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RESEARCH ARTICLE

PHYSICO-CHEMICAL AND ADULTERATION ANALYSIS OF BUFFALO MILK AT VARIOUS STAGES OF SUPPLY CHAIN IN AND AROUND PARBHANI CITY

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ABSTRACT

The percentage of whiteness of milk was higher between range of 75 to 85.4 percent whereas, the yellowish white ranges from 4.1 to 25 percent. The specific gravity of milk at farm was 1.0297 ± 0.002, during transportation 1.0269 ± 0.002 and 1.0255 ± 0.002 at retail shop. The pH of milk was found to be 6.72 ± 0.014 at farm, 6.76 ± 0.015 during transportation and 6.70 ± 0.015 at retail shop. The titratable acidity of milk was observed 0.13 percent at farm, 0.12 percent during transportation and 0.11 percent at retail shop. A total of 31.25 percent samples showed positive Clot on Boiling test at farm and transportation whereas 18.7 percent positivity was seen at retail shop. The alcohol test of milk showed 33.30 percent positivity at farm, 31.25 percent during transportation and 65.41 percent at retail shop. The water adulteration was found to be highest (81.25 percent) during transportation followed by at retail shop (75 percent) and farm (50 percent). All the milk samples at all stages of supply chain were found to be negative for presence of urea, starch and caustic soda. Physico-chemical analysis was found to be of fair quality. Water adulteration was found to be high in majority of milk samples at all levels of supply chain.

INTRODUCTION

Livestock sector is an important sector of agriculture in Indian economy. It forms an important livelihood activity for the farmers. The buffalo is an important species for milk production in the country. The 19th Livestock Census, 2012 of India estimated a buffalo population of 108.7 million with a growth rate of 3.13 percent in 12th five year plan (2007-2012). The Maharashtra state is having buffalo population of 55.94 million. India continues to be the largest milk producer in the world. The milk production showed annual growth rate of 6.27 percent. The per capita availability of milk is around 337 gm per day. The average milk yield of buffalo is found to be 5.76 kg per day during 2015-2016. The buffaloes contribute to the 36 percent of total milk production in the country (Annual Report 2015-2016 of Department of Animal Husbandry, GOI). About 10 percent of milk produced goes into organized dairy sector for processing. The milk is processed at dairy plants located in semi urban areas and then transported to cities. The balance of about 90 percent of total production is being handled by private traders.

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A total of 45 percent of milk production is consumed as fluid milk. Dairy farming is an important source of subsidiary income to small farmers and agricultural labourers (Patil and shinde, 2016). In recent times, a new trend of marketing in small scale dairy units has emerged. The consumers want fresh milk rather than processed and refrigerated milk in semi urban areas. There is high demand for buffalo milk due to its fat percentage. To maximize percentage of profit, a new business model of direct selling of buffalo milk to the consumers through retail marketing has been found to be successful. The supply chain of buffalo retail milk consists of production, transportation and retail selling. The farms are located in vicinity of around 5 kilometers of Parbhani city. The milk is being packed in polythene bags. It is important to evaluate supply chain of buffalo milk from public health point of view. Assessment of physico-chemical analysis of milk at various stages of supply chain helps in critical evaluation of hazards (Soomro *et al*. 2014). The milk contains more than hundred substances that are either in solution, suspension or emulsion in water. The nature of adulterants generally encountered in milk are water, removal of fat, addition of skimmed milk powder, thickening agents such as starch and preservatives such as sodium bicarbonate, sodium hydroxide. The adulteration of milk poses public health challenges despite of various acts. The water is an adulterant in milk which is added to increase volume of milk which in turn decreases nutritive value of milk, contaminated water poses a health risk, especially to infants and children. Detergents are added to emulsify and dissolve the oil in water giving a frothy solution, the characteristic of white colour of milk. Detergents cause gastrointestinal complications. The urea is added to milk to provide whiteness, increase consistency and for leveling the contents of SNF. The presence of urea in milk overburdens kidneys of consumers. The starch is used as an adulterant, responsible for diarrhea and if accumulated become fatal for diabetic patients. The caustic soda can cause disruption in hormone signaling that regulate development and reproduction.

MATERIALS AND METHODS

Collection of samples

A total of 4 dairy farms were selected randomly from Parbhani city. A total quantity of 200 ml of raw milk samples were collected at each farm (F1,F2,F3 and F4) during transportation (T1, T2, T3 and T4) and at retail shops (R1, R2, R3 and R4) aseptically in sterile glass bottles. The samples were transported to the laboratory on ice packs maintaining 4˚c. A total of 144 milk samples were collected twice a week for six weeks.

