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Effect of different Heat treatments on the TN, NPN, TAN, β-LgN, NCN, PPN of buffalo raw milk, neutralised milk and milk containing sucrose as additive

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ABSTRACT
Addition of some of the additives such as carbohydrates, nitrogenous substances, aldehydes etc. are known to enhance the heat stability of milk. These additives may also interact with proteins on subjecting the milk to various heat treatments. Much work has been carried out on the changes in milk proteins of buffalo milk during various heat treatments. By neutralising the developed acidity of buffalo milk with various neutralizers or by incorporating additives to enhance its keeping quality, significant changes in salt balance and milk serum proteins are expected as a result of various heat treatments given to milk. Studies on the changes of buffalo milk proteins after incorporating neutralisers or additives and then subjecting to various heat treatments have not been carried out so far. Since the use of neutralisers or additives by the organised sector has increased considerably, the study is of immense importance to the dairy industry. In the present paper, effects of different heat treatments on the TN, NPN, TAN, β-LgN, NCN and PPN of buffalo milk samples viz. raw, milk neutralised with N/10 NaOH & N/10 Na_2CO_3 and milk containing sucrose as additive, were observed, discussed well in tabular form and finally delineated precisely.
Keywords: *Pasteurisation, Boiling, Sterilisation, ANOVA, Nutritive value, Nitrogen, Sucrose, Neutralisers*

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INTRODUCTION

Milk, being the most nutritious and perishable food, is extremely susceptible to microbial contamination which adversely affects its shelf-life. Processing of milk by physical and chemical methods may reduce the microbial load considerably with concomitant increase in its shelf-life. Heat treatments find the most important place for eliminating harmful microorganisms. Due to its high nutritive value, milk is an ideal medium for growth of a variety of microorganisms. If raw milk is not immediately cooled or pasteurised within a reasonable period, the microbial load will increase tremendously, rendering it unsuitable for human consumption. Heat treatments of milk bring about various physicochemical and nutritional changes and products derived there from. These alterations are directly related to the severity of heat treatment given to milk. Heat processing of milk causes denaturation of milk proteins accompanied by alteration in solubility and other physicochemical properties of dairy products [1, 2].

Heat processing affects the ionic mineral balance of milk which may (i) readily reversible, (ii) sluggishly reversible, or (iii) irreversible. In addition to denaturation of whey proteins, on heating milk significant chemical changes involving various amino acids, sulphydryl and disulphide residues are noticeably observed. Addition of some of the additives viz. carbohydrates, nitrogenous substances, aldehydes, etc. are known to enhance the heat stability of milk. These additives may also interact with whey proteins on subjecting the milk to various heat treatments. Studies on the changes in buffalo milk proteins after adding neutralizers or additives and then subjecting it to various heat treatments viz sterilization, pasteurization, boiling…, have not been carried out so far. Since the use of neutralizes and/or additives by the organised sector has increased considerably, the study will be of tremendous importance to the dairying sector [3, 4].

Several researchers have studied the effect of heating milk at various time and temperature combinations on its individual constituents and reported various changes during such heat treatments. Protein, lactose, minerals, enzymes and vitamins in milk are the main constituents which undergo heat induced changes. Proteins undergo denaturation and are involved in browning reaction which lowers its nutritive value. Denaturaion renders these protein fractions insoluble and can be precipitated along with caseinate and the same time there is demineralisation of the undenatured casein (loss of Ca & P).heating causes slight break-down of lactose to lactic acid and various secondary products. Heating of milk also causes a change in its salt balance which considerably affects the stability of the milk products. Milk and milk products exhibit extreme sensitibility to its Maillard reaction as a result of high heat treatments due to the presence of lactose and the whey proteins [5,6].

MATERIALS AND METHODS

Collection of Milk samples

Buffalo milk samples employed for present investigation was collected from the herd maintained at NDRI, Karnal, Haryana. Composite milk sample was collected and stored at 4^0C till further use. Skim milk was obtained by warming buffalo milk to 35^o-40^oC and Alfa-Laval cream separator was employed to separate cream centrifugally. All samples were allowed to develop acidity up to 0.20, 0.25 and 3% as LA and the neutralised with N/10 NaOH, N/10 Na_2CO_3. Similar time was provided to the milk samples containing 10% additive (sucrose).

