QUALITY EVALUATION AND SAFETY ASSESSMENT OF BUFFALO MILK COLLECTED FROM MATHURA CITY

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ABSTRACT

The present study was carried out for quality and safety assessment of buffalo milk collected from five different areas of Mathura city *viz*. Holigate (H), Sadar (S), Aurangabad (A), Chungi (A) and Township (T). The milk samples were collected from dairy shops, vendors and milk producers and evaluated for various organoleptic tests, Physico-chemical properties, proximate estimation and microbiological studies by following the standard procedures. Among organoleptic tests, Chungi (80%) and Township (90%) samples had a more clear appearance than Holigate (30%), Sadar (40%) and Aurangabad (60%) areas. There were 40, 60, 80, 90 and 100% samples which were white in color, in H, S, A, C and T areas respectively. The normal consistency of milk was observed in 50, 60, 70, 80 and 90% samples in H, S, A, C, and T area. There were 40, 50, 70, 80 and 90% samples that had a normal odor in H, S, A, C, and T respectively. The COB test was positive in 40, 30, 20, 10 and 0% samples of H, S, A, C and T regions respectively. The temperature, pH and specific gravity were lower though titrable acidity was higher than the normal prescribed limit in all milk samples. All the proximate parameters showed significantly ($P<0.05$) difference between buffalo milk samples. Among proximate parameters, moisture content were comparatively higher; however other parameters (protein, ash, total solids) showed lower values than the normal prescribed range. The formalin adulteration was detected in 50, 30, 10, 10 and 0% milk samples from H, S, A, C, and T areas. All buffalo milk samples were negative for other preservatives, neutralizers, and adulterants. The microbial load of all milk samples were higher than normal prescribed limit in terms of SPC, DMC, Coliform count as well as *Staphylococcus* count.

Keywords: *Bubalus bubalis*, buffaloes, quality evaluation, organoleptic tests, physico-chemical qualities, proximate, microbiological studies

INTRODUCTION

According to FAO STATE (2021), the total milk production of the world is estimated about 883 million tonnes. However, in India milk production is estimated about 209.95 million tonne. Total milk

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contribution in India by buffalo is 51% followed by 24%, 21% and 4% by crossbred cows, non-descript cows and goats respectively (BAHS and FS, 2021). The result of increased share in total milk production by buffalo because of implementations of schemes in India like Livestock health and disease control programme and National programme for dairy development by which expansion in size of herd and improved productivity of milking animals. It is the second global milk-producing animal all over the world and India is the leading country for production of buffalo milk. Buffalo milk plays a vital role in human nutrition particularly in developing countries where malnutrition is a major problem. Buffalo milk is rich in nutrients i.e. high levels of fat, lactose, protein i.e. casein, it has 50% more protein than cow milk, 40% more energy in calories, nearly 40% more calcium and a high level of natural antioxidants like Tocopherol. Whereas, it has lower cholesterol content and is beneficial for the cardiovascular system.

However, the changing pattern of milk consumption, dynamic demography patterns, as well as growing urbanization of rural areas as well as population growth have all contributed to a demand-supply gap. Despite this, the problem of milk deficiency occurs due to a scarcity of land, manpower, as well as inadequate chilling facilities, insufficient animal feeding, and other factors. The population is rising day by day; there is increased pressure on the land resources, availability of land for cultivation of food crops and fodder crops, all these factors contributed to certain malpractices in milk and milk products. Milk adulteration is the most dangerous, hazardous practice in developing countries including India, Pakistan and Bangladesh. Today the Indian dairy sector is facing the most serious problem of adulteration, which not only causes ill-effects to human health even causes major economic losses to the dairy industry. FSSAI has conducted National survey on public health concern in 2011, revealed that almost 70% of milk sold and consumed in India is adulterated by contaminants such as detergents, preservatives, starch, sugar and vegetable oil etc, whereas water is the most common adulterant followed by detergent. Nirwal *et al.* (2013) stated that 68% of milk samples were found to be adulterated in which 31% came from rural areas. Of these 16.7% were packet or branded milk and rest were loose milk samples from dairies. In the urban areas, 68.9% of milk was found to be adulterated with water, detergent, urea and skim milk powder. Several factors like adulteration, presence of antibiotic, insecticides and pesticides residues and seasonal changes affect the Physico-chemical qualities and composition of milk (Bashir *et al*., 2013).

