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Non-Invasive Detection of Hydrogen Peroxide Adulteration in Milk using E-Tongue Analysis

[1]R. Premananda, [2] H J Jambukesh, [3]Santosh M Herur, [4] Nagaraj Bhat, [5]Hanumanthraju R K

[1]Assistant Professor, Department of ECE, Govt. Engineering College, Huvinahadagali, Karnataka, India
[2]Assistant Professor, Department of ECE, Govt. Engineering College, Haveri, Karnataka, India
[3]Professor, Department of ECE, Jain Institute of Technology, Davanagere, Karnataka, India
[4]Associate Professor, Department of ECE, KLS Vishwanathrao Deshpande Institute of Technology, Haliyal, Karnataka, India
[5]Assistant Professor, Department of ECE, GM University, Davanagere, Karnataka, India

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ABSTRACT: Milk adulteration involves deliberately compromising the quality of milk intended for sale, achieved through practices such as mixing or substituting it with inferior substances or removing essential ingredients. Contrary to common belief, milk adulterants can lead to severe health hazards, potentially causing fatal diseases. In this paper the experimental study of the prevalent milk adulterant, hydrogen peroxide (H2O2) is discussed and various methods are used for qualitative and quantitative detection. In addition to established techniques, recent advancements in detection methods are also discussed. The increasing sophistication of milk adulteration calls for cutting-edge research in identifying these adulterants. Through this review, we aim to contribute to the collective knowledge on milk adulterants and their detection techniques, fostering awareness and enabling effective measures to ensure milk quality and safety.

Milk adulteration remains a prominent concern within the food industry, with hydrogen peroxide (H2O2) being one of the frequently employed adulterants. This study introduces an innovative technique utilizing electronic tongues (E-tongue) to detect and measure hydrogen peroxide levels in milk. The E-tongue method provides a swift and non-intrusive way to assess the presence of H2O2 in raw milk samples. Through the measurement of changes in hydrogen ion concentration and conductivity resulting from the interaction between hydrogen peroxide and milk, the study offers an indirect quantitative assessment of the adulterant. Experimental analyses were conducted at 27°C and 30°C to replicate typical storage conditions, revealing notable fluctuations in E-tongue and lactometer values in response to varying temperatures and H2O2 concentrations. Significantly, higher concentrations of hydrogen peroxide displayed improved detectability, thereby presenting a promising avenue for identifying adulterated raw milk. This research contributes to enhancing the detection capabilities for H2O2 in milk, thus safeguarding the integrity and quality of dairy products in the marketplace.

Keywords: Milk analysis, E-tongue, Hydrogen peroxide, Milk adulteration.

INTRODUCTION

Milk, being a vital source of essential nutrients, is a fundamental component of the human diet. Ensuring the quality and safety of milk is of paramount importance to safeguard public health. However, the rising concern over milk adulteration poses a significant challenge for the dairy industry and regulatory authorities. One common adulterant used in the milk industry is hydrogen peroxide (H2O2), which can lead to serious health hazards if consumed in adulterated milk. Milk analysis techniques play a crucial role in detecting and quantifying adulterants, ensuring the integrity of the milk supply chain. Traditional methods for milk analysis are effective but may require time-consuming sample preparation and complex instrumentation. To address these limitations, innovative approaches, such as electronic tongues (E-tongue), have emerged as promising alternatives for rapid and accurate milk analysis.

The term "E-tongue" refers to a sensor array that mimics the human taste and smell senses, enabling the identification of various chemical components in a sample. Its application in milk analysis offers a non-destructive and real-time assessment of milk quality, providing valuable insights into its composition and detecting any potential adulteration.

This paper focuses on the use of E-tongue technology for the detection and quantification of hydrogen peroxide in milk, addressing the specific issue of milk adulteration. The method relies on measuring the changes in hydrogen ion concentration and conductivity that occur after the reaction between hydrogen peroxide and milk. By indirectly evaluating hydrogen peroxide levels, this approach offers a rapid and efficient means of identifying adulteration and ensuring milk quality. Throughout this study, we investigate the presence of hydrogen peroxide in raw milk samples, considering various concentrations and different temperatures. The results obtained from the E-tongue analysis are compared and correlated with conventional lactometer measurements, further validating the effectiveness of the E-tongue approach in detecting milk adulteration.