Heat Processing of Milk Samples

a. Pasteurisation

In a clean dry 250ml glass stoppered Erlenmeyer flask, 100ml of milk sample was taken. The flask was placed to a water bath maintained at nearly 80oC to having rise in temperature i.e, 63^oC within 5 min. After attaining required temperate, the flask was kept in another water bath where the temperature of milk was thermostatically controlled at 63^oC. Milk samples were stayed here for 30_omin with constant shaking and finally cooled under tap water.

b. **Boiling**

In a clean dry 250ml loosely glass stoppered Erlenmeyer flask, 100ml of milk sample was taken. It was heated at about 85^oC in a water bath. It was then shifted to another water bath with continuous heating employing electric device until the milk started boiling momentarily. After doing so, the flask was kept under tap water to cool it.

c. Sterilization

In a clean dry 250ml loosely glass stoppered Erlenmeyer flask, 100ml of milk sample was taken. The flask was pluged with non-adsorbent cotton and sterilised in an autoclave at a pressure of $1.02kg/cm^2/10min$. Finally, pressure was released and flask was taken out to coll under tap water.

ESTIMATION OF TN, NPN TAN B-LGN NCN PPN OF BUFFALO MILK SAMPLES

i. TN

By the method of Rowland [7], TN was estimated. Well mixed 5 ml of sample was taken in 100 ml volumetric flask and it was made 100 ml with glass distilled water. 20 ml of the diluted sample was digested in a Kjeldahl flask using 4 ml H_2SO_4 (Analar) and digestion mixture containing 2g of Na_2SO_4 (Analar) and 0.2g of $CuSO_4$. The digested material was transferred into a Kjeldahl micro distillation apparatus and 15ml of 50% NaOH added and the distillate was adsorbed in H_3BO_3 (2%) solution containing 2-3 drops of mixed indicator (80mg methyl red + 20mg methylene blue dissolved in 100ml ethyl alcohol) and titrated with N/10 H_2SO_4.

ii. NPN

In accordance with the Rowland's method, 10 ml of milk sample was taken in a 50ml volumetric flask and the total volume was made with 15%TCA. Whatman filter paper no.

40. was used to filter it. As given for N, 20 ml of the distillate was digested and distilled. The distillate was titrated with N/100 H_2SO_4.

iii. TAN

According to the method adopted by Aschaffenburg and Dowry (1957), 40 ml of milk was taken in a 100ml volumetric flask and warmed to 40°C. To this milk, 4 ml of 40 per cent Na_2SO_4 was added at 40°C and the mixture was allowed to cool to 20°C. The volume of the flask was made using 20 per cent Na_2SO_4 solution. After mixing the contents of the flask thoroughly, it was filtered through Whatman filter paper no.4. For the determination of N, 10 ml of filtrate was taken. By achieving the difference between TAN and NPN, TAN was determined.

iv. β-LgN

By employing methods of Aschaffenburg and Dowry [8], β-LgN and NPN were estimated together. From the total album determination, 40 ml of the filtrate was pipetted out into a conical flask and 1 ml of 1N HCl was added to lower the pH to 2.0. the mixture was allowed to stand for 30min and the filtered through Whatman Filter paper no.40. As described for N, 10ml of filtrate was digested, distilled and titrated with N/100 H_2SO_4. Value of β- LgN and NPN was so determined. Subtracting the value of NPN, the β-LgN was calculated.

v. NCN

Also by employing methods of Aschaffenburg and Dowry [8], NPN was determined. In 100ml 0f flask, 40ml of milk was taken having added 0ml of distilled water. The temperature of milk was raised to 35°C. After 10 min, 4ml of 10 per cent CH_3COOH was added followed by 4ml of CH_3COONa. At room temperature, volume of the mixture was made 100ml with distilled water and filtered through Whatman filter paper no. 40. With 100ml of H_2SO_4, 10ml of digested and distilled filtrate was titrated as detailed for N. The NCN content estimated.

vi. NCN minus PPN

The fraction was estimated as per the procedure adopted by Aschaffenburg and Dowry (1957). Casein free filtrate (50ml) was pippeted out from the filtrate the filtrate of casein free filtrate obtained in the NCN determination. Anhydrous Na_2SO_4 (6g) was added to the filtrate and slightly warmed to dissolve it. After filtration through Whatman filter paper no. 40. As described for TN, 10ml of filtrate was digested and distilled. Deducting this value from NCN, the value of PPN was calculated.

RESULTS AND DISCUSSION

Samples of buffalo milk were allowed to develop acidity up to 0.25 and 0.30 per cent as LA which were the neutralised with N/10 NaOH, N/10 Na_2SO_4 up to acidity of fresh milk and given different heat treatments. In othe milk samples, sucrose was added and kept at room temperature to develop acidity and analysed.

