowing the identification of species of origin even in processed foods such as dairy products (Morin, 2018).

According to EU legislation, isoelectric focusing of γ -caseins after plasmolysis should be used as a reference method to ensure that the products obtained are exactly sheep's milk, goat's milk or buffalo milk or a mixture of sheep's, goat's and buffalo milk. In this method, samples must be analysed together with reference standards containing 0% and 1% cow's milk, and the test is considered positive if both γ 2- and γ 3-cow's milk exist (obtained by plasmolysis), or the corresponding peak area ratios when densitometry is applied, are equal to or greater than the 1% reference standard level. The method may be used to detect either raw or heat-treated and caseinised cow's milk in fresh or ripened sheep's, goat's and buffalo's milk cheeses or mixtures thereof (Morin, 2018).

Recently, a commercially available kit has been developed that is based on a competitive ELISA using a mouse monoclonal antibody raised against bovine κ -casein that allows screening of both raw milk and heat-treated cow and buffalo milk in milk and cheese from other species and sources (Morin, 2018).

CONCLUSIONS

The milk adulteration represents a main global concern because milk is consumed as a healthy dairy product everywhere in the world. People are concerned about the quality and purity of milk as a result of the growing fraudulent practice of adulterating milk. As a consequence, the consumers' health may be harmed by milk adulterants like water, vegetable and animal fat, extraneous proteins, and chemical additives like melamine, urea, ammonium sulphate, formalin, acids (e.g. boric

acid, benzoic acid, salicylic acid), caustic soda, hydrogen peroxide, detergents, and sugars that are knowingly added. Various techniques have been developed over time to identify adulterants in milk, but the most accurate are instrumental. Therefore, there is a pressing need for the development of reliable, affordable and non-expensive methods and technologies that could detect and stop the practices of adulterating milk.