The findings of this research hold great significance for the dairy industry, regulatory bodies, and consumers alike. Detecting and combatting milk adulteration is essential for maintaining consumer trust and ensuring access to safe and unadulterated milk products. Additionally, the application of E-tongue technology in milk analysis opens up new possibilities for rapid and efficient quality control in the dairy sector.

Figure 1. Milk sample and its products

LITERATURE REVIEW

Milk is a crucial component of the human diet, providing essential nutrients and promoting overall health. Ensuring its quality and authenticity is paramount to safeguard public health and maintain consumer confidence. However, the widespread practice of milk adulteration, including the use of hydrogen peroxide as an adulterant, poses significant challenges for the dairy industry and regulatory authorities.

Combine 5mL of the suspected milk sample with an equal amount of raw milk and introduce 5drops of a 2% paraphenylenediamine solution into a test tube. The presence of a blue color signifies the existence of hydrogen peroxide as an adulterant in the milk. Hydrogen peroxide is a frequently encountered adulterant in milk, particularly in developing nations [1].

In the United States, the Food and Drug Administration (FDA) authorizes the presence of hydrogen peroxide exclusively in milk designated for cheese production (21 CFR 184.1366 – Hydrogen peroxide) [3]. While small quantities of hydrogen peroxide (1–2 mg/L) can occur naturally in raw milk due to internal chemical processes, its concentration needs to be ten times higher to effectively combat pathogens. Simultaneously, elevated levels of H2O2 in milk can lead to alterations in its chemical composition, potentially resulting in adverse effects on consumers [4] [5].

Researchers have placed increasing emphasis on the development of non-enzymatic electrochemical sensors to improve the detection capability of hydrogen peroxide (H2O2) [6]. Electrochemical methods have also been proposed for the determination of hydrogen peroxide in milk [7]. Additionally, various colorimetric, luminescent, and spectrophotometric techniques have been introduced for this purpose [8].

Numerous chromatographic methods have been utilized to quantify hydrogen peroxide, employing High-Performance Liquid Chromatography (HPLC) with various detection approaches. For instance, one study showcased the quantification of hydrogen peroxide in aqueous solutions via its reaction with iodide and vanillic acid, leading to the formation of iodovanillic acid. This compound was subsequently measured using HPLC equipped with a UV detector [9]. Another approach involved determining hydrogen peroxide in disinfectants containing per acetic acid by oxidizing triphenylphosphine to form triphenylphosphine oxide, which was used for quantification through HPLC [10]. This same principle was adapted for the determination of hydrogen peroxide in toothpaste [11]. These HPLC techniques, requiring standard equipment, are straightforward to set up and may be suitable for milk analysis.

Additionally, HPLC with fluorescence detection has been employed for hydrogen peroxide determination. These techniques entail the derivatization of hydrogen peroxide using 4-phenylacetic acid or methanol/fluoral.P through an enzyme or enzyme-like catalyzed process [12]. The methods employing 4-phenylacetic acid necessitate the use of a post-column derivatization system. Furthermore, HPLC with electrochemical detection enables the direct measurement of hydrogen peroxide without the need for additional reactions [13].

Hydrogen peroxide (H2O2) finds widespread use as a bleaching agent and antiseptic in the food industry. In the processing of dried bean curd, the addition of H2O2 serves to prevent the growth of microorganisms. However, excessive H2O2 residues in dried bean curds can lead to food poisoning and accelerate the aging process, posing health risks to consumers. Furthermore, damage to the human gastrointestinal tract and respiratory system can result in various diseases, including cancer [14]. China's hygienic standard for food additives (GB2760) strictly prohibits the detection of any H2O2 residue in dried bean curds. Consequently, the detection of hydrogen peroxide residues in dried bean curds has become an area of increasing concern.

Currently, common methods for detecting H2O2 include high-performance liquid chromatography, fluorescence, titrimetry, colorimetry, spectrophotometry and chemiluminescence. However, these methods generally suffer from slow detection speed, complex operation, and high costs. Therefore, there is a need for the development of a more convenient, rapid and accurate detection method for H2O2 in dried bean curds. Electrochemical sensors offer a promising solution to overcome the drawbacks of traditional detection methods and enable the swift detection of H2O2 in food[15].