Physico-chemical analysis

Colour

Sensory analysis was done for in relation to colour. Milk samples were analysed by observing colour of the milk samples as per the standard method described ISO/TC-34/SC.

Specific Gravity

Specific gravity of milk sample was calculated as per the standard method described by Gemechu *et.al.* (2014). Raw milk was sufficiently filled into the glass cylinder of 100 ml capacity. Then lactometer was hold to the tip and inserted into the milk. The lactometer was allowed to float freely until it reached equilibrium. The lactometer reading to the lower meniscus was recorded. At the same time thermometer was inserted into the milk sample and the temperature of milk sample was recorded. The following formula was used to calculate the specific gravity of milk.

$$\text{Specific gravity} = \left(\frac{L}{1000}\right) + 1$$

Where,

L = Corrected lactometer reading at given temperature, that is, for every degree above 15.56 ˚c, 0.2 was added to the lactometer reading but for every degree below 15.56˚c, 0.2 was subtracted from the lactometer reading.

pH

The pH of the milk samples was recorded based on the method described by O connor (1995).The pH of milk samples was recorded in laboratory by using a digital pH meter (Aquasol).

Titratable acidity

Titratable acidity of milk sample was determined as per the method described by Gemechu *et.al* (2014). A quantity of 9 ml of milk sample was pipetted into the beaker and 3 to 5 drops of 1% phenolphthalein indicator was added to it. The milk sample was then titrated with 0.1 N NaOH solution until faint pink colour persisted. The titratable acidity, expressed as % lactic acid, was finally calculated by using formula.

$$\text{Titratable acidity (\%)} = \frac{\frac{N}{10}\,\text{NaOH (ml)} \times 0.009}{\text{Weight of sample}} \times 100$$

Clot on boiling

The clot on boiling test was performed as per the method described by O connor (1995). A quantity of 5 ml of milk was placed in a test tube and it was placed in the boiling water for five minutes. Then the test tube was removed carefully from the water bath and observed for the precipitate formation.

Alcohol test

The alcohol test was performed as per the method described by Tassew and Seifu (2010). A quantity of 5 ml of milk sample and 5 ml of 68 percent ethanol were placed in test tube. The test tube was inverted for several times with thumb held tightly over the open end of the test tube. Then the test tube was examined for formation of curd particles.

Adulteration analysis

Water

Water adulteration was detected by method described by Navale and Gupta (2016).Presence of water in the milk sample was detected 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**.**

Urea

Urea adulteration was detected by using method described by FSSAI Lab Manual (2015). A quantity of 1 ml of milk was mixed with 1 ml of 1.6% DMAB reagent in a clean test tube. Distinct yellow colour observed in milk indicates presence of urea in milk. The control (normal milk) showed a slight yellow colour due to presence of natural urea.

Starch

Adulteration was detected by using method described by FSSAI Lab Manual (2015). About 5 ml of milk was taken in a test tube. The milk in the test was brought to boiling condition and the test tube was allowed to cool to room temperature. 1-2 drops of iodine solution was added to the test tube. Development of blue colour indicated presence of starch which disappeared when sample was boiled and reappeared on cooling.

Caustic soda

Caustic soda adulteration was detected by using method described by FSSAI Lab Manual (2015). To 10 ml of milk added equal volume of 95% alcohol in a test tube. Few drops of 0.1% alcoholic solution (w/v) rosalic acid was added. If alkali is present a rose red colour appears whereas pure milk shows only a brownish colour.

RESULTS AND DISCUSSIONS

Physico- Chemical analysis

Colour

The colour of milk was classified into whitish and yellowish white. All the 144 samples were subjected to the colour evaluation and the results are depicted in Figure 1. It was evident from the results that the percentage of whiteness of milk is higher between range of 75 to 85.4 percent whereas, the yellowish white ranges from 4.1 to 25 percent. The variations in colour might have been attributed due to differences in diet (Khan *et al.* 2008). A significant (p<0.05) effect of stage of supply chain was observed on colour of milk. A highly significant effect on colour of milk (p<0.01) effect of collection week was seen on milk colour. similar observations were reported by Khan *et al.,* 2008; Batool *et al*., 2012; Gemechu *et al.,* 2015; Kader *et al*., 2015.

