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Assessment of physico-chemical changes in UHT milk during storage at different temperatures

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Abstract

This research communication describes enzymatic and physico-chemical changes during storage of UHT milk. The UHT milk sample was stored at 5 and 30°C for 4 months and analyzed regularly at intervals of one month. During storage of UHT milk, there was a significant ($P < 0.001$) increase in non-protein nitrogen, non-casein nitrogen, soluble calcium, soluble magnesium and proteolysis, while a significant ($P < 0.001$) decrease in pH was observed. There was a slight change in the particle size and zeta potential of casein micelles. Changes were more pronounced in milk samples stored at 30°C than in those stored at 5°C. During storage, there occurred changes in pH, viscosity, salt balance and nitrogenous components which adversely affected quality. It was concluded that the proteolysis led to the acidification which had a destabilizing effect on the milk.

Ultra high temperature (UHT) processing is carried out at very high temperature for short periods of time (135–150°C for 1–10 s), resulting in production of sterile milk which when packaged aseptically is stable for about 6 months at room temperature. During storage of UHT milk, various physio-chemical changes and enzymatic reactions take place including dephosphorylation of casein micelles, breakdown of lactose, precipitation of calcium phosphate, disturbance of salt balance and proteolysis (Gaucher *et al*., 2008; Aldubhany *et al*., 2014). These changes drastically affect the quality of UHT milk as a result of gelation, which limits its shelf life and thus the consumer acceptance (Rauh *et al*., 2014). Age-gelation is a major problem in UHT milk which is related to proteolysis by action of native plasmin or psychotropic bacterial protease (Chavan *et al*., 2011). Many reasons have been proposed to explain age-gelation such as acidification, Maillard reaction, enzymatic and physico-chemical proteolysis (Gaucher *et al*., 2008; Chavan *et al*., 2011; Rauh *et al*., 2014). The gelation of the product occurs in a two-stage mechanism. In the first stage, casein micelles get dissociated from the complex formed between denatured β-lactoglobulin and k-casein during heating of UHT milk. Second stage involves exclusively the physico-chemical changes, which finally results in aggregation of casein micelles forming a 3D structure, causing gelation (Gaucher *et al*., 2008; Chavan *et al*., 2011). However, Auldist *et al*. (1996) failed to show any correlation between rate and extent of proteolysis with the onset of age-gelation, supporting the importance of the second stage gelation mechanism. The exact mechanism of gelation during storage of UHT milk is not yet clear and is still debatable. The objective of the present study was to throw light on the mechanism of first stage reaction of proteolysis, the aggregation of casein micelles and formation of a 3D structure. We investigated the susceptibility of UHT milk to proteolysis during storage. Changes in size and zeta potential of casein micelles, salt balance and nitrogenous compounds were also assessed which were correlated with proteolysis in UHT processed milk during storage at room as well as refrigerated temperature.

Materials and methods

Collection and preparation of milk sample

Nine UHT toned milk processed (by indirect method) samples were collected from Verka Dairy plant, Chandigarh, India. The collected UHT milk samples were divided into two groups; the first was stored at 5°C and the second at 30°C. Samples were examined at regular intervals of one month during 4 months of storage.

pH

The pH was determined using Lab India pH analyzer at 20 ± 2°C.

Viscosity

The viscosity was measured at 20°C using Ostwald viscometer under constant conditions.

Non-casein nitrogen (NCN) and non-protein nitrogen (NPN)

NCN and NPN content was determined according to ISO 8968-4 (2001).

Soluble calcium and soluble magnesium content

Soluble calcium and magnesium content were analyzed according to ISO 8070 (2007).

Particle size and zeta-potential of casein micelle

Particles and zeta-potential of casein micelles were determined using particle size analyser (Zetasizer Nano-ZS90, Malvern, UK) employing the method of Hooda *et al.* (2018).

Proteolysis

Proteolysis in milk samples was assessed using TNBS (Tri nitro benzene sulfonic acid) method in clarified filtrate obtained either by isoelectric precipitation or by TCA precipitation (Chove *et al.*, 2011).

Statistical analysis

The physico-chemical changes during preparation and storage of UHT milk were compared using two-way ANOVA employing Bonferroni Post-Tests to compare results of different months using Prism Graph Pad (Prism version 7.01) software.

