

QUALITATIVE DETECTION OF ADULTERANTS IN MILK SAMPLES FROM KOLKATA AND ITS SUBURBAN AREAS

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ABSTRACT

India is the largest producer and consumer of milk. Although milk production has increased substantially, there remains a gap between demand and supply of milk because of steep rise in processed dairy products. A section of greedy and dishonest milk traders has utilized this situation to increase milk supply in the market by adulterating milk. The common milk adulterants include water, skim milk powder, cane sugar (sucrose), starch, fat, ammonium sulphate, etc. to increase its volume while maintaining its specific gravity. Preservatives like hydrogen peroxide, benzoic acid, salicylic acid, carbonates, bicarbonates, formalin, caustic soda, antibiotics are also used to increase shelf life of adulterated milk. Other additives like urea, vanaspati are also used to look it natural. This paper deals with the analysis of milk samples from Kolkata and its suburban areas to find out these adulterants.

KEYWORDS: Adulteration, Fresh Milk, Pasteurized Milk, Kolkata

INTRODUCTION

India is the world's largest producer and consumer of milk. According to an estimate by Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, Government of India, all India milk production was 132.4 million tonnes in 2012-13. Of these West Bengal produced 4.8 million tonnes amounting to 3.67%. About 70% of the total milk production is used in raw form. Demand for processed dairy products has resulted in continuous increase in milk production. Unfortunately increased demand and supply ratio has resulted in the practice of adulteration by a section of milk traders.

Milk adulteration is an act of intentionally debasing the quality of food offered for sale either by admixture or substitution of inferior substances or by the removal of some valuable ingredients [1]. Milk adulteration may be intentional to increase the profit or accidental due to unhygienic and faulty production and handling practices. The most common form of adulteration is intentional addition of water to milk which may be polluted with feces, microorganisms and harmful chemicals [2].

Addition of water to increase its volume is the most common practice of adulteration in India. Cane sugar, starch, fat ammonium sulphate or other reconstituted milk powder is then added to the diluted milk to maintain its viscosity and specific gravity [3]. Urea added to increase SNF and whiten milk results in abnormal physiological activity in young children. Sodium chloride and some chemicals like hydrogen peroxide, carbonates, bicarbonates, formalin, caustic soda or antibiotics may be added as preservative. Hydrogen peroxide used to increase lacto-peroxidase activity may cause gastritis and inflammation of gastroenterocytes. Carbonate in milk may produce gastrointestinal problems including gastric ulcer,

diarrhoea and electrolytes disturbance. Other than causing vomiting, diarrhoea and abdominal pain, formalin affects the optic nerves and cause blindness and is one of the potent carcinogens. Consumption of antibiotic results in antibiotic resistance [4-14]. Another type of adulteration is addition of ice to increase the shelf life. Synthetic milk, a low-cost adulterated milk devoid of essential nutrients, produced by blending of urea, cooking oil, detergent, caustic soda, sugar, salt and skim milk powder is the third type of adulteration [15].

Recent reports of milk adulteration from different areas of India warrants the study on detection of common adulterants in different milk samples collected from Kolkata and its suburban areas.

MATERIALS AND METHODS

Collection of Samples

Non-homogenized fresh cow milk samples (N=17) were collected in pre-sterilized labeled screw capped glass bottles from dairy farms or milk vendors. Pasteurized, homogenized, packed milk samples (N=31) were purchased from dairy outlets in Kolkata and its suburban areas in the year 2014-2015. Samples were kept in icebox during transportation to the laboratory and stored at 4-8°C before analysis.

Detection of Adulterants in Milk

Detection of Ammonium Sulphate

2 ml. of each of the milk sample was taken in a test tube and 0.5 ml of NaOH (2%), 0.5 ml sodium hypochlorite (2%) and 0.5 ml phenol (5%) were added to the test tube. The mixture was heated in boiling water bath for 20 seconds. Immediate appearance of a bluish color turning deep blue afterward indicates addition of ammonium sulphate in the milk sample since pure milk shows salmon pink colour which gradually changes to bluish after 2 hours [22].

Detection of Benzoic Acid

To about 5 ml of milk sample in a test tube, 3-4 drops of concentrated sulphuric acid and 0.5% ferric chloride solution was added drop by drop and mixed well. Development of buff colour indicated the presence of benzoic acid in the milk sample [19].

