

REVIEW ARTICLE

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# Common milk adulteration and their detection techniques

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## Abstract

Food adulteration is a global concern and developing countries are at higher risk associated with it due to lack of monitoring and policies. However, this is one of the most common phenomena that has been overlooked in many countries. Unfortunately, in contrast to common belief, milk adulterants can pose serious health hazards leading to fatal diseases. This paper presents a detailed review of common milk adulterants as well as different methods to detect the adulterants both qualitatively and quantitatively. This study is organized to be an 'adulterant based' study instead of 'techniques based' one, where qualitative detection for most of the common adulterants are enlisted and quantitative detection methods are limited to few major adulterants of milk. Apart from regular techniques, recent development in these detection techniques have also been reported. Nowadays milk is being adulterated in more sophisticated ways that demands for cutting edge research for the detection of the adulterants. This review intends to contribute towards the common knowledge base regarding possible milk adulterants and their detection techniques.

**Keyword:** Milk processing, Adulteration, Product safety, Dairy, ELISA

## Introduction

Milk and dairy product adulteration came into global concern after breakthrough of melamine contamination in Chinese infant milk products in 2008 (Xin & Stone, 2008). However, history of milk adulteration is very old. Swill milk scandal has been reported in 1850 which killed 8000 infants in New York alone (How we poison our children 1858). Milk is considered to be the 'ideal food' because of its abundant nutrients required by both infants and adults. It is one of the best sources for protein, fat, carbohydrate, vitamin and minerals. Unfortunately milk is being very easily adulterated throughout the world. 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). The motivation for food fraud is economic, but the impact is a real public health concern (Ellis, Brewster, Dunn, Allwood, Golovanov, & Goodacre, 2012; Singh & Gandhi, 2015). The situation is significantly worse in developing and underdeveloped countries due to the absence of adequate monitoring and lack of proper law

enforcement. Qualitative detection of adulterants in milk can be easily performed with chemical reactions while quantitative detections are complex and diverse. Type of quantitative detection techniques depend on the nature of adulterants in milk. For example, LC (Liquid Chromatography) and ELISA (Enzyme Linked Immunosorbent Assay) are the most common techniques used to detect foreign protein; PCR (Polymerase Chain Reaction) and PAGE (Polyacrylamide Gel Electrophoresis) are usually used to detect milk from different species as adulterants in milk of a particular species. Milk adulteration detection techniques need to be very specific and rapid, because defrauders have escaped condemnation claiming less effectiveness of the conventional detection techniques (Garcia, Sanvido, Saraiva, Zacca, Cosso, & Eberlin, 2012).

## Typical adulterants in milk

Milk powder is the second most likely food item being in the risk of adulteration after olive oil (Moore et al. 2012). Adulterants in milk mainly include addition of vegetable protein, milk from different species, addition of whey and watering which are known as economically motivated adulteration (Fischer, Schilter, Tritscher, & Stadler, 2011;

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Singh & Gandhi, 2015). These adulterations do not pose any severe health risk. However, some adulterants are too harmful to be overlooked. 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). Urea, being a natural constituent of raw milk, has a maximum limit imposed by FSSAI (Food Safety and Standards Authority of India) Act 2006 and PFA (Prevention of Food Adulteration) Rules 1955 which is to be 70 mg/100 ml. Commercial urea is added to milk to increase non-protein nitrogen content (Sharma et al. 2012). Similarly, melamine is added to increase protein content falsely (Liu et al. 2012). 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 (Singh & Gandhi, 2015). 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 (Singuluri & Sukumaran, 2014).

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 (Domingo, Tirelli, Nunes, Guerreiro, & Pinto, 2014). 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 (Singuluri & Sukumaran, 2014). Urea in milk overburdens the kidneys as they have to filter out more urea content from the body (Kandpal, Srivastava, & Negi, 2012). In addition, carbonate and bicarbonates might cause disruption in hormone signaling that regulate development and reproduction (Manual of Methods of Analysis of Foods: Milk and Milk Products 2005).

