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A Review on Various Sensors Employed for Detecting Adulterants (Urea and Melamine) in Milk and Milk Products

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Abstract— Food safety is a critical concern globally, with increasing incidents of adulteration posing a significant threat to public health. Adulteration involves the addition of unauthorized substances to food products, compromising their quality and safety. The use of advanced sensor technologies for the detection of adulterants has gained prominence in recent years. There are various methods for detecting the urea and melamine used as an adulterant in milk but the use of sensor based technology has made it easy, fast, and accurate detection of food adulterants. A wide variety of biosensing approaches for the detection of urea adulteration in milk have been developed in recent years. This review article presents a comprehensive case study on various sensors used for spotting adulterants - Urea and Melamine, in milk and milk products, emphasizing their principles, applications, and effectiveness

Keywords- Food safety, Adulteration, Sensor technologies, Spectroscopy, Electrochemical sensors, Biosensors, Case study, urea, melamine.

I. INTRODUCTION

The prevalence of food adulteration has become a crucial matter affecting consumers, regulatory bodies, and the food industry. Adulterants such as chemicals, contaminants, and inappropriate additives can pose serious health risks. The use of sensors for detecting these adulterants offers a rapid and accurate solution, enhancing food safety and its quality control.

The frequency of adulteration in food products is rising quickly, which has an adverse impact on the durability and safety of consumer goods. For Food regulatory bodies, major food-producing countries across the world, securing the reliability and security of foodstuffs deemed suitable for human consumption is of utmost importance. Strict monitoring during the manufacturing process and sporadic inspections of food products at the point of delivery are used to enforce compliance with regulations. Food adulteration has become prevalent in all packaged food and drinking items. The key focus of any detection strategy hinges on identifying the type of adulterant, determining the extent of contamination, and validating these measurements.

The industry standard for testing food goods is laboratory-based analytical techniques like NMR, GC-MS, FT-IR and HPLC etc. In order to detect adulteration at the point of sale and avoid laboratory-based analysis, which would increase costs and lengthen the time needed for product withdrawal, advanced, robust sensors that can be combined with portable devices are currently necessary [1].

Analytical sensing methods often interpret changes in chemical or biological events as changes in electrical response. Biosensors, also known as biological sensors, are platforms that use immobilized receptors, like aptamers and antibodies, to transduce binding events. Analytes then interact with these receptors to alter the output of the signal. Biosensors are useful for monitoring packaged food products and determining the freshness of goods including fruits, vegetables, fish, and meat products since they have good sensitivity and specificity [2].

It is possible to produce printed electrodes that have been electrochemically modified for food analysis on a large-scale using 3D printing technology. Using the fused deposition modelling (FDM) approach, Nasir et al. created graphene-based electrodes with high catalytic activity and absorption capacity for the detection of certain analytes in food products [3].

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In human culture, food adulteration has a long history and persists to this day. The guarantee of food safety is the assurance that, when prepared and consumed as intended, food will not injure the customer. It comprises handling, preparing, and storing food in a way that keeps people from getting food-borne infections. Food production still faces significant challenges regarding quality and safety [4, 5].

2. METHODOLOGY OF THE REVIEWS

An exhaustive investigation of the body of literature was judged essential during the review process. As a result, to obtain relevant scientific evidence, a thorough search was conducted using a variety of sources, including National Library of Central (PubMed), Research gate, Google Scholar, Science Direct, Scopus, and Web of Science. To find relevant articles, specific keywords like adulteration, food adulteration, analytical approach, detection method, and health effects were used. The review was limited to words related to the given subject.

3. RESULTS AND DISCUSSION

3.1. Concept of food adulteration: The term "food adulteration" describes the intentional modification of food quality. It involves adding additives to change a food product's composition to benefit monetarily.

3.2. Types of Adulterants in Food:

To understand the significance of sensor technology in identifying adulterants, it is crucial to categorize the various types of adulterants commonly found in food products. This section provides an idea of chemical contaminants, microbial agents, and physical adulterants, highlighting their potential risks and health implications.

3.2.1 Intentional adulteration: The deliberate use of poor ingredients that have comparable characteristics to the foods they are combined with is referred to as "intentional adulteration".

As such, it is challenging to differentiate between these hazardous ingredients. Adulterants may originate from biological or physical sources. In an attempt to maximize profits, dishonest producers and sellers purposefully contaminate various meals to improve the concentration of vital nutrients after decreasing a specific level. [6, 7, 9].

Since nutrients are lost and foreign chemicals are added to food by business-oriented people who have simply forgotten the humanity behind the money-making attitude, this is the most dangerous kind of contamination [10].

