Physico-chemical changes during processing and storage of UHT milk

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Abstract: In this study, physico-chemical and heat induced changes during the preparation and storage of UHT milk were evaluated. Parameters such as pH, acidity, viscosity, sedimentation, colloidal calcium (Ca), colloidal magnesium (Mg), hydroxyl methyl furfural (HMF), lactulose and color value were evaluated. During conversion of raw milk to UHT milk, there was slight increase in viscosity, colloidal Ca, colloidal Mg, HMF, lactulose and colour value, while decrease in pH value was observed. During storage of UHT processed milk there was significant (p<0.001) increase in viscosity, a value, b value, HMF and lactulose content, whereas a significant (p<0.001) decrease in pH, colloidal Ca, colloidal Mg and L value was observed. Increase in acidity, sedimentation content and formation of Maillard browning products adversely affected the quality of UHT milk. These changes were noticed more in UHT processed milk stored at 30°C vis-à-vis 5°C.

Keywords: UHT milk, Calcium, Magnesium, HMF, Lactulose, Sedimentation

Introduction

Ultra-high temperature (UHT) processing of milk is one of the promising heat processing technique for elongating the shelf life of milk. UHT processing of milk is carried out at high temperature (135-150 °C) for a short time (1-10 seconds), resulting in production of sterile milk which when packaged aseptically is stable for about 6 months at room temperature (Al-Saadi and Deeth, 2008; Ranvir et al. 2020a). During processing and storage of UHT milk, various physio-chemical and biochemical reactions take place, including Maillard reactions, degradation of lactose, hydrolysis of lipid, aggregation and denaturation of whey proteins, formation of β-lactoglobulin and k-casein complexes and disturbance of salt balance (Corredig and Dalgleish, 1996; Richards et al. 2014; Sakkas et al. 2014). Apart from these changes, heating process causes development of undesired colors and flavour (especially cooked) which significantly affects the nutritional and sensory quality of UHT milk (Elliott et al. 2003; Elliott et al. 2005; Al-Saadi and Deeth, 2008; Sakkas et al. 2014). These changes drastically affect the quality of UHT milk, which limits its shelf life and thus the consumer acceptance (Rauh et al. 2014; Gaur et al. 2018; Sunds et al. 2018). The heat sensitive components, which are already present or develop in milk during heat processing, allow a direct and quantitative assessment of the processing impact without the detailed knowledge of the actual heat treatment history of the product are called as heat load indicators or thermal time integrator’s (TTIs) (Claeys et al. 2002). TTIs can be categorized into two groups; first one is type- I and another one is type- II indicators. Type I-indicators are heat sensitive components such as whey proteins, vitamins, enzymes etc. During processing and storage, these components undergo inactivation, degradation and denaturation. Type II-indicators are those which are not originally present or present at a low level and are developed during processing and storage of heated milk and milk products e.g. HMF and lactulose (Elliott et al. 2003; Mayer et al. 2010; Sakkas et al. 2014). Type I-indicators are more suitable parameters for the evaluation of low intensity heat processing conditions e.g. pasteurization, while type II-indicators are better for assessment of severe heat treatments, such as UHT processing (De Block et al. 1996; Sakkas et al. 2014; Meshram et al. 2018).

Mostly two types of problems occurs in UHT milk during its storage, the first one is age gelation and the second one is development of changes like off flavour, colour and nutritional loss. The rate of these changes is largely dependent on the storage time and temperature. They cause adverse impact on the quality of UHT milk, thus limiting consumer acceptance (Corredig and Dalgleish, 1996; Richards et al. 2014; Sakkas et al. 2014; Deeth and Lewis, 2017; Ranvir et al. 2020b). Some of the researchers
reported that during storage of UHT milk, there occurs reduction in sweet taste and development of sugary aromatic flavor (Clare et al. 2005; Jensen et al. 2015). The present study has been planned to assess the physico-chemical changes during conversion of raw milk to UHT milk and impact of storage time and temperature on the quality of UHT milk.

