Development of Paper Based Low Cost Milk Adulteration Testing Strips

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By

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DEDICATION

This research is dedicated to my parents who prayed for me at each and every step and my supervisor, my inspiration and my spirit who offered me kind assistance and supported me all the time, without whom the present project would have been a mere dream.

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Muhammad Nadeem

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List of Abbreviations

Abbreviations	Full Name
Conc.	Concentration
G	Group
ml	Milliliter
HCl	Hydro chloric acid
Min	Minutes
Hrs	Hours
FTIR	Fourier transform Infrared
NIR	Near infrared
GC	Gas chromatography
HPLC	High performance liquid chromatography
mmol	Millimole
PCR	Polymerase chain reaction
H_2O_2	Hydrogen peroxide
HS-GC	Headspace gas chromatography
ELISA	enzyme-linked immunosorbent assay

ABSTRACT

Adulteration of milk is a widespread problem throughout the world. Many kinds of milk adulterants such as, hydrogen peroxide, urea, detergents, formealdehyde etc exists which leads to biological hazards. Different techniques such as mass-spectrometry, chromatography like GC and HPLC, Immunological method like immunoassay and different DNA based process like PCR are being used to detect adulterants in milk. In this study we formed various kinds of paper based testing strips to detect various adulterants in milk. Potassium iodide and starch were used to form a paper-based strip to identify hydrogen peroxide, and there was an appearance of a dark blue color on the strip. Another strip was formed by using Bromocresol purple solution to detect detergents in milk; this strip gave a violet color. A urea-paper-based strip was also formed by using the p-dimethylaminobenzaldehyde reagent, 1.6% ethanol, and 37% hydrochloric acid, which resulted in a light yellowish color of the strip. The study offers the possibility to use paper-based testing strips, an efficient and affordable technology, to identify hydrogen peroxide, detergents, and urea in tainted milk.

CHAPTER 1

INTRODUCTION

1.0 Introduction:

Milk is a supply of vital elements such as glucose, proteins, lipids, vitamins, and mineral deposits, making it virtually a complete food for living beings. It is essential to keep healthy and encourage growth. However, it can also act as a means of transferring chemicals and other contaminants[1]. Due to its unique nutritional value and significant impact on both human and animal health, milk is very significant. It contains all the elements that organisms require in the most easily assimilated form [2]. Consumption of animal-sourced foods, particularly milk, has boosted linear growth and weight gain in infancy, youth, and adolescence in poor nations whose diets are frequently nutrient-deficient [3]. It is meant to provide complete nourishment for young animals; it is a particularly complex food that is of great interest. As globules of milk fat suspended in an aqueous medium containing lactose, a variety of proteins, mineral salts, and water-soluble vitamins, milk is fundamentally a complicated colloidal system. Milk and dairy products contribute significantly to the consumption of saturated fatty acids since they contain comparatively high levels of saturated fatty acids compared to other animal-derived lipids and noticeably more than lipids in chicken meat [4].

Milk and dairy products are widely regarded as highly recommended foods for consumers of all ages that are very difficult to substitute, and their consumption by children is irreplaceable. On taking into account the information provided above, that we are able to draw the conclusion that milk is a significant food because it and other dairy products are indispensable sources of nutrients required for development in humans and can be used as medicine by consumers of every age and health constraints [5].

One major issue in the food production process is the adulteration of food products. This is the method used by dishonest producers to deceive regulators and customers. The food processing industry as a whole is impacted by adulteration. Large-scale products that are manufactured in large quantities, as well as pricey goods whose adulteration generates revenue, are the most commonly contaminated [6].

In order to reduce the financial losses caused by the spoiling of milk during its transit and sale, several unethical measures are typically adopted to keep milk momentarily fresh. For instance, thickening agents such as starch, flour, skimmed milk powder, whey powder, or other substances are added to prevent the dilution effect and enhance the milk's solids content. Water is also added to increase the amount of milk. To offset the diluted milk's hazardous fat, carbohydrate, and protein levels, uses vegetable oil, sugarcane, or urea. In order to extend the lifespan of the milk, certain substances are used, including hydrogen peroxide, carbonate, antimicrobial agents, baking soda, and even the most deadly chemical, formalin. Detergents are also used to improve the cosmetic qualities of milk, giving it a foamy appearance and whitening it, which can cause gastrointestinal issues. Most of the time, adulteration is done on purpose to increase profits, but it can also occur for a variety of reasons, including inadequate awareness, inadequate detection technologies, and uncertainty about the right drug administration methods[7].

Melamine is a food additive that is falsely added to boost the appearance of protein in meals. Several human deaths from renal failure and other illnesses were brought on by melamine adulteration episodes that took place in China in 2008. This problem spurred research facilities, both public and private, to create several analytical techniques to identify melamine in food[8]. The chemical formula of melamine is $C_3H_6N_6$, (2, 4, 6-triamino-1,3,5-triazine) it is an organic substance. Liebig heated potassium thiocyanate and ammonium chloride to create it for the first time in 1834[9]. It has a highly stable heterocyclic structure and is frequently the main byproduct of several rather high-temperature reactions that are ultimately based on the ammonia and carbon dioxide reaction [10]. It is a naturally occurring substance which is used to make polymers, fertilizers & textiles [11]. Consequently, melamine-tainted milk products are now receiving major attention on a global scale [12]. Since melamine can cause significant or even deadly renal and kidney failure [13]. It may build up in the body and lead to toxicity issues, essentially harming the kidneys and then, it has been established that, depending on the circumstances and the type of metabolism creating stones. Infants who consume melamine-containing milk powder frequently will be more vulnerable to these side effects [14].

For food safety, the urea content of products like milk is essential. For instance, there have been reports of diluted milk being adulterated with urea to maintain its thickness and

viscosity. Therefore, it's crucial for food safety and health to measure urea adulteration accurately [15].

For children, the effects of drinking tainted milk are far more severe and persistent [16]. Adulteration of milk has an intricate problem that not just exerts a consequence upon the well-being of people and is highly expensive; however it also prevents our bodies from utilizing the beneficial components of milk, which are needed for healthy bodily development. Therefore, it is necessary to regularly and randomly check unpasteurized milk in markets regarding adulteration. Additionally, increasing awareness among shopkeepers as well as street sellers will significantly lower the likelihood of this issue occurring [17].