Results and discussion

Assessment of the physio-chemical changes and enzymatic reactions during storage of UHT milk is required to understand their impact on quality and shelf life of milk.

pH

It was observed that pH decreased throughout the storage period. The pH value of freshly processed UHT milk sample was observed to be 6.71 ± 0.021 which decreased significantly ($P < 0.001$) to 6.61 ± 0.014 and 6.49 ± 0.014 after storage at 5 and 30°C, respectively for 4 months (Fig. 1a). There was a higher rate of decrease in pH values for samples stored at 30°C than that stored at 5°C. Our results are in agreement with the corresponding results obtained by Aldubhany *et al.* (2014) who also reported that pH of UHT milk decreased during storage. Decrease in pH during storage of UHT milk may be due to dephosphorylation of casein micelles, breakdown of lactose, precipitation of calcium phosphate and proteolysis (Al-Saadi and Deeth, 2008).

Viscosity

Changes in viscosity during storage of UHT milk are depicted in Fig. 1b. Viscosity of freshly prepared UHT milk sample was found to be 1.64 ± 0.021 cP, which increased significantly ($P < 0.001$) to 1.92 ± 0.028 cP and 2.16 ± 0.021 cP after storage for 4 months at 5 and 30°C, respectively. Samples stored at 30°C showed higher viscosity than that stored at 5°C. Our results were in agreement with Aldubhany *et al.* (2014), who also observed a higher increase in viscosity in UHT milk sample stored at 37°C than that stored at 4°C. Increase in viscosity is directly related to the proteolysis which results in gelation during storage of UHT milk (Rauh *et al.*, 2014).

NCN and NPN

NCN content in freshly processed UHT milk was observed to be 53.3 ± 0.562 mg/100 g which was increased significantly ($P < 0.001$) to 77.9 ± 0.14 and 96.05 ± 0.913 mg/100 g after storage for 4 months at 5 and 30°C, respectively (Fig. 1c). NPN content in freshly processed UHT milk was found to be 39.97 ± 0.883 mg/100 g which was increased significantly ($P < 0.001$) to 58.2 ± 0.898 and 68.85 ± 0.777 mg/100 g after storage for 4 months at 5 and 30°C, respectively (Fig. 1d). The sample stored at 30°C showed a higher increase in NCN and NPN content as compared to that stored at 5°C. There was 1.46 and 1.80 times increase in NCN content and 1.45 and 1.72 times increase in NPN content for the UHT samples stored at 5 and 30°C, respectively after 4 months of storage. This increase in NCN and NPN content was might be due to the proteolysis which had occurred during storage of UHT milk sample. Our results are in agreement with Aldubhany *et al.* (2014) also reported increase in NCN and NPN during storage and that the increment was higher in milk samples stored at 30°C than that at 5°C. They reported that after six months of storage, there was 1.99 and 2.16 times increase in NCN content, while 1.46 and 1.56 times in NPN content of milk samples stored at 4 and 22°C, respectively.

Soluble calcium and magnesium content

The soluble Ca content significantly ($P < 0.001$) increased during storage from 524 ± 4.24 to 554.5 ± 3.72 and 569.5 ± 4.17 mg/l at 5 and 30°C, respectively after 4 months (Fig. 1e). Also, the soluble Mg content significantly ($P < 0.001$) increased during storage from 88.5 ± 0.700 to 94.4 ± 2.54 and 98.1 ± 1.37 mg/l at 5 and 30°C, respectively after 4 months (Fig. 1f). Sample stored at 30°C showed greater conversion of colloidal Ca and Mg phase to soluble Ca and Mg phase than that stored at 5°C. Comparing the soluble Mg content of freshly processed UHT milk with samples stored over a period of 2 months, it was observed that samples stored at 5°C showed non-significant changes ($P > 0.05$), while sample stored at 30°C showed a significant ($P < 0.01$) increase. The changes in soluble Ca and Mg contents may be due to decrease in pH values during storage, especially when the samples were stored at 30°C. In concomitant to our results, Aldubhany *et al.* (2014) also reported transfer of Ca and Mg from colloidal to soluble form during storage and that the changes were more pronounced in UHT milk samples stored at 37°C than that stored at 4 and 22°C. They reported that after six months of storage, there was 1.25 and 1.32 times increase in soluble Ca content, while 1.06 and 1.10 times increase in soluble Mg content of milk samples stored at 4 and 22°C, respectively.

