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Quality changes in milk adulterated with detergent, urea, ammonium sulphate and neutralizers

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ABSTRACT

Quality changes in milk adulterated with different concentrations of adulterants like urea, detergent, ammonium sulphate and neutralizers were determined using physico-chemical and microbiological quality characteristics. Addition of detergents at 0.1 to 0.9% concentrations showed an increase in the refractive index (RI). Specific gravity increased with incremental addition of detergent. Same trend was observed with total solids, total ash and pH; whereas, moisture, fat and lactose contents decreased. Microbial growth in the adulterated milk did not show much variation in the samples.

Key words: Ammonium sulphate, Detergent, Microbial analysis, Neutralizer, Urea.

INTRODUCTION

Milk consumption has been increasing globally and especially in developing countries, due to immense health benefit and improvement in living standards. India produces about 136 million tonnes of milk per annum and is currently the highest milk producing country in the world (National Dairy Development Board, 2014). Milk contains over a hundred biochemical compounds of which the majority has substantial nutritional value. Milk protein covers all essential amino acids which the human body needs on a daily basis (Omkar *et al*, 2010).

The adulteration of milk is done to derive undue profit by adding water or extraction of fat. Besides this, milk is also adulterated with urea, detergent and vegetable fat. Addition of adulterants like water, starch, salt, pulverized soap, detergents, urea, skim milk powder and preservatives like formalin and hydrogen peroxide in milk will deteriorate its overall quality. These compounds are used to prepare so called synthetic milk (Chagas *et al*, 2007). Addition of preservatives such as dichromate, salicylic acid, neutralizer and hydrogen peroxide to milk have the objective of inhibiting or delaying the growth of microorganisms in liquid milk (Barbano *et al*, 2010 and Souza *et al*, 2014). The detection methods are difficult to implement in local production and packaging. The official procedures are time consuming and expensive. (Kartheek *et al*, 2011). Sometimes to mask the appearance and to increase the specific gravity after the addition of water, some soluble substances like sugar or starch are added. In detecting adulteration in milk, the percent of the main constituents is considered with reference to or legal standards.

MATERIALS AND METHODS

Fresh homogenized milk was obtained from M/S Nandini Dairy, Mysore. Adulteration of milk was done by mixing weighed amounts of adulterants (as per % weight) and making up the volume to 100ml of milk.

Chemical analysis: Refractive index (ç) was determined using Abbe's refractometer (Advance Company, R-8, No.23017) and pH determinations were made using digital pH meter (Cyberscan 510, Eutech Instruments, Malaysia) as described by Trout *et al*. (1992).

Moisture was estimated using hot air oven method and ash content by AOAC method (2000). Similarly acidity was estimated using IS method1479 (II) 1961, fat by the Gerber method, IS: 1224, Bureau of Indian standards, New –Delhi (1979), total solids by IS: 1479 (Part II) – 1961, casein by IS: 1479 (Part II) – 1961, protein content by the BIS method (1990) and specific gravity by lactometer.

Microbiological analysis: Microbial evaluation of adulterated milk was carried out as per APHA (1992) procedures involving standard plate count (SPC), coliform, yeast and molds.

Physical analysis: The L^*, a*, b* values for colour were measured using D-65 Illuminant with a spectral range of 400-700nm with a spectral resolution of 10nm (Colour Flex, CFLX-45-2, Hunter lab, Hunter Associates laboratory Inc., Reston, VA, USA). The photo sensor was calibrated using standard black and white tiles and the sample readings were obtained using Easy Match QC Software. The output values were analyzed for the effect of adulterants on the colour changes in milk.

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RESULTS AND DISCUSSION

Milk adulterated with detergent : The moisture content of the raw milk (homogenized toned milk) was 88.37±0.06%. With the increase in the level of detergent the moisture content of the milk decreased so also the other parametrs got affected (Table 1).