### **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 1. 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 2 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 3.

### **Quantitative detection methods**

#### **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 intolerance (Kolar et al. 1979). However, soy, wheat and almond proteins are labeled as allergens by FALCPA (Food Allergen Labeling and Consumer Protection) of 2004 (Scholl et al. 2014) while pea, rice, lupin and maize proteins are clinically recognized as allergens (Sanchez-Monge et al. 2004), (Satoh and Nakamura 2011), (Saz & Marina, 2007). The fact that production cost of soy milk is 70% lesser than normal milk and soya bean protein is much more cheaper than milk protein, incites manufacturers to adulterate milk with soy milk (May, Fomon, & Remigio, 1982), (Dawson, Morrill, Reddy, Minocha, & Ramsey, 1988). 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 (Sharma et al. 2012). NIR (Near Infra Red) spectroscopy has been used for detecting milk powder adulteration with vegetable protein (Maraboli, Cattaneo, & Giangiacomo, 2002). Adulteration of pasteurized or UHT (Ultra High Temperature) milk powders with soy, pea, and wheat proteins have been reported. ELISA has been used to detect these proteins with polyclonal antibodies (Sanchez, Perez, Puyol, Calvo, & Brett, 2002). Skimmed milk powder adulterated with soy, pea, brown rice and hydrolyzed wheat protein has been successfully isolated using UHPLC (Ultra High Performance Liquid Chromatography) (Jablonski et al. 2014). Mass spectroscopy

**Table 1** Rapid qualitative detection of different edible adulterants in milk

Adulterant	Procedure	Observation	Limit of detection (w/v) (R. Sharma, Rajput, Barui, & N., 2012)	References
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 the presence of added sugar.	0.2% (w/v)	(Sharma et al. 2012); (Kamthania et al. 2014); (Arvind Singh et al. 2012)
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 the presence of starch.	0.02% (w/v)	(Sharma et al. 2012); (Arvind Singh et al. 2012), (Kumar et al. 1998)
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 reagent to the turbid solution.	Immediate appearance of deep blue color indicates the presence of glucose.	0.1% (w/v)	(Sharma et al. 2011)
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)
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); (Arvind Singh et al. 2012)

based techniques to identify milk protein structures have been reported in (Siciliano, Rega, Amoresano, & Pucci, 2000). Selectivity of tetraborate-EDTA buffer for extracting plant proteins from milk has been used to develop a rapid turbidimetric detection system to detect insoluble plant protein (Scholl et al. 2014).

#### 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 (Recio, Perez-Rodríguez, Ramos, & Amigo, 1997). 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 (Bakircioglu, Kurtulus, & Ucar, 2011; Brescia, Caldarola, Buccolieri, Dell'Atti, & Sacco, 2003). Cow milk adulteration in caprine milk has been quantified by HPLC/ESI-MS (High Performance Liquid Chromatography/Electrospray Ionization- Mass Spectrometry) (Chen et al. 2004). This method identifies molecular masses to differentiate between proteins in the milk of cow and goat. Detection of addition of cow milk to goat and ewe milk has been

described by (Abrantes et al. 2014; Romero, Perez-Andújar, Olmedo, & Jiménez, 1996; Song, Xue, & Han, 2011). 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) (Ferreira & Cacote, 2003; Guerreiro, Barros, Fernandes, Pires, & Bardsley, 2013), high resolution melting (HRM) based method utilizing specific mitochondrial primers (Ganopoulos, Sakaridis, Argiriou, Madesis, & Tsafaris, 2013) and solid-phase microextraction-mass spectrometry method (SPME-MS) based on volatile profile (Majcher, Kaczmarek, Klensporf-Pawlik, Pikul, & Jeleń, 2015). Indirect competitive ELISA has been used to detect cow milk adulteration in goat, sheep and buffalo milk (Hurley et al. 2004). Adulteration of caprine milk with cow milk has also been detected using PCR (Bania et al. 2001). In addition, PCR has been used to detect cow milk in ewe milk (López-Calleja et al. 2004), goat cheese (Maudet & Taberlet, 2001) and in buffalo mozzarella cheese (Bottero, Civera, Anastasio, Turi, & Rosati, 2002). An electronic tongue that is capable to recognize 5 basic taste standards has been used to detect caprine milk adulteration in bovine milk (Dias, Peres, Veloso, Reis, Vilas-Boas, & Machado,

