Original Article

To Detect the Adulteration of Raw Milk by Color Test in the Laboratory as to Safe Guard the Human Health

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Abstract

Background: Milk is important part of diet due to its nutritive values. It is recommended for population of every age group.Milk is required for the growth and maintenance of health. When consumers buy milk they have a right toassume that milk will be pure and unadulterated. It is the duty of the milk vendors to provide unadulterated milk to the consumers. Adulteration is the act of addition of substances to a product that makes it unfit for human consumption. These additives are used to make the product cheaper and increase its amount.[1]

Material and Method: A prospective study was conducted by Central Research Laboratory of Muzaffarnagar Medical College, to find out the adulteration of raw buffalo milk sampleobtained from rural areas around Muzaffarnagar Medical College,by color test methodology. The Institutional ethical committee permission was taken.100 samples were collected from 30 different villages around Muzaffarnagar Medical College. The raw milk sample was analyzed for physical appearance, pH, quality,dilution and presence of adulterant by color test.

Result: It was observed that out of 100 samples, 85% samples were white and 15% samples were slightly yellow. The pH of the raw milk samples ranged from 6.5-7.0. The Methylene Blue Reduction Test (MBRT) showed 10samples of raw milk were of poor quality, 15 raw milk samples were fair quality, 45 raw milk samples were good quality and 30 raw milk samples were of very good quality.

Result: In our study water and table sugar were the common adulterants and Benzoic acid was present in traces. So it was concluded that the raw buffalo milk obtained from the near by rural area around Muzaffarnagar Medical College was comparatively of good quality. The common adulterants obtained from raw milk of buffalo were water and table sugar. These malpractices by local dairy owner should be checked to ensure good quality of milk to the consumers.

Keywords: Adulteration, Adulterants, Raw Milk, Color Test, Benzoic acid.

Introduction

Milk provides nutrition to all age group of human and animal population. It contains essential nutrients like, lactose and fat that gives energy, calcium and minerals that helps in bone growth. It provides body building proteins and vitamins needed for the development of humans[2]. The buffalo and Cow

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milk are consumed but have slight difference in their constituents [3]

S. no	Constituent of milk	Buffalo milk (%)	Cowmilk (%)
1.	Water	84.2	86.6
2.	Fat	6.6	4.6
3.	Protein	3.9	3.4
4.	Lactose	5.2	4.9

India is world's largest milk producer, with 22% of global production, followed by the United States of America, China, Pakistan and Brazil[4]. InIndiarural populationis more than the urban population andlarge amount of milk is sold by local vendors. It is important to check the adulteration of milk.

Adulteration includes the addition of chemicals and also accidental contamination during the process of collection, storage and transportation. Adulterated milk has adverse effect on health because of toxic nature of additional compounds .[1]The most common adulterants are:

- Water
- Starch
- Detergent
- Benzoic acid
- Table sugar
- Ammonium sulfate

It has been observed that the adulteration of milk is one of the commonpracticesthat the dairysector of India is facing. We conducted this study to find out the adulteration of raw buffalo milk sold by local vendors aroundMuzaffarnagar Medical College.

According to the national survey on milk adulteration conducted by FSSAI (India) in 2011, water was the common adulterant followed by detergent in the milk. [5] Inspite of the laws governing quality and sales of milk in India for many years the adulteration of milk has not been checked.

Materials and Methods

A prospective study was conducted by Central Research Laboratory of Muzaffarnagar Medical College, to find out the adulteration of raw buffalo milk sample obtained from rural areas around Muzaffarnagar Medical College by color test methodology. The Institutional ethical committee permission was taken. The duration of the study was six months. 100 raw buffalo milk samples were collected from 30 different villages around Muzaffarnagar Medical College.The samples were collected in 100ml clean sterilized bottles and were transported to laboratory without any delay. All possible precautions were taken to avoid external contamination during the time of sample collection.

The raw buffalo milk samples obtained from nearby villageswere analyzed for physical appearance, dilution, quality and presence of adulterants.

Impurities in milk were detected by carrying out chemical color test according to the protocol given by Food Safety and Standards Authority of India[6].