In recent years, numerous nations have paid significant attention to the legitimacy of various animal food items. Major milk proteins analysis is the foundation of the majority of procedures used to determine the authenticity of dairy products. It is essential to conduct authenticity tests on food products, such as meat, milk, and fish, in order to avoid unfair competition and guarantee that consumers are protected from frequent fraudulent tactics in the food sector. These tests are also vital for labeling and value evaluation. The producers can help safeguard consumers from misleading marketing strategies and facilitate trade based on accurate product descriptions by boosting consumer confidence in the authenticity of the product [18].

Many techniques for identifying milk adulterants have been developed over time. Selective identification and detection of several milk adulterants have been achieved by the use of mass spectrometry in conjunction with chromatographic techniques like GC and HPLC. Several widespread milk contaminants are also specifically detected through the application of immunological methods like Immunoassay and different DNA-based processes like polymerase chain reaction. The standard for adulterant detection systems has been increased by spectroscopic techniques, specifically FTIR and NIR combined with chemo-metrics. Electronic tongue and nose devices are just a couple of the sophisticated tools utilized in the detection of adulterants in milk and other foods [19]. These methods limit the use of onsite field testing since they are labor-intensive, time-consuming, and involve expensive tools, highly specialized workers, sample preparation, and sample transportation to the laboratory [20]. Unfortunately, the majority of them are expensive, time-consuming, and lab-based methods that restrict their use [21].

One possible solution to the aforementioned issues with milk adulterant detection could be the use of paper-based micro-fluidic techniques [22]. Office paper has various advantages over plastic-based strips, including its ability to produce conducting strip for chemical interaction and load bio-hybrid tiny sensors evaluation insecticides, also minimize trash collection[23].

1.2 Objectives:

The objectives of this study mainly include to investigate the,

- Development of low-priced paper strips to test specifically milk adulteration.
- Detection of different adulterants such as hydrogen peroxide, urea and detergents in milk.

CHAPTER 2

LITRATURE REVIEW

According to Mahony & Fox, mammals, of which there are around 4500 species, are known for their distinctive milk, which is generated to satisfy all of the nutritional, physiological, and defense needs of the species' newborns. All species' milk is essentially the same; however there are notable species-specific variations. Apart from meeting the newborn's complete nutritional needs, numerous insignificant components of milk such as enzymes, immunoglobulins, oligosaccharides, and proteins that bind to metals also have defensive functions [24]. Maijala studied that animal products were shown to be full, adaptable, and healthful human foods in the first half of the 20th century, and as such, they were significant for human health. Among the various foods, milk seems to hold a special place since it is the only substance available to humans and other mammals during the first few years of their lives. Consequently, milk has all the nutrients a developing organism requires for growth and development, including an adequate amount of protein and minerals. Because they are rich in components that are crucial for adult nutrition and contain all the essential nutrients, milk and milk products have long been regarded as an essential component of a balanced diet, both in Western and many other cultures [25].

Kamthania and his partners informed that a significant source of nutrients needed for both adult health maintenance and newborn and child growth is milk. As it is easily absorbed and digested, milk is the ideal food. It is a child's and infants only natural food. It is primarily an excellent source for outstanding fat, protein, and vitamins & minerals. The essential amino acids required for a baby's and child's development can be found in proteins. For adults, it's also necessary for tissue upkeep [26]. Kundu et al., showed that because of its high nutritional content and ability to satisfy hunger in a variety of ways, milk has long been a favorite food among humans. However, due to clinical studies showing certain milk constituents are linked to harmful health effects like cow milk allergy (CMA), lactose intolerance (LI), anemia, and coronary heart disease, its consumption has become more controversial among the health-conscious and risk-averse population.1-2-3 for those seeking dairy-free options, plant-based milk can be a decent option. Customers are now more inclined to favor vegan diets over traditional dairy products

because of this [27]. According to Hansen & Holroyd with its high nutritious content, milk is a staple food consumed all over the world. Furthermore, it is used for producing significant and frequently traded food commodities [28].

Hussain et al., surveyed that the need of milk continues to increase along with Pakistan's population, which is still growing. Pakistan's primary dairy animal consists of the buffalo, and is estimated to have around 29 million heads nationwide (Govt. Pakistan, 2008-2009). Pakistan is the world's second-largest producer of buffalo milk [29].

Banti, reported that majority of the foods we eat are vulnerable to adulteration and food fraud. Food adulteration can occur when non-food ingredients are inadvertently introduced or when non-food items are purposefully added to increase the amount of raw or prepared foodstuff. Some dangerous or harmful substances which would render the meal unhealthy also have the potential to be a form of food adulteration. Food adulteration can involve adding, taking away, or replacing expensive food ingredients with less expensive (cheaper) ones in order to get unfair financial advantage. The manufacturer may profit financially from this act of food fraud, but the eventual consumers of the goods suffer losses. Customers are impacted by purchasing and consuming contaminated food for a variety of aspects. Firstly, they might not receive the proper nutrients through what they are eating, and secondly, the contaminated food might be harmful to their health or cause them to suffer financial losses [30].

Soomro et al., studied that among the greatest challenges facing Pakistan's dairy industry in current time involves contaminated milk that offers serious threats to the health of consumers in addition to causing significant losses in revenue for the milk manufacturing sector. Because a consequence of the potential for waterborne infections, milk tainted with contaminated water poses a major health risk. The chemicals added to milk as adulterants may have negative effects on consumers' health [31].

According to Minetto et al., in the recent years, there has been a steady growth in milk output worldwide. In 2020, global output levels exceeded 900 million tons. Owing to their economic significance, milk products are vulnerable to adulteration by the addition of different substances, including formaldehyde, H_2O_2, as well as urea. At this regard, numerous instances of adulterated milk have been documented across multiple nations. From 1980 to 2010, milk and

dairy products accounted for 14% of reported events worldwide, making them the second most often used food item in frauds and adulterations. Apart from resulting in financial losses, adulteration of milk can lead to serious health issues for humans, such as cancer and heart attacks [32].

Devrani & Pal reported that in many developing nations, including Bangladesh, China, India and Pakistan etc milk adulteration is raising problem. Preservatives and adulterating agents are the two primary categories of compounds added to milk to boost the economical yield [33]. According to Patari et al., human infections can result from milk adulteration, which is a prevalent issue in developing nations. The effects of many adulterants are still unknown despite numerous researches that have been conducted to identify distinct adulterants in milk samples. Hydrogen peroxide disrupts the body's natural defenses by upsetting the antioxidants, which accelerates aging. The acid-base balance in the organism and the pH of the blood are both disrupted by the chloride in milk. The negative effects make it illegal to adulterate milk. Milk's carbonate content can lead to gastrointestinal issues such as diarrhea, colon ulcers, gastric ulcers, and electrolyte imbalances [22].

