Soumis le : 02/07/2017
Forme révisée acceptée le : 22/01/2018
Auteur correspondant : debbabih@gmail.com

Nature & Technology

http://www.univ-chlef.dz/revuenatec

Correlations between quality, enzymatic activities and storage stability of UHT milk in Tunisia

FITOUHI Nahla, GMAR Mouna, SBOUI Nesma, DEBBABI Hajer*

Institut National Agronomique de Tunisie, Research Unit UR17GR01"Innovation & Tradition", University of Carthage, 43 Avenue Charles Nicolle, 1082 Tunis, Tunisia

Abstract

Destabilization of ultra-high temperature (UHT) milk (140 °C, 4s) in the form of gel or sediment may occur during storage. This phenomenon is a real industrial and scientific problem that could be caused by milk proteolysis and lipolysis. This study aimed to study the influence of storage conditions (time, temperature) on the quality of UHT milk produced under industrial conditions. The physicochemical characteristics of these different kinds of milk, including composition and stability as assessed by phosphate test, were determined. UHT heat treatment of milk resulted in a change in pH, acidity, and stability derived from phosphate test, as well as in a drop in psychrophilic bacteria counts. Proteolytic and lipolysis activities also decreased with UHT treatment. However, during UHT milk storage for 6 months, the milk proteolytic activity increased, and in consequence, an increase in acidity and non-casein nitrogen values was observed. Moreover, lipolytic activity was higher in milk stored at 30 °C, as compared to the milk kept at 4 °C. In addition, the stability values increased during UHT milk storage at 30 °C. Stability was inversely correlated with milk fat and acidity ($p<0.05$). Furthermore, it showed a strong relationship with proteolysis ($r=0.765$), but no significant relationship was found with lipolysis. The analysis of the sediment of UHT milk stored for 6 months showed levels of fat (15 %), proteins (40 %) and lactose (42 %). The composition of the sediment illustrated the complexity of the formation process.

Keywords: Type your keywords here, separated by semicolons ;

1. Introduction

In Tunisia, 70 % of total raw milk production is utilized in milk processing and dairy products manufacturing [1]. Moreover, 80 % of raw milk is produced in small, primitive family farms. Raw milks collected from these farms do not meet with standard bacteriological quality criteria [1]. Thus, a heat treatment must be conducted to ensure milk safety for consumers. Ultra-high temperature (UHT) sterilization (140 °C × 5 sec) allows preserving milk for more than five months [2]. During the UHT process, vegetative bacteria and most spore-formers are inactivated. A major concern with UHT processing is the heat stability of the milk. Indeed, heat-stable enzymes of native or bacterial origin can survive during UHT processing [3], and cause serious defects during milk storage, such gelation and sedimentation defects and the development of off-flavors [2, 4]. The most important of enzymes are proteases and lipases [5-7].

Gelation of UHT milk during storage is a major factor limiting its shelf life [8]. This gel is, in fact, a three-dimensional protein matrix. Its formation is initiated by interactions between the whey protein β-lactoglobulin and the k-casein of the casein micelle during the high heat treatment [9]. These interactions lead to the formation of a β-lactoglobulin–k-casein complex (βk-complex). A feasible mechanism of age gelation is based on a two-step process; during the first step, there is a dissociation of the βk-complexes from the casein micelles due to the breakdown of multiple anchor sites on k-casein. During the second step, the aggregation of these complexes can form a three-dimensional matrix. When a critical volume concentration of the β-k complex is attained, a gel of custard-like consistency is formed. The onset of gelation influences several factors such as the heat treatment nature, milk composition and quality, seasonal milk production factors and storage temperature, as well as enzymatic activities during storage [3, 10-12]. Several studies have suggested that levels of proteolysis appeared to be linked to the onset of gelation [8, 12]. Proteolytic

enzymes can attack caseins, leading to a bitter flavor and the skim milk can become more or less transparent [12, 13]. Lipolysis is also known to contribute in both desirable and undesirable flavors in dairy products, initially through hydrolysis of milk triacylglycerols [5, 13]. Rancid flavor deterioration caused by lipolysis creates serious stability problems in dairy products storage [4].

This study aimed to assess the influence of heat treatment and storage on the quality, enzyme activities and heat stability of milk.