1. Effect of different heat treatments on the TN of neutralised buffalo milk and milk with added sucrose

The effect of different heat treatments on the TN of neutralised buffalo milk and added sucrose is presented in table 1. TN content of raw buffalo milk was observed to have a declining decline trend proportional to the severity of heat treatments given to milk. Neutralisation of the 3 levels of developed acidizes in milk by N/10 NaOH and N/10 Na_2CO_3 and on subsequent pasteurisation exhibited marginal increase in the values. However, on adding 10% sucrose in milk on pasteurisation which was similar to that in raw milk. On boiling, raw TN was found to decrease in milk neutralised by N/10 NaOH and milk with added sucrose, whereas in milk neutralised with Na_2CO_3, it increased slightly. At 10psi/15min (sterilisation) TN in milk neutralised with N/10 Na_2CO_3 and with sucrose was found to decrease, whereas in milk neutralised with NaOH, it was found to decrease marginally.

Menefee *et al.* [9] and Hostetteler [10] did not report any significant change in TN of milk heated at various temperatures and timings. Tandon and Ganguli [11], however, observed slight reduction in TN of buffalo milk on sterilisation. The marginal reduction observed in the TN of buffalo milk may be due to (i) slight precipitation, (ii) decomposition of proteins to form NH_3 which escape out, and (iii) due to browning reaction (Maillard reaction) in which Lys was lost (Patton *et al,* 1850). These variations in the values obtained for TN may be due to the difference in the composition of bulk milk samples from different breeds of animals at various stages of lactation. The ANOVA carried out to test the significance of difference between 3 treatments and 4 acidities for both neutralisers and additive. The 3 heat treatments had significant effect P<0.01) on the TN content. The 4 acidities levels also differed significantly (P<0.01) with respect to the TN in case of neutralised milk but in milk which has sucrose as additive, the effect of acidity levels was non-significant. The significant interaction among heat treatments and acidity levels suggested that the variations affect the TN content in case of neutralised milk. However, it was non-significant in milk added sucrose (Table 1.1).

2. Effect of different heat treatments on the NPN of neutralised buffalo milk and milk with added sucrose

The effect of different heat treatments on the NPN of neutralised buffalo milk and milk with added sucrose. NPN of raw neutralised milk was observed to posses an increasing trend proportional somewhat to the severity of heat treatments given to milk. On neutralisation of 3 levels of acidities and on successive pasteurisation, NPN was found to increase in N/10 Na_2CO_3 neutralised milk and milk with added sucrose. However, milk neutralised with N/10 NaOH, it decreased marginally. On boiling NPN was found to decreased in N/10 Na_2CO_3 neutralised milk and milk with added sucrose but it increased with NaOH (Table 2). Boiling buffalo milk did not cause any difference in the NPN of buffalo milk. At higher temperatures, it was likely that the protein N breaks up to form NP compounds. An increasing trend in the NPN fraction has also been observed by Tandon and Ganguli [12]. NPN of milk increased at higher temperature of heating probably due to denaturation of WP. The ANOVA carried out to test the significance of difference between 3 treatments conferred significant effect on the NPN. The 4 acidity levels had interaction among heat treatments and acidity levels had no significant levels (Table 2.1).

3. Effect of different heat treatments on TAN of raw, neutralised buffalo milk and milk with added sucrose

The effect of different heat treatments on the TAN of neutralised buffalo milk and milk wit added sucrose is shown in Table 3. TAN in raw buffalo milk was observed to decrease after different heat treatments viz. Pasteurisation, boiling, and sterilisation. The reduction in the TAN was observed in boiled milk than sterilised milk. Neutralisation of the 3 levels of the developed acidities by N/10 NaOH and Na_2CO_3 or milk containing sucrose as additives, on successive heat treatments decreased TAN in all the cases. However, milk neutralised with N/10 after pasteurisation and boiling showed significantly more reduction when compared with raw milk. Milk containing sucrose after sterilisation exhibited slightly more reduction in TAN as observed in neutralised milk.

The denaturation of albumin and globulin was followed by appreciable hydrolysis of proteins which resuted in considerable increase in the proteose and in the NPN. The reduction in TAN was due to the denaturation of whey proteins during boiling and sterilisation or high heat treatments were observed by Ismail [13]. The ANOVA carried out to test the significance of difference between 3 treatment effects and 4 acidity levels for both neutralisers and additive is presented in table 3.1. The 3 heat treatments had significant effect (P<0.01) on the TAN. The 4 acidity levels and nitration between heat treatment and acidity levels had no significant effect.

4. Effect of different heat treatments on β-LgN **of raw, neutralised buffalo milk and milk with added sucrose**

The β-LgN of raw buffalo milk was observed to possess a declining trend on the severity of the heat treatments (Table 4). Neutralisation of 3 levels of developed acidities by N/10 NaOH, Na_2CO_3 and milk containing sucrose additive, on successive pasteurisation decreased β-LgN in all in all cases similar to that in raw milk. On boiling, more reduction (marginal) in β-LgN was observed in both the types of neutralised milks. On sterilisation, the reduction in β-LgN was slightly more in milk added sucrose, whereas it was found to decline to some extent in both the types of neutralised milks.

