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Review Article

Milk adulteration and emerging health issues in humans and animals (A review)

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ABSTRACT

Milk is a vital source of nutrients that are required for the proper growth, development, and functioning of the human body. Its adulteration is a global issue, mainly concerned in developing countries. Unfortunately, mixing milk with many toxic agents leads to various health issues in the consumers. Most of the time safety level and quality are hardly maintained. Milk adulteration is multi chain process which starts from animal owner, milk man, rural collection centers and finally to mega processing units. The main reason for the adulteration is to get maximum profit without taking in account the health of people due to poverty, lack of education and lack of law enforcement from basic to higher level. In this review, different adulterants added to the milk and the different emerging health issues due to the improper use of these adulterants have been discussed. Adulterants can be detected both qualitatively and quantitatively. These techniques are usually classified based on adulterants, as mostly used adulterants are detected by qualitative techniques and the limited major adulterants are detected by quantitative methods. This is need of time to create awareness among the community for timely stoppage of such immoral practices to avoid the alarming health issues. This review article would be helpful in creating awareness about the commonly used milk adulterants, their effects on human and animal health and the possible available ways for their detection particularly in the developing countries.

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Introduction

Milk is a complete diet which is full of nutrients and is produced by the mammals through their mammary glands. Milk is required for growth in infants, youngsters, and maintenance of health in adults. Milk could be a perfect food as it is readily digested and absorbed. It is the sole natural food for infants and youngsters (Azad and Ahmed 2016). It is chiefly a valuable source of fine quality protein, fat, carbohydrates, vitamins, and minerals (Afzal et al. 2011). Amino acids present in the proteins are required for the growth of infants and children. It is also necessary for the maintenance of tissues in adults. In short, milk is a complete package for health maintenance, if consumed properly. New Zealand, Germany and Netherlands are the largest exporters of milk products in world. In Pakistan, milk is the largest and the single most vital commodity within the livestock sector (Farooque 2017). Milk and milk products are consumed by almost six billion people worldwide (Moore et al. 2012). Its use has a great

importance because of the nutrients, energy and as a whole package of diet. It is the basic diet for the newborn child because their digestive system is not developed in the early stage of life and milk is the only diet that they can be digested. In China, "big head disease" scandal was reported in 2004. Infants were fed an unhealthy formula of milk that caused rapid weight loss from their bodies and head swelling thus called as "big head disease" (Xin and Stone 2008). It is a case of acute malnutrition, in which the consumer appears to be thin because of lack of flesh and prominence of more bony structures all over the body, comparative to the skull. Basically, the companies made bogus milk formula that contained almost negligible nutrients where protein almost counts for 1% that was not enough to fulfill the needs of children. A lot of children died because of this formula especially from the poor families that did not have enough knowledge regarding nutrition etc. (Xin and Stone 2008). Naturally PH of milk ranges from 6.4-6.8. Normally milk contains 30-35% of the proteins per liter of milk and 80% of which is comprises of "casein micelles". A mineral as chloride, potassium, citrate, sodium, calcium, magnesium and phosphate occurs in the concentration of round about 5-40 mM. Other than that milk also provides us many other vitamins that include vitamin A, D, E, K, B6, B12, C, biotin, niacin, riboflavin, thiamine, pantothenic acid and folates (Kamthania et al. 2014). Milk composition also includes lipids, sugars and carbohydrates. Out of which glucose, glactose, lactose and some other oligosaccharides are the main carbohydrates. The sweet taste of the milk is because of the lactose and it almost accounts for the 40% of calories in the whole milk (Singh and Gandhi 2015).

Adulteration means intentional removal and addition of superior and inferior substances respectively from the milk (Handford et al. 2016). This bad habit of playing with the lives of the people is just because of economic benefit (Lateef et al. 2009). They are risking the life of people and governments of especially developing countries are silent on this issue because of lack of knowledge and facilities to detect this adulteration (Javaid et al. 2009; Ramya et al. 2015; Handford et al. 2016; Memon et al. 2018).

Adulterants are the substances that are added to the milk that might include water, whey, powder milk, preservatives urea, vegetable oils and many other substances. These are added to enhance the taste, appearance and volume of the milk (Sharma et al. 2017). Some of the food adulterants are extremely dangerous and lead to the various diseases and hormonal disorders (Ingelfinger 2008; Zhang et al. 2009; Dhanashekar et al. 2012; Rahman et al. 2015). They also result in many other severe conditions and sometime death. So, it should be considered as a major issue and authorities should devise regulatory policies to protect the end consumers in the country. This problem mostly occurs in developing countries due to lack of facilities and policies (Afzal et al. 2011; Abbas et al. 2013; Faraz et al. 2013; Barham et al. 2014, 2015, 2017; Handford et al. 2016).