In a study by Zhang et al. (2017), the potential of an E-tongue was explored for milk analysis, particularly for the detection of hydrogen peroxide as an adulterant. The researchers investigated the changes in electrical properties, such as conductivity and impedance, after exposing the milk samples to hydrogen peroxide. The E-tongue successfully distinguished between adulterated and unadulterated milk samples, offering a promising approach for rapid milk adulteration detection.

A similar study by Silva et al[16] focused on the use of an E-tongue system with electrochemical sensors for milk analysis. The researchers developed a novel methodology to evaluate milk quality, detecting adulteration with hydrogen peroxide as well as other common adulterants. The E-tongue's ability to differentiate between various adulterants in milk demonstrated its potential for comprehensive milk analysis.

A study by Gupta et al. [17] explored the use of both E-tongue and lactometer for detecting hydrogen peroxide in milk. The researchers established a correlation between the variations in E-tongue response and lactometer values with different levels of hydrogen peroxide concentration. This integrated approach provided a more robust detection mechanism for milk adulteration.

A study by Rahman et al[18] employed support vector machines (SVM) to classify milk samples based on their adulteration status. The combination of E-tongue technology with SVM analysis yielded high accuracy in identifying adulterated milk samples, demonstrating the potential for automated and precise milk analysis.

In conclusion, the literature review reveals that E-tongue technology has emerged as a promising approach for milk analysis and the detection of hydrogen peroxide as an adulterant. With ongoing advancements in sensor technology and machine learning techniques, E-tongues offer a rapid, non-destructive, and accurate means of assessing milk quality, providing valuable insights for the dairy industry and regulatory bodies to combat milk adulteration effectively. Further research and validation studies are warranted to fully harness the potential of E-tongue technology in milk analysis and ensure the safety and authenticity of milk products for consumers.

METHODS AND MATERIALS

Destructive Detection of Hydrogen Peroxide

In a milk adulteration laboratory test designed specifically to detect hydrogen peroxide, technicians will employ two distinct methods. In the initial approach, a test tube will be used, into which 5 ml of silver nitrate reagent and 3 drops of Paraphenylene Diamine will be introduced. The resulting mixture will be vigorously agitated. A change in the mixture's color to blue will indicate the presence of hydrogen peroxide in the milk. Alternatively, they can opt for an alternative procedure. In this method, 10 ml of milk will be poured into a test tube, followed by the addition of 10-15 drops of vanadium pentoxide reagent. After thorough mixing, a color shift to pink or red will confirm the presence of hydrogen peroxide in the milk [14].

Non-Destructive / E-Sensing methods

Milk samples

To validate the method, samples of pasteurized Nandini cow milk with varying fat contents of 1.5% and 4.1% were used. A total of twelve commercial milk samples were examined. The introduction of biosensors has transformed the detection of adulterations in liquid and semi-solid food, enhancing sensitivity significantly. One such durable and cost-efficient biosensor operates through the hydrolysis of hydrogen peroxide, resulting in the liberation of hydrogen and carbonate ions. This process leads to a shift in the hydrogen ion concentration and electrical ion combination. In the case of potentiometric biosensors, the hydrolysis of hydrogen peroxide also liberates ions that generate a potential difference across the transducer, facilitating the detection process. Sensors based on pH rely on the catalytic reaction of enzymes with H2O2 in milk, triggering the release of hydrogen ions that cause a change in the pH value for detection purposes. While these biosensors are capable of swiftly processing a large volume of samples and achieving a detection limit of 0.0018% (wt/wt), their accuracy diminishes with prolonged use.

RESULTS AND DISCUSSION

Hydrogen peroxide exhibits strong preservative properties for milk, allowing for preservation for a minimum of 24 hours when present in raw milk at concentrations ranging from 0.07% to 0.08%. Refer to Table 1 and Table 2 for the behavior of milk with hydrogen peroxide. It has been detected by E-tongue, Lactometers and analyzed by simple Arduino UNO board.

