Milk adulteration and their detection technique

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***Abstract*: Milk may be a complex mixture and a liquid food, which may easily be adulterated. Most of the chemicals used as adulterants are poisonous that cause health hazards affecting citizenry. The Indian Council of Medical Research has described that "milk adulterants have hazardous health effects. Although many well-known methods for detection of adulteration in milk, exists, the methods compiled below during this review are not only simple and rapid but also very sensitive to detect milk adulteration. The adulterants in milk i.e. urea, formalin, detergents, ammonium sulphate, boric acid, caustic soda(NaOH), benzoic acid, salicylic acid, hydrogen peroxide, sugars and melamine used for the increasing shelf life, volume etc. lead to serious public health concern. This study is arrange to be an 'adulterant based' study rather than 'techniques based' one, where qualitative detection for many of the common adulterants are enlisted and quantitative detection methods are bounded to few major adulterants of milk.**

***Keywords*: Milk, Adulteration, Methods for Detection, Electrical Methods**

1. INTRODUCTION:

Milk in its natural form has high nutritive value as it's a good source of quality proteins, fats, carbohydrates, and vitamins. It is easily digestible and readily absorbed and thus is especially important for infants, nursing women, children and elderly people. Milk proteins also supply amino acids needed for the proper growth of adults and infants.

Figure 1: Milk

Milk is a pale liquid produced by the mammary glands of mammals. It is the primary source of nutrition for infant mammals before they are able to digest other types of food. Early-lactation milk contains colostrum, which carries the mother's antibodies to its young and can reduce the risk of many diseases. Milk also contains other nutrients including protein and lactose.
Milk is a complex mixture and liquid food, which can easily be adulterated. According to PFA-1954 (prevention of food adulteration act) definition, "Milk is the normal mammary secretion derived from complete milking of healthy milk animal without either addition there to or extraction there from. There are many methods known for detection of adulteration in milk but the methods discussed below are simple but rapid and sensitive methods to detect adulteration. Milk contains more than 100 substances that are either in solution, suspension or emulsion in water, the important being casein -the major protein of milk, lactose -milk sugar, whey and mineral salts.

Figure 2: Difference Between Real and Synthetic Milk.

Milk is the best and cheapest source of nutrition and used by all the age groups in rural as well as in urban areas. It provides appreciable amount of fats and protein and also provides body building vitamins along with furnishing energy giving lactose and

many other nutrients, therefore, an ideal food for pregnant female and infants. Milk is essential to provide nutrients to maintain health and normal growth of body.

In order to keep milk temporarily fresh, some unethical methods are usually adapted to prevent the financial losses due to the spoilage of milk during its transportation and sale. For instance, the water is added to increase volume of milk, thickening agents like starch, flour, skimmed milk powder, whey powder or other ingredients to prevent the dilution effect and extend the solids content of the milk. Vegetable oil, sugarcane or urea to compensate the fat, carbohydrate or protein content of diluted milk that leads to hazard. Some chemicals such as hydrogen peroxide, carbonates, bicarbonates, antibiotics, caustic soda and even the most lethal chemical formalin to increase the shelf quality of milk, detergents to enhance the cosmetic nature of milk which give foamy appearance and whitening of milk that leads to gastro-intestinal problem.

Milk adulteration is a very common food fraud and is posing a big social problem in today's world. Apart from the ethical and economical issue, it also creates health hazards. Some of them are renal and skin disease, eye and heart problem and may also leads to cancer. So, for preventing these, determination of milk adulteration is very important.

Adulteration of milk is rampant, a startling six per cent of the samples tested in 2015 by the Ministry of Health had presence of 'detergents' in them confirming that 'synthetic milk' is a huge problem. Different type of methods used to detect adulterants in milk by using techniques i.e. DSC, RP-HPLC, LC-GC, HPTLC, immunoassays: CE, ELISA, FAMPST, FTIR, NIR spectroscopy, PAGE, IEF, DNA-based methods and MALDI-MS that have been developed and quantitative estimation of adulterants can also be done by using these techniques.

India is the world's largest producer of milk, and is the leading exporter of skimmed milk powder, yet it exports very few other milk products. Throughout the world, there are more than six billion consumers of milk and milk products. Over 750 million people live within dairy farming households.

1.1 pH, PHYSICAL AND CHEMICAL PROPERTIES OF MILK:

The pH of milk ranges from 6.4 to 6.8 and it changes over time. Milk from other bovines and non-bovine mammals varies in composition, but has a similar pH.

Milk is an emulsion or colloid of butterfat globules within a water-based fluid that contains dissolved carbohydrates and protein aggregates with minerals. Because it is produced as a food source for the young, all of its contents provide benefits for growth. The principal requirements are energy (lipids, lactose, and protein), biosynthesis of non-essential amino acids supplied by proteins (essential amino acids and amino groups), essential fatty acids, vitamins and inorganic elements, and water.

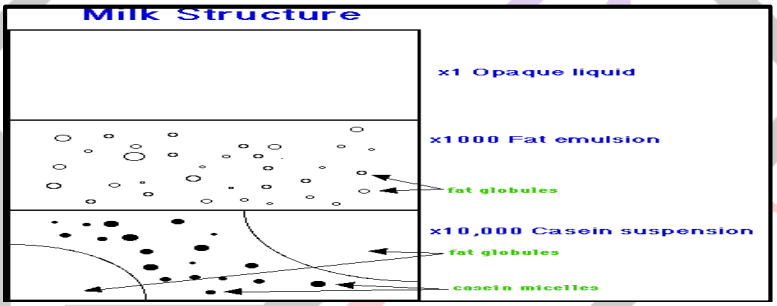

Figure 3: Milk Structure

1.2 PROCESSING OF MILK:

Processing of milk is mainly carried out in 3 ways, namely:

(a) Pasteurization:

Pasteurization is used to kill harmful microorganisms by heating the milk for a short time and then immediately cooling it. The standard high temperature short time (HTST) process produces a 99.999% reduction in the number of bacteria in milk, rendering it safe to drink for up to 3-weeks if continually refrigerated. Dairies print expiration dates on each container, after which stores remove any unsold milk from their shelves. A side effect of the heating of pasteurization is that some vitamin and mineral content is lost. Soluble calcium and phosphorus decrease by 5%, thiamin and vitamin B12 by 10%, and vitamin C by 20%.

