Milk Adulteration, Detection Methods and Its Impact on Health

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Abstract

The milk is the dietary fluid and high quality milk should have better density and is free from the adulterants. Addition of substances in a product that results in unhealthy and harmful effects when consumed is called adulteration. This is done to increase the quantity of the product to gain profit. Adulteration is very common in almost all the countries and adulteration of milk is a very serious problem. India is the largest producer of milk but due to its growing population and therefore more requirements, milk adulteration is very common here also. Adulteration is not always to make greater profits but sometimes it is due to the lack of knowledge and skill in storing and transporting the milk. It exists both in the developing and advanced countries. There are reports of various methods for milk analysis available in literature. Very popular and common methods are chemical, electrical, optical methods. Since, in developed countries advanced methods are used to adulterate milk and sophisticated methods are used to detect it therefore researchers proceed for electrical methods which are accurate, data can be stored and automated and the instrument can be made handy. In this paper different techniques for detecting adulterants in milk are reported.

Key words: Adulteration, starch, urea, conductivity.

Introduction

It is the primary source of nutrition for infants before they are able to digest other types of food. The important constituents of milk consist of carbohydrate, fat, protein, vitamins, minerals, enzymes etc. [1]. The composition of milk varies with the breed of mammal, stage of lactation, feed, season and various other factors. Because it is produced as a food source for the young ones, all of its contents provide benefits for growth. The pH of milk ranges from 6.5 to 6.8 which changes over a period of time [2-4]. Milk from different animals varies in composition, but has a similar pH. The common adulterants in milk are starch, urea, vegetable oil, sugars, whiteners etc. These adulterants make the milk tastier. Addition of these substances results in increase in quantity but quality of milk becomes low. Consumption of such type of milk results in various health problems such as heart diseases, kidney related problems and intestinal infections. Chemical method is one of the methods of detecting the adulterants. The adulterants in these methods are detected by a reaction with a specific compound which results in the formation of a compound whose different coloured appearance is the indication of whether adulterant is present or not. The chemically testing methods are very reliable and give accurate results. The conventional methods, such as chromatography, spectroscopy, osmometry, capillary electrophoresis, thermometric sensors, mass spectrometry, etc. are tedious, time consuming and expensive.

Milk conducts electricity due to the presence of ionic minerals and salts that is, chloride, potassium and sodium ions. Electrical methods are, therefore, being adopted by many researchers. The conductance, resistance and impedance can be used to detect added harmful substances and other impurities in milk [5]. The conductance and admittance methods of measurement of adulterants in milk have been reported by many researchers since few decades to measure the fat and protein content of milk. Current researches are focused on analyzing the milk purity by measuring the electric admittance of milk [6]. There are several reports of adulterant detection in milk based on fractional order sensor. The optical measurements are proposed by many researchers which involve noncontact measurement and detection method. These methods preserve the quality of milk sample so that sample can be reused for multiple testing.

Adulterants and their effects

Addition of substances in milk not only decreases its nutritional value but also pose risk to health. Some of the common adulterants in milk are discussed here. Various antibiotics are used for the treatment of diseases in cattle. Mastitis is a very commonly occurring problem in dairy herds. These antibiotics are found in large quantity in milk. Antibiotics are also added to increase shelf life of milk. These are dangerous to human health and therefore its detection in milk is very necessary. There are various detection techniques available in literature but the commonly used electrical methods are the detection using electrical conductivity, biosensor array and E-nose for the detection of antibiotics in milk [7].

One of the oldest forms of milk adulteration is through the addition of variable volumes of water for high profit. This can decrease the nutritional value of milk, and if the water added is contaminated there is a high risk to human health because of waterborne diseases [8]. Adulteration of milk with water is cheap rather than starch which may be homogenized and obviously can be detected

and discovered by the consumer. Addition of water changes specific gravity of milk. Some standard techniques have been used to detect the water content in milk, using like the freezing point of raw milk or the refraction of light when passing through whey after fats are removed [9]. These techniques require too much time and high expenditures. So, more effective methods need to be developed for fast and reliable detection. Various electrical methods are now used to detect the addition of water.