**Materials and Methods**

**Reagents**

Trifluoroacetic acid (TCA) was procured from Sisco Research Laboratories Pvt. Ltd, India. Sodium acetate was procured from Loba chemie, India. Other chemicals required in the study were procured from Sigma-Aldrich, Germany. All sample preparations and measurements were carried out using double distilled water (Cascada water purifier, UK) having conductance of 0.055 µS/cm.

**Collection of milk samples**

Raw milk and subsequently pasteurized and UHT processed (by indirect method) toned milk samples were collected from Verka dairy plant, Chandigarh, India. Raw and pasteurized milk were analyzed for their physico-chemical parameters and heat induced components. UHT milk was produced by heating milk at 140°C for 4 s by using an indirect tubular heat exchanger (STORK, Netherlands). Nine UHT milk (fat 3.0% and 8.5% SNF) samples were brought to the laboratory on the day of manufacture and divided into two groups; first group was stored at 5ºC and second group was stored at 30ºC. Samples were examined at a regular interval of one month till 4 months of storage.

**pH**

The pH was determined in UHT milk by using Lab India pH analyzer (M-420, Cyberscan pH Tutor, EUTECH Instruments, Thermo Fisher Scientific, Mumbai, India) with combined electrode at 20±2ºC. Prior to use, the pH meter was calibrated with standard buffer solution of pH 4, 7 and 11.

**Acidity**

Titratable acidity of milk samples was determined as per the procedure described in BIS (IS: 1479 (part-1) 2016).

**Viscosity**

The viscosity was measured at 20°C under constant conditions using Ostwald viscometer in athermostatically controlled water bath.

**Sedimentation**

Sedimentation test was carried out as per the method suggested by Hassan et al. (2009). The UHT milk sample was drain out from the cartons by leaving the bottom 4 cm. The cartons were then inverted for about 10 minutes, up righted and kept in the exhaust hood to dry. The cartons were allowed to dry for 48 hours after opening the bottom flaps or wings of cartons to facilitate the drying of any sediment entrapped there. The cartons were weighed and then washed thoroughly to remove any sediment or residue adhering to the container. The washed cartons were again dried and weighed.

**Colloidal Ca and colloidal magnesium content**

UHT milk samples were defatted by centrifugation (Centrifuge 5810 R, Eppendorf, Sigma-Aldrich, Germany) at 4000xg for 10 min at 4°C and skim milk was ultrafiltered with Amicon® Ultra-15, 10KDa Centrifugal Filter Devices (Merck, Germany) using centrifuge (at 5000xg, 40 min, 25°C). The permeate was discarded and retentate was analyzed for colloidal Ca and Mg content by using atomic absorption spectrometry (AA-7000; Shimadzu, Japan) as described in International Organization for Standardization (ISO, 8070 2007).

**HMF**

Total HMF content in UHT milk was determined using the method suggested by Cais-Sokolinska (2005). Ten millilitre UHT milk sample was acidified with 5 ml 0.3 N oxalic acid followed by heating at 100°C for 60 min. Content was then cooled to room temperature and mixed with 5 ml of 40% trichloroacetic acid solution. The mixture was thoroughly mixed and filtered thorough Whatman paper No. 42 filter paper. One millilitre of aqueous solution of TBA (0.05 M) was then added to 4 ml of the filtrate. The solution was mixed thoroughly and incubated in water bath maintained at 40°C for 40 min. It was then cooled to room temperature and absorbance was measured at 443 nm.

**Lactulose**

The lactulose content was measured by enzymatic method using kit (Cat No: K-LACTUL) procured from Megazyme, Bray, Wicklow.