Detection of Cane Sugar

To about 10 ml milk sample in a test tube, 1 ml concentrated HCl and 0.1 g resorcinol was added and shaken. It was then kept in a boiling water bath for 5 minute. Development of red color indicated the presence of cane sugar in the milk samples [18].

Detection of Carbonates

To about 5 ml of milk sample in a test tube, 5 ml of alcohol and a few drops of an alcoholic solution of rosolic acid (1% w/v) were added, and then mixed well. Appearance of a rose red color indicated the presence of carbonates in the milk sample [21].

Detection of Detergent

0.1 ml bromocresol purple solution (0.5%) was added to 5 ml of each of the milk samples in a test tube. Appearance of violet color indicated the presence of detergent in milk [23].

Detection of Formaldehyde

To about 10 ml sample of milk in a test tube, 5 ml concentrated sulphuric acid containing traces of ferric chloride was added slowly along the side of the test tube so that it forms a layer at the bottom. The development of a violet or blue colour ring at the junction of the two liquids indicated the presence of formaldehyde in the milk sample [25].

Detection of Glucose

1 ml Barfoed reagent was added in 1 ml of each of the milk samples and was heated in a boiling water bath for 3 min and then cooled under tap water for 2 min. After that 1 ml phosphomolybdic acid was added to it and mixed well. Development of deep blue color indicated the presence of glucose in milk [19].

Detection of Hydrogen Peroxide

To about 2 ml of milk in a test tube, 2 drops of a solution of paraphenylenediamine (2% w/v) was added. Development of a blue color indicated the presence of hydrogen peroxide [21].

Detection of Salicylic Acid

5 ml of the milk sample was taken in a test tube and 3-4 drops of concentrated sulphuric acid and 0.5% ferric chloride solution was added drop by drop in the test tube and the mixture was mixed well. Development of a violet color indicated the presence of salicylic acid in the milk sample [19].

Detection of Skimmed Milk Powder (SMP)

Each of the milk sample (5ml) was taken in a test tube and 10 drops of concentrated nitric acid was added to it. Development of orange color in milk was presumed to be positive and the yellow color for negative SMP [24].

Detection of Sodium Chloride

0.1 ml of 5% potassium dichromate and 2 ml of 0.1 N silver nitrate solution was added to 2 ml of the milk sample. Appearance of yellow precipitate indicated the presence of sodium chloride in the sample [19].

Detection of Starch

Three ml well mixed sample of milk was taken in a test tube, heated to boil over flame and then cooled to room temp. Appearance of blue color after adding two to three drops of 1% iodine solution indicated presence of starch [17].

Detection of Urea

5ml of each of the milk samples was added to an equal volume of 24% trichloroacetic acid (TCA) in order to precipitate fat and proteins of milk. After filtration, in 1 ml of the filtrate, 0.5 ml sodium hypochlorite (2%), 0.5 ml sodium hydroxide (2%) and 0.5 ml phenol solution (5%) were added and mixed well. A characteristic blue or bluish green colour developed in presence of added urea whereas pure milk remained colourless [20].

Detection of Vanaspati

3 ml of milk sample was mixed with 10 drops of hydrochloric acid and one teaspoonful of sugar. Development of red color indicated the presence of vanaspati in the milk sample [16].

Detection of added water

Presence of added water in milk samples was determined by putting a drop of milk on a polished slanting surface. The drop of pure milk flows slowly leaving a white trail behind it, whereas milk adulterated with water will flow immediately without leaving a mark [16].

RESULTS AND DISCUSSIONS

A total of 48 milk samples were tested in duplicates at room temperature. The results are summarized in Table 1.

Table 1: Number of Milk Samples Containing Adulterants

Adulterants	Type of milk	
	Fresh (n=31)	Pasteurized (n=17)
Ammonium sulphate	19	7
Benzoic acid	3	3
Cane sugar	6	9
Carbonate	21	7
Detergent	3	2
Formaldehyde	0	3
Glucose	0	0
Hydrogen peroxide	3	5
Salicylic acid	0	10
Skimmed milk powder	14	11
Sodium chloride	24	11
Starch	9	0
Urea	14	1
Vanaspati	26	0
Water	20	8

It was found that higher amount of water was present in 64.52% and 47.06% of fresh milk and pasteurized milk samples, respectively. In countries like ours where scarcity for potable water is high, there might be chance of unsafe contaminated water added to milk. This may cause gastroenteritis and chemotoxicity to the consumers. Water was found to be the predominant adulterant in milk by other workers [26 & 27]. Raw milk was found to contain starch, cane sugar, ammonium sulphate, skim milk powder to increase the lactometer reading. Percentage wise positive milk samples are represented by “Figure 1” and “Figure 2”.