Chlorine is another milk adulterant added to compensate the density of milk which changes after addition of water. Potentiometric method and Coductometric Sequential Injection Analysis are used to detect the presence of Chlorine in milk. Presence of Chlorine in milk can cause heart diseases. Non milk proteins e.g. soy milk, pea etc. and whey powder are sometimes added to milk. Milk fat is the costliest among all the milk constituents and is being removed from the milk to sell it separately. Fatty substances and vegetable oils are mixed in milk to compensate the quantity of fat. Conductivity method, electrophoresis and e-noses are used to detect presence of these adulterants in milk. Addition of various substances in milk results in change in colour of milk and therefore certain colouring materials are mixed to retain its natural colour. Capillary electrophoresis method is used to detect colour as adulterants [10].

The safety of milk has always been challenged due to illegal use of preservatives and adulterants such as hydrogen peroxide, salicylic acid, benzoic acid, water, neutralizers, melamine, and so on. Formalin is a common preservative to increase shelf life of milk. Preservatives added to food can be sometimes turned into carcinogen compounds. Some foods consist of nitrosamines, a preservative which has nitrites and nitrates which when mixed with acids in stomach can be carcinogenic. In developing countries, refrigeration is not feasible because of the high capital and running costs and problem of unreliability of electricity supply adulteration of milk by preservatives are high. Preservatives can be detected using electrical methods like, conductivity, impedimetric, capacitance methods. The major preservatives in dairy products are sodium benzoate, potassium sorbate, and natamycin. Neutralizers which are added to milk are Sodium Hydroxide, Sodium Carbonate and Sodium bicarbonate to overcome the acidic nature of milk. The acidic milk is the result of addition of some adulterants such as whey. These are not permitted additives in milk as per FSSAI and their presence in milk indicates unhygienic storage of milk. Conductivity and pH measurement are often used to analyze the neutralizers in milk and other dairy products. Milk contains bacteria which converts lactose into lactic acid. This results in change in electrical parameters. Impedance probe are used to measure bacterial content in milk [11].

Conventional methods of detection the adulterants in milk

A need for methods to detect these harmful impurities arises in order to protect the health of humankind especially children and infants. Various methods of detection are being used commercially in industries to detect adulterants. Chemical method of detection is one of the popular method of detection the adulterants. Here, the adulterant is detected by a reaction with a particular compound which results in production of another compound which can be detected by its appearance and gives the indication of presence of specific adulterant. These chemical testing are very accurate and particular to a compound and therefore false results will not be obtained. The sensitivity of the many tests used extensively is found to be very high [5].

The methods such as chromatography, spectroscopy etc. are extensively used to detect milk adulteration. The techniques that are used are analytical techniques which includes freezing point osmometry, capillary electrophoresis, thermometric sensors and mass spectrometry for detection of adulterants such as starch, whey, sucrose etc.in powdered milk. These are the conventional methods of analysis of food products that include costly and complex instruments. Such techniques are time consuming, laborious, costly and also expertise is required to judge the quality in the dairy food.

Over the last decade, several analytical procedures have been proposed for rapid screening or selective confirmation of the quality of milk such as liquid chromatography and Gas Chromatography, especially coupled with mass spectrometry (MS). The studies are often supported by a chemometric approach which allows reliable qualitative and quantitative procedure.

Amongst the parameters used in evaluating milk quality are chemical composition, physicochemical properties, microbial quality, somatic cell count and level of antimicrobial residues. One of the physicochemical parameters of milk is osmolality.

The determination of the osmolality of aqueous samples using a freezing point osmometer is popular laboratory method used in clinical and pharmaceutical laboratories but are also employed for testing quality of food. This method is also employed in determination of the osmolality of milk. The osmolality of milk is a significant value when the milk is collected from a larger group of animals. This value is used in milk processing to control the water content, based on the German Food Control Regulations for Milk. The freezing point osmometry is also in use in the food laboratory. The osmolality can be defined as the number of solute molecules that are dissolved in 1 kg of solvent (water in this case). In all these applications the solute is that which is determined by osmometry. When milk is measured, its additional water content is the most important factor for the determination of milk osmolality [6].

Capillary zone electrophoresis is an evolving technique which can be able to give rapid separations with high accuracy and good quantification. Mass Spectrometry is an analytical technique which is used to measure the mass or identify unknown molecular bodies.

Electrical techniques for detection of adulterants in milk

The electrical properties of milk under controlled circumstances depend upon different parameters such as measuring current, voltage, frequency, impulse, type of electric current, experimental conditions and different chemical components in raw material and their degree of dissociation. Milk has high water and mineral content and is characterized by good ionic conductivity. The relationship between milk constituents and their electrical properties has been investigated and applied for quality evaluation. Zhuang et.al found statistically significant coorelation between the protein content of a commercial whey powder determined using electrical conductance method [6]. Mabrook and Petty [7] used the method of electrical measurements. The performance of a constant phase angle based impedance sensor to detect different types of adulterant in milk was reported in few reports. The sensors successfully able to distinguish between the original milk and synthetic milk reconstituted by addition of whey, urea, or tap water are added to original milk [9].

