

Effects of Storage on Some Physico-Chemical Characteristics of UHT Milk Stored at Different Temperature

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ABSTRACT

Raw milk for UHT processing at commercial dairy plant was collected from well managed dairy farms. The milk was processed by direct system and heated at 143-145°C for 3-5s, then aseptically packed. Three different batches of UHT milk were used in this study. Samples of UHT milk were analysed after processing and during storage at 4, 22 and 37°C ± 2°C for 6 months. The changes in pH, acidity as lactic acid, total solids, viscosity, fat content, electrical conductivity, specific gravity non-protein nitrogen, non-casein nitrogen total and soluble calcium, magnesium, citrate, and phosphorus during storage were studied. The samples were analysed at 0,30,60,90,120,150 and 180 days for the physico-chemical properties of milk. Values indicate an increase in acidity, viscosity, NCN, NPN, soluble calcium magnesium citrate and phosphate. The results showed after statistically analysis that there is a significant affect of storage period and storage temperature on pH, titratable acidity, non – protein- nitrogen, non-casein nitrogen, viscosity, soluble and colloidal calcium and phosphate, soluble magnesium and citrate. There are a significant decrease in pH, electrical conductivity, by increasing storage period, with increasing in acidity ratio, non- protein- non-casein nitrogen and viscosity.

INTRODUCTION

Consumers demand foods that are as fresh as possible with good sensory properties, additionally being safe and having a substantial shelf life, yet without application of additives. Because of its high nutritional value, milk is an excellent medium for microbiological growth. Consequently, fresh milk necessitates a heat treatment in order to guarantee a safe and shelf stable product. The most commonly applied technique to achieve this is heat treatment (Chavan, *et al* 2011).

Ultra-high temperature (UHT) treatment of milk is a heating process at very high temperatures for short holding times, which renders the milk commercially sterile and gives a product with a long shelf life at ambient temperatures. UHT treatments are usually carried out at temperatures of 140 °C for 4-6 s. (Gaucher, *et al* 2008) During heat treatment, chemical, physical and biochemical reactions take place. These changes are significant because they influence nutritional, sensorial and microbiological aspects of

milk. Most bacteria are inactivated but heat-stable enzymes of native or bacterial origin can survive and give rise, during storage, to both gelation and off-flavours (bitter, stale and oxidised) (Burton, 1988).

Gelation is the major physical state change which usually related to protein breakdown (Harwalkar, 1982). It is manifested by a rise in viscosity before formation of a gel and loss of fluidity. This phenomenon, which is irreversible, is known as 'age gelation'. Different causes and mechanisms have been proposed to explain this defect, and proteolysis has often been suspected (Datta and Deeth 2001; McMahon 1996). Proteolysis of UHT milk during storage at room temperature is a major factor limiting the shelf life through changes in its flavour and texture (Datta *et al.*, 2002).

A certain level of proteolysis is required to induce age gelation, the relation between gelation time and extent of proteolysis is controversial (Manji and Kakuda, 1988; Newstead *et al.*, 2006).

The aim of the current study was to investigate susceptibility of UHT milk during storage at different temperature up to 180 days to proteolysis and studying the global changes of physico-chemical composition during storage.

MATERIALS AND METHODS

Materials

Raw milk samples

Three different batches of raw milk (A, B and C) were collected from several well managed dairy farms were heat treated to produce UHT milk.

Manufactured of UHT milk

The UHT milk batches were processed on famous milk factory using direct (Infusion) heating at 143-145°C for 3-5 s, homogenized downstream using a 2-stage aseptic homogenizer operating at 150 and 50 bar in the first and second stages, respectively. The UHT processed milks were filled aseptically in 1 liter Tetra Pak.

UHT milk samples

UHT samples of three batches (A, B, C) were obtained immediately after processing. Each batches was divided to three group the first was stored at refrigerator temperature (4±2 °C) the second at room

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temperature (22 ± 2 °C) and the third at incubator temperature (37 ± 2 °C).

The stored samples were examined, fresh (just after processing) and every 30 days up to 180 days.

Analytical methods

The pH values were determined either in raw milk or UHT milk by using Philips pH meter type 9418/30 with combined electrode at 20 ± 2 °C.

The total acidity (as lactic acid) and specific gravity of milk were determined according to AOAC (2005).

The fat content was determined according to the AOAC (2005) by Rose-Gottlieb process extraction method using diethyl ether, petroleum ether and ethanol.

The electrical conductivity was measured by using a Radiometer conductivity type CDM2C.