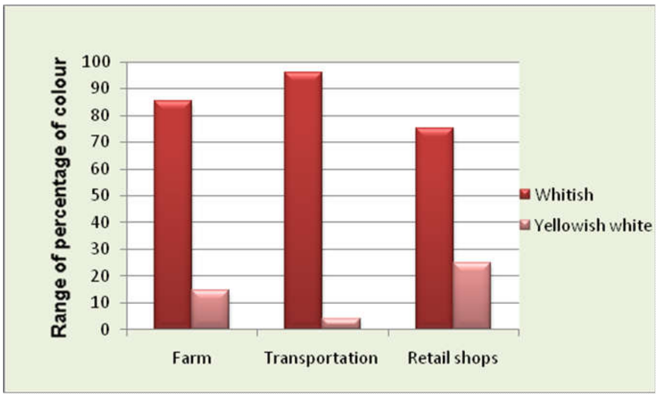

Figure 1. Results of colour evaluation of milk samples at various stage of supply chain

pH value of milk

In present study the pH values are given in Table 2. The pH values observed at farm, during transportation and retail shop were 6.72 ± 0.014, 6.76 ± 0.015 and 6.70 ± 0.015 respectively. A significant (P<0.05) effect of weeks was observed on pH values. This may be due to effect of summer heat resulting into higher acidity at each stages of supply chain. The apparent acidity of milk is measured with the help of pH. This acidity is due to casein phosphates. The pH of milk changes due to conditions like mastitis, colostrum and type of bacteria present (Herrington, 2000). The pH of buffalo milk ranges from 6.57 to 6.84 and is not influenced by month, lactation number or season of calving are co-related with solid not fat and lactose contents (Ahmed *et al*., 2013).

Titratable Acidity

The average percentages of titratable acidity observed were 0.13 ± 0.014 at farm, 0.012 ± 0.015 during transportation and 0.11 ± 0.015 at retail shop. Similar observation were reported by many workers (Bille *et al*. 2009; Saxena and Rai, 2013; Kas *et al*. 2013; Gemechu *et al*. 2015). The results of titratable acidity of milk at various stages of supply chain are given in Table 3. It is evident that a significant (P<0.05) effect of weeks on titratable acidity was observed. This may be due to advances in stages of lactation of buffaloes (Herrington, 2000).The titratable acidity is also known as true or real acidity and is due to lactic acid. It is made up of apparent acidity due to original constituent of milk and a real acidity due to lactic acid. The acidity of milk varies during course of lactation (Herrington, 2000).

Clot on boiling test (COB)

The results of COB are depicted in Table 4. The percentage of COB positive milk samples observed were 31.25 percent at farm, 31.25 percent during transportation and 18.75 percent at retail shop. It was observe that a non significant effect of supply chain was seen on COB test. This clearly indicates suitability of milk for further processing and consumption. The COB test was also successfully exploited for assessment of quality of milk by many workers (Saxena and Rai, 2013; Gemechu *et al*.,2015; Hasan and Rakib,2016). The results of present study are in agreement.

ANOVA for colour analysis of milk

Sr. no	Source	df	Sum of square	Mean square	F value	P
1	Stage of supply chain	2	0.990	0.495	4.195*	0.017
2	Week	5	2.819	0.564	4.779 **	0.000
3	Trial	1	0.000	0.000	0.004 N	0.950
4	Error	135	15.926	0.118		

*P<0.05 , **P<0.01, N- Non significant

Table 1. Results of Specific gravity evaluation of milk samples at various stage of supply chain

		*Farms				
Sr.no	Stage of supply chain	F1	F2	F3	F4	Average (gm/ml ± SE)
1	Farm	1.0298	1.0292	1.0300	1.0301	1.0297 ± 0.002
2	Transportation	1.0270	1.0263	1.0274	1.0272	1.0269 ± 0.002
3	Retail shop	1.0246	1.0259	1.0255	1.0260	1.0255 ± 0.002

* Farms- F1, F2, F3 and F4

Specific gravity

The results of specific gravity evaluation of milk samples in present study are given in Table 1. The average specific gravity at farm, during transportation and at retail shop were 1.0297 ± 0.002, 1.0269 ± 0.002 and 1.0255 ± 0.002 respectively. Specific gravity values indicate water addition to milk samples. The water is used to increase volume of milk. The higher value of specific gravity (1.035 indicate skimming off fat whereas, the lower value of specific gravity (1.020) is indicative of addition of water (Gemechu *et al*., 2015).

Alcohol test

Alcohol test is a rapid test which helps in assessment of stability of milk and its suitability for processing.