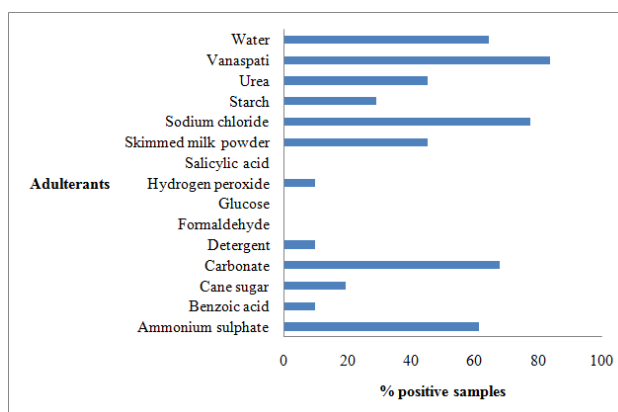


Figure 1: Percentage of Fresh Milk Samples Containing Adulterants

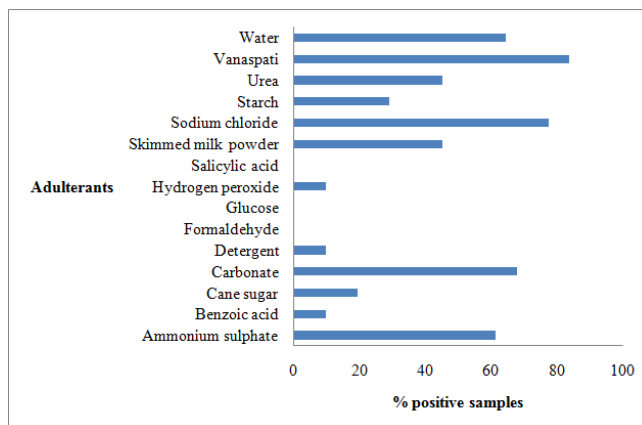


Figure 2: Percentage of Pasteurized Milk Samples Containing Adulterants

Of the seventeen fresh milk samples, 29.03% samples contained starch while 19.35% contained cane sugar. Ammonium sulphate was present in 61.29% while skim milk powder in 45.16% of the fresh milk samples. Added glucose was absent in fresh milk. Pasteurized milk samples were free from starch and glucose. Cane sugar, ammonium sulphate, skim milk powder were present in 52.94, 41.18 and 64.7% of the pasteurized milk samples. While only one (5.89%) sample of pasteurized milk was found to be contaminated with urea, fourteen (45.16%) of the samples of fresh milk were adulterated with this to mask the colour of the adulterants. It may cause renal failure and kidney damage [28]. Formaldehyde was absent but sodium chloride, hydrogen peroxide and carbonates were present in 77.42%, 9.68% and 67.74% of the fresh milk samples studied. Formaldehyde, sodium chloride, hydrogen peroxide and carbonates were present in 17.65, 64.71, 29.41 and 41.18% of the pasteurized milk samples analyzed. Cane sugar, water, formalin and starch have also been reported from other studies [29 & 30]. Starch causes diarrhea and other disturbances in the colon while formalin is a known carcinogen. Sodium chloride masks the high water content in milk. It has been reported in milk by many other workers [26]. Higher level of sodium chloride in body affects acid-base balance of body, kidney problem, speech and sensory disturbances and even decreased immunity [31]. Carbonates and bicarbonates are frequently used to neutralize the pH and acidity of milk [32]. Presence of hydrogen peroxide is in accordance with Singuluri *et al.* [33] but in contrast to Das *et al.* [34]. Salicylic acid was absent in fresh milk but 58.82% of the pasteurized milk were adulterated with this chemical. Benzoic acid was present in 17.65% and 9.68% of pasteurized milk and fresh milk, respectively. Salicylic acid, Benzoic acid and boric acid are added to milk to increase its shelf life mostly during summer season when the surrounding temperature is high. Detergent and vanaspati were found in 9.68% and 83.87% of the fresh milk samples studied. However, 11.76% of the pasteurized milk samples were adulterated with detergent while vanaspati was absent. Detergent is added to milk that has been diluted with water to give milk a foamy appearance and enhance its cosmetic nature [35 & 36].