Table 1.The behavior of milk with hydrogen peroxide@27^{0}C

Temp/ Fat%	Fat1.5		Fat4.1		H_2O_2
	pH	**Cd**	**pH**	**Cd**	**%**
27^{0}C	6.65	4.8	6.50	5.5	0
	6.62	4.7	6.48	5.3	0.02
	6.59	4.5	6.45	5.1	0.04
	6.55	4.2	6.41	4.9	0.06
	6.53	4.0	6.39	4.7	0.08
	6.50	3.9	6.35	4.6	0.1
	6.47	3.7	6.31	4.3	0.12
	6.44	3.4	6.27	4.1	0.14
	6.40	3.2	6.22	4.0	0.16

Cd : Notes conductivity in milli Siemen

Table 2. The behavior of milk with hydrogen peroxide @30^{0}C

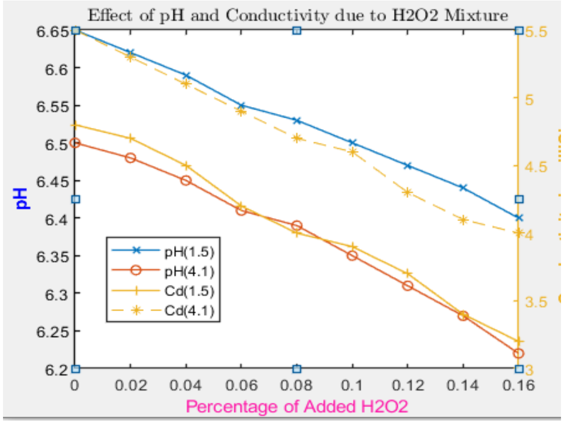
Temp/Fat%	Fat1.5%		Fat4.1		H_2O_2
	pH	**Cd**	**pH**	**Cd**	**%**
30^{0}C	6.67	4.7	6.52	5.4	0
	6.63	4.6	6.49	5.1	0.02
	6.57	4.4	6.47	5.0	0.04
	6.52	4.0	6.44	4.8	0.06
	6.50	3.8	6.42	4.6	0.08
	6.48	3.6	6.38	4.4	0.1
	6.45	3.4	6.34	4.2	0.12
	6.41	3.2	6.30	4.0	0.14
	6.38	3.0	6.26	3.8	0.16

Hydrogen peroxide is mildly acidic, and when added to milk, it might have led to a decrease in its pH. This shift in pH could be attributed to the release of protons (H+) from hydrogen peroxide into the milk solution. Lowering the pH of milk could have implications for its taste, texture, and overall quality. The changes in milk conductivity and pH observed in this study(Table 1, Table 2, and Table3) could be attributed to various factors:

a. Chemical Reactions: Hydrogen peroxide is a powerful oxidizing agent and can undergo chemical reactions with organic compounds present in milk. These reactions might lead to the formation of new ions or compounds, affecting both the pH and conductivity of the milk.

b. Microbial Activity: Hydrogen peroxide also possesses antimicrobial properties. When introduced into milk, it may have influenced the activity of bacteria and other microorganisms present. The metabolic processes of these microorganisms could result in the release of acids or bases, impacting the milk's pH.

c. **Enzymatic Reactions:** Some enzymes naturally present in milk could react with hydrogen peroxide, causing changes in the milk's composition and properties, including its electrical conductivity and pH.



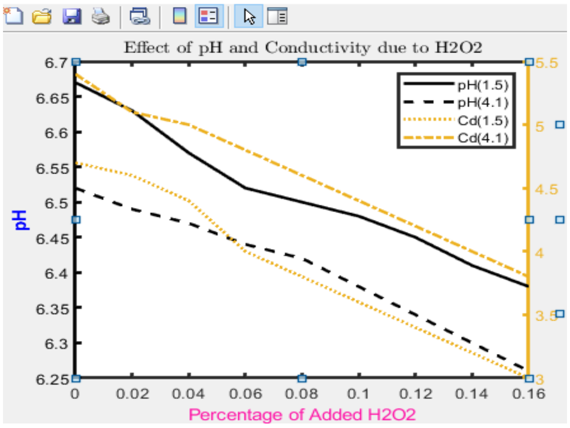
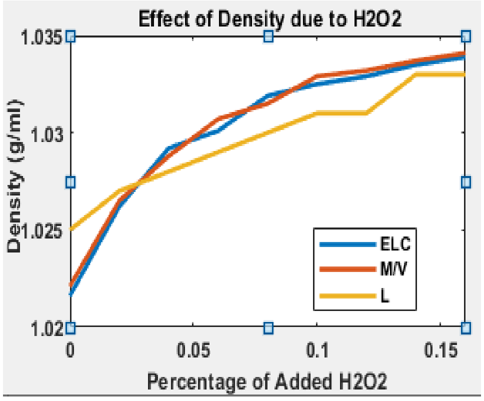