Pardeep et al., studied that adulteration encompasses accidental contamination that occurs during preparation, storage, and transportation in addition to the deliberate addition or substitution of ingredients. Because adulterated food contains hazardous substitutes or lacks nutritious components, it can have a negative impact on one's health. The most often added adulterants to milk include urea, flour, oils, and water. Urea consumption harms the liver and heart and causes renal failure. According to a Varanasi study, children make up the bulk of milk consumers, and the widespread use of urea caused headaches, vision issues, and diarrhea in these kids. Overindulgence in carbohydrates may cause nutritional displacement and obesity [34].

According to Shalileh et al., one of the most popular milk adulterants is urea, which is used to falsely indicate that milk has higher protein content. Consuming urea could result in adverse impacts on the kidneys and digestive tract. While there are some traditional techniques in tracking the concentration about the urea in milk, actually sensors offer advantages over conventional methods of identification, making them useful instruments that allow quick, simple, as well as accurate identification of dietary fake ingredients. In recent years, an extensive variety

of biosensing techniques are being established for the identification of urea corruption within milk [35].

Jaiswal et al., studied in order to give milk its white color, improve its quality, and enhance the amount of nonprotein nitrogen and solid-not-fat (SNF) that is naturally present in milk for leveling; urea is added to the milk. Urea is additionally employed in the production of synthetic milk [36]. According to Mabood et al., in order to enhance the solid-to-fat content of the milk, the urea is incorporated to serve as an additive in order to provide it brightness and stability. However, urea in excess is susceptible to renal damage and overloading [37]. According to (Chitra, 2021) when urea is added to milk, it raises its non-protein content, levels out its solid content, and thickens its consistency[38].

Lahankar et al., noticed that it is a widespread practice around the world to add hydrogen peroxide to raw milk in order to extend its shelf life. It prolongs the shelf life of milk by acting as a preservative, which stops microorganisms from rotting it. It may be the result of gastrointestinal issues, which in turn may induce gastritis and intestinal inflammation. Additionally, it disrupts the body's quantity of antioxidants, impairing immunity as well as accelerating aging [39]. According to Raturi et al., Hydrogen peroxide is used to preserve milk's freshness for a longer period of time; however, peroxides damage gastrointestinal tract cells and can cause cancer. Intestinal inflammation and ulcers may result from this. H_2O_2 upsets the body's antioxidants, which throws off the natural equilibrium and ageing is accelerated as a result. It is merely an odorless, colorless oxidizing and bleaching agent. It is mostly used in the production of other compounds, deodorants, and water and sewage treatment. It prolongs milk's shelf life and prevents bacterial growth, making it similar to formalin [40].

Navale & Gupta studied that to thin out & absorb oil into liquid; detergents are included, giving in a foamy mixture that has the distinctive white color of milk. it leads to problems with the gastrointestinal tract[41]. Dangi, evaluated that milk adulterants may pose major health risks to the general public, maybe even resulting in deadly illnesses [42].

According to Azad & Ahmed in underdeveloped nations, conventional detection methods are not always easily available or handy, which makes it challenging to handle the various methods of fraudulent adulteration in milk. In order to detect milk adulteration, it is necessary to

develop, apply, and disseminate improved methodologies. To this end, scientific groups and regulatory authorities must work together [43].

Ullah et al., reported that methods are already available and in use for both qualitative and quantitative analyses of the composition of milk and the identification of adulterants. These methods include mass spectrometry, polymerase chain reaction, chromatography, ELISA, and sensory analysis, among others. Generally speaking, these methods are thought to be lengthy, costly, and necessitate the use of qualified experts either preparing samples or execution. Researchers have been working on sensitive, dependable, and reasonably priced techniques to aid in analyzing of food and dairy products for the past 20 years [44].

According to Dou, applications for low-cost tests are numerous and include everything from environmental investigation to food safety inspection and human health examinations. So, inexpensive assays are particularly appealing for developing nations and rural areas with limited financial resources. In recent times, microfluidic devices made from paper have become an inexpensive system that significantly speeds up point-of-care examination in areas with limited funding [45].

CHAPTER 3

MATERIAL AND METHOD

3.0 Methodology

3.1 Study area:

The study was conducted in chemistry laboratory of Superior University Lahore.

3.2 Detection of Hydrogen peroxide:

3.2.1 Strip formation:

Three kinds of strips (1st, 2nd, and 3rd) were formed by using different concentrations of potassium iodide (5, 5.5, and 6 in millimole for the 1st, 2nd, and 3rd kinds of strips, respectively), and the concentration of starch was used (2 mmol, 2.5 mmol, and 3 mmol for the 1st, 2nd, and 3rd kinds of strips, respectively) by using 10 ml of water for each kind of strip. We prepared all the strips by taking water in beakers, followed by the addition of various concentrations of potassium iodide and strach. After the addition, the stirring process was done by using a magnetic stirrer to form a solution. After that, the adsorption of solution on Whatman filter paper was done by dipping Whatman filter paper in the solution for 60 seconds, and then Whatman filter paper was dried for 6 hours in the sun to make a testing strip. We tested many concentrations of potassium iodide and starch to make the required strips, but the above-mentioned concentrations of KI and starch gave positive results in the formation of the desired testing strips.

3.2.2 Shelf life of the strips:

Newly formed strips were stable for 5 months to give reasonable results if it store at ambient temperature.

3.2.3 Hydrogen peroxide test:

Three beakers labeled B1, B2, and B3 with 20 ml of a standard solution of hydrogen peroxide were taken. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for 60 seconds.

3.2.4 Non adulterant milk samples:

Various milk samples from different dairy farms surrounding the area of Lahore (Punjab, Pakistan) were collected. The samples contained the milk of cows and buffalos. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100 ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round of experimentation. Each beaker has 50 ml of natural milk, which acts as a control for comparison purposes. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for a moment to obtain the result. Three replicates of each kind of strip were used.

3.2.5 Adulterant milk samples:

In order to detect the different adulterants, raw milk samples were purchased from several Lahore sale outlets, including dairy shops, milk producers, collectors, and milk vendors. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100 ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round of experimentation. Each beaker has 50 ml of market milk. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for 60 seconds to obtain the result. Three replicates of each kind of strip were used.

Fig.3.1: Beakers (B1,B2 &B3) with milk samples

3.2.6 Tetra pack milk samples:

Tetra packs milk samples from different companies were purchased from Lahore's market. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100 ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round of experimentation. Each beaker has 50 ml of tetrapack milk. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for 60 seconds to obtain the result.