The reduction was due to the denaturation and precipitation of β-LgN along with casein due to heat treatments. The results are in conformity with the findings of Ismail *et al.* [13]. The ANOVA carried out to test the significance of difference between 3 treatment effects and 4 acidity levels for both neutralisers and additive is shown in Table 4.1. The 3 heat treatments had significant (P<0.01) effect on the total β-LgN. The 4 acidity levels and interaction due to heat treatment and acidities had no significant effect.

5. Effect of different heat treatments on NCN of raw, neutralised buffalo milk and milk with added sucrose

The effect of different heat treatments on NCN of raw, neutralised buffalo milk containing sucrose as additive is presented in table 5. The NCN of raw buffalo milk was observed to decrease proportionately on the severity of heat treatments given. The reduction in NCN after pasteurisation was comparatively too less when compared with boiling and sterilisation. Neutralisation of 3 levels of developed acidities by N/10 NaOH, N/10 Na_2CO_3 on successive pasteurisation, boiling and sterilisation exhibited comparatively lower reduction in NCN content in both the types of neutralised milks. On pasteurisation, milk added sucrose gave almost similar reduction as in both the types of neutralised milks. On boiling and sterilisation, however, marginally more reduction was observed in the NCN as compared with the neutralised buffalo milk. The reduction in NCN due to sterilisation was also observed by Ismail *et al.* [13].

As the milk was heated at increasing temperatures the amount of albumin and globulin coagulation increased and the coagulated proteins were co-precipitated with the casein causing high results for N fraction. In UCW, heat treatment caused considerable lowering of the NCN. The ANOVA carried out to test the significance of difference between 3 treatment effects and 4 acidity levels for both neutralisation and additive. The 3 treatments had significant ($P<0.01$) effect on the NCN. The 4 acidity levels and interaction between heat treatment and acidity had no significant effects (Table 5.1).

6. Effect of different heat treatments on PPN of raw, neutralised buffalo milk and milk with added sucrose

The effect of different heat treatments on the PPN of raw, neutralised milks and milk with added sucrose is exhibited in table 6. PPN of raw buffalo milk was observed to decrease on pasteurisation and boiling, whereas the N fraction increased on sterilisation. In case of neutralised milks and milk with sucrose, it was found to decrease at all the 3 temperatures viz. pasteurisation, boiling and sterilisation. The reduction in PPN was found to be comparatively more in raw milks having similar heat treatments. Various reports pertaining to the effect of heat treatments on PPN levels had been observed to be contradictory. Ismail *et al.* [13] reported no change in PPN due to heating milk at 75°C and 95°C for 15min, whereas 15.1% increase was observed at 120°C for 15min. However, Hostettler *et al.* [10], Tandon and Ganguli [11] had reported reductions in PPN on various heat treatments.

The ANOVA carried out to test the significance between 3 heat treatments effects and 4 acidity levels for both neutralisers and added sucrose is exhibited in table 6.1. The 3 heat treatments had significant ($P<0.01$) effect on the PPN. The 4 acidity levels also differed significantly ($P<0.01$) with respect to PPN in the case of neutralised milk and milk added with sucrose. The significant interaction among heat treatments and acidity levels suggest that the variations affect the PPN in all the cases.

Table 1: Effect of different heat treatments on the TN of neutralized buffalo milks & milk containing sucrose as additive

Milk samples		Pasteurized milk			Boiled milk			Sterilized milk		
		Range	Mean	Change%	Range	Mean	Change%	Range	Mean	Change%
Raw milk		590.2-679.3	636.2	-	585.2-672.8	631-4	-	580.2-664.2	625.3	-
Neutralized milk 0.20% LA	NaOH	592.4-681.8	638.0	+0.28	585.3-672.8	631.3	-0.02	590.2-680.2	636.9	+1.85
	Na_2CO_3	592.3-681.9	638.0	+0.28	593.4-682.2	638.6	+1.14	582.2-680.8	636.9	-0.07
	Sucrose (A)	590.2-679.5	636.2	-	585.3-672.8	631.4	-	580.0-664.2	625.3	-
Neutralized milk 0.25% LA	NaOH	592.4-682.4	638.1	+0.29	585.3-672.7	631.3	-0.02	590.3-680.7	636.8	+1.83
	Na_2CO_3	592.3-681.8	637.8	+0.25	593.5-692.3	638.7	+1.15	582.4-665.8	625.8	-0.07
	Sucrose (Additive)	590.3-679.4	636.1	-0.02	585.2-672.7	631.4	-	580.2-664.3	625.2	-0.20
Neutralized milk 0.30% LA	NaOH	592.3-682.0	638.1	+0.29	585.4-672.6	631.2	-0.04	590.4-680.7	636.8	+1.83
	Na_2CO_3	592.4-681.9	637.9	+0.26	592.8-682.4	638.6	+1.12	582.3-665.7	625.7	-0.06
	Sucrose (Additive)	590.2-679.5	636.1	-0.02	585.2-672.6	631.3	-0.02	580.2-664.4	625.2	-0.02

