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Physicochemical Changes during Storage of Ultra High Temprature Processed Whole and Skimmed Buffalo Milk

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Physicochemical changes in Ultra high temperature (UHT) whole and skimmed buffalo milk stored at 10, 20 and 40°C for three months were studied. A significant decrease in pH and an increase in titratable acidity occurred during storage of UHT whole and skimmed milk at 20 and 40°C after three months of storage. At 10°C, viscosity of milk increased to some extent whereas decrease in vicosity was observed at higher temperaures. After three months storage at 40°C, total ash and lactose contents decreased by 18.5% and 6.67% from UHT whole milk and 20.93% and 15.66% from UHT skimmed milk, respectively. Similarly, decrease in casein nitrogen was 14.58% and 16.66% for UHT whole and skimmed milk respectively, after three months storage at 40°C. On the other hand, non protein nitrogen and non casein nitrogen increased by 68.72% and 47.72% in UHT whole milk and 64.28% and 31.25% in UHT skimmed milk, respectively. However total nitrogen content of UHT milk remained unchanged during storage. A significant increase in hydroxy methyl furfural (HMF) values was observed depending upon the initial concentration of lactose in both types of milk and storage conditions. These changes were also observed during storage of UHT milk at 10 and 20°C but to a lesser extent. Subjective evaluation studies showed that UHT milk stored at 10 and 20°C was organoleptically acceptable after three months storage. On the other hand, sensory quality characteristics were adversely affected at 40°C after three months storag of UHT whole and skimmed milk

Key words: Physicochemical changes, UHT treated milk, Storage temperature.

Introduction

UHT and pasteurization processes are commonly used to extend the storage life of milk. However, UHT process is one of the best way of sterilization in which storage life of milk is increased to several months. Losses in nutrients have been observed to some exten during heating process of milk (Burton 1984; Sharma and Darshan 1998). Besides the losses in nutrients, new compounds are also formed due to degradation of lactose on heating of milk (Karin and Erbersdobler 1996; Reddy *et al* 1999). HMF and other browing reaction precursors appear in milk as a result of Maillard reaction between lactose and lysine during these heating processes (Van Bockel and Rehman 1987). Storage of processed milk has also been reported as a factor that can reduce the nutritional quality of milk (Nieuwenhuijse 1995). Nutritional changes in carbohydrate and lysine have been observed during storage of UHT processed milk (Schmidt and Renner 1978; Corzo *et al* 1988). Storage stability of vitamins in processed milks has also been studied by many workers. (Lavigne *et al* 1989, Sierra and Vidal-Valverde 2000). However, there is scarce information in the literature about the physicochemical changes during storage of UHT milk. Therefore, present work was undertaken to compare the physicochemical properties of UHT whole and skimmed buffalo milk during storage at elevated temperature.

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Material and Methods

UHT whole and skimmed buffalo milk sample in ½ litre tetra pak cartons were obtained directly from a commercial milk processing plant. The milk samples were grouped into three categories and stored at 10,20 and 40°C until their expiry date (3 months). The milk samples for analysis were taken out from the cartons after thorough mixing at one month's interval whereas for fat estimation after three months samples were withdrawn underneath the cream layer carefully by means of pipette. The milk samples were analyzed three times, each after one month interval for pH, titratable acidity, viscosity, ash, fat, nitrogenous components, lactose and HMF contents. pH of milk samples was determined using a glass electrode pH meter (PYE Unicam England) whereas titratable acidity of milk was measured by titration against 0.1N sodium hydroxide solution using phenolphthalein as an indicator (A.O.A.C. 1984). Viscosity was measured by means of Hoppler viscosimeter at 20°C. Fat in milk was determined by Gerber method using standard butyrometer (Pearson 1976). Total ash in the sample was estimated after ignition of the dried milk at 550°C for 6 h (A.O.A.C. 1984) while total nitrogen, casein nitrogen, non casein nitrogen (soluble at pH 4.6) and non-protein nitrogen (soluble in 15% TCA) in milk samples were determined according to standard methods of A.O.A.C. (1984). Lactose in milk samples was determined spectrophotometrically at 370nm using phenylhydrazine solution (Wahba 1965) whereas HMF values were measured

Table 1
Titratable acidity, pH and viscosity in UHT whole and skimmed buffalo milk during storage at three temperatures