3.3 Detection of detergents:

3.3.1 Strip formation:

Different concentrations of Bromocresol purple solution (0.2, 0.3, and 0.4 in millimole for the first, second, and third kinds of strips, respectively) and 20 ml of water were used to form the first, second, and third kinds of strips. We started by putting water in beakers and then adding different amounts of the Bromocresol purple solution to make all the strips. Using a magnetic stirrer, the addition was followed by stirring to form a solution. Subsequently, the Whatman filter paper # 42 was dipped in the solution for 60 seconds to absorb the solution, and the Whatman filter paper was sun-dried for 6 hours to make a testing strip. In order to get the required testing strips, we tested a variety of concentrations of Bromocresol purple solution; however, the concentrations specified above developed the desired testing strips.

3.3.2 Shelf life of the strips:

Newly formed strips were stable for 5 months to give reasonable results if it store at ambient temperature.

3.3.3 Detergents test:

Three beakers containing the standard detergent solution (20 ml) were labeled B1, B2, and B3. For sixty seconds, the first type of strip was dipped in B1, the second type in B2, and the third type in B3.

3.3.4 Non adulterant milk samples:

Various samples of milk were taken from a variety of dairy farms that surround Lahore, Pakistan (Punjab). The samples included buffalo and cow milk. By taking precautions and using care, these samples were brought into the Superior University of Lahore's Chemistry Laboratory. The collected samples were put into glass beakers holding 100 milliliters. For this experiment, three beakers with the labels B1, B2, and B3 were used. There was 50 ml of natural milk in each beaker, which served as the control (the real base). To get the result, the first type of strip was dipped in B1, the second type in B2, and the third type in B3 for 60 seconds. Three replicates of each kind of strip were used.

3.3.5 Adulterant milk samples:

In order to detect the different adulterants, raw milk samples were purchased from several Lahore sale outlets, including dairy shops, milk producers, collectors, and milk vendors. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100 ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round of experimentation. Each beaker has 50 ml of market milk. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for a moment to obtain the result. Three replicates of each kind of strip were used.

3.3.6 Tetra pack milk samples:

Tetra packet Milk's samples of different companies were purchased from Lahore's market. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100 ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round of experimentation. Each beaker has 50 ml of tetrapack milk. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for 60 seconds to obtain the result.

3.4 Detection of urea:

3.4.1 Strip formation:

Three kinds of strips (1st, 2nd, and 3rd) were formed by using different concentrations of p-Dimethylaminobenzaldehyde reagent (0.5, 0.8, and 1 in millimole for the 1^{st}, 2nd, and 3^{rd} kinds of strips, respectively), 1 ml of 70% ethanol, 37% hydrochloric acid, and 5 ml of water for each type of strip. We prepared all the strips by taking water into beakers, followed by the addition of ethanol and Hydrochloric acid to form the solution. After that, the addition of p-Dimethylaminobenzaldehyde reagent was done, and a suitable solution was formed by the stirring process using a magnetic stirrer. After that, the adsorption of solution on Whatman filter paper # 42 was done by dipping Whatman filter paper in the solution for 60 seconds, and then Whatman filter paper was dried for 6 hours in the sun to make a testing strip. We tested many concentrations of p-Dimethylaminobenzaldehyde reagent to make the required strips, but the above-mentioned concentrations of p-Dimethylaminobenzaldehyde reagent gave positive results in the formation of the desired testing strips.

3.4.2 Shelf life of strips:

Newly formed strips were stable for 5 months to give reasonable results if it store at ambient temperature.

3.4.3 Urea test:

Three beakers containing the standard solution of urea (20 ml) were labeled B1, B2, and B3. For 60 seconds, the first type of strip was dipped in B1, the second type in B2, and the third type in B3.

3.4.4 Non adulterant milk samples:

Various milk samples from different dairy farms surrounding the area of Lahore (Punjab, Pakistan) were collected. The samples contained the milk of cows and buffalos. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100 ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round of experimentation. Each beaker has 50 ml of natural milk, which acts as a control for comparison purposes. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for a moment to obtain the result. Three replicates of each kind of strip were used.

3.4.5 Adulterant milk samples:

In order to detect the different adulterants, raw milk samples were purchased from several Lahore sale outlets, including dairy shops, milk producers, collectors, and milk vendors. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100 ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round of experimentation. Each beaker has 50 ml of market milk. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for a moment to obtain the result. Three replicates of each kind of strip were used.

3.4.6 Tetra pack milk samples:

Tetra packs Milk’s samples from different companies were purchased from the local market in Lahore. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100-ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round

of experimentation. Each beaker has 50 ml of tetrapack milk. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for 60 seconds to obtain the result.

3.5 Detection of Formalin:

3.5.1 Strip formation:

Strip formation was done by taking 10 ml of Tollen's reagent in a 100 ml beaker and filter paper. Strip formation was done by the adsorption of Tollen's reagent on filter paper.

3.5.2 Formaline test:

Three beakers containing the standard solution of the formalin (20 ml) were labeled B1, B2, and B3. For 60 seconds, the first type of strip was dipped in B1, the second type in B2, and the third type in B3.

3.5.3 Non adulterant milk samples:

Various milk samples from different dairy farms surrounding the area of Lahore (Punjab, Pakistan) were collected. The samples contained the milk of cows and buffalos. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100-ml glass beakers. We put 50 ml of natural milk in the beaker, and the testing strip was dipped into the milk for a moment to obtain the result.

3.5.4 Adulterant milk samples:

In order to detect the different adulterants, raw milk samples were purchased from several Lahore sale outlets, including dairy shops, milk producers, collectors, and milk vendors. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100 ml glass beakers. We put 50 ml of market milk in the beaker, and the testing strip was dipped into the milk for a moment to obtain the result. Three replicates were used to gain reasonable results.

3.5.5 Tetra pack milk samples:

Tetra packs Milk's samples from different companies were purchased from the local market in Lahore. These samples were brought into the chemistry laboratory of Superior University Lahore by adopting preventive measures and care. The collected samples were placed into 100-ml glass beakers. Three beakers labeled B1, B2, and B3 were utilized for a single round of experimentation. Each beaker has 50 ml of tetrapack milk. The first kind of strip was dipped into B1, the second kind of strip was dipped into B2, and the third kind of strip was dipped in B3 for 60 seconds to obtain the result.

CHAPTER 4

RESULTS

4.1 Results of hydrogen peroxide

4.1.1 Chromaticity of strips:

All the strips (1st, 2nd & 3rd kinds) formed for the detection of hydrogen peroxide showed slightly pink color.