Table 2: Effect of different heat treatments on the NPN of neutralized buffalo milks & milk containing sucrose as additive

Milk samples		Pasteurized milk			Boiled milk			Sterilized milk		
		Range	Mean	Change%	Range	Mean	Change %	Range	Mean	Change%
Raw milk		38.2-42.4	40.5	-	39.2	42.0	-	40.5-45.2	43.1	-
Neutralized milk 0.20% LA	NaOH	38.2-42.2	40.3	-0.50	39.5-42.9	41.9	-0.24	40.5-45.2	43.1	-
	Na_2CO_3	38.0-42.3	40.5	-	39.5-43.3	42.0	-	39.5-44.8	42.6	-1.17
	Sucrose (A)	38.1-42.3	40.4	-0.25	39.3-43.3	42.1	-0.23	39.8-44.9	42.6	-1.17
Neutralized milk 0.25% LA	NaOH	38.1-42.1	40.2	-0.75	39.4-43.0	41.9	-0.24	40.6-45.3	43.2	+0.23
	Na_2CO_3	38.2-42.4	40.4	-0.25	39.4-43.2	41.9	-0.24	39.4-44.4	42.6	-1.17
	Sucrose (A)	38.0-42.2	40.3	-0.50	39.4-43.2	42.0	-	39.7-44.7	42.7	-0.93
Neutralized milk 0.30% LA	NaOH	38.0-42.2	40.2	-0.75	39.5-42.8	41.8	-0.48	40.6-45.3	43.2	+0.23
	Na_2CO_3	38.2-42.3	40.4	-0.25	39.4-43.3	41.8	-0.48	39.4-44.6	42.5	-1.28
	Sucrose (A)	37.9-42.2	40.2	-0.75	39.3-42.2	41.8	-0.48	39.6-44.7	42.7	-0.93

Table 3: Effect of different heat treatments on the TAN of neutralized buffalo milks & milk containing sucrose as additive

Milk samples		Pasteurized milk			Boiled milk			Sterilized milk		
		Range	Mean	Change%	Range	Mean	Change %	Range	Mean	Change%
Raw milk		85.2-94.2	88.1	-	12.5-14.2	13.3	-	14.5-16-5	15.6	-
Neutralized milk 0.20% LA	NaOH	85.1-94.4	88.1	-	12.2-14.3	13.2	-0.76	14.4-16.4	15.5	-0.65
	Na_2CO_3	84.8-94.1	87.9	-0.23	12.1-14.2	13.1	-1.15	14.5-16.6	15.7	+0.64
	Sucrose A)	85.2-94.2	88.0	-0.12	12.4-14.2	13.1	-1.51	14.2-16.2	15.6	-
Neutralized milk 0.25% LA	NaOH	84.8-94.3	87.9	-0.23	12.1-14.3	13.1	-1.51	14.3-16.3	15.5	-0.65
	Na_2CO_3	87.7-94.1	87.9	-0.23	12.0-14.1	13.0	-2.26	14.4-16.5	15.7	+0.64
	Sucrose (A)	85.3-94.3	88.1	-0.12	12.3-14.2	13.1	-1.51	14.2-16.3	15.6	-
Neutralized milk 0.30% LA	NaOH	84.9-94.2	97.9	-0.23	12.0-14.2	13.0	-2.26	14.3-16.3	15.5	-0.65
	Na_2CO_3	84.6-94.0	88.4	+0.35	12.0-14.0	13.0	-3.01	14.3-16.5	15.7	+0.64
	Sucrose (A)	85.2-94.2	88.4	+0.34	12.3-14.1	13.1	-1.51	14.1-16.1	15.5	-0.65

Table 4: Effect of different heat treatments on the β-LgN of neutralized buffalo milks & milk containing sucrose as additive