Particle size and zeta potential of casein micelles

Changes in casein particle size with storage time and temperature are presented in Fig. 2a and their distribution in Fig. 2c, d. In the present study, skimmed milk was used for analysis of particle size and zeta potential. Casein particle size of freshly processed UHT

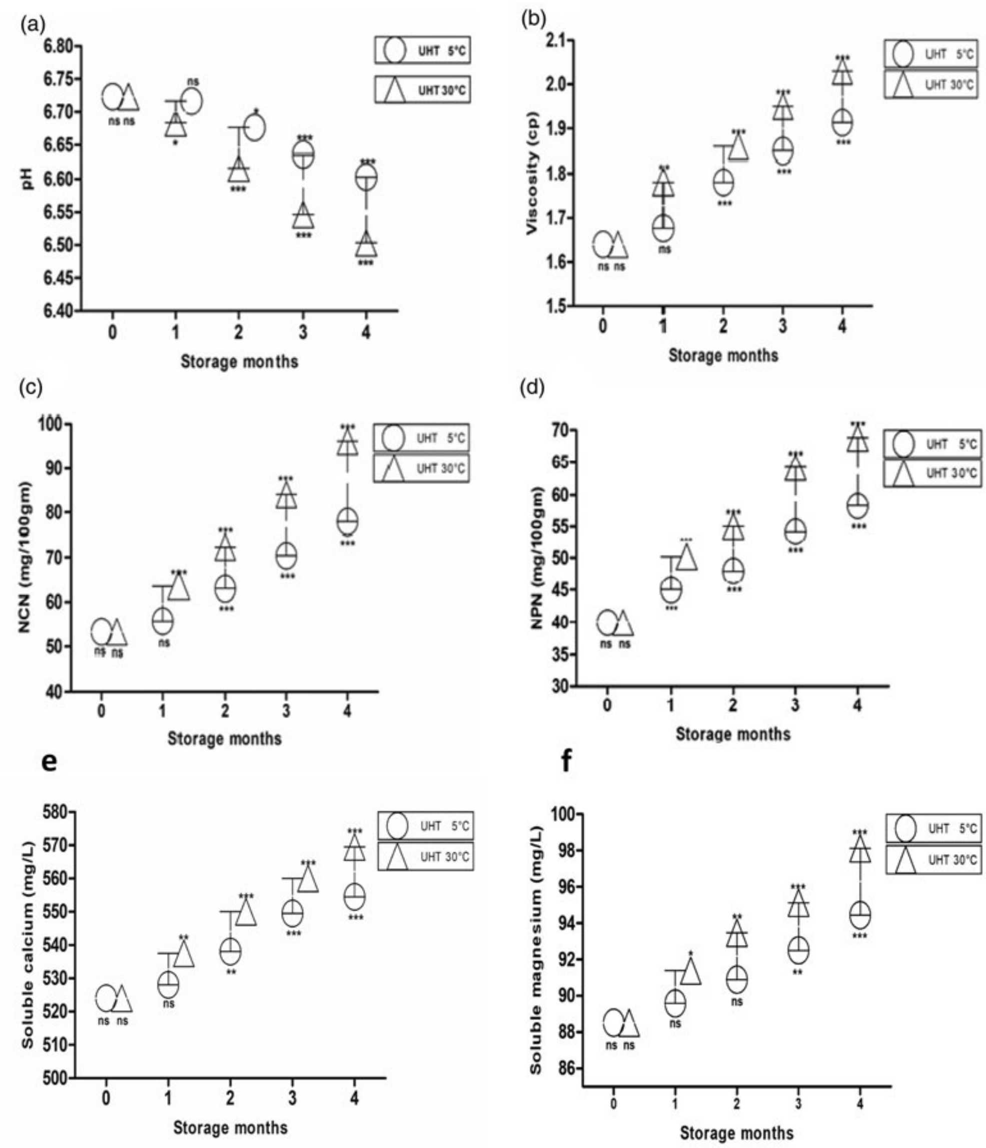

Fig. 1. Changes in pH (a). Changes in viscosity (b). Changes in NCN content (c). Changes in NPN (d). Changes in soluble calcium (e). Changes in soluble magnesium (f). All changes occurring during storage of UHT milk sample at 5°(O) and 30°C (Δ) after 0th, 1st, 2nd, 3rd, and 4th months. Centre line shows the standard error; the circle (O) shows the milk sample stored at 5°C and the triangle (Δ) shows the sample stored at 30°C. All the readings were taken in triplicates. ^{ns}P>0.05, $^{*}P < 0.05$, $^{**}P < 0.01$ $^{***}P < 0.001$.