Fig4.1: Morphology of strips before testing H_2O_2

4.1.2 Hydrogen peroxide test:

All the strips (1st, 2nd & 3rd kinds) showed dark blue color when dipped in standard solution of hydrogen peroxide; it means that milk contaminated with hydrogen peroxide should have to give blue color on testing.

4.1.3 Non adulterant milk samples:

There is no change in color of any kind of strip which indicates that milk does not have hydrogen peroxide or it show negative test for hydrogen peroxide.

4.1.4 Adulterant milk samples:

All the strips (1st, 2nd & 3rd kinds) showed dark blue color when dipped in milk which indicates that milk was contaminated with hydrogen peroxide and give positive test.

Fig4.2: Morphology of strips after testing of H_2O_2

4.1.5 Tetra pack milk samples:

There is no change in color of any kind of strip which indicates that tetra pack milk does not have any contamination or it show negative test for hydrogen peroxide.

Table 4.1: Detection of Hydrogen peroxide:

Kind of Strips	Reagent with quantity	Shelf life of the strips	Color of the strip before testing	Samples	Color of the strip after testing	Result
1st	Water = 10ml Starch = 2 mmol Potassium iodide solution = 5 mmol	150 days	Slightly pink	Standard solution	Blue	Presences of Hydrogen peroxide
				Adulterant milk	Slightly blue	
				Non adulterant milk	No change	Absence of Hydrogen peroxide
				Tetra pack milk	No change	
2nd	Water = 10ml Starch = 2.5 mmol Potassium iodide = 5.5 mmol	150 days	Slightly pink	Standard solution	Blue	Presences of Hydrogen peroxide
				Adulterant milk	Slightly blue	
				Non adulterant milk	No change	Absence of Hydrogen peroxide
				Tetra pack milk	No change	
3rd	Water = 10ml Starch = 3 mmol Potassium iodide = 6 mmol	150 days	Pink	Standard solution	Blue	Presences of Hydrogen peroxide
				Adulterant milk	Blue	
				Non adulterant milk	No change	Absence of Hydrogen peroxide
				Tetra pack milk	No change	

4.1.6 Chemistry behind change of the color:

Hydrogen peroxide reacts with potassium iodide to produced elemental iodine**.** It reacts with starch to form deep blue color due to formation of starch iodine complex

$$2KI + H_2O_2 \longrightarrow I_2 + 2KOH$$

$$I_2 + \text{Starch} \longrightarrow \text{Starch iodine complex}$$

4.2 Results of detergents

4.2.1 Chromaticity of the strips:

All the strips (1st, 2nd & 3rd kinds) formed for the detection of detergents showed yellow color as shown in given below figures:

Fig4.3: Morphology of strips before testing of detergent

4.2.2 Detergent test:

The entire all the three types of the strips showed blue color when dipped in the standard solution of detergents.

4.2.3 Non adulterant milk samples:

The color of the strip changed from yellow to lemon green, it means that natural milk does not have detergents because the strip in the standard solution of detergents gave a blue color, but here there is no appearance of a blue color, which shows that milk does not have detergents.

4.2.4 Adulterant milk samples:

The entire all the three types of the strips showed violet color when dipped in the market milk samples which indicates that milk was contaminated with detergents and gave positive test.

Fig4.4: Morphology of strips after testing of detergent

4.2.5 Tetra pack milk samples:

The strip's color changed from yellow to lemon green, indicating that natural milk is detergent free. In the standard detergent solution, the strip produced a blue color; in this case, the blue color is absent, indicating that milk is detergent free.

Table 4.2: Detection of detergents:

Kinds of Strips	Reagent with quantity	Shelf life of the strip	Color of the strip before testing	Samples	Color of the strip after testing	Result
1st	Bromocresol purple solution= 0.2 mmol Water = 20 ml	150 days	Light yellow	Standard solution	Violet	Presence of detergents
				Adulterant milk	Violet	
				Non adulterant milk	Lemon green	Absence of detergents
				Tetra pack milk	Lemon green	
2nd	Bromocresol purple solution= 0.3 mmol Water = 20 ml	150 days	Yellow	Standard solution	Violet	Presence of detergents
				Adulterant milk	Violet	
				Non adulterant milk	Lemon green	Absence of detergents

				Tetra pack milk	Lemon green	
3rd	Bromocresol purple solution= 0.4 mmol Water = 20 ml	150 days	Dark Yellow	Standard solution	Violet	Presence of detergents
				Adulterant milk	Violet	
				Non adulterant milk	Lemon green	Absence of detergents
				Tetra pack milk	Lemon green	

4.2.6 Chemistry behind change of the color:

Bromocrisol purple is a pH indicator. It has yellow color when the pH is below than 5.2 and it show violet color if pH is greater than 6.8. Bromocrisol purple in contaminated milk with detergents give pH above than 6.8 therefore the color of the strip become violet.

4.3 Results of urea

4.3.1 Chromaticity of the strips:

All the three types of strips formed for the identification of urea does not show any color or colorless strips were formed.

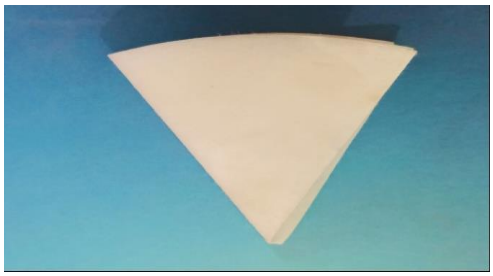

Fig4.5: Morphology of strips before testing of urea

4.3.2: Urea test:

The entire all the three types of the strips showed light yellowish color when dipped in the standard solution.

4.3.3 Non adulterant milk samples:

There is no change in color of any kind of strip which indicates that milk does not have any contamination or it show negative test.

4.3.4 Adulterant milk samples:

All the three strips showed light yellowish color when dipped in market milk samples which indicates that milk was contaminated with urea and gave positive test.

Fig 4.6: Morphology of strips after testing of urea

4.3.5 Tetra pack milk samples:

There is no change in color of any kind of strip which indicates that tetra pack milk does not have any contamination or it show negative test for urea.