CONCLUSIONS

Overall analysis of data shows that out of the fifteen types of adulterants analyzed, seven types of adulterants viz. ammonium sulphate, carbonate, sodium chloride, starch, urea, vanaspati and water were present in higher percentage of fresh milk samples while seven types of adulterants viz. benzoic acid, cane sugar, detergent, formaldehyde, hydrogen peroxide, salicylic acid and skim milk powder were present in higher number of pasteurized milk samples. Glucose was absent in both categories of milk. No fresh milk samples contained salicylic acid and formalin while starch and vanaspati were absent from pasteurized milk samples. Presence of adulterants in such a high percentage of milk samples is a severe

public health concern especially to the children, pregnant women and aged persons.

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REFERENCES

1. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 1995. Defect Action Level Handbook. Washington, DC: Government Printing Office.
2. M. S. Eman, A. A. Abd-alla, and M. Y. Elaref, Detection of raw buffalo's milk adulteration in Sohag governorate, *Assiut Vet. Med. J. Vol. 61(144)*, 2015, 38-45.
3. H. Varley, *Practical clinical biochemistry* (4th Edition, CBS Publishers, Delhi, 1969).
4. W. Haasnoot, N. G. Smits, A. E. K. Voncken and M.G. Bremer, Fast biosensor immunoassays for the detection of cows' milk in the milk of ewes and goats, *Journal of Dairy Research*, 71, 2004, 322-329.
5. D.P. Beall, and R.H. Scofield, Milk-alkali syndrome associated with calcium carbonate consumption, Reports of seven patients with parathyroid hormone levels and an estimate of prevalence among patients hospitalized with hypocalcaemia, *Medicine*. 74 (2), 1995, 89-96.
6. T.C. Rideout, Q. Liu, P. Wood and M.Z. Fan, Nutrient utilization and intestinal fermentation are differentially affected by the consumption of resistant starch varieties and conventional fibers in pigs, *British Journal of Nutrition*, (99), 2008 984-92.
7. M. Ayub, Q. Ahmed, M. Abbas, I.M. Qazi and I.A. Hattak, Composition and adulteration analysis of milk samples, *Sarhad Journal of Agriculture*, 23 (4), 2007, 1127-1130.
8. M. C. Gwin, G. Lienert and J. Kennedy, Formaldehyde exposure and asthma in children-A systematic review, *Environment Health Perspective*, 118, 2009, 313-317.
9. S. See, A. B. Salleh, F. A. Bakar, N. A. Yusof, A.S. Abdulmir and L.Y. Heng, Risk and health effect of boric acid, *American Journal of Applied Sciences*, 7, 2010, 620-627.
10. F. J. M. Mota, F. Implvo, S. C. Cunha, M. Beatriz and P.P. Oliveira, Optimization of extraction procedures for analysis of benzoic and sorbic acids in foodstuffs, *Food Chemistry* 3(82), 2003, 469-473.
11. B. Saad, M. F. Bari, M. I. Saleh, K. Ahmad and M. K. M. Talib, Simultaneous determination of preservatives in food stuffs using HPLC, *Journal of Chromatography Analysis*, (1073), 2005, 393.
12. Z. Li, L. L. Wu, Y. P. Wang, A. M. Liu, C. C Zou, and Z. Y. Zhao, Melamine contaminated milk products induced urinary tract calculi in children, *World Journal of Pediatrics*, 5 (1), 2009, 31-35.
13. S. D. Kandpal, A. K. Srivastava and K. S. Negi, Estimation of quality of raw milk (open & branded) by milk adulteration testing kit, *Indian Journal of Community Health*, 3, 2012, 188-192.