On industrial scale, if adulterants are required, then it is necessary to check the safe limit. Now days, unfortunately as the production and utilization supply gap is increasing, and due to other economic benefits, they are not considering these limits and are using the adulterants without any check and balance.

These kinds of practices are quite normal in developing countries. Such practices need to be stopped as soon as possible because they are playing with the health of the peoples.

The presence of adulterants in milk can be detected by using different reported procedures (Finete et al. 2013). We mostly check three parameters to detect the adulteration including SNF (Solid not fat) %, Freezing point and protein contents (Sharma et al. 2017).

Detection of milk adulterants is one of the most important tools that can be done by using different methods, such as LC (liquid chromatography), ELIZA (Enzyme linked immune sorbent Assay), Polymerase Chain Reaction (PCR) and Polyacrylamide Gel Electrophoresis (PAGE) etc. (Sanchez et al. 2002). ELISA and LC are mostly used to measure the foreign proteins in the milk. On the other hand, PAGE and PCR techniques can be used to check if there is mixing of milk from different species. These above four are rapid and reliable methods of detection of milk adulteration as compared to the conventional methods. Keeping in view, some conventional technical methods of detection that include Zacca, Cosso, Sanvido, Eberlin and Saraiva.

These conventional techniques are not so reliable due to that it is recommended to use ELISA, PAGE, PCR and LC techniques for the detection of the milk adulteration (Wei et al. 2009).

Adulteration

It is the intentional addition of some inferior substances within the food or the removal of some superior ingredient of any food (Rahman et al. 2015). When this action is applied to milk, it is called milk adulteration. It adversely affects the nature, quality, and taste of milk along with the health of the consumers. They are mainly hazardous to humans when used and risk the health of the consumers greatly and directly (Recio et al. 1997).

Adulterants

The substance of inferior quality being added to take advantage is named an adulterant. These adulterants when immorally mixed with milk and milk products become the source of various emerging diseases in society. The adulteration of milk is generally done in many ways, including mixing of water in milk, removal of fat, the addition of milk powder, reconstituted milk, thickening agents like starch, flour, glucose, urea, salt and chlorine (FDA 1995; Paradkar et al. 2000). The most commonly used milk adulterants used for the economic purpose includes milk from other animal species, water, whey, vegetable protein, milk powder, olive oil, etc. Preservatives like neutralizers usually consist of bicarbonate of soda, washing soda, sodium hydroxide and calcium hydrates. In rare cases, they may add animal fats and vegetable oils (Paradkar et al. 2000). Many are very harmful to health if added to the milk as boric acid, caustic acid, benzoic acid, urea, formalin, detergents, ammonium sulfate, salicylic acid, hydrogen peroxide, and melamine etc.

(Souza et al. 2011). There are various forms of adulteration of milk. We can classify the adulteration into two major categories: indirect adulteration and direct adulteration.

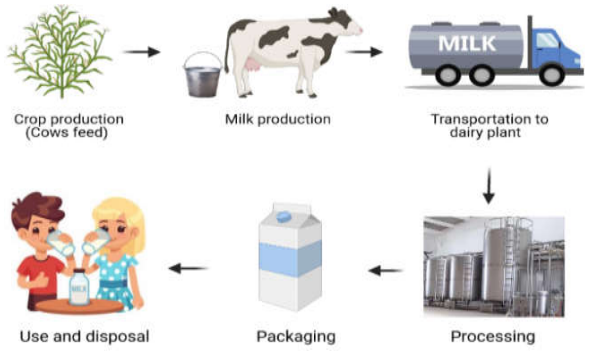

Fig. 1: The general diagrammatic description of stages involved in milk processing

Indirect adulteration includes the excessive use of fertilizers, pesticides to the crops and fodder to get maximum production in short duration. When these fodders are fed to the animals; it causes many healthproblems to the animal itself. Along with that some residues cross the blood-mammary barrier of animals and ultimately come in milk which cause several toxic problems in human and calf which includes hepatomegaly, nephropathy, intestinal villus atrophy, immunosuppression and ultimately one of leading cause of cancer (Rahman and Baxi, 1983).

