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Qualitative Detection of Some Adulterants in Milk Samples Supplied in the Twin Cities of Secunderabad and Hyderabad, Telangana

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Abstract
This study was conducted keeping in view the ever increasing reports on adulteration of natural milk with various illegal substances to increase the quantity and make more profit. Qualitative analyses for the detection of adulterants in milk samples was carried out with a standard milk adulteration kit manufactured by Himedia Laboratories, Mumbai, India. Among the different milk samples tested none of the samples were positive for cellulose (0%), benzoic acid (0%) and soap (0%). However, majority of these milk samples tested positive for maltodextrin / maltose (30%), proteins (40%), ammonium sulphate (37%), boric acid (33%) vegetable oil (43%) and pond water (nitrate-nitrogen) (50 %) respectively.
Keywords: *Milk adulteration; Qualitative analysis of milk, maltodextrin/maltose, proteins, vegetable oil and pond water (Nitrate-Nitrogen).*

Introduction

Milk is consumed by all age groups of human (e.g., growing children's, young, old people and expectant mothers) because it supplies good quality proteins, fat, carbohydrates (**Afzal** et al., 2011) (Table 1), vitamins and minerals (i.e., provides special nutritive value) in good proportions.

Table I: (Kandpal et al., 2012)

Constituent	Buffalo Milk (%)	Cow Milk (%)
Water	84.2	86.6
Fat	6.6	4.6
Protein	3.9	3.4
Lactose	5.2	4.9

Therefore, milk for human consumption should be free of any adulterant (e.g., if the product contains (i) inferior or cheaper substances in it; (ii) constituent of a product is subtracted; (iii) prepared or packed and stored under unhygienic conditions; (iv) consists of filthy, rotten, decomposed or diseased animal or vegetable or is infested with insects; (v) contains any poisonous ingredient that is prohibited or contains excessive preservatives and finally (vi) quality nor purity of the article conform to the legal standards prescribed by the Food Safety and Standards Authority of India (FSSAI). Despite the efforts of Government organizations (safety agencies) to prevent adulteration of food, fraudsters are resorting to newer ways to mimic an

original product that can be sold at a cheaper price and at the time the adulterant can go un detected.

Objective of Adulteration

Adulteration of milk may be either intentional (e.g., addition of extraneous water, nondairy proteins, melamine, urea, animal fat, reconstituted milk, synthetic milk) or unintentionally by natural means (e.g., natural entry of antibiotics from cattle treated for mastitis, or dust particles or other extraneous objects that might have entered into the milk during processing and lack of proper hygienic conditions.

Adulteration of milk

Overpopulation, rapid urbanization and scattered colonization are some of the factors that may be responsible for the increase in demand for milk production (Awan A et al., 2014). To meet the linkage between demand and supply of milk, dealers are often found to involve in milk adulteration (Mustafa MI, et al., 1991). Here are a few examples of adulterants that can be added to milk in order to maintain its freshness and market value. These adulterants can cause great harm to the consumer leaving them clueless of what direct effect these adulterants have on them.

Nature of Adulteration (table II)

Table II: Adulteration in Milk and its Harmful Effects

Adulterant	Purpose	Harmful Effects
Water (most common) (Kasemsumran, S., et al., 2007; Yu, H., et al 2007; Fourie, C. J et al., 2007 Barham GS et al., 2014).	To increase the volume of milk	Water polluted with feces, microorganisms and harmful chemicals may have deleterious effects on human health (Eman, M. S et al., 2015). In addition, there is a potential risk of waterborne diseases (Campos Motta et al. 2014 & Singuluri and Sukumaran 2014). Acute malnutrition (severe cases of malnutrition have resulted in death) (BBC NEWS., 2004; Naandi Foundation. 2011; FAO. 2013; Barham GS, et al 2014 & Soomro AA, et al 2014).
Cellulose	To increase total solids and hence the quantity of the products (Technews., 2009)	
Maltodextrin / Maltose	Maltodextrine are used in dairy foods to add flavor and reduce the cost of the products (Harding, F., (ed.), 1999). To increase solids but not fat content	
Proteins	Low priced non-milk proteins such as soy, pea and soluble wheat proteins (SWP) (Haasnoot, W., et al., 2006; Destaillats, F., et al., 2006; Lopez-Tapia, J et al., 1999 & Dziuba, J, et al., 2004 are added to compensate for protein loss when water is added to milk	
Ammonium sulphate	A chemical fertilizer, which is added to milk to raise the density of watered milk (Jivraj Makadiya and Astha Pandey., 2015).	Nausea, Vomiting, Diarrhea, Adverse effects on the gastrointestinal, respiratory system, and Skin and Sensory disturbances (Ayub et al., 2007; Barham GS, et al 2014 & Singh P, Gandhi N. 2015).
Boric acid	Used as a stable preservative	Cause nausea, vomiting, diarrhea, kidney damage, acute failure of the circulatory system and even death (Beall and Scofield, 1995; Mota et al., 2003; Haasnoot et al., 2004; Saad et al., 2005; Ayub et al., 2007[l]; Rideout et al., 2008; Gwin et al., 2009; Li et al., 2009 and See et al.,2010).
Benzoic Acid and Sodium Benzoate	Used as a common preservatives	Cause nausea, headache, asthma, urticaria, pseudoallergy, hyperactivity and behavioral disorders in children (Mota et al., 2003; Qi P et al., 2009; Barham, G. S., et al., 2014) & Singh P, Gandhi N. 2015).
Soap/ Detergents	Added to emulsify and dissolve the oil in water to give foamy appearance and	Gastrointestinal, complications, vomiting, Hypotension, respiratory irritation and cancers (Afzal A, et al 2011;