3.2.2 Unintentional adulteration: Throughout the food processing journey, ranging from harvest to consumption, unsanitary conditions and inadequate facilities stand out as primary contributors to inadvertent food adulteration.

This can manifest in acquired forms, such as bacterial or fungal contamination of foods, spoilage due to rodent activity, infiltration of dust and foreign objects, or the presence of harmful residues from packaging materials. Additionally, inherent adulteration may occur, involving the presence of certain chemicals, organic compounds, or naturally occurring radicals in foods, such as toxic varieties of pulses, mushrooms, green vegetables, and seafood. Food adulteration can occur unintentionally when substances are introduced without the knowledge or intent of producers, traders, or retailers.[8]

However, various stages of the production, handling, processing, storage, transportation, and marketing processes may serve as sites for adulteration, as any substance not integral to the product is considered extraneous.[9]

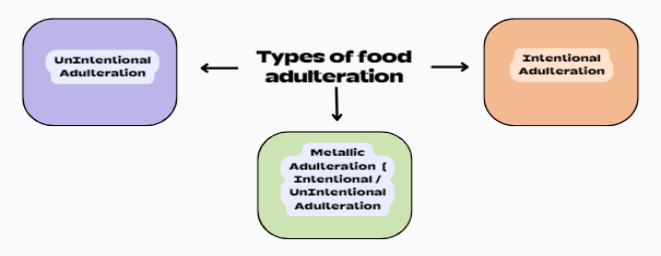

Fig. 1: Types of Food Adulterants [7,8]

4. Adulteration of Milk and milk products:

The significance of the dairy items can never be compromised but due to the need of the administrative bodies, corruption of dairy items comes about in a troublesome impact on the wellbeing of consumers. Contamination in these items is gambling human body to immunodeficiency and uncovering cancer, keeping in view the nature of the adulterants utilized. Other signs such as kidney disease, unusual development and illnesses of joints and heart related diseases might happen [9].

In a study conducted in Boditi town and its environs in Southern Ethiopia, researchers led by Ayza et al.,[9] investigated milk and milk product adulteration. They surveyed residents from both the town and nearby areas, discovering that a significant portion of respondents acknowledged the regular occurrence of intentional milk adulteration.

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The primary motivation behind this adulteration, as identified by Ayeza et al.[9], was to augment revenue or increase profit margins. They attributed the prevalence of adulteration to dishonesty and inadequate quality control measures. Additionally, Mohit et al.,[18] in their own research, identified several other factors contributing to milk adulteration. These include discrepancies between supply and demand, the physical properties of milk which allow for the incorporation of various adulterants, unethical business practices driven by profit motives, socioeconomic pressures leading impoverished individuals to adulterate milk, the perishable nature of milk prompting the use of preservatives to extend its shelf life, economic constraints of consumers, the disorganized state of the dairy industry, lax regulatory standards, and insufficient testing methods [14].

Table 1: Objective and permissible limit of mostly used adulterants in milk , impacts of food adulteration on public health [11,12,13]

Adulterant	**Objective**	**Limit**	**Reference**	**Health Issue**
Urea	It increases the non protein content of the milk	<70mg/100ml	(Khan et al. 2015; Sharma et al. 2017) [11]	degenerative and necrotic effects on liver and kidney even in short-term exposure, gastrointestinal disorders such as indigestion and ulcers [12]
Melamine	To increase protein content of the milk	1mg for infants <2.5mg/kg for adults	(Lawley 2013) [11]	Renal stone, s renal and urinary problems [13]

5. Principles of Sensor Technologies:

This section explores the fundamental principles behind sensor technologies used for detecting adulterants in food. It discusses the working mechanisms of different sensor types, including electrochemical sensors, biosensors, and molecular sensors. Understanding these principles is essential for evaluating the appropriateness of sensors for specific applications.

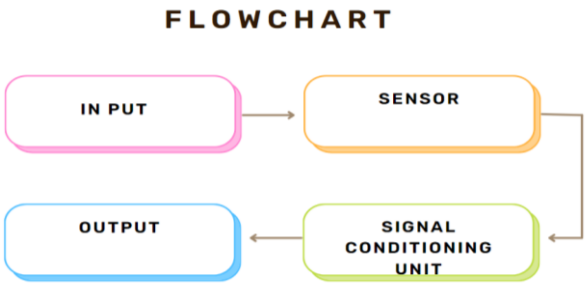

Fig. 2: Working of Sensors

6. Applications of Sensors in Food Safety for the detection of milk adulterants:

The case study aims to into specific examples of sensors utilized for the detection of adulterants - Urea and melamine in Milk products . Examples include the use of electrochemical sensors for the detection .[15]

6.1.1. Detection of urea adulteration in milk using Gas sensor: Valarmathy R. S. et al. [15] projected and tested the application of gas sensors for milk urea detection. The sensor's output is routed to a controller, where a concentration calibration (ppm) is performed. The urea concentration is shown on an LCD. Researchers found that the proposed technique, when applied to milk at 70°C, can identify urea contamination of at least 2 mg/lt.