ANOVA for specific gravity analysis of milk

Sr. no	Source	df	Sum of square	Mean square	F value	P
1	Stage of supply chain	2	0.002	0.001	3.802*	0.025
2	Week	5	0.001	0.000	0.799 N	0.552
3	Trial	1	0.000	0.000	0.754 N	0.387
4	Error	135	0.032	0.000		

*P<0.05 , N- Non significant

Table 2. Results of pH analysis of milk samples at various stage of supply chain

		* Farms				
Sr.no	Stage of supply chain	F1	F2	F3	F4	Average
1	Farm	6.75	6.71	6.7	6.725	6.72 ± 0.014
2	Transportation	6.81	6.74	6.74	6.76	6.76 ± 0.015
3	Retail shop	6.70	6.69	6.7	6.70	6.70 ± 0.015

* Farms- F1, F2, F3 and F4

ANOVA for pH analysis of milk

Sr. no	Source	df	Sum of square	Mean square	F value	P
1	Stage of supply chain	2	0.100	0.050	4.930 N	0.009
2	Week	5	0.190	0.038	3.739*	0.003
3	Trial	1	0.012	0.012	1.150 N	0.285
4	Error	135	1.369	0.010		

*P<0.05, N- Non significant

Table 3. Results of Titratable acidity evaluation of milk samples at various stage of supply chain

		*Farms				
Sr. no	Stage of supply chain	F1	F2	F3	F4	Average (%)
1	Farm	0.14%	0.13%	0.12%	0.13%	0.13%
2	Transportation	0.13%	0.14%	0.12%	0.11%	0.12%
3	Retail shop	0.11%	0.12%	0.11%	0.11%	0.11%

*Farms- F1, F2, F3 and F4

ANOVA for Titratable acidity analysis of milk

Sr. no	Source	df	Sum of square	Mean square	F value	P
1	Stage of supply chain	2	0.100	0.050	4.930 N	0.009
2	Week	5	0.190	0.038	3.739*	0.003
3	Trial	1	0.012	0.012	1.150 N	0.285
4	Error	135	1.369	0.010		

*P<0.05, N- Non significant

Table 4. Results of Clot on boiling evaluation of milk samples at various stage of supply chain

		*Farms (No. of positive samples / No. of samples tested)				Positive (%)
Sr.no	Stage of supply chain	F1	F2	F3	F4	
		Positive	Positive	Positive	Positive	
1	Farm	3/12	1/12	0/12	1/12	5/48 (31.25%)
2	Transportation	5/12	3/12	4/12	3/12	15/48 (31.25%)
3	Retail shop	4/12	2/12	2/12	1/12	9/48 (18.75%)

*Farms- F1, F2, F3 and F4

ANOVA for Clot on boiling of milk

Sr. no	Source	df	Sum of square	Mean square	F value	P
1	Stage of supply chain	2	0.769	0.385	2.386 N	0.096
2	Week	5	1.022	0.204	1.267 N	0.282
3	Tria	1	0.142	0.142	0.879 N	0.350
4	Error	135	21.767	0.161		

N-Nonsignificant

Table 5. Results of Alcohol test of milk samples at various stage of supply Chain

		*Farms (No. of positive samples / No. of samples tested)				
Sr. no	Stage of supply chain	F1	F2	F3	F4	Positive (%)
		Positive	Positive	Positive	Positive	
1	Farm	3/12 (25%)	7/12 (58.30%)	4/12 (33.30%)	2/12 (16.60%)	16/48 (33.30%)
2	Transportation	5/12 (41.60%)	5/12 (41.60%)	2/12 (16.60%)	4/12 (33.30%)	16/48 (31.25%)
3	Retail shop	6/12 (50%)	9/12 (75%)	6/12 (50%)	8/12 (66.60%)	29/48 (60.41%)

*Farms- F1, F2, F3 and F4

The test indicate about mineral balance of milk and helps in detecting abnormal milk such as colostrum, milk from animal in late lactation, milk from animals suffering from mastitis and milk with disturbed mineral balance. A positive test indicates low heat stability of milk and high acidity in milk. Earlier Tassew and Seifu, (2011) reported that around 51 percent of milk samples tested by alcohol test were found positive. It was also observed that alcohol test is more sensitive than COB.

ANOVA for Alcohol test of milk

Sr. no	Source	df	Sum of square	Mean square	F value	P
1	Stage of supply chain	2	2.400	1.200	5.381 N	0.006
2	Week	5	2.405	0.481	2.157 N	0.063
3	Trial	1	0.291	0.291	1.306 N	0.255

N- Non significant

Table 6. Results of water adulteration analysis of milk samples at various stage of supply chain

		*Farms (No. of positive samples / No. of samples tested)				Positive (%)
Sr.no	Stage of supply chain	F1	F2	F3	F4	
		Positive	Positive	Positive	Positive	
1	Farm	5/12 (41.60%)	6/12 (50%)	7/12 (58.30%)	6/12 (50%)	24/48 (50%)
2	Transportation	9/12 (75%)	11/12 (91.60%)	9/12 (75%)	10/12 (83.30%)	39/48 (81.25%)
3	Retail shop	9/12 (75%)	9/12 (75%)	9/12 (75%)	9/12 (75%)	36/48 (75%)