There is also a cotton seed cake and meal which is fed to the animals which contains residues of pesticides and fertilizers because most of cotton seed cake and meal is produced by conventional methods (Souza et al. 2011). Along with that there is mixing of some poor-quality products in cotton seed meal and cake which ultimately affect the human health. There is also a confusion regarding the use of exogenous oxytocin before milking, that can increase milk production by 3% and its effect on milk plasma activity, fat, protein, somatic cell count and lactose are non-significant (Ballou et al. 1993; Bansode et al. 1996). A simultaneous study carried out at the Dairy Cattle Physiology Division of the National Dairy Research Institute (NDRI), Karnal, Haryana, India provided the same results (Sharma et al. 2012). It is considered that oxytocin residues come in milk which exert side effect on human body. In dairy practice, oxytocin is mostly used at high dosages to treat the irregular or incomplete milk ejection from the cows and the residues of oxytocin remain in the body mixed with the milk, that milk when consumed by the consumers leads to many reproductive disorders in females (Bruckmaier 2003). However, in the recent years, some studies have reported the considerable bad effects of oxytocin administration on the composition and quality of milk and reproductive health of milking animals, therefore, authors suggested to discourage the use of oxytocin in dairy sector (Faraz et al. 2020). The selection of desired nutritive package for animals, under the supervision of animal nutrition experts, is best scientific approach to increase the milk production and to get the desired milk composition according to the trend of consumers (Walker et al. 2004).

In direct adulteration method, people simply add the chemicals, water and preservatives like substances directly in the milk instead of doing changes in diets and style of animals in order to increase milk quantity (Corbel 2006).

Main reasons for milk adulteration

The problem of milk adulteration is found worldwide. The main reasons for milk adulteration have been discussed in the following sections.

1) Economic aspect

Mainly people add water to milk to increase its quantity and other items so that the product looks desirable to the buyer. The main purpose of this is the economic gain. They charge more but they are playing with the health of people for this very selfish reason. One of the leading causes in developing countries is the lack of knowledge and appropriate policies (Azad and Ahmed 2016).

2) Demand and supply gap

There is a huge gap between supply and demand regarding milk. Milk is a whole diet but in developing countries there is less supply of milk and there is a dire need to increase its demand for a huge population (Anderson 1889). So, they are taking advantage of people's needs by using low quality adulterants. Despite doing well by increasing quantity, they are just harming their health.

3) Lack of testing facilities

In developing countries, there is a lack of facilities to test milk adulterants. Such techniques are only available to some high authorities but not for a common person. If they are available then the prices would be very high, that everyone cannot afford. Lack of testing authorities leads to the spread of harmful diseases through adulterants (Poonia et al. 2017). The purpose and maximum limit of mostly used adulterants in milk are given below in the Table 1.

Health hazards due to milk adulteration

Water is the most commonly added adulterant in the milk, it not only reduces the nutritional value of the milk, but the contaminated water further can cause significant health-related problems. Excessive use of urea fertilizers as an adulterant in milk results in kidney exertion as they have to remove the extra amount of urea from the body (Kaplan et al. 2012). The presence of urea in the body is the source of many organ failures in the body. Its consumption also causes vomiting. Detergents and peroxides are also widely in use usually result in gastrointestinal complications. Detergents are extremely harmful when added as adulterants (Smith-Slatas et al. 2006). They contain sodium and are considered slow poison for those who are suffering from heart disease

and hypertension (Boukary et al. 2012). Melamine can cause renal failure and even death. Addition of starch results in diarrhea. Diarrhea is basically due to the excessive undigested starch in milk (Smith-Slatas et al. 2006). Starch solid milk paste can also cause stomach problems. Adulterants lead to fatal conditions in diabetic patients. Carbonates and bicarbonates mainly result in hormone disruption when added in milk immorally.