**Table 2** Rapid qualitative detection of different hazardous chemicals in milk

Adulterant	Procedure	Observation	Limit of detection (v/v) (Sharma et al. 2012)	References
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%	(Arvind Singh et al. 2012); (Kamthania et al. 2014); (Sharma et al. 2012)
	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%	(Sharma et al. 2012)
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.		(Arvind Singh et al. 2012); (Kamthania et al. 2014)
	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%	(Sharma et al. 2012)
	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%	(Sharma et al. 2012)
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	A bluish colour forms immediately, which turns deep blue afterward. Pure milk shows salmon pink colour which gradually changes to bluish after 2 hours.		(Kumar et al. 2002)
	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.	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)	(Sharma et al. 2012)
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.	A characteristic blue or bluish green colour develops in presence of added urea whereas pure milk remains colourless.		(Meisel 1995)
	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%)	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.		(Sharma et al. 1993); (Arvind Singh et al. 2012),
	C. 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 whereas formation of slight yellow color indicates natural urea in milk.	0.2% (w/v)	(Sharma et al. 1993); (Arvind Singh et al. 2012); (Bector et al. 1998); (Kavita, 2000)
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)

**Table 2** Rapid qualitative detection of different hazardous chemicals in milk (*Continued*)

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.	(Arvind Singh et al. 2012)
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.	(Arvind Singh et al. 2012)

2009). This is an alternative method to classical analytical methods. PAGE has been employed to analyze the individual protein group (Strange, Malin, Van Hekken, & Basch, 1992). Addition of bovine milk in ewe yoghurt (Kaminarides & Koukiassa, 2002) and goat milk (Tamime, Barclay, Law, Leaver, Anifantakis, & O'connor, 1999) have been quantified with PAGE too. Using IES (Isoelectric Focusing) instead of PAGE is more advantageous in these applications (Borková & Snášelová, 2005). Immunochemical and DNA based methods have been combined as PCR- LCR- EIA (Polymerase Chain Reaction - Ligase Chain Reaction- Enzyme Immunoassay) to detect one specific nucleotide present in bovine milk as adulterant in ewe, goat and buffalo milk (Klotz & Einspanier, 2001). Hydrophobic interactive chromatography is used for separation and determination of different caseins in bovine, ewe and goat milk (Bramanti et al. 2003).

### 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 (Lawley, 2013). This was later adopted by United Nation's food standard body, Codex Alimentarius Commission through a new rulings in 2010 (Domingo, Tirelli, Nunes, Guerreiro, & Pinto, 2014; FAO, 2010; B. Liu, Lin, & Li, 2010). Melamine is not only added to milk powder as adulterant, but also in many other foods like wheat gluten, chicken feed, and processed foods (Ingelfinger, 2008; Lin et al. 2008). Though it is not carcinogenic, it causes renal failure and infant death in

**Table 3** Rapid qualitative detection of different mixed adulterants in milk

Adulterant	Procedure	Observation	Limit of detection (v/v) (Sharma et al. 2012)	References
Detergent	A. Take 5 ml in a test tube and add 0.1 ml 0.5% Bromocresol Purple (BCP) solution.	Appearance of violet colour indicates the presence of detergent. Unadulterated milk shows faint violet color.		(Singhal, 1980); (Arvind Singh et al. 2012)
	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.	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%	(Rajput, Sharma, & Kaur)
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.		(Arvind Singh et al. 2012); (Kamthania et al. 2014); (Ghodekar 1974)
Coloring matter	A. 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)
	B. Make the milk sample alkaline with sodium bicarbonate. Dip a strip of filter paper for 2 hours.	Appearance of red color on filter paper indicates the presence of annatto. Treatment of this paper with stannous chloride gives pink color.		(Lechner and Klostermeyer 1981)
	C. Add a few drops of hydrochloric acid to milk sample.	Appearance of pink color indicates azo dyes.		(DE Souza et al. 2000)