The viscosity was measured at 20°C under constant conditions using a Hoppler viscometer type BH. The viscosity expressed in centipoises (cp) and calculated by using special formula and table supplied with the viscometer.

Nitrogen fractions (total nitrogen, non-casein nitrogen and non-protein nitrogen) of milk, total calcium, magnesium, citrates and phosphates content were determined according to the method described in AOAC (2005).

The resulting filtrate of non-protein nitrogen was used to determine the soluble calcium magnesium, citrates and phosphates content following the method described by A.O.A.C. (2005).

Statistical Analysis

The data were analyzed according to statistical procedure of analysis of variance (ANOVA), and in case of significant differences, the mean were further computed using least significant difference (LSD) at 0.05 level of probability through computerized statistical package i.e, Student Edition of Statistics (MSTAT-C), version 8.1 (Copyright 1996, Analytical software, USA).

RESULTS AND DISCUSSION

The chemical analysis of UHT milk during storage

The gross chemical composition of UHT milk samples in this study presented in table 1. The changes that have taken place during storage dependent on temperature of storage. The pH values were decreased gradually during storage from 6.73 to 6.53, 6.71 to 6.50 and 6.70 to 6.36 at 4 ± 2 , 22 ± 2 and 37 ± 2 °C respectively after 6 month of storage (table 1). This data indicated that the highest pH decrease was reported at 37 °C. Correspondingly, the decrease in pH values of UHT

milk from powder milk was reported by Ernani *et al.*, (1997), the authors showed that the rate of decrease was greater at 25 ± 1 °C than at 3 ± 1 °C and after 6 months of storage. Gaucher *et al.*, 2008, Venkatachalam *et al.*, 1993, Celestino *et al.*, 1997 and Telles *et al.*, 2007 found that decrease in pH during 6 months of storage at 4, 20 and 40 °C and the highest changes was at 40 °C. The changes in pH values started after 30, 60 and 90 days of storage at 4, 22 and 37 ± 2 °C, respectively and the massive pH decrease was observed after 180 days of storage at 22 and 37 ± 2 °C. Similar results were reported by El-Dakhakhny (1990), who found that the pH decreased with increasing the storage heat temperature and the highest decrease was after 180 days after incubation at room temperature. While Kawady (2004) concluded that the milk type and storage temperature had no significant effect on pH value, while storage period had significant effect on pH. Putative solution for pH decrease illustrated by Vujicic *et al.*, (1991) who mentioned that adjusting pH from 6.4 to 6.6 with NaOH improved the UHT-sterilized recombined concentrate stability during the first 30 days of storage. Highly significantly changes in pH, acidity non protein nitrogen (NPN) and non casein nitrogen (NCN) ($p \leq 0.05$) were observed during storage at 37 ± 2 °C. Meanwhile, no significant changes were recorded for fat and total solids determination for all tested samples at temperature/times of storage (table 1).

Consequently, the titratable acidity was increased gradually during storage from 0.142 to 0.154, 0.143 to 0.160 and 0.146 to 0.164 at 4 ± 2 , 22 ± 2 , and 37 ± 2 °C respectively after 6 month of storage (table 1). Moreover, the statistical analysis indicated that the storage period had a significant effect on titratable acidity in all batches these results were in agreement with Fink and Kessler, (1986), Mehanna Gone (1988) they found that there was a progress relation between the acidity of UHT milk samples and storage time. Rerkrai *et al.*, (1997) reported that the increase in acidity were slightly greater in UHT samples stored at room temperature than that stored under refrigeration. While Kawady (2004) concluded that the milk type and storage temperature had no significant effect on acidity, while storage period had significant effect on acidity.

Also table 1 showed that total solids (TS) content were ranged between 11.69 to 12.47% among the samples. Present results showed no significant differences in the total solids values of UHT milk samples during storage at different temperatures. The statistical analysis showed that the storage period had no significant effect on total solids in all batches. These results were agreed with Barbano *et al.*, (2006). (1987). While Telles *et.al.*, (2007) performed a trial to characterize 30 samples of raw milk and