	characteristic white color of milk. (Centre for Science and Environment, 2006).	Mudgil D and Barak S. 2013; Tay M et al 2013 & Singuluri H, Sukumaran MK. 2014. Octylphenol and nonylphenol parts of detergents cause breast cancer (Ali et al. 2005[l]).
Vegetable oil	Milk fat is the natural source of variable variety of fatty acids diversified in nature. It is separated to make the cream and sold at high prices. People separate the cream from the milk and add vegetable fat into the milk and then sell it after homogenization.	Vegetable fat is unsaturated and it gets oxidized and becomes rancid when exposed to air so become hepato-toxic and may cause liver cirrhosis. http://www.pakdairyinfo.com/MAdulteration.htm
Pond water	Sodium and potassium nitrates are oxidizing agents and hence act as preservative Pond water also contains appreciable quantities of nitrates and such water is usually admixed with milk by rural milk producers or vendors. (Ashok Kumar Maurya et.al May 2013).	Potential risk of waterborne diseases (Campos Motta et al. 2014 & Singuluri and Sukumaran 2014). Acute malnutrition (severe cases of malnutrition have resulted in death) (BBC NEWS., 2004; Naandi Foundation. 2011; FAO. 2013; Barham GS, et al 2014 & Soomro AA, et al 2014).

Materials and Methods

A standard milk adulteration kit manufactured by HIMEDIA laboratories, Mumbai, India was used. The tests for adulteration were carried out on milk samples supplied in the twin cities of Secunderabad and Hyderabad, Telangana. Samples were collected in clean, dry and sterilized glass bottles. The milk samples were tested for the following adulterants – cellulose, maltodextrin / maltose, proteins, ammonium sulphate, boric acid, benzoic acid, soap, vegetable oil and pond water (Nitrate-Nitrogen).

Results and Discussion

A total of 30 milk samples were tested in duplicates. All tests were carried out at room temperature (29^0C). For convenience, the adulterants are categorized into III groups. Adulterants in group I are, cellulose, maltodextrin/ maltose and proteins; group II includes, ammonium sulphate, boric acid and benzoic acid; while group III is classified as other compounds where soap, vegetable oil and pond water (Nitrate-Nitrogen) are included.

Determination of the Extent of Different Adulteration in Milk Samples

The percent of different adulterant varied significantly for each of the adulterant tested. The results of group I adulterants are shown in **table. III. and figure.1.** As evident from the table I and figure 1 all the samples tested negative for cellulose (Figure 2). In comparison, the extent of adulteration of milk sample with maltodextrin /maltose (Figure 3) and proteins were 30% and 40% respectively. Presence of maltodextrine indicates that it might have been used either to add flavor to the milk or reduce the cost of the products. Generally in watered milk, milk powder and other dairy products are often adulterated by low priced non-milk proteins such as soy, pea and soluble wheat proteins (SWP).

Table III: Adulteration of Milk Samples **(Group I adulterants)**

	Cellulose		Maltodextrin / Maltose		Proteins	
	Positive	Negative	Positive	Negative	Positive	Negative
%	0	100	30	70	40	60