	Initial values	10°C Storage time (months)			20°C Storage time (months)			40°C Storage time (months)		
		1	2	3	1	2	3	1	2	3
UHT Whole Milk										
pH	6.77^{a} ±0.88	6.77^{a} ±0.72	6.75^{a} ±0.67	6.73^{a} ±0.77	6.72^{a} ±0.69	670^{b} ±0.70	6.60^{c} ±0.70	6.68^{a} ±0.61	6.38^{b} ±0.60	6.10^{c} ±0.62
Titratable acidity %	0.14^{a} ±0.02	0.15^{a} ±0.03	0.15^{a} ±0.03	0.15^{a} ±0.02	0.15^{a} ±0.01	0.16^{b} ±0.02	0.18^{c} ±0.01	0.15^{a} ±0.02	0.18^{b} ±0.04	0.26^{c} ±0.03
Viscosity Cp	1.29^{a} ±0.06	1.42^{b} ±0.07	1.45^{b} ±0.06	1.42^{c} ±0.05	1.26^{a} ±0.03	1.22^{a} ±0.07	1.20^{b} ±0.05	1.30^{a} ±0.05	1.24^{b} ±0.06	1.12^{c} ±0.06
UHT Skimmed Milk										
pH	6.70^{a} ±0.80	6.68^{a} ±0.57	6.69^{a} ±0.72	6.70^{a} ±0.70	6.68^{a} ±0.59	6.64^{b} ±0.77	6.60^{b} ±0.55	6.66^{a} ±0.82	6.44^{b} ±0.68	6.15^{c} ±0.64
Titratable acidity %	0.14^{a} ±0.02	0.16^{a} ±0.01	0.17^{a} ±0.01	0.16^{a} ±0.02	0.16^{a} ±0.03	0.17^{b} ±0.02	0.17^{b} ±0.01	0.15^{a} ±0.02	0.17^{b} ±0.03	0.24^{c} ±0.02
Viscosity Cp	1.26^{a} ±0.06	1.36^{b} ±0.04	1.38^{b} ±0.07	1.40^{c} ±0.80	1.25^{a} ±0.05	1.24^{a} ±0.09	1.20^{b} ±0.07	1.24^{b} ±0.06	1.12^{c} ±0.08	1.06^{c} ±0.04

Mean values± SD n=3. Figures in a row followed by different superscripts indicate significantly ($p<0.05$) different values determined by Duncan's Multiple Range Test

on spectrophotometer at 443 nm after developing colour with thiobarbituric acid as described by Keeny and Bassette (1959).

Organoleptic analysis. UHT whole and skimmed milk after three months storage was evaluated for sensory qualities by a trained taste panel of ten persons. Samples were assessed for colour, taste, flavour, texture and overall acceptability using a nine point hendonic scale ranging from 1 for dislike extremely to 9 for like extremely.

Statistical analysis. Results were carried out in triplicate and standard deviation (SD) was calculated according to the method of Steel and Torrie (1980). Duncans multiple range test was used to determine significant differences ($P<0.05$).

Results and Discussion

Physicochemical changes in UHT whole and skimmed buffalo milk occurred to various extents on storage at three different temperatures. The range of temperature included in this study (i.e. 10,20,40°C) covered the atmospheric temperature of Pakistan.

It is apparent from Table-data that pH and titratable acidity of UHT whole and skimmed milk did not change at all during storage at 10°C. However, decrease in pH and increase in titratable acidity was observed during storage of both types of milk at 20 and 40°C. The increase in titratable acidity was 42.82% for UHT whole milk and 28.57% for UHT skimmed milk after three months storage at 40°C (Tabl 1). The increase in acidity during storage of milk could be attributed to increase in the concentration of free fatty acids, lactic acid and other organic acids which resulted from degradation of milk components mainly fat and lactose. Changes in calcium phosphate equilibrium might be also responsible for increased acidity and reduced pH of stored UHT milk as suggested by Schmidt & Renner (1978). These results are consistent with the findings of earlier workers who found a small reduction in pH of UHT milk stored at higher temperature (Zadow and Chituta 1975).

