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Adulteration of milk and its detection: A review

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Abstract
Milk adulteration is a global concern and social problem. It exists both in the backward and advanced countries. Developing countries are at higher risk due to lack of monitoring and policies. Consumption of adulterated milk causes serious health hazards leads to fatal diseases and a great concern to the food industry. Increased demand, growth in competition in dairy industry and financial gain makes the some producers to adulterate the milk. This paper presents a detailed review of common milk adulterants as well as different methods to detect the adulterants and their health hazards.

Keywords: Milk Adulteration, Detection Techniques, Electrical methods, Health Hazards

Introduction
Milk is considered to be the 'ideal food' because it is having abundant nutrients required for both infants and adults. It supplies body building proteins, bone forming minerals, health giving vitamins, minerals and furnishes energy giving lactose, milk fat and also supplying certain essential fatty acids. Milk adulteration came into global concern after breakthrough of melamine contamination in Chinese infant milk products. Unfortunately milk is being very easily adulterated throughout the world and significantly worse in developing and underdeveloped countries due to the absence of adequate monitoring and lack of proper law enforcement. Apart from the ethical and economical issue, it also creates health hazards. Most of the times, the adulteration is intentional to make greater profit, but sometimes it may be due to the lack of proper awareness. Possible reasons behind it may include- demand and supply gap, perishable nature of milk, low purchasing capability of customer and lack of suitable detection tests (Kamthania *et al.,* 2014**) [9].** Chemical adulterants are used for different purposes. The common adulterants are sugar, water, salt, starch, chlorine, hydrated lime, sodium carbonate, formalin, ammonium sulphate, H_2O_2 and non-milk proteins etc. To meet the deficit of milk, some people are preparing synthetic milk by mixing urea, caustic soda, refined oil and common detergents which has poisonous effect. Hence, the current review highlights the milk adulterants, their detection and their hazards on health of consumers.

Typical Adulterants and their health hazards on humans
A) Water
Water is the most common adulterant added to increase the volume of milk which in turn decreases the nutritive value of milk. But if contaminated water is added to milk, it is a serious health concern to the milk consuming community.

B) Melamine
Melamine is added to milk and milk powder to increase protein content falsely. It causes renal failure and deaths in extreme cases (Cheng *et al.,* 2010) [2].

C) Urea
Urea is added to milk to provide whiteness, increase the consistency of milk, increase non-protein nitrogen content and for levelling the contents of solid-not-fat (SNF) as are present in natural milk. Urea is also used to prepare synthetic milk. Health hazards associated are acidity, indigestion, ulcers and cancers. Urea is harmful to heart, liver especially for kidneys as the kidneys have to do more work to remove urea from the body (Kandpal *et al*., 2012) [10]. Ammonia in milk develops regression, loss of acquired speech and sensory disturbances.

D) Detergents
Detergents are added to emulsify and dissolve the oil in water giving a frothy solution, the characteristic white colour of milk (Singuluri & Sukumaran, 2014) [18]. They enhance the cosmetic nature of milk. Detergents cause gastro – intestinal complications.

E) Hydrogen peroxide (H_2O_2)
Hydrogen Peroxide is added to milk to prolong its freshness, but peroxides damages the gastro intestinal cells which can lead to gastritis and inflammation of the intestine. H_2O_2 disturbs the antioxidants in the body disturbing the natural immunity hence increasing aging.

F) Starch
Starch is used to increase solid-not-fat (SNF) and if high amounts of starch are added to milk, this can cause diarrhoea due to the effects of undigested starch in colon. Its accumulation in the body may prove very fatal for diabetic patients (Singuluri & Sukumaran, 2014) [18]. Apart from the starch, wheat flour, arrowroot, rice flours are also added.

G) Sugar
Generally sugar is mixed in the milk to increase the solids not fat content of milk *i.e.* to increase the lactometer reading of milk, which was already diluted with water.

G) Neutralizers
NaOH is often used in synthetic milk to neutralize the acidic effect. Synthetic milk is a common problem in India which is prepared by adding urea, caustic soda, refined oil and common detergents. Caustic soda contains sodium, acts as slow poison for those suffering from hypertension and heart ailments. Caustic soda deprives the body from utilizing lysine, an essential amino acid in milk, which is required by growing babies. Such artificial milk is danger for all, but is more harmful for pregnant women. As a substitute of milk fat, refined oil is mixed; and to dissolve the oil in water and to give a frothy solution, detergents are used. Carbonates and bicarbonates are added to milk which can cause disruption in hormone signalling that regulate development and reproduction (Manual of Methods of Analysis of Foods: Milk and Milk Products 2005) [12]. Carbonates in milk produce gastrointestinal problems including gastric ulcer, diarrhoea, colon ulcer and electrolytes disturbance.

H) Chlorine
Chlorine is added to compensate the density of the diluted milk after addition of water. Chlorinated milk can cause clogging in arteries and develop heart problem (Hattersley, 2000) [7]. Chloride in the milk disturbs the acid base balance in the body and also blood pH.