REFERENCES

- Aparnathi, K. D., Shaikh, A. I., Patel S.I. (2020). *Qualitative Tests for Detection of Common Adulteration in Milk*. Directorate of Research Anand Agricultural University. <https://www.studocu.com/in/document/university-of-delhi/introduction-to-food-technology-i/qualitative-tests-for-detection-of-common-adulterants-in-milk-oct2021/63712688>
- Aquino, L. F. M. C., Silva A.C.O, Freitas, M.Q., Felicio, T.L., Cruz, A. G., & Conte-Junior, C.A. (2014). Identifying cheese whey an adulterant in milk: Limited contribution of a sensometric approach. *Food Research International*, 62, 233-237. <https://doi.org/10.1016/j.foodres.2014.03.001>
- Barui, A. K., Sharma, R., Rajput, Y. S., & Singh S. (2013). A rapid paper chromatographic method for detection of anionic detergent in milk. *Journal of Food Science and Technology*, 50(4), 826-829.
- Chaudhry, H.R., Khushi, M.Z., Rabbani, M. (2015). *Laboratory Manual Quality Control of Milk: Quality Control of Milk*. University of Veterinary and Animal Science Punjab, Pakistan.
- Gawali, S. P. (2021). Common milk adulteration and their detection techniques: A review. *International Journal of Multidisciplinary Educational Research*, 10 (2/5).
- Gilmanov, K., Vafin. R., Bliadze, V.G., & Michailova, I. (2020). *The problem of falsification of milk species appliance*. Moscow, Russia: All-Russian Dairy Research Institute. DOI: 10.37442/978-5-6043854-1-8-2020-1-125-129
- Handford, C. E., Campbell, K., & Elliott, C. T. (2015). Impacts of Milk Fraud on Food Safety and Nutrition with Special Emphasis on Developing Countries. *Comprehensive Reviews in Food Science and Food Safety*, 130-142. <https://doi.org/10.1111/1541-4337.12181>
- Hanganu, A., & Chira N.A. (2021). When detection of dairy food fraud fails: An alternative approach through proton nuclear magnetic resonance spectroscopy. *Journal of Dairy Science*, 104(8), 8454-8466.
- Ivanova, A. S., Merkuleva, A. D., Andreev, S.V., & Saharov, A. K. (2019). Method for determination of hydrogen peroxide in adulterated milk using high performance liquid chromatography. *Food Chemistry*, 283, 431-436. <https://doi.org/10.1016/j.foodchem.2019.01.051>
- Jalili, M. (2017). A Review Paper on Melamine in Milk and Dairy Products. *Journal of Dairy & Veterinary Sciences*, 1(4). DOI:10.19080/JDVS.2017.01.555566
- Kedjia, H. M. (2018). *Milk adulteration. Option to maintain a quality product*. Addis Ababa University. <https://www.amazon.com/adulteration-Options-maintain-quality-product/dp/3346004848>
- Kumar, V., & Dash, S. (2021). Evaporation-Based Low-Cost Method for the Detection of Adulterant in Milk. *ACS Omega*, 6, 41, 27200-27207. <https://doi.org/10.1021/acsomega.1c03887>
- Mabood, F., Hussain, J., Moo, Al. N., Gilani, S. A., Foaooq, S., Naureen, Z., Jabeen, F., Ahmed, M., Hussain, Z., & Al-Harrasi, A. (2017). Detection and Quantification of Formalin Adulteration in Cow Milk Using Near Infrared Spectroscopy Combined with Multivariate Analysis. *Advances in Dairy Research*, 5(1), <https://www.longdom.org/open-access/detection-and-quantification-of-formalin-adulteration-in-cow-milk-using-near-infrared-spectroscopy-combined-with-multiva-24078.html>
- Mafrá, I., Honrado, M., & Amaral, J. S. (2022). Animal Species Authentication in Dairy Products. *Foods*, 11(8). <https://doi.org/10.3390/foods11081124>
- Marin, M.P., Marin, I., & Vidu, L. (2019). Learning about the reduction of food waste using Blockchain technology. *13th annual International Technology, Education and Development Conference*, Valencia, Spain, 3274-3277. doi: 10.21125/inted.2019.0856
- Morin, J. L., & Lees, M. (2018). *Food Integrity Handbook. A guide to food authenticity issues and analytical solutions*. *Eurofins Analytics France*. <https://doi.org/10.32741/fihb>
- Nascimento, C. F., Santos, M. P., Pereira-Filho, E. R., & Rocha, F. R. P. (2017). Recent advances on determination of milk adulterants. *Food Chemistry*, 221, 1232-1244. <https://doi.org/10.1016/j.foodchem.2016.11.034>
- Ntakatsane, M.P., Liu, X.M., & Zhou, P. (2013). Short communication: *Rapid detection of milk fat adulteration with vegetable oil by fluorescence spectroscopy*. State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University. <https://doi.org/10.3168/jds.2012-6417>
- Qi, Ping., Hong, H., Liang, X., & Liu, D. (2009). Assessment of benzoic acid levels in milk in China. *Food Control*, 20(4), 414-418. <https://doi.org/10.1016/j.foodcont.2008.07.013>
- Rupak, N., Sharma, A., Kumar, D., Chawla, P., & Kumar, A. P. (2021). Milk adulterant detection: Conventional and biosensor based approaches: A review. *Sensing and Bio-Sensing Research*, 33, 100433. <https://doi.org/10.1016/j.sbsr.2021.100433>
- Slane, C. (2019). *Fake milk is real news, as synthetic alternatives threaten traditional dairy farms*. The University of the West of England. <https://www.nbcnews.com/business/business-news/fake-milk-real-news-synthetic-alternatives-threaten-traditional-dairy-farms-n973236>

- Smirnova, A., Konoplev, G., Mukhin, N., & Stepanova, O. (2020). Milk as a Complex Multiphase Polydisperse System: Approaches for the Quantitative and Qualitative Analysis. *Journal of Composites Science*.
<https://doi.org/10.3390/jcs4040151>
- Tanzina, A., & Shoeb, A. (2016). Common milk adulteration and their detection techniques. *International Journal of Food Contamination*, 24(1), 127-139.
- Tessema, A., & Tibbo, M. (2009). *Milk Quality Control*. Technical Bulletin No. 2, International Center for Agricultural Research in the Dry Areas, Pakistan
- Vujadinovic, D., Beribaka, M., Vukic, M., & Marjanović-Balaban, M. (2017). Comparison of methods for determining the falsification milk. *Journal of Hygienic Engineering and Design*.
<https://keypublishing.org/jhed/wp-content/uploads/2020/07/02-Full-paper-Dragan-Vujadinovic.pdf>.