Figure.1: Adulteration of Milk Samples **(Group I adulterants)**

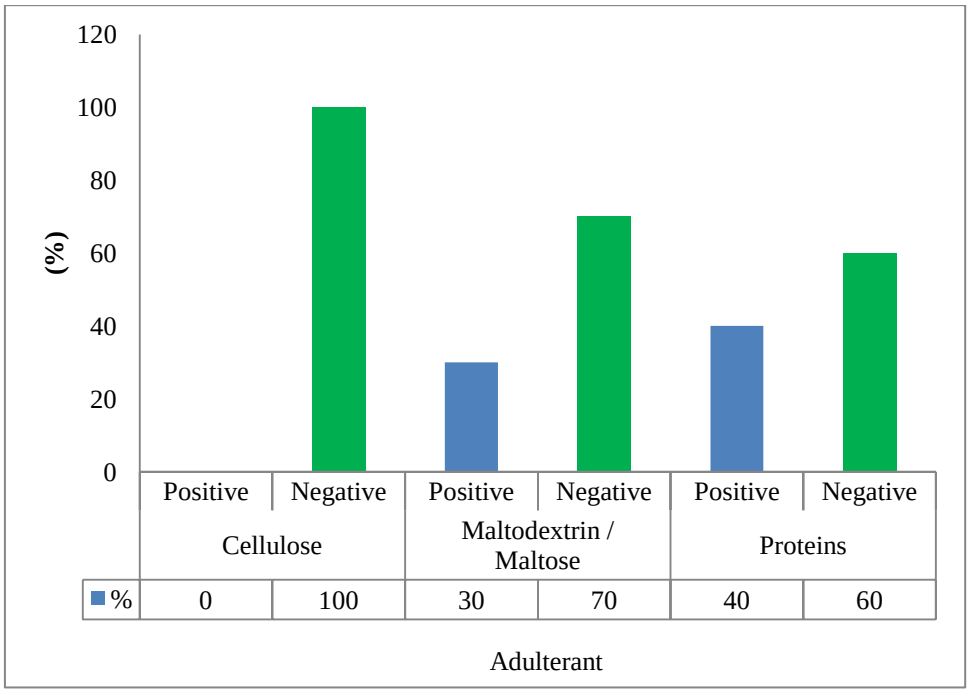
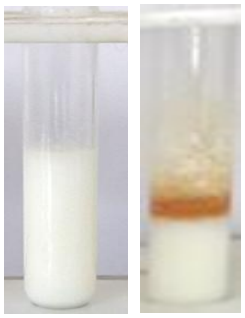


Figure 2
Cellulose Adulteration
Sample Test

Negative

Figure 3
Maltodextrin / Maltose Adulteration
Sample Test



Positive

Extent of group II adulterants is summarized in **table IV** and depicted in **figure 4.** In these milk samples the extent of adulteration with ammonium sulfate and boric acid were 37% (Figure 5) and 33% (Figure 6) respectively. In contrast, all the samples tested negative for benzoic acid. Ammonium sulfate is added to milk to raise the density of watered milk. Boric acid and benzoic acid are used as preservatives which increase the shelf life of fresh milk. All these samples tested negative for benzoic acid (Figure 4).

Table IV: Adulteration of Milk Samples **(Group II adulterants)**

Adulterant	Ammonium sulphate		Boric acid		Benzoic acid	
	Positive	Negative	Positive	Negative	Positive	Negative
%	37	63	33	67	0	100

Figure 4: Adulteration of Milk Samples **(Group II adulterants)**

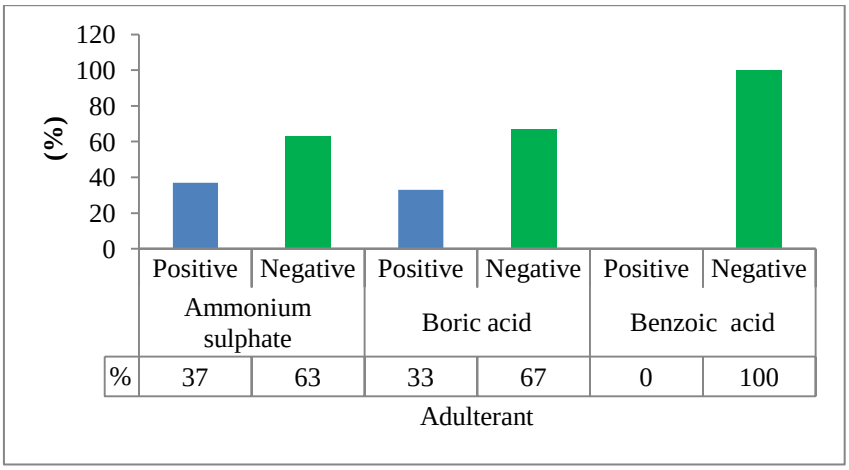
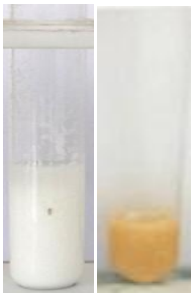


Figure 5
Ammonium Sulphate Adulteration Sample Test



Positive

Figure 6
Boric acid Adulteration Sample Test

Positive

Results of group III adulterants are presented in table V and figure 7. In this group, 43% of milk samples were positive for vegetable oil. Similarly, 50% of milk samples were positive for pond water (Nitrate-Nitrogen). However, none of the samples tested positive for soap (Figure 8).