Table 4.3: Detection of urea

Kinds of Strips	Reagent with quantity	Shelf life of the strips	Color of the strip before testing	Samples	Color of the strip after testing	Result
1st	Ethanol= 1 ml Water = 5 ml 30 % Hydrochloric acid = 1 ml p-Dimethylaminobenzeldehyde = 0.5 mmol	150 days	Colorless	Standard solution	Yellow	Presence of urea
				Adulterant milk	Light yellowish	
				Non adulterant milk	No change	Absence of urea
				Tetra pack milk	No change	
	Ethanol= 1 ml			Standard solution	Yellow	Presence of urea
				Adulterant milk	Light yellowish	

2nd	Water = 5 ml 30 % Hydrochloric acid = 1 ml p-Dimethylaminobenzeldehyde = 0.8 mmol	150 days	Colorless			
				Non adulterant milk	No change	Absence of urea
				Tetra pack milk	No change	
3rd	Ethanol= 1 ml Water = 5 ml 30 % Hydrochloric acid = 1 ml p-Dimethylaminobenzeldehyde = 1 mmol	150 days	Colorless	Standard solution	Yellow	Presence of urea
				Adulterant milk	Yellow	
				Non adulterant milk	No change	Absence of urea
				Tetra pack milk	No change	

4.3.6 Chemistry behind change of the color:

p-Dimethylaminobenzaldehyde react with urea at ambient temperature producing a chromogen that emit a light yellowish

4.4 Results of Formalin

4.4.1 Chromaticity of strips:

Colorless strips formed for the detection of Formalin.

4.4.2 Formalin test:

The color of the strip changed into cloudy black when it was dipped in the standard solution of formalin.

4.4.3 Non adulterant milk samples:

There is no change in color of the strip which indicates that milk does not have any contamination or it show negative test.

4.4.4 Adulterant milk samples:

There is no change in color of the strip it does not means that milk do not have formalin. Usually formalin as adulterant present in limited concentration which does not able to give color with strip therefore the testing strip does not show any color. Strip can show color if formalin present at higher concentration which normally not present in milk.

4.4.5 Tetra pack milk samples:

There is no change in color of any strip or it gave negative test for tetra pack milk.

Table 4.4: Overall results of various adulterants in milk samples:

Sr.No	Name of adulterants	Color of the strip before testing	Color of the strip after testing	Result
1	Hydrogen peroxide	Slightly pink	Blue	Presence of Hydrogen peroxide
2	Detergents	Yellow	Violet	Presence of detergents
3	Urea	Colorless	Light yellowish	Presence of urea
4	Formalin	Colorless	Colorless	Not confirmed

CHAPTER 5

DISCUSSION

5.0 Discussion:

In this work, paper-based testing strips were developed utilizing an inexpensive technology. These strips are affordable, suitable for the detection of various milk adulterants, and have stability for more than five months and this investigation is according to Savaliya et al., they created various strips for the detection of adulterants in milk that have validity six months to give reasonable results; they stored these strips at ambient temperature [46].

In our investigation, the testing strip's color was light yellowish prior to the test and turned dark blue when it was dipped into milk tainted with hydrogen peroxide. This result is based on research by Mohanty et al., who demonstrated that when milk contaminated with hydrogen peroxide was tested, a blue tint appeared [47] this result also relates with Sharma et al., they proved that if a milk have hydrogen peroxide as a adulterant, it gave blue color when experiment was done for the detection of hydrogen peroxide they utilized 24 hours to detect the adulterant while in our work the detection of hydrogen per oxide was done within minutes. Our approach to detecting H_2O_2 in milk was significantly faster than other approaches employed by earlier studies [48]. In our research there was appearance of dark blue color of the testing strip when it was used to detect hydrogen peroxide in the milk this investigation also relates with Debnath et al., who experimented that milk contaminated with hydrogen peroxide gave blue color when experiment was carried to detect hydrogen peroxide in milk[49].

Several approaches or methods that have been previously employed by scholars can be employed to detect hydrogen peroxide in milk. A few of these approaches are included below:

Costa et al., proposed that hydrogen peroxide in milk can be detected by using calorimetric method coupled with smart phone images this method is not suitable for poor people’s which don’t have a smart phone, our work represents a low cost method someone can detect hydrogen peroxide easily, here we used paper based strips which are very cheap any one can performed this action due to its simplicity and low cost properties[50]. Another technique by Ivanova et al., is listed they used high performance liquid chromatography (HPLC) with a diode

array detection as an indirect method for the detection of hydrogen peroxide in milk. This technique required a proper setup and certain chemicals with suitable conditions, which are normally not available. Our new approach, on the other hand, has advantages over previously mentioned methods since it can be readily adopted and all requirements can be met [51]. Hurely et al., suggested that the adulterants present in milk can be detected by using enzyme-linked immunosorbent assays (ELISAs) they revaled that ELISA should be used in combination with polymerase chain reaction (PCR) if we look at our current method for the detection of various adulterants in milk is a very simple and cost effective method as compared to enzyme-linked immunosorbent assay (ELISA) and PCR which are more complicated and expensive methods than that of paper based testing strips method [52].

During our investigations, we found that when tests were carried out to find the detergents in the milk, the contaminated milk turned violet in color and seemed clear, white, and fresh. Prior to testing, the color of the strip was yellow; however, it turned violet after it came into contact with detergent-contaminated milk this is according to Kamthania et al., they showed that violet color appeared when milk was exposed to detergents [26]. According to kumar and his colleagues detergents can be detected by unmodified gold nanoparticles by using different chemicals, this method was costly, required a proper laboratory and its equipments it also required great time while our current method is cost effective or low cost, don't required a proper laboratory and instant results can be gained or it requires very less time as compared to other methods adopted in previous studies by different researchers[53]. Kimbahune et al., reported that detergents in milk can be detected by hyperspectral radiometry technology, this method is difficult to use and involves several critical stages to carry out the experiment for the detection of hydrogen peroxide in milk for example, here data is collected using a portable spectroradiometer and machine learning techniques are used to create a model to capture the different adulterants in the milk this method also required a professional and trained person to perform the testing while in our work we can detect detergents in milk in simple and easy way as it does not required the above mentioned conditions in Kimbahune and his colleagues work[54].

Our research revealed that, when urea-contaminated milk was tested using paper-based testing strips, the results showed a light yellowish color. This finding is consistent with the

findings of Aslam et al., who proved that, under the right circumstances, urea-contaminated milk can produce a yellow colorss [55] this result is also consistent with the findings of Patari et al., who found that urea-contaminated milk turned yellow when tested using a 3D paper-based device. However, in their work, they used a paper-based device that was more complex than the simple paper-based testing strips that we used in our research. In the current work, however, we designed testing strips using Whatman filter paper, which was less expensive than the paper-based device which they used [22]. Our findings about the urea detection are consistent with those of Brindha et al., who demonstrated that urea contaminated milk, gave yellowish color when an experiment was conducted to determine whether urea was present in the milk as an adulterant [56]. Renny et al., reported that urea in milk can be detected by building a manometric biosensor this method require a proper setup and trained personnel to carry out the experiment to obtain perfect results. Generally, these conditions are not met and should face numerous challenges in order to detect adulterants in milk. Therefore, an easy and approachable method for the detection of urea in milk is required. Our current method is simple and can be performed by a common individual[57]. Another approach the Headspace-Gas Chromatography (HS-GC) method was developed by Xie et al., to detect urea in milk according to their protocol, this method takes 40 minutes to produce satisfactory results and requires a fixed temperature of 35 degrees Celsius for the experiment in contrast, our method yields good results whithin 5 minutes and does not require a fixed temperature to be carried out therefore our method can be used to detect urea instantly in the milk which act as a adulterants [58].