**Color Value**

Tristimulus spectrophotometer Hunter Lab model Color Flex® [Hunter Associates Laboratory Inc., VA, U.S.A with software (version 4.10)] was used for measuring the color of UHT milk and the results were expressed in terms of the CIE-LAB system according to the method of Popov-Raljic et al. (2008). The instrument was standardized in day light at reflectance angle of 10° (illuminant D65/10° standard observer). The instrument was calibrated with standard black and white tiles as specified by the manufacturer. The light source was dual beam xenon flash lamp. Measurements were made on the milk sample taken in a glass sample cup (10 cm height and 6 cm diameter) supplied with the instrument by filling it to a fixed level (up to 3 cm) for each sample.
Statistical analysis

The results of physico-chemical changes during preparation and storage of UHT milk were compared using two-way ANOVA and that obtained between raw, pasteurized and freshly processed UHT milk through one-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 changes in physico-chemical parameters and production of heat induced components during pasteurization and UHT treatment is required to understand their impact on quality of milk. The changes in physico-chemical and heat induced parameters of raw milk, subsequently pasteurized and UHT processed milk are given in Table 1. During heating of milk, different chemical, physical and biochemical reactions take place. Comparing raw, pasteurized and UHT milk, there was a non-significant (p>0.05) change in acidity and viscosity, while the a value, HMF and lactulose content showed a significant (p<0.001) increase. HMF, colloidal Ca and colloidal Mg content was increased significantly (p<0.05) in pasteurized and UHT milk as compared to that of raw milk (Table 1). Thus, pasteurization and UHT treatment cause several physico-chemical and heat induced changes in milk. Similar results were also obtained by Jeurnink and De Kruif (1993), Elliot et al. (2003), Pestana et al. (2003) Gaucher et al. (2008) and Oh and Deeth (2017). Ritota et al. (2017) reported increase in pH, viscosity, HMF, lactulose, colloidal Ca, colloidal Mg and colour value and decrease in acidity during heating of milk. It is worth mentioning here that a small amount of lactulose (7.09 ± 0.071 mg/L) was also observed in raw milk. Previous researchers (Elliott et al. 2003; Lan et al. 2010) have also reported this important indicator of heat treatment in raw milk. Increase in viscosity during heating may be due to the denaturation of whey proteins (Jeurnink and De Kruif, 1993) and increase in pH may be due to lower whey protein associating with the micelles (Pestana et al. 2003). Burton (1984) reported that increase in colloidal Ca because of ionic Ca combined with the phosphates or the denatured proteins turns into the colloidal Ca form while the Ca moves to the inside of casein micelles, reduced the content of the ultrafiltrable Ca accordingly. There was also increase in colloidal Mg due to their migration into the calcium phosphate microgranules as evidenced by the increase in the size of these particles (Oh and Deeth, 2017). Maillard reaction formed HMF, Lactulose during heating of UHT milk (Sakkas et al. 2014) and also formed brown-coloured pigments (pyralysins and melanoidin) which caused change in colour value (Popov-Raljic et al. 2008).

| Milk Type | Acidity (%L/L) | pH | Viscosity (cp) | Colloidal Ca (mg/100mL) | Colloidal Mg (mg/100mL) | HMF (mg/L) | Lactulose (mg/L) | Colour |
|-----------|----------------|----|--------------|--------------------------|-------------------------|------------|------------------|--------|
| Raw       | 6.61±0.026     | 6.95±0.028 | 1.61±0.022    | 7.41±0.024               | 3.56±0.024             | 7.09±0.071 | 3.56±0.024       |        |
| Pasteurized| 6.65±0.028     | 7.02±0.019 | 1.63±0.044    | 7.52±0.017               | 4.02±0.017             | 7.09±0.071 | 4.02±0.017       |        |
| UHT       | 6.67±0.019     | 7.02±0.022 | 1.64±0.029    | 7.72±0.028               | 4.24±0.186             | 7.09±0.071 | 4.24±0.186       |        |

Data are presented as mean ± SEM (n = 3).