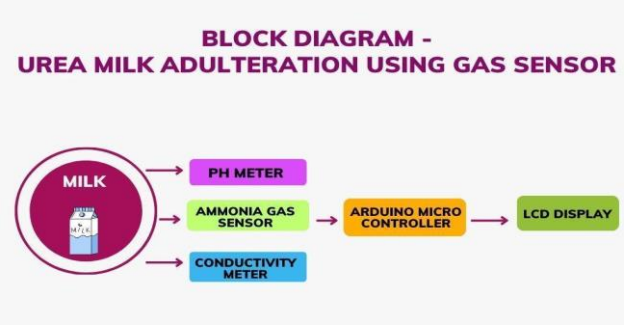

Fig. 3: Block diagram detection of Urea adulterated milk using gas sensor [15]

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Working of gas sensor: Valarmathy R. S. et al. [15] set up this detection technique, where at the top of the milk-filled beaker, an ammonia gas sensor was mounted as one of the components in the circuit. The Arduino and sensor were interfaced. The digital pin was linked to the LCD. The Arduino receives the data from the sensor, which it uses to show the values on the LCD.

The beaker was placed onto the magnetic stirrer for heating and agitation of the milk. To initiate the program, the Arduino and PC were connected. The primary sensing was conducted using the ammonia gas sensor (MQ135). As the temperature reaches approximately 70°C, the escaping ammonia gas is detected by the sensor [15]. The sensor records the value and transmits it to the Arduino. The analog signal from the sensor is then digitized by the Arduino board. Through programming, the voltage output of the Arduino can be converted into any desired unit. In this case, the values are converted into parts per million (ppm). The converted signal is then displayed as the required value on the LCD screen.

This proposed method is capable of detecting a minimum of 2 milligrams per liter of urea adulteration in milk at 70°C. Furthermore, this method can potentially be further developed into a handheld device, making it accessible for domestic use in identifying urea adulteration in milk [15].

6.1.2. Constant phase element (CPE) sensor for Urea adulteration in milk: Siuli Das et al., [16], have used a sensor utilizing a constant phase element (CPE) which comprises a stick-type two-terminal device. When this sensor is immersed in a substance, the phase angle between its terminals remains consistent, thus earning it the designation "constant phase element" (CPE). But it was observed that alterations in the measuring medium's properties can cause changes in the phase angle. Consequently, disparities in phase angles between unadulterated and adulterated milk samples are observed. This phase angle shift is identified through a phase detector circuit, with indicator LEDs employed to signify the type of adulteration. The benefits of employing such sensors include their poly-methyl methacrylate (PMMA) coating, rendering them biocompatible and ensuring that the milk's properties remain unaltered upon immersion. Furthermore, their stick-type rigid design facilitates easy insertion into the measuring medium, a critical requirement for automated detection [16].

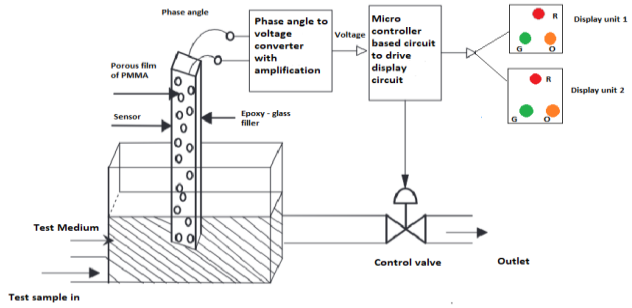

Fig. 4: Block diagram of the CPE instrumentation automatic sensing system [16]

This study presents a very simple, inexpensive instrument to detect milk adulteration, discriminating between pure milk and water-urea mixtures. Using this sensor for water adulteration in milk, noticeable differences are observed between pure milk and samples adulterated with 10% water, but the distinction diminishes at higher concentrations of adulteration. Further investigation is entitled to enhance sensor sensitivity through the application of various polymer coatings. Researchers observed similar findings for urea adulteration, where a maximum slope is observed at 0.6 mg/ml but diminishes with higher levels of adulteration. Notably, the phase detector circuit accurately registers changes in phase angle and produces corresponding output voltages [16].