Table 1. Chemical analysis of UHT milk stored at different temperatures

Storage temp.(°C)	Storage time(days)	pH	Acidity%	Fat%	Total Solids (TS %)
4±2 °C	0	6.73	0.1417	3.263	11.697
	30	6.72	0.1417	3.263	11.697
	60	6.70	0.1427	3.263	11.727
	90	6.63	0.1463	3.223	11.800
	120	6.58	0.1487	3.237	11.877
	150	6.55	0.1507	3.187	12.060
	180	6.53	0.1537	3.167	12.240
22±2 °C	0	6.71	0.1430	3.263	11.697
	30	6.67	0.1450	3.253	11.698
	60	6.63	0.1478	3.233	11.757
	90	6.59	0.1500	3.22	11.850
	120	6.55	0.1533	3.203	11.920
	150	6.52	0.1570	3.187	12.130
	180	6.48	0.1603	3.156	12.470
37±2 °C	0	6.70	0.1463	3.263	11.680
	30	6.54	0.1527	3.263	11.698
	60	6.51	0.1540	3.233	11.777
	90	6.49	0.1563	3.233	11.850
	120	6.45	0.1590	3.207	12.037
	150	6.41	0.1613	3.197	12.193
	180	6.36	0.1638	3.154	12.244
L.S.D.* _{0.05}		0.0784	0.0034	ns	ns

LSD: Least significant difference. TS: Total solids, ns: not significant.

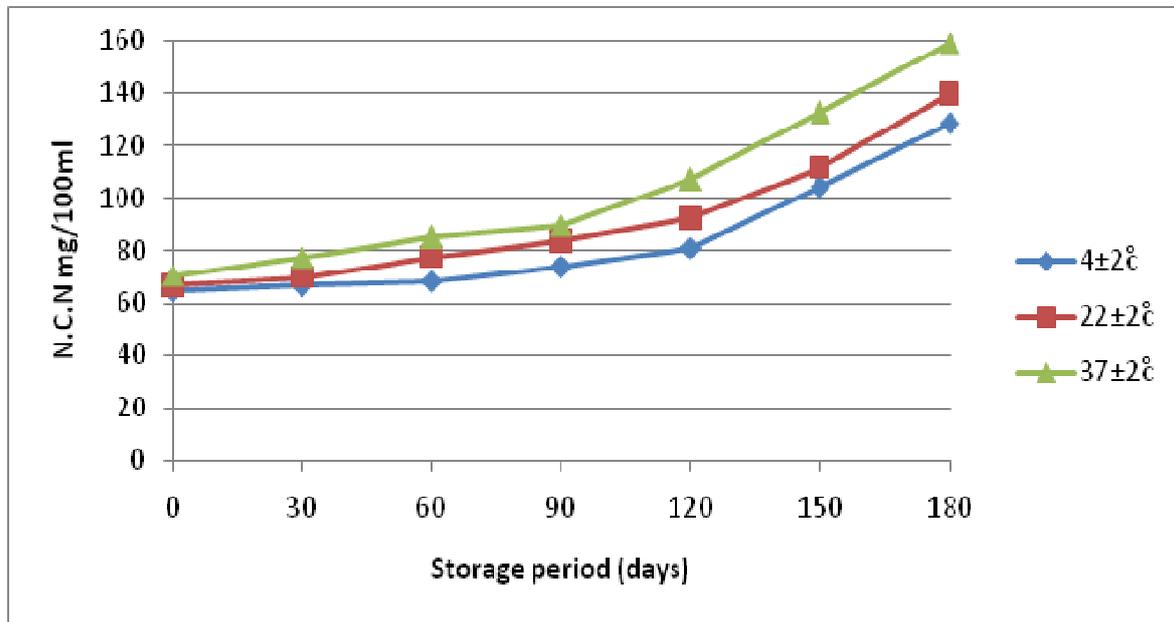


Fig.1. Effect of different storage periods and different storage temperature on NCN* content of different UHT milk samples

NCN*: non casein nitrogen.

the corresponding UHT whole milk obtained by direct heating in Sea Paulo, the authors found significant decrease in measured total solids during storage.

NCN average in freshly processed UHT milk was ranged between 64.66 to 70.39 mg /100 ml (Fig. 1), After 180 days of storage, NCN content increased gradually to 128.91, 139.96 and 159.61 mg /100 ml for milk samples stored at 4, 22 and 37±2 °C, respectively (Fig. 1). These increases in NCN content were significant for all tested storage temperatures. The higher storage temperature (37±2 °C) had the higher NCN content generally.