Means within column with different lower case superscript are significantly different (p<0.05) from each other.
Viscosity

Viscosity is an important property of any liquid food because of its direct impact on its appearance and consumer acceptance (DePeters and Cant, 1992). Casein micelles play an important role in determining the viscosity of skim milk (Clare et al. 2005); however, factors like addition of NaCl, pressure, reaction with sugars, ionic strength, pH, and heat also affects the aggregation of the micelles which influences resistance to flow (Broyard and Gaucheron, 2015). Changes in the viscosity during storage of UHT milk are depicted in Fig. 2(a). Viscosity of freshly prepared UHT milk sample was found to be 1.64±0.029 cP which increased significantly (p<0.001) to 1.87±0.005 and 2.01±0.009 cP after storage for 4 months at 5 and 30°C, respectively. It was observed that samples showed non-significant (p>0.05) decrease in pH value at 5°C, while samples stored at 30°C showed a significant decrease (p<0.01) after one month of storage period. Decrease in pH during storage of UHT milk may be due to the dephosphorylation of casein micelles, breakdown of lactose, precipitation of calcium phosphate, and proteolysis (Al-Saadi and Deeth, 2008). Our results are agreeing with the corresponding results obtained by McMahon (1996) and Hassan et al. (2009). Aldubhany et al. (2014) also reported that pH of UHT milk decreased during storage. Decrease in pH value might be a major factor for causing gelation during storage of UHT milk. As previously reported by Hassan et al. (2009) at neutral pH (6.7), the casein micelles are stable, while the lowering of the pH leads to aggregation of casein micelles and formation of a gel.

Acidity

The acidity in UHT milk samples was determined by titration method and it was expressed in terms of percent lactic acid. The results of acidity in UHT milk sample stored at 5 and 30°C are presented in Fig. 1 (b). The acidity value of freshly prepared UHT milk sample was observed to be 0.124±0.025 % lactic acid which increased significantly (p<0.001) to 0.144±0.017 and 0.164±0.0006 % after storage for 4 months at 5 and 30°C, respectively. Moreover, the statistical analysis indicated that the storage period had a significant effect on titratable acidity. The acidity percentage increased significantly (p<0.001) during storage at both 5 and 30°C temperature. The sample stored at 30°C showed higher rate of increase as compared to that stored at 5°C. The Maillard reaction takes place during processing and storage, which degrade the lactose into acids. Formic acid is responsible for increasing titratable acidity of milk during storage. Our results are in agreement with earlier study carried out by Aldubhany et al. (2014), who also reported that a progressive relation exists between the acidity of UHT milk samples and storage time and the rate of increase in acidity was slightly higher in UHT samples stored at room temperature as compared to sample stored under refrigeration.

Sedimentation

The quality of sediment depends on the raw milk and on the type and severity of the heat treatments. For any one type of process, the amount of sediment increases with the severity of the heat treatment (Sweetser et al. 1975; Vankatachalm and MacMahon, 1991). The amount of sediment decreases with homogenization pressure (Robinson, 1994). Sedimentation results of UHT milk samples during storage at 5 and 30°C are illustrated in Fig 2 (b). Sedimentation content in freshly processed UHT milk was observed to be 0.102±0.006 g which was significantly (p<0.001) increased to 1.937±0.018 and 3.417±0.026 g after storage for a period of 4 months at 5 and 30°C, respectively (Fig. 2b). The higher increase in sedimentation value was observed after 2 months of storage at 30°C. It was noted that sample stored at 30°C showed higher rate of increase of sedimentation than the sample stored at 5°C. The increase in sedimentation value during storage of sample may be due to the aggregation of proteins or protein particles of various sizes (Malmgren et al. 2017; Gaur et al. 2018). Similar to our findings, Ramsey and Swartzel (1984), Malmgren et al. (2017) and Gaur et al. (2018) also reported that sediment formation during storage of UHT milk was increased with storage period.