Impedance spectroscopy

Impedance spectroscopy is used as very popular technique for monitoring of complex electrochemical processes [1]. Food technology and dairy industries have adopted impedance microbiology to detect the microbial activity in food. The simple construction and performance of the CPE based on poly-methyl-methacrylate (PMMA) has been reported in [6]. Its phase characteristics also remain fairly *constant* over a wide range of frequencies. It was observed phase angle variation over a considerable range. The variation and the range depend on the ionic concentration of milk. In impedance methods the measurement is done in three different ways. The three methods are measurement of equivalent conductance of the probe, equivalent capacitance and the total impedance across the two terminals [4].

Conductance measurement

Conductivity (or electrolytic conductivity) is defined as the ability of substance to conduct electric current. It is the reciprocal of resistance. Conductivity of cow milk lies between 4 to 6 mS/cm and that of buffalo milk lies between 3 to 5 mS/cm, both at 18^0 C. To eliminate the effect of electrode polarization, the conductance of milk was measured at high frequency [5].

The conductivity of milk depend on two major parameters namely impedance, the main component of which is resistance and admittance, the main component of which is conductance. The electrical conductivity is also been studied to detect freshness and adulteration of milk. The salt content of pure milk is constant. The addition of adulterant changes the salt concentration which results in change in conductance. Conductance of milk decreases with the increase in temperature. Sadat et al detects adulteration of detergents and synthetic milk using alternating current conductance from 20 Hz to 1Mz [7]. Natural milk shows higher conductance at 100 KHz. The addition of synthetic milk decreases the conductance with respect to the concentration added to natural milk. Admittance spectroscopy is used to study the water and fat content of milk. Capacitance measurements are reported to detect the evaluation of microorganism in Milk. This paper reported that the change of capacitance of the sensor with the growth of bacteria in raw milk [8].

Impedance measurements

Bacteria convert lactose into lactic acid when raw milk is kept for some time. This results in change electrical parameter e.g. conductance and impedance etc. therefore impedance probes are used and conductivity measurements are done in order to monitor the bacterial growth.

The impedance technique to detect microbial growth is very old. The method used to sense the change in electrical parameters of the medium when microbial growth occurs [7]. The signal is expressed as a curve similar to the microbial growth curve, as obtained by other methods reported in literature, is a rapid and sensitive means of detecting active microorganisms. This is the basic technique in the field of microbiology to design an automated system. Some researchers have proposed impedance measuring techniques in different ways in the field of impedance microbiology [4].

Biosensing Techniques

A biosensor is an analytical device, used for the detection of a chemical substance that combines a biological component with a physicochemical detector. The biological element, e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids etc., acts as a the transducer or the detector element, which transforms one signal into another one, works in a physicochemical way: optical, piezoelectric, electrochemical, electrochemiluminescence etc., resulting from the interaction of the analyte with the biological element, to easily measure and quantify. The biosensor reader device connects with the associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way. The biological elements of the biosensor are enzymes, whole cells, tissues, microorganisms, receptors, and antibodies etc. acts as biomimetic

component that interacts with, or recognizes the analyte under study. Many biosensors are connected with the associated electrical sensors and electronic processors to detect milk adulteration [7].

Potentiometric methods

In potentiometric biosensors the ion-selective electrodes are used in order to transduce the biological reaction into an electrical signal. It is basically consists of an immobilized enzyme membrane surrounding the probe from a pH meter, where the catalyzed reaction generates or absorbs hydrogen ions [1]. The reaction occurring next to thin sensing glass membrane causes a change in pH which may be read directly from pH meter. Typical use of such electrode is that the electrical potential is determined at very high impedance allowing effectively zero current flow and causing no interference with the reaction. Potentiometric biosensors are used to detect presence of urea in milk. Amperometric biosensors are used to detect lactose level in milk.

The potentiometric electronic tongue is used to detect the quality of milk. Data from electronic tongue is processed by Principal Component Analysis or ANN. Potentiometric sensor arrays are widely used in food analysis namely, for milk recognition, wine recognition etc. and its correlation with human sensory perception [8], beverage analysis. In these reports, different types of sensors have been tested in the potentiometric devices e.g. lipid membranes, chalcogenide sensors, ion-sensitive PVC based membranes [5, 11], and polymer membranes formed on solid conducting supports.