(b) Microfiltration:

Microfiltration is a process that partially replaces pasteurization and produces milk with fewer microorganisms and longer shelf life without a change in the taste of the milk. In this process, cream is separated from the whey and is pasteurized in the usual way, but the whey is forced through ceramic microfilters that trap 99.9% of microorganisms in the milk (as compared to 99.999% killing of microorganisms in standard HTST pasteurization). The whey then is recombined with the pasteurized cream to reconstitute the original milk composition.

(c) Creaming and homogenization:

Milk often is homogenized, a treatment that prevents a cream layer from separating out of the milk. The milk is pumped at high pressures through very narrow tubes, breaking up the fat globules through turbulence and cavitation. A greater number of smaller particles possess more total surface area than a smaller number of larger ones, and the original fat globule membranes cannot completely cover them. Casein micelles are attracted to the newly exposed fat surfaces. Nearly one-third of the micelles in the milk end up participating in this new membrane structure. The casein weighs down the globules and interferes with the clustering that

accelerated separation. The exposed fat globules are vulnerable to certain enzymes present in milk, which could break down the fats and produce rancid flavors. To prevent this, the enzymes are inactivated by pasteurizing the milk immediately before or during homogenization.

Homogenized milk tastes blander but feels creamier in the mouth than unhomogenized. It is whiter and more resistant to developing off flavors. Creamline or cream-top milk is unhomogenized. It may or may not have been pasteurized. Milk that has undergone high-pressure homogenization, sometimes labeled as "ultra-homogenized," has a longer shelf life than milk that has undergone ordinary homogenization at lower pressure**.**

Figure 4: A milking machine in action.

2. ADULTERATION:

What Is Food Adulteration?

Food Adulteration is an act for debasing the quality of food with an admixture or through the substitution of inferior substances or by removing some valuable ingredients from the food product. Food Adulterants are the substances which are added to food items for economic and technical benefits. Such substances reduce the value of nutrients and also causes the food contaminated and not fit for consumption.

Adulteration:

This term denotes the act of mixing something impure with something pure, as, to mix inerior liquor with wino; au inferior article with coffee, tea, .and the like.

Full definition of adulterate:

1) Transitive verb

2) To corrupt, debase, or make impure by the addition of a foreign or inferior substance or element; *especially:* to prepare for sale by replacing more valuable with less valuable or inert ingredients.

What is adulterant of milk?

Any material which is or could be employed for making the milk unsafe or misbranded is known as adulterant of milk.

What is adulteration of milk?

Milk adulteration may be define as any change caused in the natural level of milk ingredients. These changes may be brought about by addition of some foreign matter to milk or by removing some more valuable ingredients eg.fat.

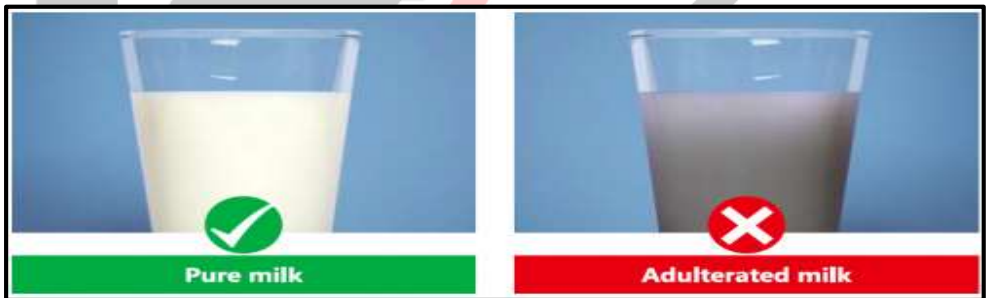

Figure 5: Detection of Adulteration Milk

As per Food Safety and Standards Authority of India, a food article could be declared adulterated if:

1. When a substance is added which depreciates or injuriously affects it.
2. Cheaper or inferior substances are substituted wholly or in part.
3. Any valuable or necessary constituent has been wholly or in part abstracted.
4. It is an imitation.
5. It is colored or otherwise treated, to improve its appearance or if it contains any added substance injurious to health.
6. For whatever reasons its quality is below the standard.

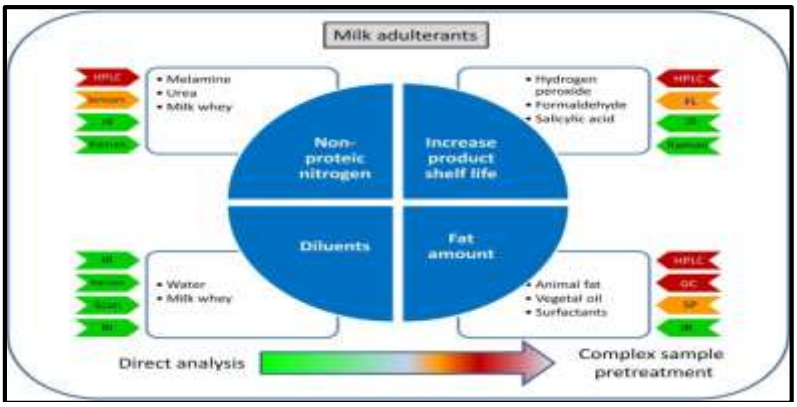

Figure 6: Adulteration and Detection Test

2.1 COMMON ADULTERANTS OF MILK:

1. Water
2. Separated milk
3. Separated milk and water
4. Starch
5. Cane-sugar
6. Egg white
7. Condensed milk
8. Colouring agent
9. Colostrum
10. Common salt
11. Sodium bicarbonate
12. Addition of buffalo milk into cow milk
13. Urea
14. Detergents
15. Blotting paper

3. METHODS FOR DETECTION OF COMMON ADULTERANTS IN MILK:

Table 1: Testing of common adulterants in milk.

S.NO.	FOOD ARTICLE	ADULTERANT	METHOD OF DETECTION
1	MILK	Water	Place a drop of milk on a polished slanting surface: a) Pure milk flows slowly leaving a white trail behind it. b) Milk adulterated with water will flow immediately without leaving a mark.
2		Urea	5 ml milk in a test tube + 5 ml dimethyl amino benzaldehyde solution, shaken Well. Yellow colour develops. It shows the presence of added urea.
3		Soda	10 ml milk in a test tube + 10 ml alcohol & shaken well + few drops of Rosalic acid. Red rose colour shows in the presence of soda.
4		Synthetic milk	Synthetic milk has a bitter after taste. Gives a soapy feeling on rubbing between the fingers. Turns yellowish on heating.
5		Detergent	5 ml milk + few drops of bromocresol purple solution. Appearance of faint violet colour indicates the presence of detergent in milk.
6		Starch	5 ml milk in test tube, boil than cool + 1 to 2 drops of iodine Solution. Appearance of blue colour which indicates the presence of starch.
7		Glucose/ Invert Sugar	5 ml milk in test tube, boil than cool + 1 to 2 drops of iodine Solution. Appearance of blue colour which indicates the presence of starch.