In summary, we can state that, in comparison to other earlier methods used by the researchers, our current approach for the detection of various adulterants in milk is very simple, quick to use, economical, and time-saving therefore our method can be used for the detection of various adulterants in milk.

CHAPTER 6

CONCLUSION

6.0 Conclusion:

The present study significantly concludes that paper based strips can be used to detect adulterants in milk and these strips have low cost, it also have time saving or instant method to identify adulterants in milk. The study concluded that paper strips formed for the detection of hydrogen peroxide showed slightly pink color, have validity more than 90 days and the color become changed into dark blue in the presence of hydrogen peroxide in the contaminated milk while there was no change in the color of the strip in the natural or pure milk which also acts as a control group. This study also concluded that strips formed for the detection of detergents gave yellow color, have validity more than 150 days and the color become changed into violet in the presence of detergents in the contaminated milk while there was no change in the color of the strip in the natural or pure milk. This investigation also revealed that paper strips formed for the detection of urea does not show any color or a colorless strip was formed, have validity more than 150 days and the color become changed into light yellowish in the presence of urea in the contaminated milk while there was no change in the color of the strip in the natural or pure milk. It is also concluded that we can detect different kind of adulterants by adopting this technique at domestic level.

REFERENCES:

1. Nirwal S, Pant R, Rai N. Analysis of milk quality, adulteration and mastitis in milk samples collected from different regions of Dehradun. International Journal of PharmTech Research. 2013;5(2):359-64.

2. POPESCU A, ANGEL E. Analysis of milk quality and its importance for milk processors. Scientific Papers Animal Science and Biotechnologies. 2009;42(1):501-.

3. Dror DK, Allen LH. The importance of milk and other animal-source foods for children in low-income countries. Food and nutrition bulletin. 2011;32(3):227-43.

4. Givens DI, Shingfield K. Optimising dairy milk fatty acid composition. Improving the fat content of foods: Elsevier; 2006. p. 252-80.

5. Kubicová Ľ, Predanocyová K, Kádeková Z. The importance of milk and dairy products consumption as a part of rational nutrition. Potravinarstvo. 2019;13(1).

6. Borková M, Snášelová J. Possibilities of different animal milk detection in milk and dairy products–a review. Czech J Food Sci. 2005;23(2):41-50.

7. Chauhan SL, Priyanka KDM, Paul BR, Maji C. Adulteration of milk: A Review. International Journal of Chemical Studies. 2019;7(1):2055-7.

8. Ritota M, Manzi P. Melamine detection in milk and dairy products: traditional analytical methods and recent developments. Food analytical methods. 2018;11:128-47.

9. Tyan Y-C, Yang M-H, Jong S-B, Wang C-K, Shiea J. Melamine contamination. Analytical and bioanalytical chemistry. 2009;395:729-35.

10. Bann B, Miller SA. Melamine and derivatives of melamine. Chemical reviews. 1958;58(1):131-72.

11. Hau AK-c, Kwan TH, Li PK-t. Melamine toxicity and the kidney. Journal of the American Society of Nephrology. 2009;20(2):245-50.

12. Wicaksono WP, Putri AP, editors. Electrochemical immunochromatographic strip test for melamine biosensor in milk products using silver nanoparticles as probe. Applied Mechanics and Materials; 2014: Trans Tech Publ.

13. Pei X, Tandon A, Alldrick A, Giorgi L, Huang W, Yang R. The China melamine milk scandal and its implications for food safety regulation. Food policy. 2011;36(3):412-20.

14. Lu C, Xiang B, Hao G, Xu J, Wang Z, Chen C. Rapid detection of melamine in milk powder by near infrared spectroscopy. Journal of Near Infrared Spectroscopy. 2009;17(2):59-67.

15. Liu J, Siavash Moakhar R, Sudalaiyadum Perumal A, Roman HN, Mahshid S, Wachsmann-Hogiu S. An AgNP-deposited commercial electrochemistry test strip as a platform for urea detection. Scientific reports. 2020;10(1):1-11.

16. Tripathy S, Ghole AR, Deep K, Vanjari SRK, Singh SG. A comprehensive approach for milk adulteration detection using inherent bio-physical properties as 'Universal Markers': Towards a miniaturized adulteration detection platform. Food chemistry. 2017;217:756-65.

17. EMAN M, A ABD-ALLA A-E, Y ELAREF M. Detection of raw buffalo's milk adulteration in Sohag Governorate. Assiut Veterinary Medical Journal. 2015;61(144):38-45.

18. Stănciuc N, Râpeanu G. Identification of adulterated sheep and goat cheeses marketed in Romania by immunocromatographic assay. Food and agricultural immunology. 2010;21(2):157-64.

19. Nagraik R, Sharma A, Kumar D, Chawla P, Kumar AP. Milk adulterant detection: Conventional and biosensor based approaches: A review. Sensing and Bio-Sensing Research. 2021;33:100433.

20. Verma M, Naqvi TK, Tripathi SK, Kulkarni MM, Dwivedi PK. Paper based low-cost flexible SERS sensor for food adulterant detection. Environmental Technology & Innovation. 2021;24:102033.

21. Yadav AK, Gattupalli M, Dashora K, Kumar V. Key Milk Adulterants in India and their Detection Techniques: a Review. Food Analytical Methods. 2023;16(3):499-514.

22. Patari S, Datta P, Mahapatra PS. 3d paper-based milk adulteration detection device. Scientific Reports. 2022;12(1):13657.

23. Cioffi A, Mancini M, Gioia V, Cinti S. Office paper-based electrochemical strips for organophosphorus pesticide monitoring in agricultural soil. Environmental Science & Technology. 2021;55(13):8859-65.