Now a day's consumers are more concerned towards nutritionally enriched organic milk, so it is very important to screen the quality of milk and milk products. By taking all these points into consideration, the present study titled as "Quality and safety evaluation of buffalo milk collected from different areas of Mathura city" was envisaged.

MATERIALS AND METHODS

The experiments were carried out in the Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura. For the present study, total 50 milk samples each from buffalo procured from local market/milk vendors etc. from 5 different areas of Mathura *i.e*., Holigate (H), Sadar (S), Aurangabad (A), Chungi (C) and Township (T). The 250 ml raw milk sample was procured in

sterile plastic bottle from bulk milk container of milk vendors and then put into the ice box. The chemicals and culture media used in the present study were procured from Hi Media Laboratories (P) Ltd, Mumbai and Tulip Diagnostics (P) Ltd., Goa.

Analytical procedure

Platform tests

Under platform test general appearance, odor, color, texture and clot on boiling test was performed (IS, 2007).

Physico-chemical properties

The physico-chemical parameters i.e., pH was determined by using digital pH meter (WTW, Germany, model pH 330i). The temperature of milk samples was determined with the help of a thermometer by immersing the same in the milk (100 ml) contained in measuring cylinder. Specific gravity of milk sample was observed by method of as per the Mahoney (1988). Titratable acidity of the milk sample was determined as per the method of the AOAC (1990).

Proximate estimation

Among the proximate parameters, the fat content was determined by using Gerber's method (APHA, 1985), whereas moisture, protein, fat and ash content were evaluated by following AOAC (1995) method. The SNF and Total solids contents were determined by IS (2007) method.

Adulteration and preservatives detection

The qualitative test for different preservatives i.e., formalin, hydrogen peroxide and boric acid and adulterants i.e. urea, starch, neutralizers like carbonate/bicarbonates and cane sugar, in milk were evaluated by following ISI (1960) methods.

Microbiological parameters

The Direct microscopic count (DMC), standard plate count (SPC), *Coliform* count and *Staphylococcus* count were assessed by following the procedure described in APHA (1984).

Statistical analysis

Data were analyzed statistically on 'SPSS-19.0' software package as per standard methods (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSIONS

Platform tests

The milk samples procured from H, S, A, C and T were 30, 40, 60, 80 and 90% clear in appearance respectively. The visible dirt was observed in 50, 50, 30, 20 and 10% of buffalo milk sample from H, S, A, C and T respectively. However, H, S and A had 20, 10 and 10% milk sample with dirt in them. There was no milk sample with dirt in C and T area. The buffalo samples procured from Chungi and Township had comparatively more clear appearance than the samples procured from other three areas. The nearby areas of Chungi and Township are Acharya Nagar and Refinery Town respectively. The consumers of these areas have higher socio-economic status, conscious towards their health and ready to pay higher price, which might lead to hygienically and proper handling of milk.

Out of total milk samples of buffalo, 40, 60, 80 and 90 samples were of pure white color whereas 60, 40, 20 and 10% samples had light yellow color in H, S, A and C regions respectively. Cent percent milk samples of T area were pure

white in color. There was no sample of pale yellow dark yellow color. The lower carotene content (0.25 to 0.48/ug/g) and converting of carotene to vitamin A might be the reason of white color of buffalo milk (Sakumar, 1991). Islam *et al.* (2013) reported golden yellow, yellowish white and white color of buffalo milk from three different local markets Dharchuni, Atani and Khamar Bazar of Bangladesh.

There were 50, 60, 70, 80 and 90% buffalo milk samples which had normal texture/ consistency, however 30, 30, 20, 20 and 10% samples were adulterated with water in H, S, A, C and T areas respectively. The watery consistency of milk samples in different areas might be due to the adulteration of milk with water to increase the volume. The collected milk samples had no thick and ropy texture; however, samples of H, S and A areas were having slimy consistency in 20, 10 and 10% samples. The ropy consistency of milk might be due to presence of aerobic microorganisms because of increase in time interval between milking and distribution. Islam *et al.* (2013) studied that all milk samples procured from different local market of Muktagacha Upazila had normal texture.