Table 2: Tests to be done in laboratory

S.NO.	FOOD ARTICLE	ADULTERANT	METHOD OF DETECTION
1	MILK	Formalin	5ml milk in a test tube + few dros of phloroglucinol solution and mix properly + few drops of sodium hydroxide solution. Flashy pink colour, shows the presence of formalin.
2		Hydrogen peroxide	10 ml milk in a test tube + 10-20 drops vanadium pentaoxide solution. Development of pink/red colour indicates the presence of hydrogen peroxide.
3		Vanaspati	3 ml milk in a test tube + 10 drops of HCl +Mix one teaspoonful of sugar. After 5 min examine the mixture. The red colouration indicates the presence of vanaspati in the milk.
4		Salt	5 ml Silver Nitrate Reagent in a test tube + 2-3 drops of Potassium Dichromate Reagent + 1 ml of milk and mix thoroughly. Occurrence of yellow colour indicates the presence of salt in the milk.
5		Sugar	3 ml of milk in a test tube + 2 ml of HCl. Heat the test tube after adding 50 mg of resorcinol. The red colouration indicates the use of sugar in the milk.
6		Sodium bicarbonate/ Neutralizer	3 ml of milk in a test tube + 5 ml of rectified spirit + 4 drops of rosalic acid solution. Appearance of red/rosy coloration indicates the presence of sodium bicarbonate in the milk.
7		Boric acid	3 ml of milk in a test tube + 2 drops of HCl and shake the test tube or mix up the contents thoroughly. Dip a yellow paper strip and remove it after 1 min. A change in colour from yellow to red, followed by the change from red to green, by the addition of one drop of ammonia solution takes place. It indicates the presence of boric acid in the milk.
8		Fat	The Lactometer reading will go above 26.

4.MILK ADULTERANT AND ITS DETECTION:

(a) Typical adulterants in milk:

Milk powder is the second most likely food item being in the risk of adulteration after olive oil. Some of the major adulterants in milk having serious adverse health effect are urea, formalin, detergents, ammonium sulphate, boric acid, caustic soda, benzoic acid, salicylic acid, hydrogen peroxide, sugars and melamine

Common parameters that are checked to evaluate milk quality are- fat percentage, SNF (Solid-not-Fat) percentage, protein content and freezing point. Adulterants are added in milk to increase these parameters, thereby increasing the milk quality in dishonest way. For example, cane sugar, starch, sulfate salts, urea and common salts are added to increase solid-not-fat (SNF). Commercial urea is added to milk to increase non-protein nitrogen content. Similarly, melamine is added to increase protein content falsely. Ammonium sulphate is added to increase the lactometer reading by maintaining the density of diluted milk. Formalin, Salicylic acid, Benzoic acid and Hydrogen peroxide act as preservatives and increase the shelf life of the milk. Since milk fat is very expensive, some manufacturers of milk and dairy products remove milk fat for additional financial gain and compensate it by adding non-milk fat such as vegetable oil. Detergents are added to emulsify and dissolve the oil in water giving a frothy solution, which is the desired characteristics of milk.

Unfortunately, some of the adulterants have severe health impact, sometimes in the long run. The ingestion of melamine at levels above the safety limit can induce renal failure and death in infants. Both peroxides and detergents in milk can cause gastro-intestinal complications, which can lead to gastritis and inflammation of the intestine. Excessive starch in the milk can cause diarrhea due to the effects of undigested starch in colon, however, accumulated starch in the body may prove very fatal for diabetic patients.

(b) Qualitative detection methods:

Qualitative detection of adulterants in milk are simple color based chemical reactions. These can be performed in any Biosafety Level 1 Laboratory with availability of chemical reagents and necessary precautions. Major drawbacks of these techniques are the facts that these are valid for a limited range of concentrations and are not sufficiently precise.

However, qualitative detections are advantageous because these are simple, rapid and very easy to perform. Some of the edible compounds are often used as adulterants to improve the taste of the milk. Presence of those in milk can be detected rapidly as discussed in **Table 3.** However, there are some hazardous chemicals added in milk to improve the physical appearances and shelf life. Some of those are very hazardous and can lead to fatal diseases. **Table 4** shows fast, yet simple hazardous chemicals detection techniques in milk. In addition, some other mixed chemicals such as soap, detergents and coloring compounds are sometimes added

to the milk to improve appearance. Qualitative detection of some of those common adulterants in milk have been discussed in **Table 5**.

Table 3: Rapid qualitative detection of different edible adulterants in milk

Adulterant	Procedure	Observation	Limit of detection
Sugar	Take 5 mL milk sample in a test tube. Add 1 mL conc. HCl and 0.1 g resorcinol solution. Place the test tube in water bath for 5 min.	Appearance of red color indicates he presence of added sugar.	0.2% (w/v)
Starch	Take 3 mL sample in a test tube. After boiling it thoroughly, cool it to room temperature. Add 1 drop of 1% iodine solution.	Appearance of blue color indicates he presence of starch.	0.02% (w/v)
Glucose	Take 1 ml of milk sample in a test tube. Add 1 ml of modified Barfoed's reagent. Heat the mixture for exact 3 min in a boiling water bath. Rapidly cool under tap water. Add one ml of phosphomolybdic acid agent to the turbid solution.	Immediate appearance of deep blue color indicates the presence of glucose.	0.1% (w/v)
Common salt	Take 5 ml of milk sample into a test tube. Add 1 ml of 0.1 N silver nitrate solution. Mix the content thoroughly and add 0.5 ml of 10% potassium chromate solution.	Appearance of yellow color indicates the presence of added salts, whereas, brick red color indicates the milk free from added salt.	0.02% (w/v)
Buffalo milk	Dilute the milk 1/10. Put a drop of diluted milk on the centre of a glass slide. Now place a drops of Hansa test serum (duly preserved) on the drop of milk and mix together with a glass rod or clean tooth pick.	Curdy particles develop within half a minute in milk containing buffalo milk.	