I) Antibiotics
Antibiotics are used mainly to treat a variety of diseases and 80% of veterinarians use antibiotics for treatment of mastitis disease. These antibiotics in the form of antimicrobial residues are found in milk. Presence of tetracycline, aromatic amines, gentamicin residue after mastitis treatment, neomycin residues, sulfamethazine residues, chloramphenicol residues, aflatoxin M1 contamination etc. are also a deep concern as milk adulterants (Das *et al*., 2016) [3]. Intramammary infusion of antibiotics for mastitis therapy is a major reason for milk contamination. Residues of these drugs in milk poses serious health hazards such as allergic reactions, increase in the number of antibiotic resistant, interference in intestinal flora and some of them (such as sulfamethazine residues) may have carcinogenic properties. It may also cause tissue damage. It interferes in the bacterial fermentation process which produces important losses in fermented products. Very low amount of penicillin residue is a potential cause of urticaria (Das *et al.*, 2016) [3].

J) Food colours
Many food colorants are also added to improve the appearance and have hazardous effects on health.

K) Milk powder
Sometimes milk powder as an adulterant is added in fresh milk. This is done for economic advantage when a country has milk powder in excess or subsidy is provided for dried powder milk (Guan *et al*., 2005) [4].

L) Non-Milk Proteins & fats
Milk, milk powder and other dairy products are often adulterated by low priced non-milk proteins such as soy, pea and soluble wheat proteins. Bovine rennet whey powder is sometimes mixed in milk powder (Haasnoot *et al*., 2006) [5]. Sometimes milk fat is replaced by fat from other sources which may also pose a risk to human health. 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 (e.g., sunflower oil) (Jha and Matsuoka 2004) [8].

M) Low valued Milk
Milk is adulterated by mixing lower valued milk to the higher valued milk. For example often goat milk is adulterated with cow milk for greater profit. It has been found that health hazards related to this practice are not well defined (some may have allergy in cow milk) but from commercial and ethical point of view this is a great concern in food industry (Das *et al.*, 2016) [3]. Low priced cow milk is often added in the milk of ewes, goats, buffalos (Haasnoot *et al*., 2004) [6] and in sheep milk (Pappas *et al*., 2008) [13].

N) Preservatives
Development of micro organism spoils the milk and spoiled milk is not good for health. Boric acid, Formalin, Sodium carbonate (Na_2CO_3), Sodium bicarbonate ($NaHCO_3$), Salicylic acid, Benzoic acid, Sodium azides can preserve the milk for long time and has poisonous effect which can lead to death (Poisonous milk for pupils;1905) [14]. It develops abdominal pain, diarrhoea, vomitions and other poison related symptoms.

O) Whey
Addition of liquid-whey is a very common practice to increase the volume of milk. Low priced rennet whey is sometimes mixed with liquid milk and milk powder. Whey, rennet whey solid are often found in milk (Recio *et al*.; 2000) [19]. Addition of rennet whey solid in UHT milk causes lowering of blood pressure. Some businessmen, for greater profit, use cheap muriatic acid to prepare whey which causes serious health problem.

P) Pesticides
Pesticides are also used to kill the microorganisms present in milk and to resist its further growth or in other words it is

used to preserve milk. Their presence in milk poses serious health hazards due to its toxicity or carcinogenicity.

Detection of milk adulteratants: Various milk adultrants and the method used to detect those adulterants are presented in table 1.

Table 1: Different milk adulterants and the method used to detect those adulterants.

S. No	Type of Adulterant	Method used to detect adulterant
1	**Chlorine**	Sequential Injection Analysis (SIA) Flow Injection Analysis (FIA) Potentiometric detection Conductometric sequential injection analysis
2	**Antibiotics**	Electrical conductivity BRT Test (Test kit) Spot Test Penzyme milk test, SNAP test and LACTEK test (kits) Chromatography (HPLC) Liquid chromatography mass spectrometry Somatic cell count (SCC) Screening test Biosensor assay based on surface plasmon resonance (SPR) E-Nose
3	**Non-milk proteins**	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
4	**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
5	**Milk powder**	FAST (Fluorescence of advanced maillard products and soluble tryptophan)
6	**Color**	Color Capillary electrophoresis
7	**Preservatives**	Conductivity Impedance Capacitance Piezoelectric transducer Impedimetric E-Tongue Thermoacoustic analysis Rosalic acid test
8	**Neutralizers**	Conductivity or pH measurement
9	**Urea**	Potentiometric biosensor pH measurement Durable NH+ 4 sensitive CHEMFET based sensor pH sensitive field effect transistor Manometric biosensor Ion selective electrode Calorimetric method Biosensors
10	**Whey/Liquid whey**	Reverse phase HPLC method Capillary Electrophoresis ELISA Fourth derivative spectroscopy Blot immunoassay method Phosphor partition NIR spectroscopy Immunochromatographic assay
11	**Water**	Frequency admittance measurements E-nose Electrical conductivity Ultrasonic transmitter receiver system NIR measurement Freezing point osmometry and freezing point cryoscopic method

Source: Das S, Goswami B and Biswass K 2016. Milk Adulteration and Detection" A Review. *Sensor Letters,* 14:4-18 [3].