The initial value for the NPN content was ranged between 37.65 to 40.52 mg/100 ml, and gradually increased to 55.25, 58.71 and 63.84 mg /100 ml after storage for 180 days at 4, 22 and 37±2 °C respectively (Fig. 2). The significant changes of NPN were observed after 30 and 90 days of storage at 4 °C, 22 and 37±2 °C respectively. These increases, suggest proteolysis during storage, which also reported by Gaucher *et al.*, 2008 whereas, El-Din *et al.*, 1991 found that both NCN and NPN where increased during 150 days of storage, and the increments were larger at 30 than 5°C. In the other side, Kawady (2004), concluded that the milk type and storage period had significant effect on total nitrogen, non-protein nitrogen, non-casein nitrogen, casein nitrogen, while storage temperature had no significant effect on total nitrogen, non-protein nitrogen casein nitrogen and non –casein nitrogen.

The determination of minerals (calcium, phosphate, magnesium and citrate) contents and stability for tested UHT milk samples during storage at various temperatures is presented in table 2. The minerals values for freshly processed UHT milk were 40.44 ± 0.27, 36.43 ± 0.04, 7.25 ± 0.07 and 126.37 ± 0.05 mg / 100 mL of soluble calcium, phosphate, magnesium and citrate respectively and 82.58 ± 0.02, 55.48 ± 0.01, 2.11 ± 0.04 and 10.9 ± 0.0 for the colloidal form of the same minerals. The equilibrium of calcium between soluble and colloidal phase is very important phenomenon for milk stability and bioavailability (Singh 2007). Therefore, the partitioning of calcium between the dissolved and colloidal phases was studied during storage of the UHT milk samples at different temperatures. Normally the distribution of original calcium of milk is about one-third of the natural calcium presents in the dissolved state in cow milk. The results in table 2 revealed that calcium content in fresh UHT milk samples was 40.44 ± 0.27 mg / 100 mL and the colloidal contents was 82.58 ± 0.02 mg / 100 mL these results are agree with the corresponding results obtained by Singh (2007) and El-Dakhakhny (1990). This ratio

was changed during the storage as the soluble calcium was increased gradually during storage from 40.20 to 50.87, 40.38 to 53.88 and 40.74 to 54.88 mg / 100ml at 4±2, 22±2 and 37±2 °C respectively after 6 month of storage (table 2).

The determination of soluble and colloidal phosphate presented in table (2) revealed an increasing in soluble phosphate during storage from 36.40 to 36.90, 36.41 to 37.15 and 36.47 to 37.33 mg / 100 ml at 4±2, 22±2 and 37±2 °C respectively after 6 month of storage. While the colloidal phosphate recorded a slight decline almost does not mention gradually during the 180 days of storage (table 2) and did not affect by the storage temperatures. The changes in soluble calcium and phosphate contents may attributed to the high heat treatment of 140 °C, used in this study and the decreased of the pH values during storage especially at the elevated temperature of 37 °C. pouliot *et al.*, (1989), found that increasing the temperature used in the preparation of UHT milk results in the transfer of the soluble calcium and phosphate to the colloidal phosphate, with a concomitant decrease in milk pH. Also, El-Dakhakhny (1990) showed that no alteration in total calcium and total phosphorus contents by heat treatment while a clear changes are observed in soluble calcium and phosphore during storage. The author proved a progressive decrease in soluble calcium and phosphorus contents with increasing time and temperature of storage.

The physical analysis of UHT milk

Table (3) presents the physical changes of UHT milk samples during storage under different temperatures. The statistical analysis showed that there are no significant differences in viscosity between batches (data not shown), while the viscosity were increased gradually during storage from 1.337 to 1.877, 1.382 to 2.07 and 1.393 to 2.237 cp after 6 month of storage at 4±2, 22±2 and 37±2 °C respectively. The significant increase in viscosity started after 30 days of storage at all storage temperatures while the highest changes were reported after 120 and 90 days of storage at 22 and 37 °C respectively (table 3). These results concluded that the storage period had a great significant effect ($p \leq 0.05$) on the viscosity of stored UHT milk samples even at refrigerated temperature. These results are agree with the corresponding results determined by Ernani *et al.*, (1997). Also, Kawady (2004) concluded that the milk type and storage period had significant effect on viscosity, while the storage temperature had no significant effect on viscosity. El-Dakhakhny (1990), found that the different storage temperatures and storage periods had clear effect on viscosity.