Table 4: Rapid qualitative detection of different hazardous chemicals in milk:

Adulterant	Procedure	Observation	Limit of detection (v/v)
Hydrogen peroxide	A. Add to 5 mL of suspected milk sample in attest tube, an equal volume of raw milk and 5 drops of 2% solution of paraphenylenediamine.	Appearance of blue color indicates the presence of hydrogen peroxide as adulterant.	0.025%
	B. Take 1 mL milk sample in a test tube and add 1 mL of potassium iodide-starch reagent solution and mix well.	Appearance of blue color indicates the presence of hydrogen peroxide as adulterant.	0.004%
Formalin	A. Take 10 mL milk sample in attest tube. Add 5 mL conc. sulfuric acid with a little amount of ferric chloride without shaking.	Appearance of violet or blue color at the junction of two liquid layers indicates the presence of formalin.	
	B. Take about 5 ml of milk in a test tube. Take 1 ml of 10% ferric chloride solution in a 500 ml volumetric flask and make up the volume using concentrated hydrochloric acid. Add 5 mL from this solution to the sample in test tube. Keep the tube in boiling water bath for about 3-4 min.	Appearance of brownish pink color indicates the presence of formalin.	0.1%
	C. Take 1 mL of sample milk in a test tube. Take saturated solution of 1,8- dihydroxynaphthalene-3, 6- disulphonic acid in about 72% sulfuric acid to make chromotropic acid solution. Add 1 mL of chromotropic acid solution to the sample in test tube.	Appearance of brownish pink color indicates the presence of formalin.	0.05%

Ammonium sulfate	A. Take 2 ml. milk in a test tube and add 0.5 ml NaOH (2%) 0.5 ml sodium hypochlorite (2%) and 0.5 ml phenol (5%) Heat in boiling water bath for 20 sec. B. Take 10 ml of milk in a 50 ml stoppered test tube. Add 10 ml of TCA solution. Filter the coagulated milk through Whatman filter paper Grade 42. Take 5 ml of clear filtrate. Add few drops of barium chloride solution.	A bluish colour forms immediately, which turns deep blue afterward. Pure milk shows salmon pink colour which gradually changes to bluish after 2 hours. Formation of milky-white precipitates indicates the presence of added sulfates like ammonium sulfate, sodium sulfate,zinc sulfate and magnesium sulfate etc.to milk	0.05% (w/v)
Urea	A. Take 5 mL milk sample in a test tube. Add equal volume of 24% TCA to precipitate fat and proteins of milk. Take 1 mL filtrate and add 0.5 mL 2% sodium hypochlorite, 0.5 mL 2% sodium hydroxide and add 0.5 mL 5% phenol solution, then mix. B. Take 5 ml milk in a test tube, add 0.2 ml urease (20 mg/ml) Shake well at room temperature and then add 0.1 ml Bromothymol Blue (BTB) solution (0.5%) C. Take 5 mL milk sample in a test tube. Add 5 mL p-Dimethyl Amino Benzaldehyde reagent.	A characteristic blue or bluish green colour develops in presence of added urea whereas pure milk remains colourless. Appearance of blue colour after 10-15 min. indicates the presence of urea in milk. Normal milk shows faint blue colour due to natural urea present in milk. Appearance of distinct yellow color indicates presence of added urea whereas formation of slight yellow color indicates natural urea in milk.	0.2% (w/v)
Nitrate	Take 10 ml sample milk in a beaker. Add 10 ml mercuric chloride solution to it. After mixing, filter through what man No 42 filter paper. Take 1 ml filtrate in a test tube and add 4 ml of diphenyl amine sulphate or diphenyl benzidine reagent.	Appearance of blue colour indicates the presence of nitrates. Pure milk sample will not develop any color.	0.2%
Benzoic and salicylic acid	Take 5 mL milk sample in a test tube. Upon acidification with sulfuric acid, 0.5% ferric chloride solution is added to it drop by drop. Mix it. Five ml of milk is taken in a test tube and acidified with concentrated sulphuric acid. 0.5% ferric chloride solution is added drop by drop and mixed well. Development of buff colour indicates presence of benzoic acid and violet colour indicates salicylic acid.	Appearance of buff color indicates the presence of benzoic acid whereas that of violet color indicates salicylic acid.	
Borax and boric acid	Take 5 mL milk sample in a test tube. Add 1 mL conc. HCl to it. A turmeric paper is dipped and it is dried in a watch glass at 100 °C.	If the turmeric paper turns red, it indicates the presence of borax or boric acid.	

Table 5: Rapid qualitative detection of different mixed adulterants in milk

Adulterant	Procedure	Observation	Limit of detection (v/v)
Detergent	A. Take 5 ml in a test tube and add 0.1 ml 0.5% Bromocresol Purple (BCP) solution. B. Take 5 mL of milk sample into a 15 mL test tube. Add 1 ml of Methylene blue dye solution and 2 ml chloroform. Vortex the contents for about 15 sec and centrifuge at about 1100 rpm for 3 min.	Appearance of violet colour indicates the presence of detergent. Unadulterated milk shows faint violet color. Relatively, more intense blue color in lower layer indicates presence of detergent in milk. Relatively more intense blue color in upper layer indicates absence of detergent in milk.	0.0125%
Pulverized soap	Take 10 ml milk sample in a test tube. Add equal quantity of hot water to it, then add 1 – 2 drops of phenolphthalein indicator.	Appearance of pink color indicates presence of soap.	
Coloring matter	A. Take 10 mL milk sample in attest tube. Add 10 ml diethyl ether. After shaking, allow it to stand. B. Make the milk sample alkaline with sodium bicarbonate. Dip a strip of filter paper for 2 hours. C. Add a few drops of hydrochloric acid to milk sample.	Appearance of yellow color in ethereal layer indicates the presence of added color. Appearance of red color on filter paper indicates the presence of annatto. Treatment of this paper with stannous chloride gives pink color. Appearance of pink color indicates azo dyes.	

(c) Quantitative detection methods:

(1) Milk adulteration with foreign proteins:

Soy, rice and almond proteins are intentionally processed into milk-like products and sold as milk supplements for consumers with lactose. However, soy, wheat and almond proteins are labeled as allergens by FALCPA (Food Allergen Labeling and Consumer Protection) of 2004 while pea, rice, lupin and maize proteins are clinically recognized as allergens. Reported detection techniques for soy milk in milk are polarimetric method, isoelectric precipitation, SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis), HPLC (High Performance Liquid Chromatography) and immunodiffusion method. NIR (Near Infra Red) spectroscopy has been used for detecting milk powder adulteration with vegetable protein. Adulteration of pasteurized or UHT (Ultra High Tempearture) milk powders with soy, pea, and wheat proteins have been reported. ELISA has been used to detect these proteins with polyclonal. Skimmed milk powder adulterated with soy, pea, brown rice and hydrolyzed wheat protein has been successfully isolated using UHPLC (Ultra High-Performance Liquid Chromatography). Mas spectroscopy based techniques to identify milk protein structures have been reported.