A slight increase in the viscosity of UHT whole and skimmed milk samples was recorded during three months storage at 10°C (Table 1). However, decreae in viscosity was observed during storage of milk at 20 and 40°C. Increase in viscosity could be the result of dissociaion of beta casein at low temperature due to hydrophilic property of milk proein (Burton 1984). However, decrease in viscosity could be attributed to formation of sediment at the bottom of carton during storage as observed by Mehanna and Gonc (1988).

A gradual decrease in fat and ash contents was also observed during storage of UHT milks (Table 2). Decrease in fat contents was 8.57% and 25.71% after three months storage of UHT whole milk at 20 and 40°C respectively. In fact, de-

Table 2
Fat, ash, lactose and HMF contents in UHT whole and skimmed buffalo milk during storage at three temperatures

	Initial values	10°C Storage time (months)			20°C Storage time (months)			40°C Storage time (months)		
		1	2	3	1	2	3	1	2	3
UHT Whole Milk										
Fat %	3.5^{a} ±0.71	3.5^{a} ±0.62	3.5^{a} ±0.74	3.5^{a} ±0.74	3.5^{a} ±0.64	3.5^{a} ±0.72	3.2^{a} ±0.62	3.3^{a} ±0.63	3.0^{b} ±0.77	2.6^{c} ±0.42
Total ash %	0.81^{a} ±0.12	0.80^{a} ±0.14	0.78^{a} ±0.15	0.78^{a} ±0.11	0.78^{a} ±0.11	0.74^{b} ±0.12	0.70^{b} ±0.14	0.78^{a} ±0.15	0.70^{b} ±0.11	0.66^{c} ±0.10
Lectose %	4.72^{a} ±0.26	4.72^{a} ±0.24	4.68^{a} ±0.27	4.66^{b} ±0.26	4.66^{b} ±0.21	4.62^{b} ±0.20	4.55^{c} ±0.20	4.60^{b} ±0.27	4.54^{c} ±0.23	4.40^{c} ±0.22
HMF µ mole/l	7.78^{a} ±0.42	7.80^{a} ±0.60	7.88^{b} ±0.44	7.92^{b} ±0.56	9.85^{c} ±0.60	10.38^{c} ±0.70	13.00^{d} ±0.46	10.26^{c} ±0.34	15.70^{d} ±039	18.00^{e} ±0.40
UHT Skimmed Milk										
Total ash %	0.86^{a} ±0.21	0.85^{a} ±0.30	0.84^{a} ±0.28	0.82^{a} ±0.21	082^{a} ±0.26	0.78^{b} ±0.22	0.73^{b} ±0.27	0.82^{a} ±0.22	0.75^{b} ±0.27	0. 68^{c} ±0.22
Lectose %	4.98^{a} ±0.24	4.92^{a} ±0.28	4.92^{a} ±0.42	4.90^{a} ±0.32	4.74^{b} ±0.33	4.62^{b} ±0.29	4.56^{c} ±0.40	4.70^{b} ±0.52	4.40^{c} ±0.38	4.20^{d} ±0.50
HMF µ mole/l	10.58^{a} ±0.60	10.68^{a} ±0.51	10.91^{b} ±0.42	11.50^{c} ±0.48	12.70^{c} ±0.50	14.00^{c} ±0.51	18.40^{d} ±0.44	20.00^{d} ±0.68	24.80^{e} ±0.70	28.00^{e} ±0.49

Mean values ± SD n=3. Figures in a row followed by different superscripts indicate significantly ($p<0.05$) different values determined by Duncan's Multiple Range Test

crease in milk fat was about three times more at 40°C which could be attributed to decomposition of milk fat into fatty acids as a result of lipolysis process occurring at higher temperature. Similarly, decrease in ash contents for UHT whole milk was 13.58% at 20°C and 18.51% at 40°C whereas 15.11 and 20.93% decrese in ash contents was observed at 20 and 40°C respectively during storage of UHT skimmed milk for three months (Table 2). In fact, visual examination of packages showed more pronounced sediment and fat separation after three months storage, which might be one of the reasons for decreac in the amounts of minerals (ash) and fat contents in milk during storage. In fact, this sediment contains protein, fat, lactose and minerals in varying proportions. The total amount of sediment also increased during storage but the protein content decreased over the first five weeks and then rose again (Robinson 1994).