Milk adulterated with neutralizer: Neutralizers such as alkaline carbonates or even hydroxides are added during storage to nullify the effect of developed acidity in milk. Sodium bicarbonate causes digestive disorders and is known to destroy ascorbic acid (Amit kumar *et al*, 2010). Milk was adulterated with 0.1, 0.5, 1 and 2% of neutralizer which led to a decrease in the moisture content; while the total solids, pH and ash values increased with increasing levels of adulterants (Mohit Kamthania *et al*, 2014). (Table 2)

Milk adulterated with urea: Milk was adulterated with 0.5, 1, 3, 5 and 7% of urea. leading to increased total solids content and reduced moisture. Lactose gets partially hydrolyzed to D-glucose and D-galactose reacting with urea to yield lactosylurea at low pH (Janis Cerbulis *et al*, 1978). However slowly pH of milk increased with increase in urea content and therefore does not favour lactoseylurea formation. Thus we obtained a slight increase in lactose content at higher levels of urea addition. Variation was also observed in the casein percent in milk. The normal casein content of raw milk was 2.69±0.07%, which showed an increased with urea addditions. (Table 3)

Milk adulterated with ammonium sulphate: Ammonium sulphate is a chemical substance used in milk for its preservative effect. In the study, milk was adulterated with 0.5, 1, 3, 5 and 7% of ammonium sulphate. Acidity and lactose content decreased in the adulterated samples with increasing ammonium sulphate content. Specific gravity was not significantly affected with addition of ammonium sulphate. (Table 4)

Colour: L*, a*, b* values for the milk adulterated with detergent at different concentrations were measured using Hunter Color LAB and are tabulated in Table 5 given below. As per Table 5, Hunter color coordinate pattern clearly revealed a change in the milk color after adulteration. *L* values showed a gradual decrease after the addition of adulterants indicating a drift in the color coordinate values from lightness to darkness (*L* = 100 - pure white, *L* = 0 - pure black). Lightness of milk was more affected in samples adulterated with neutralizer and low quantities of added neutralizer were sufficient enough to bring a reduction in

TABLE 1: Quality characteristics of milk adulterated with detergent

Parameters	Raw milk (Homogenized toned milk)	Adulterated milk with detergent (%)				
		0.1	0.3	0.5	0.7	0.9
Moisture	88.37±0.06	87.98±0.01	87.79±0.02	87.53±0.05	87.16±0.09	86.62±0.19
Fat	3.1±0.04	3.2±0.02	3.2±0.04	3.2±0.07	3.2±0.05	3.2±0.10
Acidity	0.169±0.01	0.112±0.00	0.105±0.05	0.060±0.02	0.028±0.25	0.018±0.01
SNF	8.53±0.01	8.82±0.02	9.01±0.02	9.27±0.01	9.64±0.03	10.18±0.01
T S S	11.63±0.00	12.02±0.12	12.21±0.25	12.47±0.05	12.84±0.09	13.38±0.05
pH	6.65±0.04	6.67±0.05	7.05±0.05	7.36±0.05	7.77±0.01	7.85±0.05
Specific gravity	1.030±0.00	1.030±0.02	1.030±0.01	1.031±0.02	1.032±0.14	1.032±0.04
R .I.	1.344±0.00	1.345±0.05	1.345±0.05	1.346±0.051	1.346±0.02	1.348±0.01
Casein	2.69±0.07	2.88±0.01	3.02±0.05	3.33±0.020	3.33±0.02	3.56±0.23
Ash	0.72±0.03	0.73±0.04	0.91±0.05	1.08±0.006	1.19±0.015	1.23±0.07
Lactose	4.73±0.03	4.68±0.01	4.59±0.15	4.51±0.005	4.39±0.020	4.35±0.01

TABLE 2: Quality characteristics of milk adulterated with neutralizer

Parameters	Raw milk (Homogenized toned milk)	Adulterated milk with neutralizer (%)			
		0.1	0.5	1	2
Moisture	88.37±0.06	88.14±0.08	87.82±0.25	87.48±0.06	87.12±0.02
Fat	3.1±0.04	3.1±0.00	3.1±0.00	3.1±0.00	3.1±0.00
Acidity	0.169±0.01	0.142±0.02	0.123±0.01	0.127±0.02	0.127±0.01
SNF	8.53±0.01	8.76±0.06	9.08±0.01	9.42±0.05	9.78±0.08
T S S	11.63±0.00	11.86±0.12	12.18±0.04	12.52±0.02	12.88±0.03
pH	6.65±0.0	6.74±0.03	6.9±0.01	6.91±0.15	6.93±0.01
Specific gravity	1.030±0.00	1.030±0.02	1.031±0.05	1.034±0.05	1.035±0.05
R .I.	1.344±0.00	1.345±0.01	1.345±0.00	1.345±0.00	1.345±0.05
Casein	2.69±0.07	2.66±0.03	2.72±0.03	2.87±0.02	2.89±0.00
Ash	0.72±0.03	0.73±0.03	0.74±0.08	0.81±0.01	0.89±0.06
Lactose	4.73±0.03	4.74±0.02	4.77±0.05	4.80±0.03	4.83±0.01