Milk samples		Pasteurized milk			Boiled milk			Sterilized milk		
		Range	Mean	Change %	Range	Mean	Change %	Range	Mean	Change%
Raw milk		40.2-44.5	42.1	-	8.8-10.6	9.5	-	6.5-7.5	7.0	-
Neutralized milk 0.20% LA	NaOH	40.5-44.3	42.2	-0.24	8.7-10.5	9.4	-1.06	6.5-7.5	7.0	-
	Na_2CO_3	40.5-44.3	42.0	-0.24	8.6-10.4	9.3	-2.11	6.4-7.3	7.0	-
	Sucrose (A)	40.2-44.4	42.0	-0.24	8.7-10.6	9.4	-1.06	6.2-7.3	6.9	-1.43
Neutralized milk 0.25% LA	NaOH	40.3-44.4	42.0	-0.24	8.7-10.6	9.3	-2.11	6.4-7.2	7.0	-
	Na_2CO_3	40.4-44.2	42.2	-0.24	8.3-10.7	9.3	-2.11	6.3-7.3	6.9	-1.43
	Sucrose	40.1-	42.0	-0.24	8.6-	9.5	-	6.0-	6.8	-2.86

	(A)	44.4			10.7			7.2		
Neutralized milk 0.30% LA	**NaOH**	40.3-44.2	41.9	-0.48	8.5-10.4	9.2	-3.16	6.4-7.3	7.0	-
	Na2CO3	40.2-44.1	41.5	-1.43	8.7-10.5	9.4	-1.06	6.3-7.4	6.9	-1.43
	Sucrose (A)	40.4-44.5	42.0	-0.24	8.5-10.7	9.4	-1.07	5.8-7.4	6.9	-1.43

Table 5: Effect of different heat treatments on the NCN of neutralized buffalo milks & milk containing sucrose as additive

Milk samples		Pasteurized milk			Boiled milk			Sterilized milk		
		Range	**Mean**	**Change%**	**Range**	**Mean**	**Change%**	**Range**	**Mean**	**Change%**
Raw milk		120.5-136.3	130.3	-	51.5-62.5	57.6	-	48.7-60.2	55.6	-
Neutralized milk 0.20% LA	**NaOH**	121.2-137.2	130.6	+0.23	52.4-62.8	58.0	+0.69	49.2-60.4	55.9	+0.53
	Na2CO3	120.4-137.3	130.7	+0.30	52.6-62.8	58.2	+1.04	49.4-60.4	56.0	+0.898
	Sucrose (A)	120.4-136.7	130.2	-0.18	51.6-62.4	57.6	-	48.7-60.2	55.2	-0.72
Neutralized milk 0.25% LA	**NaOH**	121.5-137.3	130.7	+0.30	52.6-62.7	58.1	+8.6	49.4-60.4	56.0	+0.71
	Na2CO3	121.5-137.3	130.7	+3.0	52.6-62.7	58.2	+1.04	49.5-60.5	56.1	+0,89
	Sucrose (A)	120.3-136.5	130.1-0.16	51.7-62.2	57.5-62.2	57.5	-0.28	47.4-59.8	55.1	-0.90
Neutralized milk 0.30% LA	**NaOH**	121.3-137.2	130.6	+0.23	52.6-62.8	58.2	+1.04	49.5-60.5	56.1	+0.89
	Na2CO3	121.4-137.2	130.6	+0.23	52.6-62.8	57.5	-0.28	47.4-59.8	55.1	-0.90
	Sucrose (A)	120.3-136.5	130.1	-0.16	51,7-62.2	57.5	-0.28	47.4-59.8	55.1	-0.90

Table 6: Effect of different heat treatments on the PPN of neutralized buffalo milks & milk containing sucrose as additive

Milk samples		Pasteurized milk			Boiled milk			Sterilized milk		
		Range	**Mean**	**Change%**	**Range**	**Mean**	**Change%**	**Range**	**Mean**	**Change%**
Raw milk		9.0-14.0	11.5	-	6.5-8.5	7.6	-	13.2—17.4	15.6	-
Neutralized milk 0.20% LA	**NaOH**	8.5-14.0	11.2	-2.61	6.4-8.4	7.5	-1.32	5.8-9.5	7,7	-50.6
	Na2CO3	8.4-13.8	11.2	-2.61	6.6-8.5	7.5	-1.32	6.2-8.6	7.8	-50.0
	Sucrose (A)	9.0-14.0	11.4	-08.7	6.4-8.4	7.5	-1.32	6.2-8.6	7.8	-50.0
Neutralized milk 0.25% LA	**NaOH**	8.4-13.9	11.2	-2.61	6.3-8.4	7.4	-2.64	5.7-9.4	7.7	-50.6
	Na2CO3	8.4-13.7	11.1	-2.61	6.3-8.4	7.5	-1.32	6.2-8.7	7.8	-50.6
	Sucrose (A)	8.9-13.9	11.4	-8.70	6.3-8.4	7.6	-	6.2-8.6	7.7	-50.6
Neutralized milk 0.30% LA	**NaOH**	8.2-13.8	11.1	-3.48	6.3-8.3	7.4	-2.64	5.7-9.3	7.6	-5.12
	Na2CO3	8.5-13.7	11.1	-3.48	6.4-8.2	7.5	-1.32	6.1-8.7	7.7	-50.6
	Sucrose (A)	8.9-13.8	11.4	-0.87	6.3-8.4	7.6	-	6.1-8.7	7.8	-50.0