Table 1: Purpose and maximum limit of mostly used adulterants in milk

Adulterant	Objective	Limit	Reference
Urea	To increase the non-protein nitrogen content of the milk	<70mg/100ml	(Khan et al. 2015; Sharma et al. 2017)
Melamine	To increase the protein content of the milk	<2.5mg/kg for adults <1mg for infants	(Lawley 2013)
Ammonium Sulphate	To maintain the density of the diluted milk and increase the lactometer reading	0.79 g per liter	(Virginia de Lourdes et al. 2013)
Detergent	To emulsify the non-dairy fat	5-10 ml of sample	(Santos et al. 2013)
Vegetable oil	To replace the actual milk fat	1/s teaspoon	(Garcia et al. 2012)
Hydrogen peroxide, salicylic acid, Formalin and benzoic acid	To increase the shelf life of the prepared milk	0.5 ml per kg	(Qin et al. 2013)

A high level of alkaline is responsible for the damage of tissues and proteins in the body and causes the lethargic condition. Caustic soda addition is also very harmful. It damages the mucosa of food pipes mostly in children (Kaplan et al. 2012). Formalin if used as adulterant can be very harmful for liver damage. Carbonate in milk produces gastrointestinal problems including peptic ulcers, diarrhea, and colon ulcer, and electrolytes disturbance (Bamberg et al. 2007). The oxide disturbs the antioxidants within the body disturbing the innate immunity hence increasing aging. Chloride within the milk disturbs the acid-base balance within the body and also blood ph. Ammonia when added to the milk causes regression and disturbances in speech and sensory functions. Urea in milk can cause damage to the kidneys and liver. Kidneys are the organs used for the excretion of metabolic wastes and toxins. When urea is added to milk, kidneys are overburdens to filter more urea content than normal (Khan et al. 2014). Along with these carbonates and bicarbonates which are present in milk can alter the hormonal balance and can directly affect growth and reproduction. Headaches are common symptoms found in children from age 6 to 18 years. As well as Eyesight problems are also commonly reported in all age groups (Virginia de Lourdes et al. 2013).

Detection of milk adulterants

Different parameters are used to measure the milk quality, for example, solid not fat (SNF) percentage, freezing point and total protein. As the main reason of milk adulteration is to increase the above mentioned parameters in milk (Sharma et al. 2017). Milk starch, different salts, and urea are mixed to enhance the SNF content of the milk while melamine is used to enhance the contents of proteins in milk (Siciliano et al. 2000). The milk sugar which is lactose is increased by adding sugars in milk to give a milky and frothy appearance to milk along sweeten taste. On the other hand, detergents are added which emulsify the oils and fats added in milk (Smith-Slatas et al. 2006). Several methods are available for the detection of milk quality and adulterants which are going to be discussed in the following sections (Hurley et al. 2004; Jha and Matsuoka 2004; Borin et al. 2006; Kandepal et al. 2012; Faraz et al. 2013; Guerreiro et al. 2013; Jablonski et al. 2014; Melame et al. 2014)

Detection methods

The indirect adulteration can be detected by complete feed analysis. This is not economical for local farmer. Government institutions should play their role in this regard and prophylactic use of these chemicals should be checked by proper source. The detection of milk adulterants can be classified in the form of qualitative and quantitative detection methods.

A- Qualitative detection method

The qualitative methods used for the detection of milk adulterants are quite simple and easy. They require specific chemical reagents, and the results are interpreted manually by observing the color changes. For each type of milk adulterant, a specific qualitative test is performed. The main benefit of using these qualitative detection methods is that they are simple, rapid, and easy to perform. However, there are also some drawbacks of these qualitative methods as they are not so much precise and can only detect adulterants up to a certain concentration (Azad and Ahmed 2016).

The detection of sugar as an adulterant in milk can be detected qualitatively by adding 0.1g resorcinol solution and 1ml concentrated HCL to the milk sample and then placing the test tubes in water bath for five minutes. The resulting red color will confirm the occurrence of sugar in milk. The limit of detection in this qualitative test will be 0.2 % w/v (Kamthania et al. 2014; Sharma et al. 2017). For the detection of hydrogen peroxide, 2 drops of Paraphenylenediamine