6.2. Detection of adulteration in milk by Melamine using sensor: Melamine ($C_3H_6N_6$) is a nitrogen-rich compound known for its intentional adulteration in food and milk. Its purpose is to boost the protein level in milk by artificial means. It's detection and measurement is usually done from the total nitrogen concentration using the Kjeldahl method [17].

Huanan Wu and colleagues [18] have introduced a portable miniaturized surface plasmon resonance (mini-SPR) biosensor for the rapid detection and quantification of melamine. This biosensor operates through an immunoassay (a procedure for detecting or measuring specific proteins or other substances through their properties as antigens or antibodies) based on the binding interaction between melamine and anti-melamine antibody (anti-MEL), exhibiting high selectivity to melamine. Three immunoassay types, including direct, displacement, and competitive assays*, were employed. The displacement and competitive assays, utilizing bovine serum albumin conjugated melamine (BSA-MEL), demonstrated sensitivity enhancements of approximately 14 times and 60 times, respectively, compared to the direct assay. The competitive assay achieved a detection limit of 0.02 g/ml and could be completed within 15 minutes. The effectiveness of testing real

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samples, particularly infant formula following simple pretreatment, was validated. This SPR biosensor, in conjunction with the proposed analysis assays, offers rapid, convenient, and cost-effective detection of melamine [18].

Surface plasmon resonance (SPR) biosensors, a widely utilized type of biosensor, operate on a non-invasive, label-free principle, utilizing polarized electromagnetic waves to explore interactions between an analyte in solution and immobilized biomolecular recognition elements in real-time. SPR occurs under conditions of total internal reflection at a sensor surface coated with semi-transparent noble metal [19].

In this investigation, the researchers explore the efficacy of a portable mini-SPR biosensor for the rapid detection and quantification of melamine via immuno reactions. Alongside the direct assay, which relies on antibody-antigen binding, they employ a BSA-MEL conjugate to elicit more pronounced changes in the displacement and competitive assays. In the displacement assay, an excess of the BSA-MEL conjugate is introduced over the sensor surface to saturate binding sites. Upon the subsequent addition of molecular melamine, displacement of the BSA-MEL conjugate occurs. Conversely, the competitive assay entails the introduction of a mixture of molecular melamine and BSA-MEL over the sensor surface, allowing them to compete for binding sites. The sensitivity of the three assays is assessed and compared based on the obtained results [18].

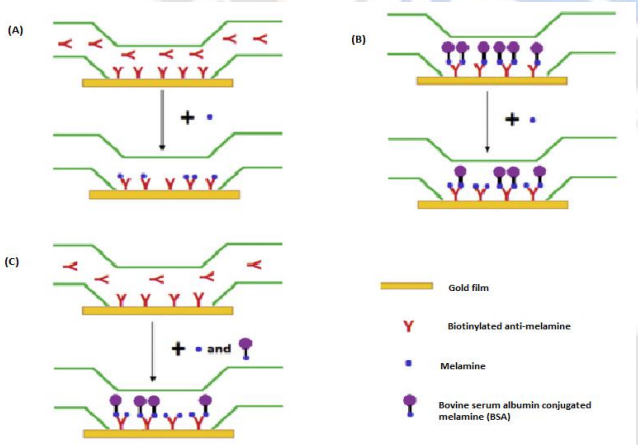

Fig. 5: Schematic illustration of the three formats of immunoassay. (A) Direct immunoassay, (B) Displacement immunoassay and (C) Competitive immunoassay [18].

Melamine solutions with concentrations ranging from 0 to 60.0 g/mL were prepared in PBS. The experimental setups for the three immunoassay methods are depicted in Fig. 1. Antibodies are immobilized on the sensor surface for all methods. In the direct assay, 95 L of melamine in PBS is injected into the flow cell at a flow rate of 10 L/min. This procedure is repeated for cyanuric acid to assess sensor selectivity. For the displacement assay, 95 L of 5 mg/mL BSA-MEL in PBS is injected into the flow cell at a flow rate of 5 L/min, followed by the introduction of melamine solutions of varying concentrations at a flow rate of 10 L/min to displace the immobilized BSA-MEL. In the competitive assay, 50 L of melamine solutions is individually mixed with 50 L of 1 mg/mL BSA-MEL in PBS to obtain a 1:1 mixture. 95 L of each mixture is injected into the flow cell at a flow rate of 10 L/min using a freshly modified SPR sensor. Real-time measurements of the interactions between the antibodies and melamine/BSA-MEL are recorded. For all assays, PBS buffer serves as a running buffer to remove loosely bound analytes after the introduction of each sample. Sensor responses are recorded after the PBS buffer yields equalized baselines.