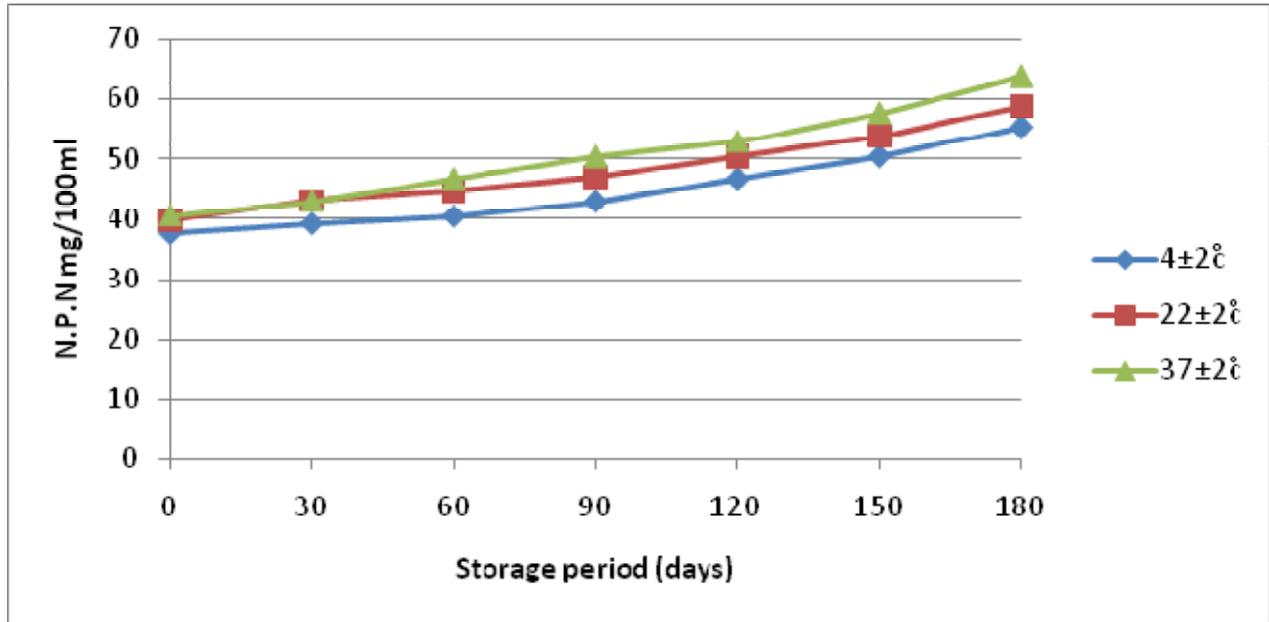


Fig.2. Effect of different storage periods and different storage temperature on NPN* content of different UHT milk samples

NPN*: non protein nitrogen.

Table 3. Physical analysis of UHT milk storage at different temperature

Storage temp. (°C)	Storage time(days)	Physical characters		
		Viscosity (cp)	Electrical conductivity (EC) (Umhos/cm)	Specific gravity
4±2 °C	0	1.337	6.800	1.032
	30	1.475	6.733	1.032
	60	1.587	6.433	1.033
	90	1.760	5.867	1.033
	120	1.673	5.800	1.034
	150	1.803	5.267	1.035
	180	1.877	4.600	1.036
	22±2 °C	0	1.382	6.600
30		1.677	6.670	1.033
60		1.793	5.367	1.033
90		1.807	5.267	1.034
120		1.880	4.800	1.034
150		2.030	4.267	1.035
180		2.070	4.030	1.036
37±2 °C		0	1.393	6.570
	30	1.810	5.567	1.033
	60	1.823	6.500	1.033
	90	1.900	5.333	1.034
	120	2.017	5.000	1.034
	150	2.127	4.470	1.035
	180	2.237	4.220	1.039
	L.S.D.* _{0.05}		0.134	0.317

LSD: Least significant difference. cp: centipoises. Umhos/cm: Micromhos /cm

Electrical conductivity determination showed no significant differences in electrical conductivity between batches of fresh UHT milk then decreased gradually from 6.80 to 4.60, 6.60 to 4.03 and 6.57 to 4.22 after 6 month of storage at 4 ± 2 , 22 ± 2 and 37 ± 2 °C, respectively (table 3). The statistical analysis indicated that the storage period had significant effect on conductivity in all batches. The specific gravity were increased gradually during storage from 1.032 to 1.035, 1.032 to 1.036 and 1.032 to 1.039 after 6 month of storage at 4 ± 2 °C, 22 ± 2 °C and 37 ± 2 °C, respectively.

According to the physico-chemical characteristics of UHT milk, it can be noticed that storage period effect is more than the effect of storage temperature. The appearance of undesirable changes were started in UHT milk stored at 37 ± 2 °C, 22 ± 2 °C and 4 ± 2 °C after 90, 120 and 180 days of storage, respectively.

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