hydrochloride solution are added in 10ml of milk sample. The emergence of intense blue color is the indication of hydrogen peroxide addition in milk up to 0.025% v/v (Arvind et al. 2012). According to Arvind et al. (2012), the adulteration of milk with urea can be detected by adding 5ml p-Dimethyl Amino Benzaldehyde reagent in a test tube containing a 5ml milk sample. The appearance of distinct yellow color is the indication for urea presence up to a limit of 0.2% w/v (Arvind et al. 2012). The emergence of violet color after adding 0.1ml of bromocresol purple solution in the milk sample will confirm the detergents in the milk (Arvind et al. 2012). Kumar et al. (2002) described a qualitative test to determine the presence of ammonium sulfate. According to Kumar et al. (2002), ammonium sulfate in milk can be detected by adding 0.5ml of 2% NaOH and sodium hypochlorite along with 0.5ml phenol (5%) in a test tube containing a 2ml milk sample. The immediate formation of bluish color in the test tube and gradually turning into dark blue color will confirm the ammonium sulfate addition (Kumar et al. 2002). As discussed earlier, formalin is added to the milk as a preservative to increase the shelf life of milk. To detect the presence of formalin in a milk sample, 5ml of concentrated sulphuric acid and a little amount of ferric chloride are added in a test tube containing 10ml of milk sample. If blue or violet color is noticed at the junctions of layers of these two liquids, the presence of added formalin is confirmed (Arvind et al. 2012). Melamine is also added in milk as an adulterant which can be detected qualitatively by Enzyme-Linked Immunosorbent Assay (ELISA) (Yin et al. 2010).

B- Quantitative detection method

The quantitative methods for adulterated milk detection are usually more complex and time-consuming but can provide accurate results. Quantitative methods like ELISA, Liquid Chromatography, Polyacrylamide Gel Electrophoresis (PAGE) and Polymerase Chain Reaction (PCR) are used to detect adulterants in milk. Unfortunately, these quantitative detection methods can detect some limited types of milk adulterants (Azad and Ahmed 2016).

The quantitative estimation of other chemical (hydrogen peroxide) in milk was determined through spectrophotometric analysis of milk samples (Amin and Oslon 1967). They precipitated the proteins in the milk sample by using trichloroacetic acid (70% w/v) and adding titanium salts. After measuring the sample on specific wavelengths using spectrophotometry, the standard curves were used for determining the levels of hydrogen peroxide in the milk sample (Amin and Oslon 1967). Medium Infra-Red (MIR) and Near Infra-Red (NIR) spectroscopy are also being used for quantitative detection of hydrogen peroxide and some other adulterants in milk. According to Santos et al. (2013), hydrogen peroxide can be measured in milk by using a specific portable spectrometer. According to their studies, MIR showed better performance for detecting hydrogen peroxide and other adulterants like urea, synthetic urine, water, and whey in milk (Santos et al. 2013).

According to a study (Khan et al. 2015), the presence of urea in milk can be determined directly by using NIR spectroscopy. Quantification of urea in milk has also been done by using liquid chromatography (Dai et al. 2012) and Gas Chromatography/Isotope Dilution Mass Spectrometry (Dai et al. 2010). Virginia de Lourdes et al. (2013) have reported that the melamine, urea, and ammonium sulfate adulteration in milk can be detected by using Kjeldahl and spectrophotometric methods in combination. A gas sensor (Inaba et al. 2013) and enzyme-based pizo-electric sensor have been developed for detecting the ammonia levels in milk which are further used for urea quantification (Renny et al. 2005). The detection and quantification of anionic detergents and formalin in milk have been carried out by using Attenuated Total Reflectance or Fourier Transform Infrared Spectroscopy (Jaiswal et al. 2017; Balan et al. 2020). Melamine detection in milk can be checked by using High-Performance Liquid Chromatography (HPLC) (Azad and Ahmed 2016). According to Zhang et al. (2010), Surface Enhanced Raman Spectroscopy (SERS) can quantitatively detect melamine in the milk. Moreover, quantitative detection of melamine in both liquid and powder milk can be measured by different techniques as reported earlier (Jawaid et al. 2013).

Conclusion

Adulteration of milk is a common problem in developing countries due to economic gain, the demand and supply gap, and the lack of testing facilities. Milk adulteration is a core reason for many diseases in humans like gastrointestinal diseases, heart diseases, hormonal disturbances and many others. Variety of adulterants cause adverse health impacts when consumed for longer period. Melamine can cause adverse effects in infants by renal failure leading to death. Starch is not easily absorbed by the colon, so it can induce diarrhea and it can be fatal for diabetic patients. Milk evaluation can be qualitative or quantitative which checks protein content, freezing point, and SNF%. To avoid this problem, we need a proper check and balance and appropriate policies.

Declaration of competing interests

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contribution

SOS, RZA, RA designed and conceived the idea of the review. SY, AM, BS, HRF helped in collection of the literature and developing the layout of the review. MTA, LAM and MM helped in arranging the information. Finally, SOS and RZA made the critics,

conclusions and proofread to give final shape to this review article.

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