E-Nose and E-Tongue

An electronic nose is a device that identifies the specific component of an odour and analyzes its chemical form to identify it. An electronic nose consist of a mechanism for chemical detection, such as an array of electronic sensors and a mechanism of pattern recognition e.g. PCA and ANN. E-noses can monitor the ageing of milk .There are reports in which e-nose is used to measure the development of rancidity in pasteurized milk [9]. E-noses are also used to detect antibiotics and non milk proteins. Electronic nose once calibrated, has the advantage of assessing of aroma throughout the whole production process at minimum costs. The instrumental techniques can be time-consuming and expensive, and require expertise. The electronic nose and electronic tongue are used to overcome these problems occur in instrumental measurements and are inexpensive [11].

Similar to electronic noses, different sensing principles can be used with electronic tongues or taste sensors. A wide variety of chemical sensors can be employed into their design, electrochemical (potentiometric, voltammetric, impedimetric), optical and biosensors. Ion- Selective electrodes and Ion-selective field effect transistors are nowadays used in developing e-tongues. Potentiometric electrodes are most widely used sensors in electronic tongue system. The sensing mechanism of most of the potentiometric sensor is based o the membrane made of inorganic or organic materials. Electronic tongues are sensor arrays for milk as well as analyzing the quality of liquids using several chemical sensors with high stability and sensitivity and ion-selective sensors. The electronic tongues are used for qualitative analysis, like, classification or identification of samples. These analysis are based on the composition of the sensor array and the mathematical methods used. An electronic tongue device is usually tested by evaluating its capability to differentiate between the basic tastes like sweet, bitter, salty [12-14].

Ultrasonic detectors and Piezoelectric sensor

Ultrasound is defined as the sound waves having frequency more than human hearing range i.e. beyond 20KHz. Ultrasound wave is one of the emerging technologies that were developed to minimize processing, maximize quality and ensure safety of the food. Chemical additives are added to increase its shelf life. The common additives are sodium carbonate, bicarbonate and formalin. These are also used to prevent curdling of milk. Mohanan et al. reported the study of thermo acoustic analysis to detect chemicals by measuring density and ultrasonic velocity.

Piezoelectric sensor is a device that uses the piezoelectric effect to measure changes in pressure, strain and force etc. An enzyme based sensor for detection of urea in milk was constructed using a piezo electric sensor, which measures the pressure of the gas evolved from the sample. The sensor showed linear behaviour for varying concentration of urea in the samples and the result showed that the sensor can be used to detect urea content in milk [16].

Optical methods

Conventional method like lactometer for measuring the specific gravity is not considered reliable when weight of the milk does not change by addition of adulterant. The methods described here are presently very common and adopted commercially but involve wastage of milk in preparing sample and are contact based methods and the milk of samples cannot be used again. The optical measurements are proposed by many researchers which involve noncontact measurement and detection method. These methods preserve the sample quality of milk sample so that sample can be reused for multiple testing. Refractive index is the principle parameter involved in detecting the adulteration. As the amount of water adulteration changed, the refractive index changed and these relations were used to configure the system for detecting adulteration of a random milk sample [17]. The scattered light by the milk contains information about adulterated particles and can be used for analyzing the quality if milk, therefore, a numerous spectroscopy optical methods have been proposed by many researchers. These techniques have advantages of being non-invasive, possibility of online implementation, rapid estimation, and cost-effective [18].

Conclusion

Detection of adulterant in milk is very complicated process. Since advanced methods are used to adulterate milk therefore it is necessary to use sophisticated method for detection. Researches are going on to develop new technologies to improve the quality and prevent the mankind from the health as well as economic loss due to this fraud. These chemical testing methods are very accurate and specific to a particular compound and therefore false results will not be obtained. The sensitivity of the many tests used extensively is very high. The sensor used for adulterant detection should be designed and fabricated in such a manner so that they can be easily dipped inside the milk or the sample under test. These should not be expensive, bio-compatible and it will not contaminate the sample medium. The materials use in sensor fabrication should not react with the medium. The performance of the sensor should not be affected by the environmental changes like temperature or humidity. Sensors should be reliable and have long life with no drift. The methods discussed here are nowadays very commonly used and can be made portable which can be used easily everywhere. New techniques like ANN, fuzzy logic and finite element methods should be used in order to improve these methods. **References**

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