24. O'Mahony J, Fox P. Milk: an overview. Milk proteins. 2014:19-73.

25. Maijala K. Cow milk and human development and well-being. Livestock Production Science. 2000;65(1-2):1-18.

26. Kamthania M, Saxena J, Saxena K, Sharma D. Milk Adultration: Methods of Detection &Remedial Measures. International Journal of Engineering and Technical Research. 2014;1:15-20.

27. Kundu P, DhANKhAr J, ShArMA A. Development of non dairy milk alternative using soymilk and almond milk. Current Research in Nutrition and Food Science Journal. 2018;6(1):203-10.

28. Hansen PW, Holroyd SE. Development and application of Fourier transform infrared spectroscopy for detection of milk adulteration in practice. International Journal of Dairy Technology. 2019;72(3):321-31.

29. Hussain M, Ghafoor A, Saboor A. Factors affecting milk production in buffaloes: a case study. Pakistan Vet J. 2010;30(2):115-7.

30. Banti M. Food adulteration and some methods of detection, review. International Journal of Nutrition and Food Sciences. 2020;9(3):86-94.

31. Soomro AA, Khaskheli M, Memon MA, Barham GS, Haq IU, Fazlani SN, et al. Study on adulteration and composition of milk sold at Badin. Intl J Res Appl Nat Social Sci. 2014;2(9):57-70.

32. Minetto TA, França BD, da Silva Dariz G, Veiga EA, Galvao AC, da Silva Robazza W. Identifying adulteration of raw bovine milk with urea through electrochemical impedance spectroscopy coupled with chemometric techniques. Food Chemistry. 2022;385:132678.

33. Devrani M, Pal M. How to detect adulteration of maltodextrin in milk. Food Beverage Process. 2018;5:22-3.

34. Pradeep S, Lakshminarayana P, Varsha R, Kota SK. Screening of adulterants in milk. International Journal of Current Research and Review. 2016;8(12):25.

35. Shalileh F, Sabahi H, Dadmehr M, Hosseini M. Sensing approaches toward detection of urea adulteration in milk. Microchemical Journal. 2023:108990.

36. Jaiswal P, Jha SN, Kaur J, Borah A. Detection and quantification of anionic detergent (lissapol) in milk using attenuated total reflectance-Fourier Transform Infrared spectroscopy. Food chemistry. 2017;221:815-21.

37. Mabood F, Ali L, Boque R, Abbas G, Jabeen F, Haq QMI, et al. Robust Fourier transformed infrared spectroscopy coupled with multivariate methods for detection and quantification of urea adulteration in fresh milk samples. Food Science & Nutrition. 2020;8(10):5249-58.

38. Chitra P. Milk adulteration: An overview on adulterants, detection tests and effects on human health. Advances in Applied Research. 2021;13(2):63-8.

39. Lahankar S, Chavan U, Gore N. A thorugh review on milk adulteration, its effect on health and detection tests. World Journal of Pharmaceutical Research. 2019;8(2):636-46.

40. Raturi N, Aman J, Sharma C. Study Of Adulteration in Milk and Milk Products And Their Adverse Health Effects. Octa Journal of Biosciences. 2022;10(1).

41. Navale D, Gupta S. To test an adulteration present in milk Sample. Int J Latest Technol Eng Manag Appl Sci. 2016;5:86-9.

42. Dangi S. Common milk adulterants in developing country Nepal: A mini-review. Aceh Journal of Animal Science. 2021;6(3):91-6.

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

44. Ullah R, Khan S, Ali H, Bilal M. Potentiality of using front face fluorescence spectroscopy for quantitative analysis of cow milk adulteration in buffalo milk. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2020;225:117518.

45. Dou M, Sanjay ST, Benhabib M, Xu F, Li X. Low-cost bioanalysis on paper-based and its hybrid microfluidic platforms. Talanta. 2015;145:43-54.

46. Savaliya RR, Shaikh AI, Chauhan MP, Parmar SC, Modi RB. Development of a strip-based test for detection of urea adulteration in milk. Journal of Food Processing and Preservation. 2022;46(7):e16722.

47. Mohanty TJ, Sahoo JP, Samal KC. Common Milk Adulteration in India and Rapid Detection Techniques. Food Sci Reports.1:59-62.

48. Sharma R, Seth R, Bauri AK. Rapid methods for detection of adulterants in milk. Winter School Training Programme Manual, National Dairy Research Institute …; 2011. p. 184-5.

49. Debnath A, Banerjee S, Rai C, Roy A. Qualitative detection of adulterants in milk samples from Kolkata and its suburban areas. International Journal of Research in Applied, Natural and Social Science. 2015;3:81-8.

50. Costa RA, Morais CL, Rosa TR, Filgueiras PR, Mendonça MS, Pereira IE, et al. Quantification of milk adulterants (starch, H2O2, and NaClO) using colorimetric assays coupled to smartphone image analysis. Microchemical Journal. 2020;156:104968.

51. Ivanova AS, Merkuleva AD, Andreev SV, Sakharov KA. Method for determination of hydrogen peroxide in adulterated milk using high performance liquid chromatography. Food chemistry. 2019;283:431-6.

52. Hurley IP, Elyse Ireland H, Coleman RC, Williams JH. Application of immunological methods for the detection of species adulteration in dairy products. International journal of food science & technology. 2004;39(8):873-8.

53. Kumar P, Kumar P, Manhas S, Navani NK. A simple method for detection of anionic detergents in milk using unmodified gold nanoparticles. Sensors and Actuators B: Chemical. 2016;233:157-61.

54. Kimbahune S, Ghouse SM, Mithun B, Shinde S, Jha AK, editors. Hyperspectral sensing based analysis for determining milk adulteration. Hyperspectral Imaging Sensors: Innovative Applications and Sensor Standards 2016; 2016: SPIE.

55. Aslam SBSKM, Anmol S, Hamza FSARA, Khan FA, Akhter R. Qualitative Analysis of Milk Adulterants in Various Samples of Milk, Available In Local Market of Lahore, Pakistan. 2019.

56.Brindha N, Chitra P, Janarthanan R, Murali A. A Study on Detection of Adulteration in Milk Samples from Different Regions of Thuraiyur District in Tamil Nadu, India. Int J Curr Microbiol App Sci. 2017;6(12):3303-10.

57.Renny E, Daniel D, Krastanov A, Zachariah C, Elizabeth R. Enzyme based sensor for detection of urea in milk. Biotechnology & Biotechnological Equipment. 2005;19(2):198-201.

58. Xie W-Q, Yu K-X, Gong Y-X. Rapid and quantitative determination of urea in milk by reaction headspace gas chromatography. Microchemical Journal. 2019;147:838-41.

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