(2) Milk adulteration with milk from different sources:

Though mixing milk from random sources and different animal species is the easiest means to adulterate milk, its quantitative detection is much more complex due to genetic and nongenetic polymorphism. Determination of geographical origin of milk has been possible using ICP-OES (Inductively Coupled Plasma Emission Spectroscopy). Along with isotope ratio mass spectrometry (IRMS), this method determines the mineral contents (inorganic metals and nonmetals) of the food and identifies geographical differences utilizing chemometric techniques based on multivariate statistical methods. Cow milk adulteration in caprine milk has been quantified by HPLC/ESIMS (High Performance Liquid Chromatography/Electrospray Ionization- Mass Spectroscopy). This method identifies molecular masses to differentiate between proteins in the milk of cow and goat. Since, PDO (Protected Denomination of Origin) cheeses are products of high commercial value confined according to legislative and proper labelling rules, different analytical techniques have been developed to evaluate the authenticity. Quantification and adulteration measurement of bovine, ovine and caprine milk mixtures in commercial PDO cheeses have been quantified by using RP-HPLC (Reverse Phase High Performance Liquid Chromatography), high resolution melting (HRM) based method utilizing specific mitochondrial primers and solid-phase microextraction-mass spectrometry method (SPME-MS) based on volatile profile. Indirect competitive ELISA has been used to detect cow milk adulteration in goat, sheep and buffalo milk. Adulteration of caprine milk with cow milk has also been detected using PCR.

(3) Milk adulteration with melamine:

Since melamine is neither a permitted additive nor a food ingredient, its limit had not been set in food legislation until the melamine contamination reported in China in 2008. Both the European Commission and the United States Food and Drug Administration (USFDA) have applied a maximum acceptable limit of 2.5 mg/kg for melamine in imported foods, and 1 mg/kg in infant formula. Melamine is not only added to milk powder as adulterant, but also in many other foods like wheat gluten, chicken feed, and processed foods. Though it is not carcinogenic, it causes renal failure and infant death in extreme cases Quantitatively melamine detection has been possible using SERS (Surface Enhanced Raman Spectroscopy). A portable sensor based on SERS has been also developed to detect melamine instantly. SB-ATR FTIR (Single Bounce Attenuated Total Reflectance - Fourier transform infrared spectroscopy) has been used to quantify melamine in both liquid and powder milk. Different types of mass spectroscopy have been employed to detect melamine in milk products including LC-MS/MS, APCIMS (Atmospheric Pressure Chemical Ionization-Mass Spectroscopy) and EESI-MS (Extractive Electrospray Ionization Mass Spectrometry. HPLC is another choice of technique to quantify melamine in milk and dairy products. Using Raman band at 676 cm-1, melamine in dried milk powder has been immediately detected without extracting melamine from milk (Okazaki et al. 2009).

(4) Milk adulteration with urea:

Urea, being a natural constituent of milk, constitutes the major portion of non-protein nitrogen in milk. According to FSSAI (Food Safety and Standards Authority of India) act 2006 and PFA (Prevention of food adulteration) rules 1955, maximum allowable limit for urea in milk is 70 mg/100 mL. Milk can be adulterated with urea in two ways – by intentional addition of urea and by addition of unspecified synthetic milk to natural milk. Near infrared Raman spectroscopy has been used to quantify the presence of urea without requiring any preprocessing. LC has been used to quantify urea as adulterant in milk. A method based on GC/IDMS (Gas Chromatography/Isotope Dilution Mass Spectrometry) has been used to quantify urea present in. HPLC has been reported to detect the presence of natural urea in milk with a suggestion to convert the urea into a derivative containing a chromophore before HPLC analysis. A combination of kjeldahl and spectrophotometric method has been suggested to detect milk adulteration by melamine, urea and ammonium sulphate. EISCAP (Electrolyte Insulator Semiconductor Capacitor), a potentiometric biosensor based on enzymatic reaction has been developed to detect urea Some other biosensors based on various principles like manometry, enzyme, potentiometry have already been developed to detect urea in milk.

(5) Milk adulteration with other compounds:

NIR (Near Infrared Spectroscopy) (1100-2500 nm) has been used to quantify water and whey in cow milk. In a comparative experimental study between NIR and MIR (Medium Infra Red) spectroscopy, developed a portable spectrometer and commented that MIR performed better than NIR to detect the adulterants such as tap water, whey, hydrogen peroxide, synthetic urea and urine.Presence of urine has also been reported by observing change in the concentration of sodium and calcium in samples undergoing flame atomic absorption spectroscopy. MALDI-QTOF MS (Matrix-assisted Laser Desorption/ Ionization Time of Flight Mass Spectroscopy) has been used to quantify vegetable oil in milk. It has also been possible to detect multiple adulterants including ammonium sulfate, dicyandiamide, melamine and urea in milk powder usingr aman chemical imaging. Milk fat adulteration is also a very common concern. however, several techniques have been developed to detect the adulteration based on Butyro Refractometer, fluorescence, derivative spectroscopy and Raman spectroscopy.

5. ELECTRICAL METHODS TO DETECT MILK ADULTERANTS:

In these section different electrical methods to detect milk adulteration is summarized. Electrical methods are comparatively simpler than other methods and easy to process as the signal is in electrical domain. The other advantages are, the measurement and data storage can be automated and the equipment can be made portable for field use.