There were 40, 50, 70, 80 and 90% milk samples that had normal odor in H, S, A, C and T areas respectively. There were no milk samples that had rancid/oxidized in any areas. It might be because of lower temperature of milk maintained in cans at 18±2ºC and milking of cows at early morning. The oxidized odor of milk is because of oxidation of due to longer storage and subsequently growth of lipolytic microorganisms. H, S, A, and C samples had 40, 30, 20 and 10% weedy odor but samples of T were free from weedy odor. Weedy odor of samples might be due to the feeding of concentrate to the animals. Out of total samples, 20, 20, 10, 10 and 10% samples were detected with absorbed odor in H, S, A, C and T respectively which might be because of storage of milk in poorly contaminated plastics/metal cans.

Among all samples procured from different areas of Mathura city, 40, 30, 20 and 10% samples were positive for COB tests in H, S, A and C areas, however 100% samples of T area were negative for Clot on boiling test. Positive COB test shows higher bacterial load and titrable acidity as well as poor quality of milk. Bashir *et al.* (2013) evaluated quality characteristics of buffalo milk and reported 29, 38 and 38% samples were positive for COB test collected from household, milkmen and restaurant respectively.

Physico-chemical properties

The significant difference ($P<0.5$) was found in temperature and specific gravity but there is no significant difference in pH and titrable acidity of buffalo milk samples procured from different areas of Mathura city. The samples of T were having significantly ($P<0.05$) higher temperature value than C. Temperature of H, S and A was comparable to both C and T areas. The temperature of all collected milk samples was lower than normal range (36 to 38ºC) which might be due to storage of milk-by-milk vendors in cool cans under lower temperature. The differences in temperature value of milk might be due to certain other factors i.e., difference in collection time, seasonal variations and chilling of milk before sale.

The significant difference was not found in pH values of all collected buffalo milk samples. However, all samples had lower pH values than normal standard value. The normal range of pH of buffalo milk sample is 6.7 to 6.8 (Sakumar, 1991). The lower pH value of all samples might be due to the fermentation of lactose which lead production

of lactic acid, resulting from due to increase time interval between milking and sale. Indumathi and Reddy (2015) also revealed in their study that lower pH of milk samples than normal prescribed values collected from milk vendors and retail shops.

The specific gravity of C areas samples was significantly (P<0.05) higher than A, S and H areas, however T area had comparable specific gravity with C and A. The specific gravity of A was also comparable with S area. The buffalo milk samples collected from all five areas had lower specific gravity than standard value. The normal range of specific gravity of buffalo milk is 1.030 to 1.032 (Sakumar, 1991). It might be due to the milk adulterated with water having lighter weight. Shaker *et al.* (2013) also observed that lower specific gravity of market milk samples procured from dairy shop, street vendor and farmers' house of Sohag district of Egypt.

The titrable acidity of buffalo milk samples had no significant difference; however, samples were having lower titrable acidity than the normal range. It might be due to fermentation of milk sugar which may lead production of lactic acid from with the passage of time during storage at higher temperature (>50ºC). Bashir *et al.* (2013) observed in their study that the acidity of milk was significantly higher (P<0.5) than the standard values in both milk vendors and restaurant milk samples collected from Rawalakot district of Jammu and Kashmir.

Proximate estimation

All the proximate parameters showed significantly (P<0.05) difference between buffalo milk samples procured from five different areas of Mathura city. The moisture content of H areas was significantly higher (P<0.05) than sample procured from other areas. However, the samples of C and T areas were not significantly differing. The moisture percent of A was comparable to S and C and T. The significantly (P<0.05) higher moisture content in H might be associated with lower specific gravity. All collected milk samples had higher moistur content than normal prescribed moisture content, which is given as 84.5% (Sakumar, 1991). It might be due to by addition of water in milk by vendors to increase volume and to gain profit. Soomro *et al.* (2014) also observed higher moisture content of milk from milk producer, milk vendor and dairy shops than the prescribed limit in Badin (Tandojam) area.