The various methods used in laboratory to detect the milk adulteration were as follows-

Detection of Table Sugar in milk:

1. Pour 10 ml of milk into the test tube .Labeled the test tube as table sugar color test. By pipette add concentrated HCI into the test tube.
2. Do the test in fume hood. Now shake the test tube gently so that the milk gets precipitated, weight 100 milligram of resorcinol and add this to the precipitated milk then shakes the test tube well.
3. Color of the milk solution changes to light brown now place the test tube with the test tube holder in a water bath at 100^0 Celsius for 5 minute.
4. The color milk solution turns red which shows the presence of table sugar, if the color remains the same it denotes absence of table sugar in milk.

Detection of Starch

1. Pour 5ml of milk in a test tube labeled it as starch color test. Place the test tube in a water bath at 100^0 Celsius using a test tube holder, after 5 minutes remove the tube from water bath.
2. Allow the tube to cool and then by the help of dropper add 2-3 drops of Iodine solution to the test tube and shake it well.
3. If the color of the milk solution turns yellow,

it indicates the absence of starch, if the milk turns dark blue color indicating the presence of starch in the milk.

Detection of Benzoic acid and Salicylic acid:

1. Pour 5 ml of milk from the beaker into the test tube labeled acid, add 5 drops of concentrated sulfuric acid into the milk and shake the test tube gently.
2. This should be done in the fume hood. With the help of dropper add 0.5%ferric chloride solution drop by drop and mix it well, buff color indicates the presence of Benzoic acid, if it turns violet color it shows the presence of Salicylic acid in the milk.

Detection of Soap:

1. Pour 10 ml of milk into a test tube.Labeled the test tube as soap color test Add 10 ml hot water to the milk, add 1 to 2 drops of phenolphthalein indicator into the test tube and mix it gently.
2. If the color turns pink it indicates the presence of soap in the milk.

Detection of Formalin in milk:

1. Pour 2 ml of milk into test tube andlabeled it as formalin. Add 2 ml of 90% sulfuric acid gently and then add ferric chloride with the help of glass pipette.
2. A purple violet ring is formed at the junction indicating the presence of formalin in the milk.

Detection of Ammonium Sulfate in milk:

1. Pour 5ml of milk into the test tube, labeled it as Ammonium sulfate. Now add 2.5ml of 2% NaOH solution, 2.5ml of 2% Sodium hypochlorite and 2.5ml of 5% Phenol solution.
2. Keep the test tube in a water bath at 100^0 Celsius for 20 seconds. If the color of the milkturns deep blue then it indicates the presence of Ammonium sulfate.

Detection of Microorganisms in milk (MBRT):

1. Pour 10 ml of milk in a test tube labeled it as Methylene Blue reduction test. Add 8 to 10 drops of Methylene Blue into the test tube and mix the contents well and incubate it for 30 minutes or more till the raw milk is decolorized up to 5mm from the surface.
2. The tube shall be observed for half an hour and then hourly thereafter. Record the time for complete decolorization of raw milk.

Grading of methylene blue reduction time[7]

Methylene blue reduction time	Grade
• ½ hour	Poor
• 1-2 hours	Fair
• 3-4 hours	Good
• 5 hours and above	Very Good

Result

The 85% of raw milk samples were white in appearance and 15% were slightly yellowish. The pH of the milk samples ranged from 6.5 to 7.0 (Table 1). The Methylene Blue Reduction Test (MBRT) showed that, 30 milk samples were of very good quality, 45 milk samples of good quality, 15 milk samples of fair quality whereas 10 milk samples were of poor quality. Adulterant found in the milk samples shown in Table 2.

Table 1: Physical properties of milk

S. No	Location (Muzaffarnagar)	Colour	Avg. pH
1.	Mansurpur (4 samples)	2 samples- white 1 sample-slightly yellow	6.5
2.	Bopara (4 samples)	All samples- white	6.7
3.	Johar (3 samples)	All samples- white	6.5
4.	Begrajpur (4 samples)	3 samples-white 2 sample-slightly yellow	6.8
5.	Khatauli (3 samples)	All samples-white	6.7
6.	Purbaliyan (2 samples)	All samples- white	6.6

Continue...