5.1 Different electrical methods to detect milk adulteration.:

(a) Potentiometric Conductance measurement
(b) Conductivity
(c) Ultrasonic
(d) E-nose
(e) E-tongue
(f) Capacitance growth
(g) Piezoelectric sensor
(h) Impedance probe

Table 6. Different milk adulterants and the method used to detect those adulterants:

Adulterants	Methods
Chlorine	Sequential injection analysis Flow injection analysis Potentiometric detection Conductometric sequential injection analysis
Antibiotics	Electrical conductivity BRT Test Spot Test SNAP test and LACTEK test Chromatography (HPLC)

	Liquid chromatography mass spectrometry Somatic cell count (SCC) Screening test Biosensor assay based on surface plasmon resonance (SPR) E-Nose
Non-milk protiens	Fluorescence spectroscopy Analysis of triacylglycerols using gas liquid Chromatography NIR spectroscopy Electrical conductivity and capacitive reactance Reversed Phase HPLC method in combination with fluorescence detector Sulfate capillary electrophoresis and chromarography E-nose
Low-valued milk	Optical biosensor (BIACORE 3000) tool Duplex polymerase chain reaction Electrophoretic, chromatographic and PCR techniques Gas chromatography ELISA and PCR techniques Reverse-phase high performance liquid chromatography and urea-polyacrylamide gel electrophoresis TaqMan real time PCR E-tongue HPLC method Sandwich IgG ELISA
Milk powder Color Preservatives	FAST (Fluorescence of advanced maillard products and soluble tryptophan) Capillary electrophoresis Conductivity Impedance Capacitance Piezoelectric transducer Impedimetric E-Tongue Thermoacoustic analysis Rosalic acid test
Neutralizers Urea	Conductivity or pH measurement Potentiometric biosensor pH measurement Durable NH+ 4 sensitive CHEMFET based sensor pH sensitive field effect transistor Manometric biosensor Ion selective electrode Calorimetric method Biosensors
Whey/Liquid whey	Reverse phase HPLC method Capillary Electrophoresis ELISA Fourth derivative spectroscopy Blot immunoassay method Phosphor partition NIR spectroscopy Immunochromatographic assay
Water	Frequency admittance measurements E-nose Electrical conductivity Ultrasonic transmitter receiver system NIR measurement Freezing point osmometry and freezing point cryoscopic method

(a) Potentiometric Sensors:

Potentiometry allows the determination of a wide spectrum of ions and inexpensive, portable equipment can be developed. Trivedi et al. has reported a potentiometric biosensor to detect urea adulteration in milk. It uses a NH+ 4 ion sensitive electrode as the transducer. It is a disposable type urea sensitive enzymatic biosensor system and has been developed by immobilizing the urease

enzyme, through entrapping, onto the ion sensitive membrane using a polymer matrix. The sensor exhibited a detection limit of 2.5×10−5 mol/L.

Conzuelo et al. has reported an amperometric biosensors to detect the lactose content of milk. Often lactose concentration is used as a basic marker for the evaluation of milk quality and the detection of abnormalities. It has been found that milk from cows suffering mastitis has low lactose levels.

Renny et al. has reported a piezo-electric sensor to detect the urea content in milk. It is an enzyme-based sensor and detects pressure of the gas, evolved in the sample when the reaction takes place in the presence of urease.

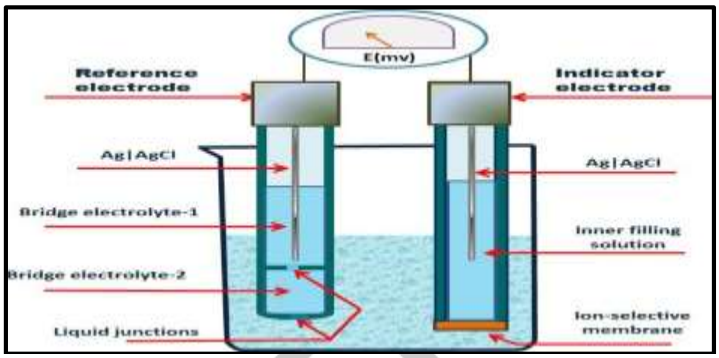

Figure 7: Potentiometric Sensors

Mirjana et al. have reported a potentiometric electronic tongue to detect the quality of milk. The potentiometric electronic tongue reported by them includes the automatic sampling system, the sensor array with the reference electrode. The sensor array consist of seven sensors coated with lipid/polymer material and Ag/AgCl electrode was used as reference. The potential is generated by the interaction of compounds in the sample and the sensitive coating of sensors. The data obtained from the electronic tongue is processed by principal components analysis (PCA) to get the variance in the experimental data.

(b) Conductance Measurement:

The conductance measurement between two electrodes is a well-known technique to detect adulteration. Most of the times the electrical equivalent model of the electrodes immersed in the sample is evaluated to identify the adulterated milk.

Anwar et al. has reported a pair of individual platinum electrodes along with a temperature unit for cyclic cooling to measure the ac conductance of milk adulterated with synthetic milk.

Siuli et al. used a constant phase element and evaluated its electrical equivalent circuit using LEVMW software where the change in the parameters of the electrical equivalent circuit reflects different kinds of adulteration.

Lawton et al. has reported to determine the fat content of the milk by measuring electrical conductivity and capacitive reactance of milk. Measurement was carried out at 100 kHz to avoid electrode polarization. The measurement requires careful temperature control.

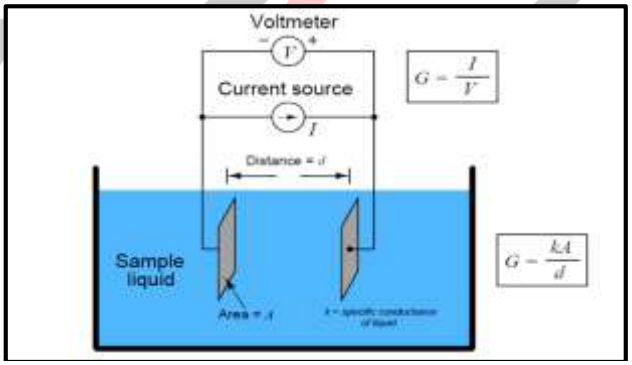

Figure 8: Conductance Measurement

(1). Mastitis Detection by Electrical Conductivity Method:

Mastitis causes increased conductivity. This is due to increased Sodium and Chloride ions in milk which in turn gives the change in the conductivity measurement and is an well known method to detect mastitis in milk.

Figure 9: Digital Mastitis detector

Fernando et al. has reported that the accuracy of electrical conductivity detection of sub clinical mastitis is better than all other indirect methods. Moreover the adaptability of this measurement is more in both manual and automatic cow-side mastitis detection systems.

(c) Ultrasonic Detectors:

Many brands of milk contain chemical additives such as sodium carbonate (Na2CO3), sodium bicarbonate (NaHCO3), formalin (HCHO)etc. These chemicals are added to milk to preserve it for longer time or as a neutraliser to prevent curdling.