Colloidal Ca and colloidal Mg content

The equilibrium of Ca and Mg between soluble and colloidal phase in milk is important for their bioavailability and stability (Aldubhany et al. 2014). Colloidal Ca and colloidal Mg content in milk are around 66.5 and 33%, respectively (Tewari and Juneja 2007; Huppertz et al. 2017; Singh et al. 2019). The changes in colloidal fraction of Ca and Mg content in UHT milk samples stored at 5 and 30°C are presented in Fig.3 (a) and (b). Colloidal Ca was decreased significantly (p<0.001) during storage from
77.2±0.282 to 73.4±0.351 and 71.3±0.312 mg/100 mL at 5°C and 30°C, respectively after 4 month of storage (Fig 3a). Colloidal Ca also significantly (p<0.001) decreased during storage from 4.22±0.186 to 3.41±0.326 and 2.93±0.214 mg/100mL, at 5 and 30°C, respectively after 4 month of storage (Fig. 3b). Sample stored at 30°C showed greater conversion of colloidal Ca and Mg phase to soluble Ca and Mg phase than that stored at 4°C. The changes in colloidal Ca 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 that transfer of Ca and Mg from colloidal to soluble form occurs...
HMF is an important component used for assessing the intensity of heat treatment. It is also used as an indicator for degree of progress of the Maillard reaction. It is not present in raw milk or present only at trace level and formed during heating and storage of high heat treated milk and milk products (Albalá-Hurtado et al. 1997; Morales and Jiménez-Pérez, 1999; Morales et al. 2000; Murata et al. 2007; Ritota et al. 2017). In earlier stage of Maillard reaction, there occurs condensation of carbonyl group of lactose and [-amino group of lysine residue, followed by development of intermediate product HMF (Morales et al. 2000; Cais-Sokolińska, 2005). Amadori rearrangement product (1-amino-1-deoxy-2-ketoses) formed during severe heating is rapidly converted into HMF under acid conditions (Nursten, 2005). In this study, extent of HMF content was determined by using the method suggested by Cais-Sokolińska, (2005) and results are illustrated in Fig. 4 (a). The HMF content was significantly

**Fig. 3** (a) Changes in colloidal calcium (b) changes in colloidal magnesium content during storage of UHT milk sample at 5°C (Ο) and 30°C (△) after 0th, 1st, 2nd, 3rd, and 4th months.

Centre line shows the standard error; the circle (Ο) shows the milk sample stored at 5°C and the triangle (△) 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.

**Fig. 4** (a) Changes in HMF content (b) changes in lactulose content during storage of UHT milk sample at 5°C (Ο) and 30°C (△) after 0th, 1st, 2nd, 3rd, and 4th months.

Centre line shows the standard error; the circle (Ο) shows the milk sample stored at 5°C and the triangle (△) 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.

with storage and that the changes were more pronounced in UHT milk sample stored at 37°C than that stored at 4 and 22°C.
(p<0.01) increased during storage from 8.03±0.035 to 13.74 ±0.091 and 18.94±0.106 µmol/L at 5 and 30°C, respectively after 4 month of storage. It was observed that there was about 1.7 times increase in the HMF content of the sample stored at 5°C and around 2.3 times increase in the sample stored at 30°C. Higher content of HMF in sample stored at 30°C may be due to the higher rate of Maillard reaction at higher temperature. Our results are in agreement with the previous study carried out by (Birlouez-Aragon et al. 1998; Morales et al. 2000; Elliott et al. 2003; Elliott et al. 2005; Sakkas et al. 2014) who found that the lactulose content in UHT milk is in the range of 50–850 mg/L. Lactulose content up to 15 mg/L in pasteurized milk and 80 mg/L in high temperature pasteurized milk has been reported (Marconi et al. 2004; Feinberg et al. 2006). Similar trend of considerable increase in the lactulose content during storage was also reported (Morales et al. 2000; Elliott et al. 2005; Feinberg et al. 2006). Morales and Jimenez-Perez (1999) observed that the maximum increase in lactulose content was at high temperatures and the highest value was recorded when milk was stored at 40 and 50°C for 90 days.

**Color value**

Heating process, storage condition and heat induced reaction like Maillard’s reaction, causes physio chemical changes in milk, which impacts the color of milk (Popov-Raljic et al. 2008). The color determination denoted in $L$, $a$, $b$ value. $L$ represent for the index of lightness, $b^{(+)}$ represent for yellow color while $b^{(-)}$ represents for blue color. $A^{(+)}$ represent values for red color whereas $a^{(-)}$ represents for green color (Manzi et al. 2013). Changes in the $L$, $a$ and $b$ values of UHT milk samples during storage was also reported (Morales et al. 2000; Elliott et al. 2005; Feinberg et al. 2006). Morales and Jimenez-Perez (1999) observed that the maximum increase in lactulose content was at high temperatures and the highest value was recorded when milk was stored at 40 and 50°C for 90 days.