Table V: Adulteration of Milk Samples **(Group III adulterants)**

Milk sample	Soap		Vegetable oil		Pond water (Nitrate-Nitrogen)	
	Positive	Negative	Positive	Negative	Positive	Negative
%	0	100	43	57	50	50

Figure 7: Adulteration of Milk Samples **(Group III adulterants)**

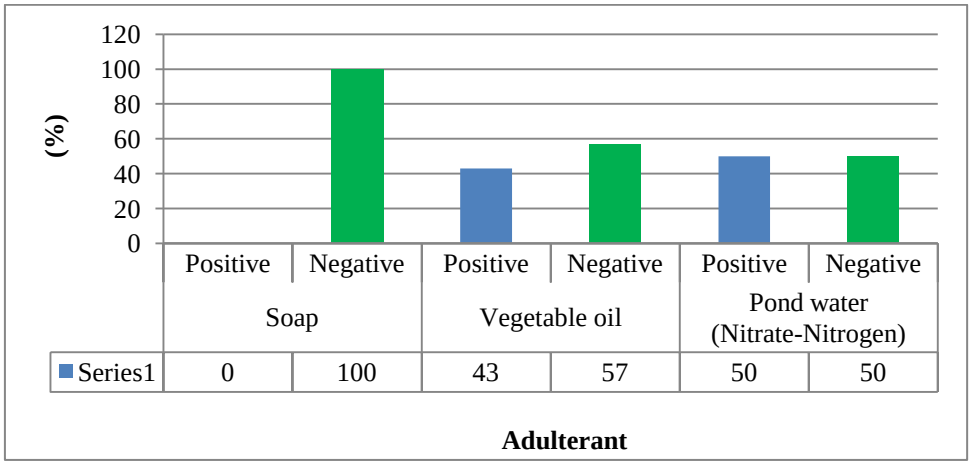


Figure 8

Soap Adulteration

Sample Test

Negative

Review of Literature

Extent of different adulterant present in milk samples

1. Asrat Ayza and Zelalem Yilma., 2014.
   Among the extraneous substances added into milk and milk products; vegetable oil accounted for 80.8%.
2. Ananya Debnath., et al 2015.
   Ammonium sulphate was present in 61.29%, while skim milk powder in 45.16% of the fresh milk samples.
   Benzoic acid was present in 17.65% and 9.68% of pasteurized milk and fresh milk, respectively.
   Vanaspati was found 83.87% of the fresh milk samples studied.
3. Jivraj Makadiya., and Astha Pandey., 2015.
   Ammonium sulphate was found to an extent of 96.66, in the milk samples studied. In contrast, none of the samples were positive for benzoic acid, detergent and vanaspati.
   The extent of adulteration varied significantly with highest for ammonium sulphate (96%).
   Nitrates (pond water) (0%); Boric acid (0%) and Cellulose (0%)
4. Singh, J., et al.
   Maltose was present in all the collected samples (open as well as branded).
5. Geeta Kumari Wasupalli., et al 2015.
   Cellulose 0%; Maltose 33.3%; Ammonium sulphate 0%; Proteins 100; Boric acid 0% and Nitrates (pond water) 53.3%.
6. Nida Shaikh, et al 2016.
   Boric acid 0%.
7. Ruqyia Shehzadi1., et al 2016.
   Boric acid was not found in any sample.
8. Rajesh Pavan, A., et al 2016.

None of the milk samples were adultered with, pulverized soap, ammonium sulphate and nitrates.

It is apparent from the findings in literature that the adulteration of milk is not confined to a particular region within a state, among different states in a country and among different countries in the World. Thus adulteration is a global issue and it is not confined to a particular region, state or country. In addition, the extent of these adulterants varied among the different milk samples.

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Conclusion

It is evident from the analyses that a large number of milk samples did not conform to the legal standards prescribed by the Food Safety and Standards Authority of India (FSSAI). Among the different milk samples tested, none of the samples were positive for cellulose (0%), Benzoic acid (0%) and Soap (0%). However, majority of these milk samples tested positive for maltodextrin / maltose (30%), proteins (40%), ammonium sulphate (37%), boric acid (33%) vegetable oil (43%) and pond water (nitrate-nitrogen) (50 %) respectively.

Conflict of interest statement : Author declares that there is no conflict of interest.

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5. Asrat Ayza and Zelalem Yilma., 2014. Patterns of milk and milk products adulteration in Boditti town and its surrounding, South Ethiopia, Scholarly Journal of Agricultural Science Vol. 4(10), pp. 512-516.
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