Mohanan et al. has reported the study of thermoacoustic analysis to identify the chemicals. In this method, the density and ultrasonic velocity are determined for different samples while the temperature is kept fixed for a particular measurement. Ultrasonic velocities were measured by a single crystal ultrasonic interferometer at a frequency of 2 MHz Parameter called Rao's specific sound velocity (*r*) has been derived from the ultrasonic velocity and the density. The Rao's parameter changes with the change of the chemical additives.

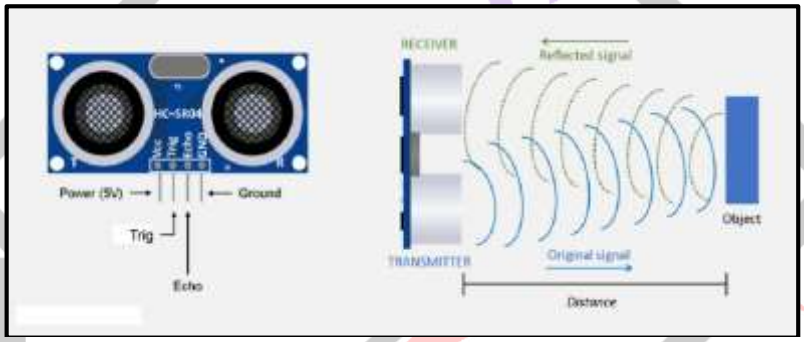

Figure 10: Ultrasonic Detectors

Anwar et al.has reported an ultrasonic transmitter receiver system to detect milk adulteration. The acoustic wave passing through the milk sample gets attenuated. The level of attenuation varies with the amount of adulteration.

(d) E-Nose:

There has been extensive research in evaluating electronic noses for monitoring the quality of milk. The two main components of an electronic nose (E-nose) are the sensing system and the automated pattern recognition system. The common pattern recognition systems are either principal component Analysis (PCA), linear discriminant analysis (LDA) or Artificial Neural Network (ANN).

Yu et al. reported an E-nose containing ten different metal oxide semiconductor sensors which can monitor the adulteration of milk by water.

Figure 11(A): E-Nose

Benedetti et al. studied the detection of Aflatoxin M1 content in milk by E-Nose system containing 12 metal oxide semiconductors (MOS) sensors and 12 MOSFETs. It has been claimed that the E-nose classification was in complete agreement with Aflatoxin M1 content measured by an ELISA procedure.
E-noses can monitor the aging of milk and can detect milk volatile compounds. Capone et al. have used an E-nose to measure the development of rancidity in UHT and pasteurized milk during 8 and 3 days with five different SnO2 thin films, prepared using sol–gel technology. The claim is, the sensors could distinguish between both types of milk as well as determine the degree of rancidity of milks. Similar results have been reported by Labreche et al.with an E-nose containing 18 MOS sensors.

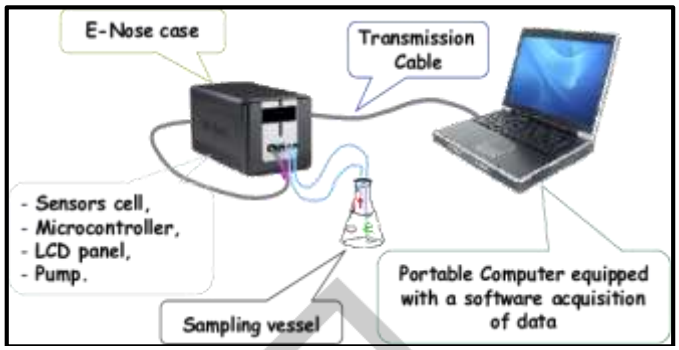

Figure 11(B): E-Nose

(e) E-Tongue:

Electronic tongues or test sensors has become an interesting tool to detect milk adulteration. It collects information by an array of sensor and can classify the milk providing the information whether it is consumable or not. Electronic tongues can be of potentiometric or voltametric.

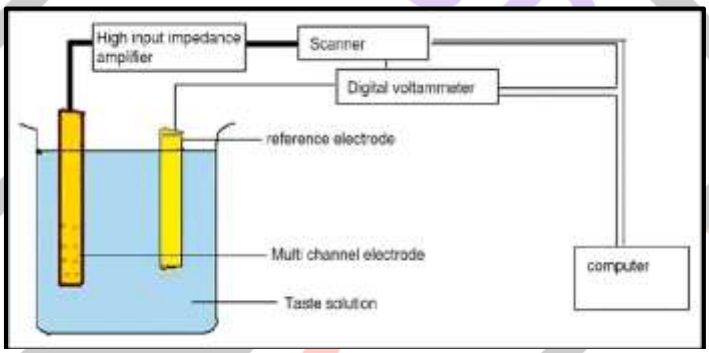

Figure 12(A): E-Tongue

Thiago et al. have reported the manufacturing of the sensors of the array using film of Prussian Blue (PB). The sensing mode is voltametric in which current is measured by varying the potential. The E-tongue has been used to detect hydrogen peroxide and fat content of the milk.
Dias et al. and laura et al. have reported an electronic tongue with 36 cross-sensibility sensor to detect goat milk adulteration with bovine milk. The system constitutes of solid-state potentiometric sensors (polymeric mixtures are applied on solid conducting silver-epoxy supports) along with the linear discriminant data analyser.

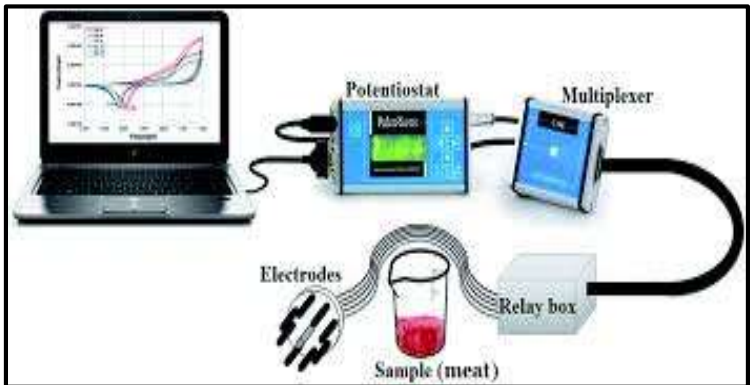

Figure 12(B): E-Tongue

(f) Capacitance Growth Curve to Detect the Development of Micro Organism in Raw Milk:

Felice et al. has reported the change of interface capacitance of the sensor with the evaluation of milk bacterial content. According to the paper it has shorter detection time and can capture bigger growth variations.