### Table 2 Color ($L$, $a$, $b$) value of UHT milk sample stored at 5°C and 30°C for 4 months

| Storage period (months) | Storage Temperature | $L$      | $a$       | $b$       |
|------------------------|---------------------|----------|-----------|-----------|
| 0                      | —                   | 87.62±0.091*** | -1.80±0.042m** | 9.33±0.042m** |
| 1                      | 5°C                 | 87.27±0.155*   | -1.04±0.021*** | 9.41±0.018m** |
|                        | 30°C                | 86.52±0.127*** | -0.81±0.077*** | 9.80±0.049*** |
| 2                      | 5°C                 | 86.22±0.12***  | -0.95±0.027*** | 9.55±0.023*** |
|                        | 30°C                | 84.91±0.092*** | -0.65±0.056*** | 9.90±0.029*** |
| 3                      | 5°C                 | 85.81±0.144*** | -0.71±0.028*** | 9.69±0.042*** |
|                        | 30°C                | 80.22±0.106*** | 0.030±0.023*** | 10.15±0.022*** |
| 4                      | 5°C                 | 84.57±0.106*** | -0.55±0.027*** | 9.81±0.017*** |
|                        | 30°C                | 79.84±0.098*** | 0.18±0.018***  | 10.42±0.070*** |

Superscript are significantly different, *p >0.05, **p<0.05, ***p<0.01 ***p<0.001 from each other.

### Lactulose

Lactulose is a semi-synthetic disaccharide consisting of galactose and fructose molecule. During heating of milk and milk product lactose gets isomerized with the formation of lactulose (4-O-ß-D-galactopyranosyl-D-fructofuranose) by Lobry de Bruyn–Alberda van Ekenstein (LA) transformation (Hashemi and Ashtiani, 2010). Lactulose is considered to be a suitable indicator for differentiating UHT milk and pasteurized milk (Montilla et al. 2005). Fig. 4 shows that the increase in lactulose concentration is greatly dependent on the storage time and temperature. Samples stored at 30°C showed increase in lactulose content than samples stored at 5°C. Lactulose content in freshly processed UHT milk was observed to be 251±3.531 mg/L which was significantly (p<0.001) increased to 284±3.535 mg/L and 345±2.121 mg/L after storage for a period of 4 months at 5 and 30°C, respectively. Comparing lactulose content of freshly processed UHT milk with samples stored over a period of 1 month, 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 increase (p<0.001) in lactulose content. Our results are in agreement with the previous study carried out by (Birlouez-Aragon et al. 1998; Morales et al. 2000; Elliott et al. 2003; Elliott et al. 2005; Sakkas et al. 2014) who found that the lactulose content in UHT milk is in the range of 50–850 mg/L. Lactulose content up to 15 mg/L in pasteurized milk and 80 mg/L in high temperature pasteurized milk has been reported (Marconi et al. 2004; Feinberg et al. 2006). Similar trend of considerable increase in the lactulose content during storage was also reported (Morales et al. 2000; Elliott et al. 2005; Feinberg et al. 2006). Morales and Jimenez-Perez (1999) observed that the maximum increase in lactulose content was at high temperatures and the highest value was recorded when milk was stored at 40 and 50°C for 90 days.
40°C. Popov-Raljic et al. (2008) reported that the average psychometric chroma b* value was significantly higher (p<0.01) during storage of UHT milk with 3.2% milk fat.

Conclusion

In agreement with other studies, pasteurized milk showed less heat damage than the UHT processed milk sample. There was increase in acidity, viscosity, sedimentation value, while decrease in colloidal Ca and Mg during storage of UHT milk. There was continuous rise of heat induced components such as lactulose, HMF and colour value during storage of UHT milk. All these changes affected the quality of UHT milk during storage. Minimal changes were observed in that milk stored at 5°C rather than that stored at 30°C. So, the study indicated that it is better to store UHT milk at refrigeration temperature to preserve its quality.

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