The mini-SPR-based portable biosensor developed is a good system for conducting various immunoassays for melamine detection, a contaminant with low molecular weight. In this sensor the sensing surface has two layers: avidin monolayer and biotinylated anti-melamine antibody, three types of immunoassays are successfully performed for the detection and quantification of melamine. The direct assay achieves a limit of detection of 1.13 g/ml and a quantifiable range from 3.76 to 30.00 g/ml [18].

Table 3: Analysis of melamine concentrations in the spiked milk samples by SPR biosensor (n = 3). [18]

Sample	Amount of ine used for g (µg/ml)	Amount ed in g/ml)	Standard on	Rela rd ion
1	4.0	4.41	0.43	9.7
2	10.0	10.61	0.75	7.1
3	20.0	21.96	1.52	6.9

The sensor developed by researchers exhibits high selectivity towards melamine and demonstrates excellent applicability for testing milk samples. Huanan Wu et al., [18] has increased the detection sensitivity through displacement and competitive assays and got good results by lowering the limit of detection (LOD) to 0.08 g/mL and 0.02 g/mL, respectively. The cost of the mini-SPR system is 30% of the cost of the rest of the techniques as well as its size is very small as compared to other instruments. significantly lower without using any organic solvent.. This proposed method offers much shorter processing times which is nearly 30 minutes. The proposed method, employing the mini-SPR biosensor, holds significant

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promise for widespread applications in onsite and rapid detection of various low molecular weight contaminants in milk safety, industrial and environmental monitoring, and clinical diagnostics [18].

8. Comparative Analysis of Sensor Technologies:

A comparative analysis is conducted to assess the strengths and limitations of different sensor technologies. Factors such as sensitivity, selectivity, cost-effectiveness, and ease of use are considered to provide insights into the suitability of sensors for specific applications in the food industry.

Table 6: A comparative analysis of different sensor technologies

Sr. No.	Milk Adulterant	Name of Biosensor	sensitivity, selectivity, cost-effectiveness	Special feature	Scope of research	Reference
1	Urea	Ammonia Gas sensor	milk at 70°C, can identify urea contamination of at least 2 mg/lt.	The technique has the potential to be transformed into a handheld device, making it accessible for use by consumers at home to detect urea adulteration in milk.	Artificial intelligence can be combined to get more accurate results	Valarmathy R. S. et al. [15]
2	Water and Urea	Constant phase element (CPE) CPE sensor for detecting urea adulteration in milk.	Regarding water adulteration, it has been observed that there is a notable difference between pure milk and milk adulterated with 10% water.	A low-cost automatic sensing system	1.Further investigation is entitled to enhance sensor sensitivity through the application of various polymer coatings 2. The change in water adulteration is less significant for higher concentrations. 3. Additional research is needed to improve the sensor's sensitivity for detecting urea adulteration. At 0.6 mg/ml, the slope is maximal but diminishes for higher concentrations.	Siuli Das et al., [16]

3	Melamine	surface plasmon resonance biosensor	The displacement assay demonstrates ease of execution alongside high sensitivity.	1. The detection time was reduced to approximately 10 minutes. 2. The direct assay, displacement assay, and competitive assay all demonstrated proficiency in detecting low molecular weight melamine.	The SPR biosensor, along with the proposed analysis assays, offers rapid, convenient, and cost-effective detection of melamine.	Huanan Wu et al., [18]

8. Conclusion:

This article examines two milk adulterants Urea and Melamine, their impact on public health, and detection methods and scope of future research. Milk adulteration involves intentionally compromising the quality of purchased milk and milk products by adding or substituting inferior materials or removing valuable ingredients. This practice has posed dangers to humanity throughout history and is now a growing global concern, with consequences ranging from public health issues to economic losses. The lack of strict laws and their implementation is a primary driver behind the rapid increase in milk adulteration, underscoring the need for robust action to protect consumers' health. Consumers, as ultimate users of food products, must be educated about prevalent adulteration practices and how to protect themselves. Awareness campaigns can help inform individuals about the risks of food and milk adulteration and empower them to safeguard their health. Moreover, efforts should be made to enhance food safety regulations and enforcement mechanisms.

More biosensors need to be developed with artificial intelligence combination, which can detect the adulteration at micro level, more precision and at an affordable rate for the daily consumers.

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DATA AVAILABILITY STATEMENT: The data used to support the findings of this study are manuscript.

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