During storage of UHT whole and skimmed milk at different temperatures, decrease in lactose and increase in HMF (Table 2) was observed to various extents The changes were more pronounced during storage of milk at 40°C. After three months of storage at 40°C, decrease in lactose contents was 6.77% for UHT whole milk, 15.66% UHT skimmed milk whereas these values were 3.60% and 7.83% at 20°C for UHT whole and skimmed milk, respectively. However, decrease in lactose contents was found to be only 1.27% for UHT whole milk and 1.60% for UHT skimmed milk after three months storage at 10°C (Table 2). It is evident from these results that decrease in lactose content was about two times more at 40°C than at 20°C on storage for three months. Decrease in lactose contents was confirmed by the formation of HMF during storage of milk. Initially, HMF values for UHT whole and skimmed milk were 7.78 and 10.58 µ mole l^{-1}, respectively (Table 2). The variation in HMF values of UHT milk may be attributed to different levels of lactose in these milk samples as reported by Akalin and Gonc (1997). There was gradual increase in HMF values during storage of UHT milk at these three temperatures which became more significant at 40 °C. HMF values become 13 and 18 µ mole l^{-1} for UHTwhole milk, 18.40 and 28.00 µ mole l^{-1} for UHT skimmed milk after three months storage at 20°C and 40°C respectively (Table 2). These HMF values were found to be related to temperature and time period of storage. Formation of HMF has also been reported by Morales *et al* (1997) during storage of UHT cow milk.

Data presented in Table 3 indicates changes in nitrgenous components during storage of UHT whole and skimmed buf-

Table 3

Nitrogenous components in UHT whole and skimmed buffalo milk during storage at three temperatures

	Initial values	10°C Storage time (months)			20°C Storage time (months)			40°C Storage time (months)		
		1	2	3	1	2	3	1	2	3
UHT Whole Milk										
Total nitrogen mg $100 ml^{-1}$	554^{a} ±1.25	550^{a} ±1.20	545^{a} ±1.11	540^{a} ±1.20	548^{a} ±1.20	537^{a} ±0.98	536^{a} ±1.22	545^{a} ±1.40	532^{a} ±1.32	528^{a} ±1.25
Casein nitrogen mg 100 ml^{-1}	466^{a} ±1.27	455^{a} ±0.40	455^{a} ±1.38	427^{a} ±1.44	450^{a} ±1.32	427^{a} ±1.20	420^{b} ±1.26	445^{a} ±1.20	415^{b} ±1.27	398^{c} ±1.26
Non Casein nitrogen mg 100 ml^{-1}	88^{a} ±1.41	95^{a} ±1.44	100^{b} ±1.29	113^{b} ±1.36	98^{a} ±1.27	110^{b} ±1.11	116^{b} ±1.19	100^{b} ±1.08	117^{c} ±1.07	130^{c} ±1.09
Non protein nitrogen mg 100 ml^{-1}	32^{a} ±0.98	35^{a} ±0.76	40^{a} ±0.68	42^{a} ±0.87	35^{a} ±0.80	43^{a} ±0.72	47^{b} ±0.76	38^{a} ±0.77	50^{b} ±0.92	54^{c} ±0.82
UHT Skimmed Milk										
Total nitrogen mg 100 ml^{-1}	476^{a} ±1.22	472^{a} ±1.36	465^{a} ±1.42	462^{a} ±1.33	474^{a} ±1.08	462^{a} ±1.21	455^{a} ±1.09	475^{a} ±1.27	464^{a} ±1.11	458^{a} ±1.08
Casein nitrogen mg 100 ml^{-1}	348^{a} ±0.21	346^{a} ±0.28	325^{b} ±0.42	309^{c} ±0.32	344^{a} ±0.33	322^{b} ±0.29	310^{c} ±0.40	340^{a} ±0.52	314^{b} ±0.38	290^{c} ±0.50
Non casein nitrogen mg 100 ml^{-1}	128^{a} ±1.16	126^{a} ±1.70	140^{b} ±1.28	153^{c} ±1.22	130^{a} ±1.09	140^{b} ±1.46	145^{c} ±1.09	135^{a} ±1.17	150^{b} ±1.27	168^{c} ±1.33
Non protein nitrogen mg 100 ml^{-1}	28^{a} ±0.45	30^{a} ±0.51	32^{a} ±0.39	35^{b} ±0.46	30^{a} ±0.78	35^{b} ±0.77	40^{c} ±0.49	29^{a} ±0.56	40^{b} ±0.59	46^{c} ±0.69