extreme cases (Cheng et al. 2010) Quantitatively melamine detection has been possible using SERS (Surface Enhanced Raman Spectroscopy) (Zhang, Zou, Qi, Liu, Zhu, & Zhao, 2010). A portable sensor based on SERS has been also developed to detect melamine instantly (Kim, Barcelo, Williams, & Li, 2012). SB-ATR FTIR (Single Bounce Attenuated Total Reflectance - Fourier transform infrared spectroscopy) has been used to quantify melamine in both liquid and powder milk (Jawaid et al. 2013). Different types of mass spectroscopy have been employed to detect melamine in milk products including LC-MS/MS, APCI-MS (Atmospheric Pressure Chemical Ionization-Mass Spectroscopy) and EESI-MS (Extractive Electrospray Ionization Mass Spectrometry) (Yang et al. 2009; Zhu et al. 2009). LC-MS/MS has been used frequently to detect melamine in milk and variety of infant formulas (Chang & Arora 2008; Guelph 2008; Michael Smoker, 2008; Sherri et al. 2008). HPLC is another choice of technique to quantify melamine in milk and dairy products (Gopalakrishnan Venkatasami, 2010; Ruicheng Wei et al. 2009). Using Raman band at  $676\text{ cm}^{-1}$ , melamine in dried milk powder has been immediately detected without extracting melamine from milk (Okazaki et al. 2009). A portable screening system based on Laser Raman spectroscopy has been developed to quantify melamine (Cheng et al. 2010). Gold nanoparticles have been developed whose surface is grafted with melamine and cyanuric acid derivative. Upon binding to melamine these nanoparticles change color from red to blue instantly which can be used as an on-site melamine detection method (Ai, Liu, & Lu, 2009). Use of oxidized polycrystalline gold electrode has been reported to detect melamine, along with conventional approach like GC-MS (Tsai, Thiagarajan, & Chen, 2010; Veyrand et al. 2009). Recent developments in melamine detection have been discussed in (Liu et al. 2012).

#### **Milk adulteration with urea**

Urea, being a natural constituent of milk, constitutes the major portion of non-protein nitrogen in milk. According to FSSAI act 2006 and PFA rules 1955, maximum allowable limit for urea in milk is 70 mg/100 mL (Sharma et al. 2012). 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 (Khan et al. 2014). LC has been used to quantify urea as adulterant in milk (Dai et al. 2013). A method based on GC/IDMS (Gas Chromatography/Isotope Dilution Mass Spectrometry) has been used to quantify urea present in milk (Xinhua Dai et al. 2010). 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 (Czauderna &

Kowalczyk, 2009). A combination of kjeldahl and spectrophotometric method has been suggested to detect milk adulteration by melamine, urea and ammonium sulphate (Virginia de Lourdes et al. 2013). Six different detection methods of urea have been discussed with their advantages and disadvantages by (Banupriya et al. 2014). Infrared ray absorbs ammonia at a characteristic wavelength of 1530 nm – this principle is used in a PCB-integrated optical waveguide sensor that enables the detection of multiple analytes including ammonia (Bamiedakis et al. 2013). A gas sensor based on a FET (Field Effect Transistor) with a graphene channel and IL (Ionic Liquid) gate has been developed that can detect at least 30 ppm of ammonia and 4000 ppm of carbon dioxide at gate voltage below 1 V, which can be used to detect urea in milk (Inaba et al. 2013). An enzyme based pizo-electric sensor has been developed to detect urea in milk where a linear behavior was found between equivalent electrical signal and amount of ammonia gas present (Renny, Daniel, Krastanov, Zachariah, & Elizabeth, 2005). EISCAP (Electrolyte Insulator Semiconductor Capacitor), a potentiometric biosensor based on enzymatic reaction has been developed to detect urea (Indranil Basu et al. 2004). Some other biosensors based on various principles like manometry, enzyme, potentiometry have already been developed to detect urea in milk (Renny et al. 2014; Mishra et al. 2010; Jenkins and Delwiche 2002; Trivedi et al. 2009).