milk was found to be about 290 ± 6.36 nm. It was observed that there was a slight change in casein micelles size during storage. The casein micelles size increased to 367 ± 5.21 and 342 ± 7.84 nm in UHT milk samples stored at 5 and 30°C, respectively after 4 months of storage. It was found that percentage of larger size particles increased with time in both the stored samples as indicated by the D (4,3) values. The sample stored at 5°C showed formation of larger size particles as compared to that stored at 30°C (Fig. 2c, d). Gelation phenomenon of UHT milk results in changes in the free energy of casein micelles. Differences in potential energy promote aggregation of casein micelles, the extent of which depends upon the probability of contact and number of micelles with small size, both of which increased with storage time (Chavan *et al.*, 2011). Sinaga *et al.* (2017) reported that there occurred a decrease in casein micelle size when pH was decreased from 6.6 to 5.5. The greater decrease was in pH value of UHT milk samples stored at 30°C than that at 5°C, hence there was larger casein micelle size of the sample stored at 5°C than that stored at 30°C. Similar to our results, Rauh *et al.* (2014) also reported that population of the larger particles increased in UHT milk sample after storage for 11 weeks. The zeta potential of freshly processed UHT milk was observed to be −20.5 ± 0.424 mV (Fig. 2b). There was a little change in the absolute value of charge in UHT milk sample stored at 5 and 30°C for 4 months of storage. In the present investigation, zeta potential slightly increased which may be due to Maillard reaction, whereby lactose molecules react with the positively charged group (lysyl and arginyl amino acid), which makes the charge more negative. Decrease in pH value with neutralisation of the negative charges and proteolysis with release of negatively charged groups such as glutamyl, aspartyl, phosphoseryl and glycosidic residues (Gastaldi *et al.*, 2003) can reduce the zeta-potential of micelles.