TABLE 3: Quality characteristics of milk adulterated with urea

Parameters	Raw milk (Homogenized toned milk)	Adulterated milk with urea (%)				
		0.5	1	3	5	7
Moisture	88.37±0.06	87.85±0.02	87.53±0.05	86.26±0.15	84.77±0.08	83.48±0.02
Fat	3.1±0.04	3.2±0.04	3.2±0.01	3.2±0.01	3.2±0.04	3.1±0.00
Acidity	0.169±0.01	0.153±0.01	0.148±0.01	0.142±0.01	0.139±0.02	0.112±0.03
SNF	8.53±0.01	8.95±0.03	9.25±0.04	10.54±0.02	12.03±0.05	13.42±0.04
T S S	11.63±0.00	12.15±0.06	12.47±0.06	13.74±0.04	15.23±0.04	16.52±0.04
pH	6.65±4.04	6.67±0.01	6.79±0.05	7.21±0.03	7.63±0.01	8.35±0.03
Specific gravity	1.030±0.00	1.0313±0.07	1.0325±0.01	>1.035	>1.035	>1.035
R .I.	1.344±0.00	1.354±0.05	1.355±0.03	1.355±0.07	1.355±0.02	1.356±.002
Casein	2.69±0.07	2.80±0.03	2.84±0.01	3.06±0.05	3.24±0.01	3.24±0.01
Ash	0.727±0.03	0.79±0.12	0.86±0.15	0.98±0.01	1.64±0.03	1.76±0.07
Lactose	4.73±0.03	4.73±0.05	4.73±0.05	4.83±0.01	5.05±0.02	5.21±0.02

TABLE 4: Quality characteristics of milk adulterated with ammonium sulphate

Parameters	Raw milk (Homogenized toned milk)	Adulterated milk with ammonium sulphate (%)				
		0.5	1	3	5	7
Moisture	88.37±0.06	87.89±0.03	87.13±0.04	86.66±0.04	86.22±0.03	85.76±0.08
Fat	3.1±0.040	3.1±0.05	3.1±0.05	3.1±0.03	3.1±0.02	3.0±0.02
Acidity	0.169±0.01	0.175±0.09	0.210±0.02	0.263±0.01	0.340±0.01	0.376±0.02
SNF	8.53±0.01	9.01±0.02	9.76±0.03	10.24±0.02	10.68±0.04	11.24±0.02
T S S	11.63±0.00	12.11±0.05	12.87±0.02	13.34±0.03	13.78±0.04	14.24±0.03
pH	6.65±0.040	6.82±0.06	6.91±0.02	7.03±0.03	7.13±0.02	7.17±0.01
Specific gravity	1.030±0.00	1.034±0.09	>1.035	>1.035	>1.035	>1.035
R .I.	1.344±0.00	1.348±0.05	1.350±0.01	1.351±0.01	1.354±0.07	1.359±0.08
Casein	2.69±0.07	2.85±0.05	2.89±0.05	3.05±0.05	3.23±0.01	3.22±0.01
Ash	0.727±0.03	0.801±0.02	0.834±0.04	0.841±0.03	0.906±0.05	0.929±0.02
Lactose	4.73±0.03	4.51±0.01	4.43±0.02	4.33±0.01	4.25±0.03	4.07±0.06