The fat percent of procured samples from T and A areas was significant (P<0.05) higher than of milk of H area, however samples of S and C were comparable to T, A and H. The normal value of buffalo milk fat is 6%, however all milk samples of buffalo collected in present study showed lower fat percent than normal prescribed value. It might be because of skimming of fat and adulteration of milk with water. Shaker *et al.* (2013) observed that fat % of Egyptian buffalo milk collected from the dairy shop, street vendor and farmer's house had lower fat than the prescribed limit.

The SNF content of C areas milk was significantly (P<0.05) higher than A, S and H region. The SNF of T region was referring comparable to C and A areas, however the value of A region was also comparable to S region. The prescribed SNF content of buffalo milk sample is 9% (Sakumar, 1991), but all samples of buffalo milk had a lower SNF content than prescribed value which might be due the adulteration of milk with water. Mansour *et al.* (2012) studied the milk samples of cow and buffalo milk procured from dairy shops and milk vendors and observed a lower SNF content in their milk due to adulteration of water (Harding, 1995).

The collected milk samples from of C and T area had significantly (P<0.05) higher

protein content than the milk samples of S and H area, however protein content of A region milk was comparable to C, T and S region. All procured samples had lower protein content than normal prescribed value that is 3.9%. Soomro *et al.* (2014) also observed in their study that 95% of milk samples collected from milk vendors and 100% from retail shop showed variation in protein content with that of control milk. It might be due to the addition of extraneous water in milk.

The collected milk samples from of A, C and T area showed significantly (P<0.05) higher ash content than the samples from H and S area, however there was no significant difference between samples from H and S areas, as well as between A, C and T. The deviation in ash content of buffalo milk sample might be due to the variation in breed, feeding, nutritional condition of animal etc. Soomro *et al.* (2014) reported that the ash content of collected milk samples from milk producers showed relatively similar ash content of control milk, while ash content of milk samples from vendors and retail shops were significantly (P<0.05) higher.

The significant difference (P<0.05%) was observed in total solid content of collected buffalo milk sample from H, S, A, C and T areas. The milk samples of C and T areas had significantly (P<0.05) higher total solid content than the samples of S and H areas, however there was no significance differences observed in the sample of C and T areas. The sample of A area was comparable with the sample of S, C and T areas. All procured milk samples were having lower total solid content than the normal prescribed range. The normal value of Total solid content of buffalo milk is 16.5%. It might be due to the, the skimming practice has been done in milk as well as adulterated with water. Mansour *et al.* (2012) observed that the total solid content of milk from dairy shops and street vendors were lower than the milk samples of dairy farm of Egypt.

Screening for various adulterants and preservatives

Screening of preservatives

The milk samples procured from H, S, A and C region were 50, 30, 10 and 10% positive for adulteration with formalin which might have been done for preservation of milk for longer time period. There was no positive sample for formalin in T area. All samples procured from different five areas were negative for boric acid as well as for hydrogen peroxide. Islam *et al.* (2013) observed all milk samples procured from Dharchuni, Atani and Khamar of Muktagacha upazila were negative for starch and formalin.

Screening of adulterants

All procured milk samples from H, S, A, C and T areas were negative for neutralizer i.e., carbonate/ bicarbonate and adulterants i.e., starch, cane sugar and urea. Neutralizers are generally used to mask the pH and titrable acidity of preserved milk passing it off as fresh milk (Faraz *et al*., 2013). Though, starch is used to increase the thickness of milk after skimming of milk. Urea is used to maintain the texture and whitish color of milk. Indumathi and Reddy (2015) also reported that the collected milk samples from milk vendors and retail shops of Tirupati were adulterated with starch, sugar, neutralizer, and urea however, samples from milk vendors, milk producers and retail shops were nit adulterated with Glucose and dextrose, Hydrogen peroxide and formaldehyde.

Microbiological studies

The SPC and *Staphylococcus* count

Table 1. Platform tests of raw milk of buffalo collected from Mathura city.