Table 1.1 ANOVA of TN

Sources of Variation	df	NaOH		Na2CO3		Additive (Sucrose)	
		M S S	F-Value	M S S	F-Value	M S S	F-Value
Replicate	3	16122.08	12498.94**	15646.28	6967.11**	15686.78	9827.89**
Heat treatment (A)	2	152.89	118.58**	708.26	313.15**	876.81	299.68**
Acidity (B)	3	57.16	44.31**	29.58	18.17**	0.0207	0.0181
Interaction(A) x (B)	6	39.13	30.33**	12.90	5.74**	0.0064	0.0040
Error	33	1.29	-	2.24	-	1.59	-

** Significant at 1% level (P<.01)

Table 2.1 ANOVA of NPN

Sources of Variation	df	NaOH		Na_2CO_3		Additive (Sucrose)	
		M S S	F-Value	M S S	F-Value	M S S	F-Value
Replicate	3	38.87	225.33**	44.28	285.32**	46.20	186.95**
Heat treatment (A)	2	38.31	193.11**	21.76	140.21**	23.97	97.00**
Acidity (B)	3	**0.0580**	**0.3365**	**0.2292**	**1.48**	**0.2436**	**0.9856**
Interaction(A) x (B)	6	**0.0391**	**0.2266**	**0.0766**	**0.4927**	**0.880**	**0.3560**
Error	33	0.1725	-	0.1552	-	-	-

** Significant at 1% level (P<.01)

Table 3.1 ANOVA of TAN

Sources of Variation	df	NaOH		Na_2CO_3		Additive (Sucrose)	
		M S S	F-Value	M S S	F-Value	M S S	F-Value
Replicate	3	51.49	14.05**	51.70	14,31**	47.85	12.55**
Heat treatment (A)	2	28974.79	7904.58**	28875.15	799.02**	29065.32	7621.61**
Acidity (B)	3	0.1080	0.0296	0.1447	0.0400	0.0257	0.0067
Interaction(A) x (B)	6	0.0119	0.0032	0.0470	0.0130	0.0549	0.0144
Error	33	3.66	-	3.61	-	3.81	-

** Significant at 1% level (P<.01)

Table 4.1 ANOVA of β-LgN

Sources of Variation	df	NaOH		Na_2CO_3		Additive (Sucrose)	
		M S S	F-Value	M S S	F-Value	M S S	F-Value
Replicate	3	11.49	26.18**	11.37	25.48**	13.31	31.44**
Heat treatment (A)	2	6121.40	13988.67**	6087.80	13635.37**	6137.56	14492.12**
Acidity (B)	3	0.0564	0.1283	0.1440	0.3227	0.1638	0.3869
Interaction(A) x (B)	6	0.0158	0.0359	0.1308	0.2928	0.0103	0.0244
Error	33	0.4351	-	0.4465	-	0.4235	-

** Significant at 1% level (P<.01)

Table 5.1 ANOVA of NCN

Sources of Variation	df	NaOH		Na_2CO_3		Additive (Sucrose)	
		M S S	F-Value	M S S	F-Value	M S S	F-Value
Replicate	3	386.98	223.35**	382.11	216.27**	417.68**	268.84**
Heat treatment (A)	2	28880.59	16668.62**	28910.23	16363.34**	29029.49	18649.63**
Acidity (B)	3	0.6518	0.3762	0.7517	0.4254	0.1313	0.0844
Interaction(A) x (B)	6	0.0344	0.0198	0.0189	0.0108	0.0620	0.0844
Error	33	1.73	-	-	-	-	-

** Significant at 1% level (P<.01)

Table 6 .1 ANOVA of PPN

Sources of Variation	df	NaOH		Na_2CO_3		Additive (Sucrose)	
		M S S	F-Value	M S S	F-Value	M S S	F-Value
Replicate	3	32.30	77.26**	25.73	53.21**	25.36	62.50**
Heat treatment (A)	2	57.44	137.41**	55.22	114.19**	59.52	146.70**
Acidity (B)	3	23.50	56.21**	22.42	46.36**	20.80	51.28**
Interaction(A) x (B)	6	19.60	46.88**	19.21	39.72**	20.15	49.67**
Error	33	0.4180	-	0,4835	-	0.4057	-

** Significant at 1% level (P<.01)

SUMMARY

The N of raw buffalo milk was observed to have a declining trend proportional to the severity of the heat treatments given to milk. Neutralisation of 3 levels of acidities in milk by N/10 NaOH and Na_2CO_3 incorporated subsequent pasteurisation exhibited marginal increase in its values. However, on adding sucrose (10%) in milk as additive, marginal reduction its values was observed on pasteurisation which showed similarity to that in raw milk. On boiling, TN was found to decrease in milk neutralised by N/10 NaOH and milk added sucrose whereas in milk neutralised with N/10 Na_2CO_3 it increased slightly. At temperatures of sterilisation TN in milk neutralised with N/10 Na_2CO_3 and with added sucrose was found to decrease, whereas in milk neutralised with N/10 NaOH, it was found to increase marginally. The 3 levels of developed acidities and its neutralisation had no significant effect.