TABLE 5: Change in colour values of adulterated milk

Sample		L	a*	b*
Raw milk(Homogenized toned milk)		92.69±0.01	-1.31±0.01	12.53±0.01
Milk adulterated with Urea (%)	0.5	91.03±0.04	-2.02±0.115	12.04±0.05
	1	91.17±0.02	-2.32±0.015	12.83±0.04
	3	90.80±0.12	-2.37±0.051	13.21±0.04
	5	90.38±0.14	-2.48±0.080	13.48±0.04
	7	89.69±0.20	-2.39±0.077	13.74±0.10
Milk adulterated with detergent (%)	0.1	83.46±0.20	-2.35±0.18	10.65±0.20
	0.3	82.68±0.18	-2.04±0.02	11.36±0.14
	0.5	82.39±0.12	-2.32±0.05	11.13±0.05
	0.7	82.19±0.07	-2.15±0.33	11.04±0.31
	0.9	81.99±0.11	-2.26±0.03	11.03±0.17
Milk adulterated with ammonium sulphate (%)	0.5	91.17±0.05	-1.55±0.02	13.85±0.07
	1	90.94±0.01	-1.64±0.01	13.94±0.05
	3	90.02±0.01	-1.79±0.07	14.74±0.06
	5	88.67±0.05	-1.88±0.01	15.73±0.05
	7	87.61±0.01	-2.07±0.03	16.66±0.06
Milk adulterated with neutralizer (%)	0.1	90.86±0.02	-1.83±0.03	12.66±0.18
	0.5	90.81±0.04	-1.80±0.02	12.73±0.14
	1	90.42±0.03	-1.71±0.02	13.10±0.02
	2	89.71±0.07	-1.70±0.02	13.13±0.11

the natural color of milk when compared to the effect of other adulterants at the same levels. The a* values of adulterated samples showed different reactions to the adulteration substances and concentration. Values showed decrease in the samples adulterated with detergent and urea, whereas an increase in the a* values with the increasing concentrations of neutralizer. The *b** values representing the yellowness of milk decreased with the addition of detergent and neutralizer whereas showed an increase in the samples adulterated with urea.

Microbial analysis

Effect of detergent: The microbial load in raw milk was found to be $2.6x10^3$ cfu/g. Milk adulterated with 0.1% detergent was $37x10^2$cfu/g, where as the milk adulterated with 0.3, 0.5, 0.7 and 9% showed a reduction in microbial load ($19x10,^2$ $5x10^1$, $2x10^1$ and $8x10^1$ cfu/g respectively). Yeast and molds were not found in adulterated milk samples. The coliform count in raw milk was found to be $1.2x10^1$ cfu/g and adulterated milk with detergent was found to decrease from $4x10^1$ at 0.1% to $2x10^1$ with 0.3% adulteration. The coliform count was also nil in all the adulterated milk samples.

Effect of neutralizers: Milk adulterated with 0.1% neutralizer showed $6.0x10^3$ cfu/g where as the milk adulterated with 0.5, 1 and 2% of neutralizer showed $4.5x10^3$, $5x10^1$, and $3x10^1$ cfu/g respectively. This indicates the growth of colonies decreased, with increasing percentage of neutralizers. The coliform count of adulterated milk with neutralizer batch was found as nil at 0.1 and 0.5%. Yeast and molds also decreased with increasing levels of neutralizers, from $1x10^1$ to nil at 0.1 and 2% respectively.

Effect of urea: Milk samples adulterated with 0.5% urea, showed $8.2x10^2$ cfu/g. whereas the milk adulterated with 1, 3, 5 and 7% of urea showed $3.05x10^2$, $8x10^1$ $7x10^1$, and $4x10^1$ cfu/g respectively. This indicates the growths of colonies decreased, with increasing percentage of urea. The coliform count in the milk batch was found as nil while yeast and molds counts were $3x10^1$ cfu/g. The coliform count of the adulterated milk with urea was found to decrease from $5.5x10^1$ cfu/g at 0.5% to $3.0x10^1$ cfu/g with 7% adulteration. Yeast and molds also decreased with increasing urea adulteration from $2.5x10^1$ cfu/g to nil at 0.5 and 7% respectively.

Effect of ammonium sulphate: Milk adulterated with 0.5% ammonium sulphate showed microbial load of $5.2 x10^2$cfu/g. whereas the milk adulterated with 1, 3, 5 and 7% of ammonium sulphate showed $3.35x10^2$, $2.1x10^2$, $1.1x 10^2$, $8x 10^1$ cfu/g. The coliform counts were $6.5 x10^1$, $4.5x 10^1$ and $3 x10^1$ cfu/g at 0.5, 1 and 3% respectively with adulteration. Yeast and mold counts were nil in the adulterated samples.

CONCLUSION

The present study revealed a significant and direct relation between various physico-chemical and microbiological quality characteristics of milk against different adulterants and their concentration levels. Urea, neutralizers, detergents and ammonium sulphate when added in fresh milk affected its natural and overall composition and contaminated the sample with hazardous constituents. Prolonged accumulation of the chemicals in the form of synthetic milk causes various disorders and diseases in human beings. Milk quality deviates after adulteration from the natural milk constituents; moreover these harmful chemical substances are dangerous for health as well.

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