(g) Piezoelectric Sensor:

Jiali et al. has reported determination of bacteria counts in fresh milk in real time using piezoelectric transducer. The detection system consists of cell for detection, oscillator, frequency counter and computer where self developed software is installed to capture the transducer response with the change of culture media during bacteria growth. The transducer could acquire sufficient data rapidly and enabled real-time monitoring of bacteria growth.

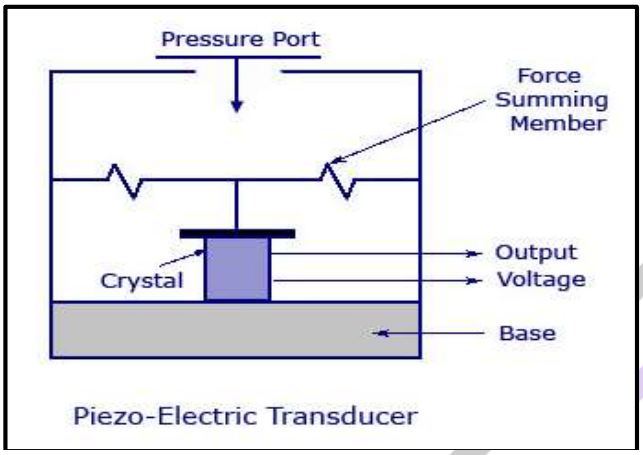

Figure 13(A): Piezoelectric Sensor

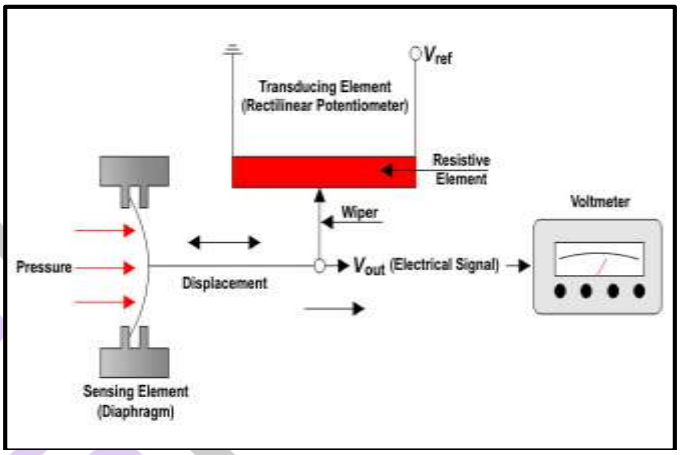

Figure 13(B): Piezoelectric Sensor

(h) Impedance Probe:

Milk containing bacteria convert lactose into lactic acid over the time. This in turn changes the electrical parameter of the milk. Conductivity is an easy parameter to detect bacteria but it is strongly infiuenced by fat content of the milk and primarily used for detection of mastitis.

Fourie et al. has presented stainless steel rod-based impedance probe for bacterial content measurement of milk. It has been described that the probe must be thoroughly cleaned after testing and the measurement is dominated by the impedance of the electrode-electrolyte interface. The stainless-steel rod has the advantage of cleaning over copper and brass probes. The sensor has been integrated with microcontroller circuit for automatic measurement and data storage.

Figure 14(A): Impedance Probe

Bishop et al. have described a impedimetric method to determine the shelf-life of pasteurized whole milk. They have considered 100 samples of pasteurized whole milk and studied different parameters such as organoleptic evaluation, standard plate count (SPC), psychrotrophic bacteria count (PBC), modified psychrotrophic bacteria count (mPBC), Moseley test (MSPC), and impedance detection time (IDT) at 18 and 21 degrees centigrade. The correlation between the shelf life and the direct count methods are not adequate to make any prediction. Moseley test (MSPC) apart from impedance method possesses significant relationships to shelf-life. And it has been claimed that impedance method has advantages over the Moseley test as it is better predictor of shelf-life, it is less labor intensive, and requires only 1–2 days, as opposed to 7-9 days to complete.

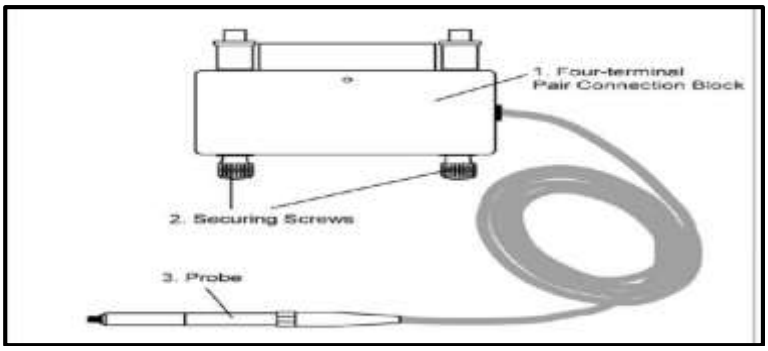

Figure 14(B): Impedance Prob

Karabi et al.has reported a constant phase element (CPE) sensor for detecting bacterial growth in milk. The bacterial growth changes the ionic property of the medium hence, the conductivity of the milk changes. A constant phase element (CPE) based sensor also has been reported by the authors to detect milk adulteration. A CPE essentially has the impedance whose phase angle remains constant over a wide range of frequencies. It has been observed that the constant phase angle (CPA) changes with the change of physical property of the medium (e.g., ionic concentration). And this property of the CPE can be used for sensing purpose. This means the phase angle of the CPE will be different for pure milk and the milk with different ionic concentration or with some impurity. An electronic circuit will measure the phase angle change and give the output in electrical signal.

6. Conclusion:

Adulteration in milk is normally present in its most crude form. Milk is the nutrient rich food for not only the young one's of animals but also for human babies. It is the major food for infants/ babies up to the occurrence of milk teeth, so that they can start feeding on solid food too. But adulteration of milk and milk products now-a-days is a major issue to think on, because it effects not only the health of baby and mother but also effects the mental condition of the young ones who are responsible for making up of tomorrow's generation.

Consumption of adulterated milk may lead to serious human health issues due to adverse effects of chemicals. Hypertension, renal diseases, skin, eye, heart problem and cancer are some of the common disease caused by consuming adulterated milk.

Hence detection of adulterants in milk is compulsory. Though several methods are available for the detection of adulteration of milk, due to increase in adulteration by means of several external agents, because of various reasons like increase in population growth detection of milk adulterants has become complicated now-a-days. Thus, the challenge is to develop simple and cost-effective techniques for detecting adulteration in milk, which could be used with high degree of repeatability.

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