#### **Milk adulteration with other compounds**

NIR spectroscopy (1100-2500 nm) has been used to quantify water and whey in cow milk (Kasemsumran et al. 2007). In a comparative experimental study between NIR and MIR (Medium Infra Red) spectroscopy, Santos et al. (2013a) 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. Like urea, synthetic urine is also used in milk to increase the nitrogen content. In a case study performed in Brazil, urine was detected in all samples of UHT milk by following a chemometric approach (Souza et al. 2011). Presence of synthetic urine up to the concentration of 0.78 mg/L of milk can be identified by a method using infrared microspectroscopy and chemometric analysis (Santos et al. 2013b). Similar approach has been reported to be used in developing a portable and hand-held infrared spectrometer for milk analysis (Santos et al. 2013c). Presence of urine has also been reported by observing change in the concentration of sodium and calcium in samples undergoing flame atomic absorption spectroscopy (Santos et al. 2012). MALDI-QTOF MS (Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectroscopy) has been used to quantify vegetable oil in milk (Garcia, Sanvido,

Saraiva, Zacca, Cosso, & Eberlin, 2012). It has also been possible to detect multiple adulterants including ammonium sulfate, dicyandiamide, melamine and urea in milk powder using Raman chemical imaging (Qin et al. 2013). Milk fat adulteration is also a very common concern, however, several techniques have been developed to detect the adulteration based on Butyro Refractometer (Arora et al. 1996), fluorescence spectroscopy (Ntakatsane, Liu, & Zhou, 2013), derivative spectroscopy (Jirankalgikar & De, 2014) and Raman spectroscopy (Uysal, Boyaci, Genis, & Tamer, 2013).

## Conclusion

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. This problem is more acute in the developing and under developed countries due to lack of adequate monitoring and law enforcement. Existing common detection techniques are not always convenient and accessible in these countries making it difficult to address the diverse ways of fraudulent adulteration in milk. This calls for combined efforts from scientific communities and the regulatory authorities through the development, implementation and dissemination of better techniques for the detection of milk adulteration. In addition, awareness and access to information can play vital role in these regions to overcome this issue. Some of these easy detection methods at the consumer level and state of the art techniques at the authority level can bring this problem to an end for the victims, including millions of children in the developing countries.

## Abbreviations

EISCAP: Electrolyte insulator semiconductor capacitor; ELISA: Enzyme linked immunosorbent assay; FALCPA: Food allergen labeling and consumer protection; FSSAI: Food Safety and Standards Authority of India; FTIR: Fourier transform infrared spectroscopy; HPLC ESI-MS: High Performance liquid chromatography electrospray ionization mass spectroscopy; HPLC: High performance liquid chromatography; ICP-OES: Inductively coupled plasma emission spectroscopy; IES: Isoelectric focusing; MALDI-QTOF MS: Matrix-assisted laser desorption/ionization time of flight mass spectroscopy; MIR: Medium infra-red; MS: Mass spectroscopy; NIR: Near infra-red; PAGE: Polyacrylamide gel electrophoresis; PCR: Polymerase chain reaction; PCR-LCR-EIA: Polymerase chain reaction - ligase chain reaction- enzyme immunoassay; PDO: Protected denomination of origin; PFA: Prevention of food adulteration; RP-HPLC: Reverse phase high performance liquid chromatography; SB ATR: Single bounce attenuated total reflectance; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; SERS: Surface enhanced raman spectroscopy; SNF: Solid not fat; UHPLC: Ultra high performance liquid chromatography; UHT: Ultra high temperature

## Authors' contributions

SA contributed to conception and design of the study, TA performed all the literature study, both authors contributed in manuscript preparation. Both authors read and approved the final manuscript

## Competing interests

The authors declared that they have no competing interests.

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