NPN of raw buffalo milks was observed to have an increasing trend proportional to the severity of heat treatments given to milk. On neutralisation of the 3 levels of developed acidities and on successive pasteurisation, NPN was found to increase in N/10 Na_2CO_3 neutralised milk and milk with added sucrose. However, in milk neutralised with N/10 NaOH, it decreased marginally. On boiling, NPN was found to increase in all the 3 cases similar to that in raw milk. At the temperature of sterilisation, NPN was found to decrease in N/10 Na_2CO_3 neutralised milk and milk with added sucrose but it increased in milk neutralised with N/10 NaOH. TAN in raw buffalo milk was observed to decrease after different heat treatments viz. Pasteurisation, boiling and sterilisation. Neutralisation of 3 levels of developed acidities by N/10 NaOH and N/10 Na_2CO, on successive pasteurisation, boiling and sterilisation exhibited comparatively lower reduction in NCN content in both the types of neutralised milks. Milk containing sucrose as additive, on pasteurisation gave almost similar reduction, whereas on boiling and sterilisation, marginally more reductions were observed in NCN as compared with the neutralised buffalo milk.

The β-LgN of raw buffalo milk was observed to have a decreasing trend on the severity of the heat treatments. Neutralisation of 3 levels of acidities by N/10 NaOH and Na_2CO_3 and milk with added sucrose, on successive pasteurisation, decreased β-LgN in all the 3 cases similar to that in raw milk. On boiling, more reduction (marginal) in β-LgN was observed in both the types of neutralised milks. On sterilisation, the reduction was slightly more in milk added with sucrose, whereas it was found to decrease to a similar extent in both the types of neutralised milks. NCN of raw buffalo milk was observed to decrease proportionately to the severity of heat treatments given. The reduction in NCN after pasteurisation was comparatively too less when compared at boiling and sterilisation.

Neutralisation of 3 levels of developed acidities by N/10 NaOH and N/10 Na_2CO_3, on successive pasteurisation, boiling and sterilisation exhibited comparatively lower reduction in the NCN content in both the types of milks. Milk containing sucrose as additive, on pasteurisation gave almost similar reduction, whereas on boiling and sterilisation, marginally more reductions were observed in NCN as compared with the neutralised buffalo milk. PPN of raw buffalo milk was observed to decrease on pasteurisation and boiling, whereas it decreased on sterilisation. In case of neutralised milks and milk with added sugar, it was found to decrease at all the 3 temperatures viz. Pasteurisation, boiling and sterilisation. The reduction in PPN was found to be comparatively more in neutralised milks as compared with raw milk having similar heat treatments.

ABBREVIATIONS

WP-Whey Proteins; UCW-Ultra Centrifugal Whey; AC- Acid Casein; ANOVA- Analysis of Variance; AW-Acid Whey; BM; Buffalo Milk; CM-Cow Milk; HTST-High Temperature Short Time; MSS-Mean Sum of Squares; NCN- Non Casein Nitrogen; NPN- Non Protein Nitrogen; PPN-Proteose Peptone Nitrogen; TAN- Total Albumin Nitrogen; TN- Total Nitrogen; β-LgN- Beta lactoglobin ; α-La- Alfa Albumin; Nitrogen; NaCl-Sodium Chloride; NaOH- Sodium Hydroxide; Na_2CO_3- Sodium Carbonate; N/10 Decinormal; A- Additive; Lys-Lysene; NH_3- Ammonia; H_2SO_4- Sulphuric Acid; Na_2SO_4- Sodium Sulphate; $CuSO_4$-Copper Sulphate; B (OH_3)- Boric Acid; CH_3COOH- Acetic acid; CH_3COONa- Sodium Acetate; TCA- Triochloro Acetic Acid; LA- Lactic Acid; KN-Casein Nitrogen; EDTA- Ethyelene Diamine Tetra Acetate; KCl- Potassium Chloride; M- Molarity; psi-per square inch; d.f- Degree of Freedom; NDRI- National Dairy Research Institute; ICAR- India Council of Agriculture Research

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