Parameter		Holigate (H)		Sadar (S)		Aurangabad (A)		Chungi (C)		Township (T)	
		General appearance									
		No.	%	No.	%	No.	%	No.	%	No.	%
i	Clear	3	30	4	40	6	60	8	80	9	90
ii	Visible dirt	5	50	5	50	3	30	2	20	1	10
iii	Foreign matter	2	20	1	10	1	10	0	0	0	0
	Color										
i	White	4	40	6	60	8	80	9	90	10	100
ii	Light yellow	6	60	4	40	2	20	1	10	0	0
iii	Pale yellow	0	0	0	0	0	0	0	0	0	0
iv	Dark yellow	0	0	0	0	0	0	0	0	0	0
	Texture										
i	Normal	5	50	6	60	7	70	8	80	9	90
ii	Watery	3	30	3	30	2	20	2	20	1	10
iii	Thick	0	0	0	0	0	0	0	0	0	0
iv	Ropy	0	0	0	0	0	0	0	0	0	0
v	Slimy	2	20	1	10	1	10	0	0	0	0
	Odor										
i	Normal	4	40	5	50	7	70	8	80	9	90
ii	Rancid/oxidized	0	0	0	0	0	0	0	0	0	0
iii	Weedy	4	40	3	30	2	20	1	10	0	0
iv	Absorbed odor	2	20	2	20	1	10	1	10	1	10
	COB test										
i	Positive	4	40	3	30	2	20	1	10	0	0
ii	Negative	6	60	7	70	8	80	9	90	10	100

Table 2. Physico-chemical properties (Mean±SE) of raw milk of buffalo collected from Mathura city.

Parameter	Holigate (H)	Sadar (S)	Aurangabad (A)	Chungi (C)	Townshi (T)	Treatment mean
Temperature	19.60^{ab}±0.46	19.59^{ab}±0.49	18.40^{ab}±0.66	17.23^{b}±0.85	19.81^{a}±0.56	18.92±0.30
pH	5.96±0.02	5.93±0.30	5.76±0.12	5.78±0.03	5.91±0.03	5.87±0.02
Sp gravity	1.020^{d}±0.01	1.023^{c}±0.01	1.024^{bc}±0.01	1.028^{a}±0.01	1.026^{ab}±0.01	1.024±0.01
Titrable acidity	0.18±0.03	0.17±0.04	0.18±0.01	0.17±0.05	0.18±0.08	0.17±0.03

Mean±SE with different superscripts in a row differ significantly ($P<0.05$); n =10.

Table 3. Proximate estimation (Mean±SE) of raw milk of buffalo collected from Mathura city.

Parmeter (%)	Holigate (H)	Sadar (S)	Aurangabad (A)	Chungi (C)	Township (T)	Treatment mean
Moisture	88.43^{a}±0.44	87.32^{b}±0.18	86.51^{bc}±0.16	85.99^{c}±0.12	86.13^{c}±0.20	86.88±0.16
Fat	4.29^{b}±0.20	4.49^{ab}±0.14	4.95^{a}±0.10	4.73^{ab}±0.12	4.93^{a}±0.13	4.67±0.07
SNF	6.91^{d}±0.31	7.74^{c}±0.12	8.16^{bc}±0.08	8.89^{a}±0.08	8.62^{ab}±0.14	8.06±0.12
Protein	1.90^{c} ±0.15	2.47^{b}±0.12	2.93^{ab}±0.14	3.16^{a}±0.08	3.08^{a}±0.08	2.71±0.08
Ash	0.60^{b}±0.014	0.63^{b}±0.17	0.70^{a}±0.018	0.74^{a}±0.013	0.72^{a}±0.015	0.68±0.010
Total solids	11.19^{c}±0.43	12.23^{b}±0.18	13.11^{ab}±0.16	13.62^{a}±0.10	13.55^{a}±0.18	12.74±0.16

Mean ± SE with different superscripts in a row differ significantly ($P<0.05$); n = 10.

Table 4. Screening of raw milk samples of buffalo for various preservatives.