Figure 2. The behavior of milk with hydrogen peroxide@27^{0}C

Figure 3. The behavior of milk with hydrogen peroxide @30^{0}C

Table 3.Milk Fat and H2O2 measurement through milk density using Different measuring methods

		1.5% Fat			4.1% Fat		
Temp	**H_2O_2 %**	**Density (g/ml) ELC**	**Density (g/ml) M/V**	**Density (g/ml) L**	**Density (g/ml) ELC**	**Density (g/ml) M/V**	**Density (g/ml) L**
27^{0}C	0	1.0216	1.0221	1.025	1.0316	1.0321	1.032
	0.02	1.0262	1.0265	1.027	1.0322	1.0325	1.033
	0.04	1.0292	1.0288	1.028	1.0328	1.033	1.033
	0.06	1.0301	1.0307	1.029	1.0341	1.0347	1.035
	0.08	1.0319	1.0315	1.030	1.0359	1.0355	1.036
	0.1	1.0325	1.0329	1.031	1.0365	1.0369	1.038
	0.12	1.0329	1.0332	1.031	1.0379	1.0372	1.038
	0.14	1.0335	1.0337	1.033	1.0385	1.0377	1.039
	0.16	1.0339	1.0341	1.033	1.0389	1.0381	1.039



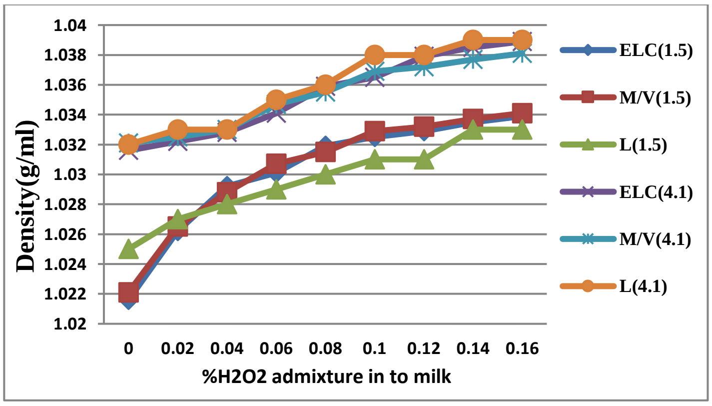


Figure 4. Effect of density due to admixture of H2O2

Figure 5. H2O2 measurement through milk density using Different measuring methods

CONCLUSION

The study presented outlines an indirect approach for determining hydrogen peroxide levels in cow milk, employing high-performance E-tongue and Lactometers. The data collected under controlled conditions reveals a lack of interaction among the constituents of cow milk with varying fat percentages. The proposed optimized and validated method demonstrates satisfactory linearity within the 0.01% to 0.06% range of H2O2. The findings illustrate that the impact of hydrogen peroxide on milk conductivity, pH, and lactometer readings is contingent upon the milk's fat content. In instances of high-fat milk, the changes in electrical conductivity and pH tend to be more moderate compared to low-fat or skim milk when exposed to hydrogen peroxide.

The alterations observed in conductivity and pH could be ascribed to the interplay between hydrogen peroxide and milk components, such as proteins, minerals, and fats. These interactions may influence the ion concentration and chemical composition of the milk, consequently leading to fluctuations in electrical conductivity and pH values. Concerning lactometer readings, the presence of hydrogen peroxide may induce subtle shifts in high-fat milk but more pronounced changes in low-fat or skim milk, owing to the direct correlation between fat content and specific gravity in milk.

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