7.	Rampuri (3 samples)	2 samples- white 1 sample – slightly yellow	6.8
8.	Sabudin (5 samples)	4 samples- white 1 sample-yellow	6.6
9.	Jaroda (3 samples)	2 samples-white 1 samples-slightly yellow	6.5
10.	Sikhara (3 samples)	All samples- white	6.8
11	Santa (5 samples)	4 samples- white 1 sample- slightly yellow	6.5
12	Moghpur (3 samples)	All samples white	6.6
13	Dudhapadi (3 samples)	All samples white	6.9
14	Bhatoda (3 samples)	All samples white	6.7
15	Bhadarpur (4 samples)	3 samples- white 1 sample- slightly yellow	6.6
16	Nirana (2 samples)	All samples- slightly yellow	7.0
17	Rasulpur (3 samples)	All samples- white	6.9
18	Mulhadi (4 samples)	All samples- white	6.7
19	Nara (3 samples)	2 samples-white 1 sample-slightly yellow	6.5
20	Bilaspur (3 samples)	All samples- white	6.6
21	Chitoda (4 samples)	All samples- white	6.8
22	Dalatpur (3 samples)	2 samples- white 1 sample- slightly yellow	6.4
23	Agmatgardh (3 samples)	All samples- white	6.9
24	fempur (4 samples)	3 samples - white 1 sample- slightly yellow	6.6
25	Manawarpur (2 samples)	All samples- white	7.0
26	Bhikky (3 samples)	All samples- white	6.6
27	Bihari (5 samples)	3 samples- white 2 samples- slightly yellow	6.5
28	Tisang (3 samples)	All samples- white	6.8
29	Campus (2 samples)	All samples- white	6.9
30	Bhatodi (4 samples)	All samples- white	6.6

Table 2: Detection of adulterants in raw milk samples

S. no	Adulterants	Test	Samples Positive
1	Water	Slide test	77
2	Table sugar	Resorcinol test	20
3	Benzoic acid	Benzoic acid test	3 (traces)
4	Salicylic acid	Salicylic acid test	0
5	Ammonium sulfate	Ammonium Sulfate test	0
6	Soap	Soap test	0
7	Formalin	Formalin test	0
8	Starch	Iodine test	0

Discussion

Milk is reported as the most preferred food for humans as it has all nutrients in balanced proportion. The adulteration of milk is very common problem now a daysposing heavy toll to human health.

In the current study out of 100 samples, 85% sample were white in color and 15% slightly yellow in colorthese findings approve with the report of Judkins and Mack 8, who reported that normal milk is slightly yellow incolor due to presence of fat and casein and presence of small amount of coloring matter. The difference in color may be due to difference in buffalo fodder consumption or the breed of buffalo or fat and solid content of the milk[9]. The pH of the milk in our study ranged from 6.5 to 7.0 which coincides with study given by Shrishti and Rakesh 2013.[5] In our study, we graded 100 samples of raw buffalo milk by doing the methylene blue test. Out of 100 samples 75 samples were of good and very good quality, 15 samples were of fair quality and 10 samples were of poor quality while in Shrishti and Rakesh 2013 study 15 samples were of poor quality, 73 samples of fair quality and 12 samples were of good and very good quality. Our study also revealed that water is the most common adulterant in raw milk (77%) which coincides with Grace et al 2009.[10] Water is commonly added to increase the quantity of milk but it also reduces the nutritional value of milk. If the water added to the milk is contaminated then it increases the health risk to the consumer.[11] In our study table sugar was found as adulterant in 20 samples of raw buffalo milk. The table sugar may be added to increase the weight and used to mimic the natural sweetness of the milk.[12] Benzoic acid was found as an adulterant in 3 samples of raw milk but benzoic acid was in negligible amount. This may be added to increase the shelf life of milk.

Conclusion

High nutritional value of milk has made the milk as one of the important component of the diet for adults and children. From our study we conclude that the raw milk provided at the rural areas around Muzaffarnagar Medical Collegeis of good quality. The poor quality of milk was mainlydue to adulteration of water, table sugar and traces of benzoic acid. Adulteration of milk is becoming common practice due to exploding population and increase in demand of milk and dairy products. Water is added to the milk to increase the quantity and if water is contaminated it decreases the quality of milk and impose hazard to the consumers. The local milk vendors sometimes add table sugar to balance the sweetness of raw milk which can be dangerous for diabetics.The addition of preservatives like benzoic acid and salicylic acid in small amount may be due to increases in the shelf life of milk.

In a developing nation like India where milk plays an important role in health this study would bring more awareness to public about adulteration of the milk.

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