Mean values ± SD n=3. Figures in a row followed by different superscripts indicate significantly ($p<0.05$) different values determined by Duncan's Multiple Range Test

falo milk. It is clear from these results that changes in nitrogenous components in these two types of milk followed the same trend but it was more pronounced in UHT whole milk. After three months storage at 40°C, decrease in casein nitrogen was 14.58% for UHTwhole milk and 16.66% for UHT skimmed milk whereas total nitrogen remained unchanged during storage. On the other hand, increase in non protein nitrogen was 68.72% and 64.28% for UHT whole and skimmed milk, respectively. However, increase in non-casein nitrogen was 47.72% for UHT whole milk and 31.25% for UHT skimmed milk after three months storage at 40°C. These changes in nitrogenous components of both types of milk were almost the same during the first month of storage at all these three temperatures. These changes could be attributed to proteolysis by native milk proteases resistant to UHT heat treatment or to reactivation of proteases during storage (Bengtsson *et al* 1973; Bjorck 1973; Snoeren *et al* 1979). Corzo *et al* (1988) observed the changes in carbohydrate and protein fraction of common UHT and sterilized milk during storage at room temperature. These changes included dissociation of the casein/whey protein complexes, conformational changes of casein molecules including break down of micelle structure, interaction of beta lacto globulin and K-casein, disulphide (S-S), exchange reactions, phosphorylation of casein and interaction of casein and carbohydrates. These changes were accelerated by increased storage temperatures (Robinson 1944).

Sensory qualtiy characteristics of UHT whole and skimmed milk were accepteable after two months storage (Table 4). Deterioration in milk started just after two months storage at 40°C. Fat separation in whole milk and coagulation of proteins appeared during this month at 40°C which adversely affected the sensory qualities of milks. After three months, taste, texture, odour, colour and over all acceptability scores of both types of milk at 40°C were significantly ($P<0.05$) less than the milk samples stored at 10 and 20°C (Table 4). However, score ratings for sensory qualities of UHT whole and skimmed milks stored at 10 and 20°C were not distinctly different from each other. These results coincide with the findings of earlier workers who reported undersirable changes in organoleptic characteristics of UHT milk due to

Table 4
Organoleptic evaluation of UHT whole and skimmed buffalo milk after three months storage

	UHT Whole Milk			UHT Skimmed Milk		
Sensory Attributes	10°C	20°C	40°C	10°C	20°C	40°C
Taste	^{a} 8.45 ±0.26	^{a} 8.02 ±0.22	^{b} 05 ±0.21	^{a} 8.56 ±0.19	^{a} 8.12 ±0.27	^{b} 5.68 ±0.32
Odour	^{a} 8.58 ±0.33	^{a} 8.16 ±0.29	^{b} 6.02 ±0.26	^{a} 8.66 ±0.24	^{a} 8.40 ±0.18	^{b} 6.48 ±0.27
Texture	^{a} 8.62 ±0.34	^{a} 8.33 ±0.30	^{b} 5.11 ±0.36	^{a} 8.75 ±0.32	^{a} 8.52 ±0.32	^{b} 5.06 ±0.29
Colour	^{a} 8.26 ±0.25	^{b} 7.69 ±0.21	^{c} 6.44 ±0.29	^{a} 8.33 ±0.33	^{b} 7.77 ±0.24	^{c} 6.21 ±0.22
Overall acceptability	^{a} 8.44 ±0.27	^{a} 8.24 ±0.41	^{b} 5.66 ±0.22	^{a} 8.50 ±0.32	^{a} 8.20 ±0.32	^{b} 5.72 ±0.31

Mean values ± SD n=3. Figures in a row followed by different superscripts indicate significantly ($p<0.05$) different determined by Dunan's Multiple Range Test

decomposition of milk fat and protein as a result of lipolysis and proteolysis processes (Al-Kanhal *et al* 1994; Garcia-Risco *et al* 1999). Other workers found that the reaction products of Maillard reaction might be responsible for adverse changes in sensory qualities during extended storage (Hansen *et al* 1980). Keeping in view, these facts, it is suggested that UHT milk should not be stored at 40°C for longer periods under hot climatic conditions during the months of summer.

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