Preservatives	Holigate (H)		Sadar (S)		Aurangabad (A)		Chungi (C)		Township (T)	
Preservative	+	-	+	-	+	-	+	-	+	-
Formalin	5	5	3	7	1	9	1	9	0	10
Boric acid	0	10	0	10	0	10	0	10	0	10
Hydrogen peroxide	0	10	0	10	0	10	0	10	0	10

Table 5. Screening of raw milk samples of buffalo for various adulterants.

Adulterant	Holigate (H)		Sadar (S)		Aurangabad (A)		Chungi (C)		Township (T)	
Adulterant	+	-	+	-	+	-	+	-	+	-
Carbonate/ Bicarbonate	0	10	0	10	0	10	0	10	0	10
Starch	0	10	0	10	0	10	0	10	0	10
Cane sugar	0	10	0	10	0	10	0	10	0	10
Urea	0	10	0	10	0	10	0	10	0	10

Table 6. Microbiological studies (Mean ± SE) of raw milk of buffalo collected from Mathura city (log_{10}cfu/ml).

Parameter	Holigate (H)	Sadar (S)	Aurangabad (A)	Chungi (C)	Township (T)	Treatment mean
DMC	6.892±0.02	6.982±0.10	6.845 ±0.02	6.799±0.03	6.957±0.11	6.895±0.03
SPC	7.490^{b}±0.11	7.976^{ab}±0.26	8.494^{a}±0.17	7.825^{ab}±0.13	7.423^{b}±0.11	7.841±0.09
Coliform count	4.960±0.19	5.311±0.20	5.439±0.10	4.869±0.19	5.001±0.17	5.116±0.08
Staphylococcus count	5.415^{b}±0.17	5.451^{a}±0.09	5.247^{a}±0.03	5.190^{a}±0.02	4.709^{b}±0.13	5.202±0.05

Mean ± SE with different superscripts in a row differ significantly ($P<0.05$); n = 10.

showed significant (P<0.05) difference in procured buffalo milk samples from H, S, A, C and T region but there was no significant difference was observed in DMC and coliform count. SPC of collected samples from A areas were significantly (P<0.05) higher than H and T areas, however SPC of S and C areas were comparable to both H and T areas. *Staphylococcus* count of A, C and S areas was significantly (P<0.05) higher than H and T areas. There was no significant difference between milk samples procured from S, A and C as well as between H and T areas. The microbial load of all milk samples was significantly higher than normal prescribed range in terms of SPC, DMC, Coliform count as well as *Staphylococcus* count. The recommend level of DMC and SPC for good milk <500000 and <200000 respectively. The Coliform and *Staphylococcus* should be absent in 10-2 dilution of raw milk. The higher microbial load of collected milk samples was due to poor hygienic condition and sanitation during the milking, handling practices, transportation and uses of uncleaned utensils. Poor herd hygiene, contaminated water, unsanitary milking practices, and improperly washed and maintained equipment may also lead to higher Coliform counts in raw milk (CDFA, 2016). Minj and Behera (2012) observed in their study that the total plate count and coliform count in both the rural and urban milk samples collected from Sambalpur (Odisha) was more than the prescribed limit. Hadrya *et al.* (2012) reported growth of *Staphylococcus aureus* (1.4×10^5 cfu/ml) within accepted limit in raw milk samples procured from Kenitra City of Morocco.

CONCLUSION

This can be concluded that milk of buffalo milk procured from dairy shops, milk vendors and producers from five different areas of Mathura city was adulterated with water and preservative like formalin. Due to skimming practice of milk as well as adulterated with water, the proximate parameters varied from the normal range. The microbial load in the milk was higher than the prescribed limits. The consumption of poor-quality milk may cause serious human health problems. Therefore, consumers must be more careful against adulteration of milk to eradicate this malpractice followed by local dairy owners as well as vendors, consumers which are deeply rooted in cities. It is utmost important to have a quality control system and stringent implementation of FSSAI standards for regular check to ensure that only good quality milk to be sold. The development of infrastructures like chilling centres, well equipped milk distribution vehicles etc. and establishment of quality control laboratories as well as collective efforts of producers, vendors as well as consumers are the essential steps to decrease the adulteration practices which will help in availability of wholesome milk to the people of nation.

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