14. H. Singuluri and M. K. Sukumaran, Milk Adulteration in Hyderabad, India – A comparative study on the levels of different adulterants present in milk, *Journal of Chromatography Separation Techniques*, 5 (1), 2014, 1-3.
15. P. Bansal and N. Bansal, Synthetic Milk: Genesis, Current Status and Options, *Current Science*, 73 (11), 1997, 904
16. Food Safety and Standards Authority of India. Quick Test for some Adulterants in Food, *Instruction Manual-Part-1*, 3-8-2012, 6-9.
17. R. Kumar, D. K. Singh, and N. K. Chawla, Adulteration / Contamination of milk demystified, *Indian Dairyman* 50, 1998, 25-33.
18. B. Miralles, B. Bartolomé, L. Amigo, and M. Ramos, Comparison of three methods to determine the whey protein to total protein ratio in milk, *Journal of dairy science*, 83(12), 2000, 2759-2765.
19. www.sitaramdixit.4t.com, www.dixitsitaram.itgo.com.
20. H. Meisel, Application of fourth derivative spectroscopy to quantitation of whey protein and casein in total milk protein, *Milchwissenschaft* 50, 1995, 247–251.
21. J. Draaiyer, B. Dugdill, A. Bennett and J. Mounsey, (*Milk testing and payment systems resource book: a practical guide to assist milk producer groups*, 2009 FAO, Rome, Italy).
22. Kumar, D. Lal, R. Seth, and R. Sharma, Recent Trends In Detection Of Adulteration In Milk Fat- A Review, *Indian J. Dairy Sci.* 55, 2002, 319-330.
23. O. P. Singhal , Adulterants and methods for detection, *Indian dairyman*, 32(10), 1980, 771-774.
24. M. Khaskheli, Dept of Animal Products Technology, Faculty Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, (*Dairy laboratory manual*, 2010).
25. D. Panda and M. P. Bindla, Detection of adulteration in ghee with animal body fats and vegetable oils using opacity test, *J. Dairying Foods & Home Sci.*, 17, 1998, 31-36.
26. G. S. Barham, M. Khaskheli, A. H. Soomro and Z. A. Nizamani, Extent of extraneous water and detection of various adulterants in market milk at Mirpurkhas, Pakistan, *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 7(3), Apr. 2014, 83-89.
27. Beniwal and N. Khetarpaul, Knowledge of consumers regarding the nature and extent of adulteration of Indian foods, *Nutrition Health*, 13 (3), 1999, 153-60.
28. S. D. Kandapal, A. K. Srivastava and K. S. Negi, Estimation of quality of raw milk (open & branded) by milk adulteration testing kit, *Indian Journal of Community Health*, 24(3), 2012, 188-192.
29. M. Lateef, A. Faraz, M. I. Mustafa, P. Akhtar, and M.K. Bashir, Detection of adulterants and chemical composition of milk supplied to canteens of various hospitals in Faisalabad city, *Pakistan Journal of Nutrition*, (9), 2009, 139-142.
30. J. Prasad, A. K. Tyagi and Neeraj, *Principles and practices of animal nutrition*, (1st Edition, 1999, Kalyani

Publishers, New Delhi).

31. M. Ayub, Q. Ahmed, M. Abbas, I. M. Qazi and I.A. Hattak, Composition and adulteration analysis of milk samples, *Sarhad Journal of Agriculture*, 23 (4), 2007, 1127-1130.
32. R. S. Singhal, P. R. Kulkarni and D.V. Rege, *Handbook of indices of food quality and authenticity*, (Wood Head Publishing Limited, Cambridge England, pp.168- 174, 1997).
33. H. Singuluri and M. K. Sukumaran, Milk Adulteration in Hyderabad, India—a comparative study on the levels of different adulterants present in milk, *Journal of Chromatography Separation Technique*, Vol. 5(1), 2014, 212.
34. S. Das, G. K. Debnath, T. Chanda, M. A. Alim and S. Sarker, A study on quality of milk from farm to shop in Chittagong metropolitan area, *Int. J. Anim.Fish. Sci.*, Vol. 3, 2010, 252-256.
35. G. P. Walker, F. R. Dunshea and P. T. Doyle, Effects of nutrition and management on the production and composition of milk fat and protein, *Australian Journal of Agriculture Research*, 55, 2004, 1009-1028.
36. M. P. Manish, R. S. Singhal and P. R. Kulkarni, An approach to the detection of synthetic milk in dairy milk, *International Journal of Dairy Technology*, 53(3), 2000, 987-91.