Table 2: Different electrical methods to detect milk adulteration.

S. No.	Electrical Method
1	Potentiometric Sensors
2	Conductance measurement
3	Electrical Conductivity method
4	Ultrasonic Detectors
5	E-nose
6	E-tongue
7	Capacitance growth curve
8	Piezoelectric sensor
9	Impedance probe

Source: Das S, Goswami B and Biswass K 2016. Milk Adulteration and Detection" A Review. *Sensor Letters*, 14:4-18 [3].

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

Adulterant	Procedure	Observation	Limit of detection	Reference
Sugar	Take 10 ml milk sample in a test tube. Add 5 ml conc. HCl and 0.1 g resorcinol. Place the test tube in water bath for 5 min.	Appearance of red color indicates the presence of added sugar.	0.2% (w/v)	(Sharma *et al*. 2012) [15] (Kamthania *et al*. 2014) [9] (Singh *et al*. 2012)
Starch	Take 3 ml milk sample in a test tube. After boiling it thoroughly, cool it to room temperature. Add 2 to 3 drops of 1% iodine solution.	Appearance of blue color indicates the presence of starch.	0.02% (w/v)	(Sharma *et al*. 2012) [15] (Singh *et al*. 2012) [17] (Kumar *et al*. 1998) [11]
Glucose	Take 1 ml of milk sample in a test tube. Add 1 ml of modified Barford's reagent. Heat the mixture for exact 3 min in a boiling water bath. Rapidly cool under tap water. Add 1ml of phospomolybdic acid and shake well.	Immediate appearance of deep blue color indicates the presence of glucose.	0.1% (w/v)	(Sharma *et al*. 2011) [16]
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)	(Sharma *et al*. 2012) [15]
Buffalo milk	Dilute the milk 1/10. Put a drop of diluted milk on the centre of a glass slide. Now place a drop 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.		(Kamthania *et al*. 2014) [9] (Singh *et al*. 2012) [17]
Skim milk powder	Add nitric acid drop by drop in to the test milk sample.	The development of orange colour, it indicates the milk is adulterated with skim milk powder. Samples without skim milk powder shows yellow colour.		Singh *et al*., 2012 [17]

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

Adulterant	Procedure	Observation	Limit of detection	Reference
H_2O_2	Take 10 ml of sample and add 2 drops of paraphenylene diamine hydrochloride solution.	Intense blue color indicates presence of H_2O_2 in milk	0.025%	(Singh *et al*. 2012) [17] (Kamthania *et al*. 2014) [9] Sharma *et al*. 2012) [15]
Formalin	Take 10 m milk sample. Add 5 m conc. sulphuric 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.	0.1%	(Singh *et al*. 2012) [17] (Kamthania *et al*. 2014) [9]
Borax and boric acid	Take 5 ml milk sample. Add 1 ml conc. HCl. 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.		(Singh *et al*. 2012) [17]
Neutralizers	Take 5 ml of milk in a test tube and add 5 ml alcohol followed by 4-5 drops of rosalic acid	If the colour of milk changes to pinkish red, then it is positive for sodium carbonate /bicarbonates		(Singh *et al*. 2012) [17]
Urea	Take 5 ml milk sample in a test tube. Add 5 ml p-Dimethyl Amino Benzaldehyde reagent.	Appearance of distinct yellow color indicates presence of added urea	0.2% (w/v)	(Singh *et al*. 2012) [17]

Ammonium Sulphate	5 ml of hot milk is taken in a test tube and added with a suitable acid for e.g. citric acid and the whey thus separated is filtered. Collect the whey in another test tube and add 0.5 ml of 5% barium chloride.	Appearance of precipitate indicates the presence of ammonium sulphate in milk.	0.05% (w/v)	(Singh *et al*. 2012) [17]
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 diphenylbenzidine reagent.	Appearance of blue colour indicates the presence of nitrates. Pure milk sample will not develop any color.	0.2%	(Sharma *et al*. 2011) [16]
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.		(Singh *et al*. 2012) [17]
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.		(Singh *et al*. 2012) [17] (Kamthania *et al*. 2014) [9]
Detergents	Take 5 ml of milk in a test tube and add 0.1 ml of bromocresol purple solution.	Appearance of violet colour indicates the presence of detergent.		(Singh *et al*. 2012) [17]
Coloring matter	Take 10 mL milk sample in attest tube. Add 10 ml diethyl ether. After shaking, allow it to stand.	Appearance of yellow color in ethereal layer indicates the presence of added color.		(Batis *et al*. 1981) [1]

Conclusion

On the basis of above review, we can conclude that, the milk adulteration is becoming serious problem. Although financial gain is considered to be one of the major reasons for milk adulteration, inadequate supply for the increasing population all over the world has paved the ground for this as well. About 68% milk delivered to consumer is not as per standards. Consumption of lower quality adulterated milk may lead to serious human health issues. Hence it is important to have an efficient and reliable quality control system that will regularly monitor, combined efforts from scientific communities and the regulatory authorities. The human and technology interface, awareness and access to information can play vital role in irradication of the milk adulteration.

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