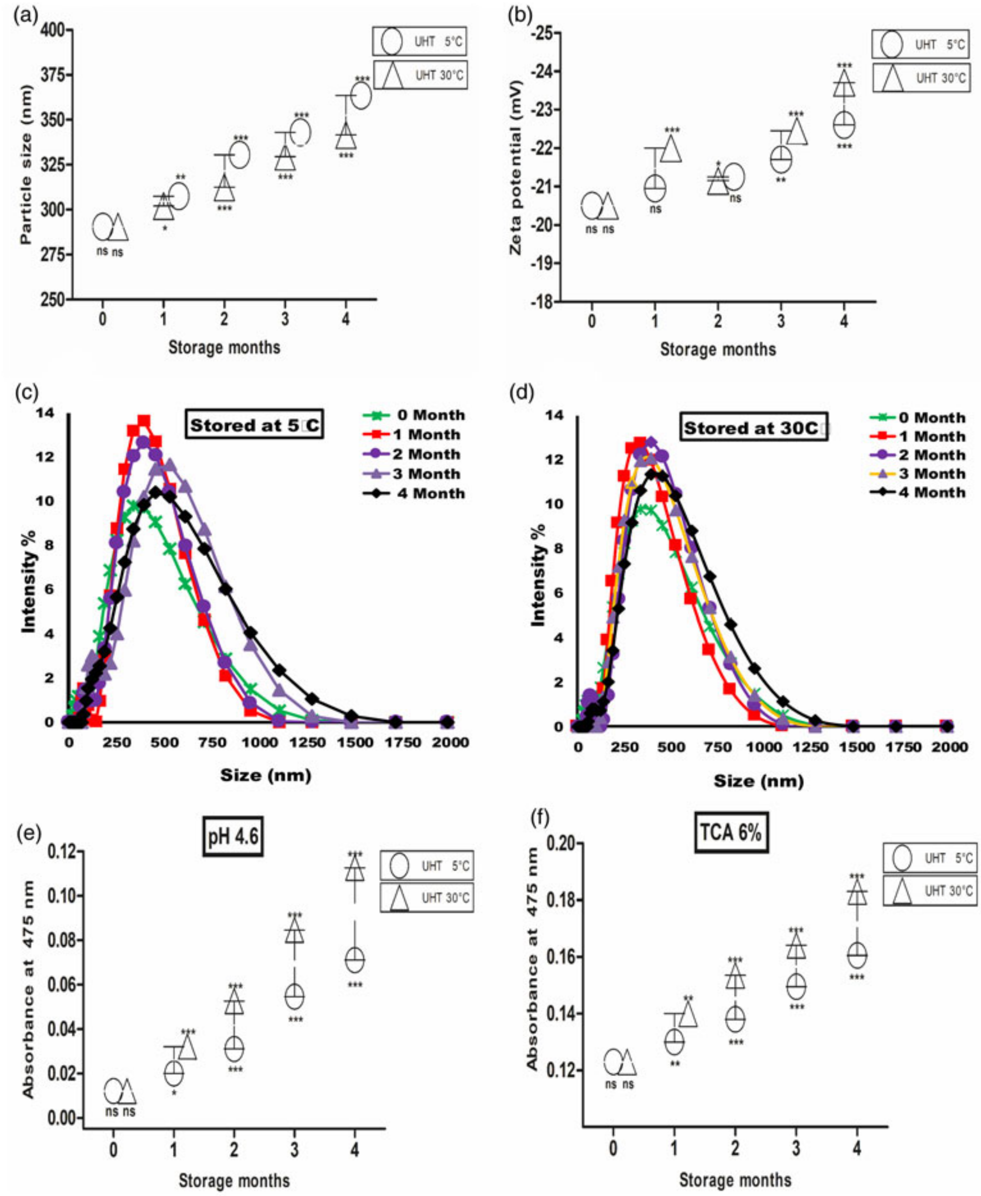

Fig. 2. Changes in particle size (a). Changes in zeta potential (b). Changes in particle size distribution (c) & (d). Changes in proteolysis (e) & (f). All changes occurring during storage of UHT milk sample at 5°(O) and 30°C (Δ) after 0th, 1st, 2nd, 3rd, and 4th months. Centre line shows the standard error; the circle (O) shows the milk sample stored at 5°C and the triangle (Δ) shows the sample stored at 30°C. All the readings were taken in triplicates. $^{ns}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$ $^{***}P < 0.001$.

Proteolysis

The absorbance of extract obtained by isoelectric precipitation of freshly prepared UHT milk sample was 0.012 ± 0.0014 which increased significantly ($P < 0.001$) to 0.072 ± 0.0028 and 0.112 ± 0.0035 after storage for 4 months at 5 and 30°C, respectively (Fig. 2e). Similarly, in TCA extract, absorbance of freshly prepared UHT milk sample was 0.124 ± 0.0042 which significantly increased ($P < 0.001$) to 0.161 ± 0.0021 and 0.184 ± 0.0042 after storage for 4 months at 5 and 30°C, respectively. It was noted that TCA soluble extracts gave higher readings than isoelectric precipitate extract. In isoelectric precipitated extract, there was 7 times increase in absorbance after storage period of 4 months at 5°C in comparison to the freshly processed samples, whereas there was 9 times increase in sample stored at 30°C. For TCA extracts, the absorbance showed 1.32 times increase, whereas there was 1.45 times increase in sample stored at 30°C. Chove *et al.* (2011) advocated TNBS method for prediction of shelf life of UHT milk, since it could detect proteolysis prior to development of off-flavors which is crucial for quality assessment. Increase in the absorbance during storage was may be due to proteolysis.

In conclusion, we have shown that proteolysis is directly linked to changes in particle size, zeta potential, NCN and NPN. All these changes in UHT milk are influenced by storage time and temperature. More proteolysis was observed in the samples stored at 30°C than that stored at 5°C. There was increase in NCN and NPN during storage while a slight change in particle size and charge of casein micelles size was observed. The proteolysis mechanism indicated that the increase in casein particle size and zeta potential may be due to aggregation and formation of larger casein particles during storage of UHT milk. Other factors such as pH and change in phase of Ca and Mg may also be responsible for gelation during storage of UHT milk. These factors limit the shelf life of UHT milk and thus the consumer acceptance. The present study also indicated that it is better to store UHT milk at a lower temperature to preserve its quality.

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