*Farms- F1, F2, F3 and F4

ANOVA for Water adulteration

Sr. no	Source	df	Sum of quare	Mean square	F value	P
1	Stage of supply chain	2	1.588	0.794	3.808*	0.025
2	Week	5	0.265	0.053	0.254 N	0.937
3	Trial	1	0.153	0.153	0.736 N	0.392
4	Error	135	28.147	0.208		

*P<0.05, N- Non significant

Table 7. Results of Urea adulteration analysis of milk samples at various stage of supply chain

		*Farms (No. of positive samples / No. of samples tested)				Positive (%)
Sr. no	Stage of supply chain	F1	F2	F3	F4	
		Positive	Positive	Positive	Positive	
1	Farm	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)
2	Transportation	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)
3	Retail shop	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)

*Farms- F1, F2, F3 and F4

Table 8. Results of starch adulteration analysis of milk samples at various stage of supply chain

		*Farms (No. of positive samples / No. of samples tested)				Positive (%)
Sr. no	Stage of supply chain	F1	F2	F3	F4	
		Positive	Positive	Positive	Positive	
1	Farm	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)
2	Transportation	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)
3	Retail shop	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)

*Farms- F1, F2, F3 and F4

Table 9. Results of Caustic soda adulteration analysis of milk samples at various stage of supply chain

		*Farms (No. of positive samples / No. of samples tested)				Positive (%)
Sr. no	Stage of supply chain	F1	F2	F3	F4	
		Positive	Positive	Positive	Positive	
1	Farm	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)
2	Transportation	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)
3	Retail shop	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/48 (0%)

*Farms- F1, F2, F3 and F4

Such milk is not suitable for heat processing as it will curdle on heating (Garg and Jadhav 2012). The results of alcohol test of milk samples at various stages of supply chain found that there is non-significant difference of alcohol test results. The highest percentage 60.41 percent of positive alcohol test was observed at retail shops. This may be due to development of acidity in milk upon storage. The results are shown in Table 5.

Adulteration analysis of milk

Water adulteration

The results of water adulteration are depicted in Table 6. It was observed that about 50 percent milk samples at farm, 81.25 percent during transportation and 75 percent at retail shops. Earlier Adulteration of milk by water in about 35.35 percent milk samples was observed by (Ali, 2009). A total of

20 milk samples at various stages of supply chain were screened for detection of water adulteration by Soomro *et al*., (2014).

Urea adulreration

Urea is being added to milk for whitening of milk and giving genuine look. However, adulteration of milk by urea pose adverse effect on human health (Barham *et al.*, 2015). The urea is added to milk to provide whiteness, increase consistency and for leveling the contents of SNF. The presence of urea in milk overburdens kidneys of consumers (Singuluri and Sukumaran, 2014). It was good to note that all tested samples (144) at various stages of supply chain were found to be negative for urea adulteration. The results are shown in (Table 7). Earlier Singuluri and Sukumaran, (2014) reported adulteration of milk in 60 percent samples.

Starch Adulteration

The starch is used as an adulterant, responsible for diarrhea and if accumulated become fatal for diabetic patients (Singuluri and Sukumaran, 2014). The solid contents of milk are increased by addition of various adulterants like vegetable oil, starch, sugarcane, whey powder, skim milk powder etc. It is resulting into a lowering of nutritive value of milk and posing health hazards (Barham *et al.*, 2015). In the present study all the 144 milk samples screened were found negative for starch adulteration (Table 8). Earlier Barham *et al*., (2015) reported adulteration of milk in 16 percent of samples. In the present study all the 144 milk samples screened were found negative for starch adulteration (Table 8). Earlier Barham *et al*., (2015) reported adulteration of milk in 16 percent of samples.

Caustic soda adulteration

The results of caustic soda adulteration are shown in Table 9. All the 144 collected at all the stages of supply chain were found to be negative for caustic soda adulteration. Barham *et al.* (2015) reported that 11 percent samples (N=100) collected from 20 farms were found to be positive for caustic soda adulteration.

Conclusion

It was concluded that physico-chemical analysis of buffalo milk sold in the Parbhani city was found to be of fair quality and within the permissible limits. Adulteration of the milk with water was found to be high in majority of milk samples at each stage of supply chain and all tested samples (144) at various stages of supply chain were found to be negative for urea, starch and caustic soda adulteration.

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