2. Materials and Methods

2.1. Milk sampling and processing

Milk sampling and heat treatment procedures were carried out in a dairy industry (Vitalait, Mahdia, Tunisia). Raw milk previously stored at 4 °C to 7 °C was standardized with milk whey powder. Heat treatment of raw milk included pasteurization at 78ºC for 30 s and a UHT sterilization step at 140 ºC for 4 s. Four UHT milk productions were followed. For each production, triplicate milk samples of the four lots used were collected at three different stages during processing, namely: a) refrigerated raw milk stored in tanks; b) pasteurized milk; and c) UHT sterilized milk. UHT samples were stored at 4 °C and 30 °C. Sediment samples were collected after storage through 6, 12 and 24 months at room temperature.

2.2. Physicochemical analysis

Milk samples were assayed for titratable acidity, by titration method using a solution of sodium hydroxide (N/9) in the presence of phenolphthalein 1 % as an indicator. The pH was monitored using a digital pH meter. Total solids, proteins, and fat contents analyses followed standard procedures [14].

Phosphate stability was used as an indicator of the heat stability of milk [adapted from 15]. Milk samples (10mL) were placed in sealed Pyrex tubes at room temperature, and volumes of 0.5M KH_2PO_4 were gradually added to each tube. After mixing, tubes were heated at 100 °C for 10 min. After the heat treatment, visual destabilization was noted and the smallest volume of phosphate solution inducing milk destabilization was the result of the phosphate stability. The experimental error was ± 0.2 mL.

2.3. Microbiological analysis

Microbiological analyses were performed on samples of raw, pasteurized and UHT sterilized milk samples. One mL of sample was homogenized with 9 mL of 0.1 % peptone saline solution. Then, according to each sample, tenfold serial dilutions up to 10^{-6} were prepared using the same diluents and subsequently plated onto standard plate count agar medium (PCA). Psychrotroph counts (colony forming unit CFU/mL) were determined after incubation at 7 ºC for 10 days [16].

2.4. Protease activity by agar-well diffusion assay

Agar was prepared along with 10 % of sterile milk and poured in Petri dishes. After the plate solidification for 30 min, holes (3 mm diameter) were punched and 30 µl of the sample was added. Incubation proceeded at 30 °C for 72 h. A cleared zone of proteolysis was detected on the agar plates.

2.5. Lipase activity assay

Lipolytic activity of milk samples was determined using the modified method of Parry *et al.* [17] using as substrate a 10 % olive oil-gum Arabic solution emulsion. One mL of milk was added to 3 ml of 10 % olive oil gum Arabic solution emulsion, 1 mL buffer Tris-HCl (0.2 M, pH 7.5) and incubated at 37 °C for 2 h with rapid stirring. Ten mL of 95 % ethanol were added to stop the reaction and the free fatty acid produced was quantified by titration using pH meter with 0.1N sodium hydroxide (NaOH). Blanks with 1.0 ml of boiled milk served as the control. Lipase specific activity was defined as the micromoles of NaOH used in the titration per minute per mg of protein.

2.6. Statistical analysis

Data analyses by One-way ANOVA, Tukey and Pearson correlation were performed using statistical software (GraphPad Prism 3).

3. Results and Discussion

3.1. Effect of heat treatments on enzyme activities and milk quality

Table 1 showed changes in milk quality parameters after heat treatments. Titrable acidity and pH were affected by milk heat treatments ($p < 0.05$).

Table 1
Milk quality and enzyme activities of raw and heat-treated milks (n=3)

Milk heat treatment	pH	Titrable Acidity (°D)	Fat (%)	Psychrophilic counts (CFU/mL)	Proteolytic activity	Lipase activity (IU/mg of proteins)
Raw	6.66 [a]	14.5 [a]	29.0 [a]	7.4 x 10 [5a]	+	4.0 [a]
Pasteurised	6.63 [a]	15.0 [a,b]	15.0 [b]	5.0 [b]	+	2.7 [b]
UHT sterilised	6.48 [b]	15.5 [b]	15.7 [b]	<1 [c]	-	0.0 [c]

[a,b,c] *different letters in the same column indicate significant differences (p < 0.05)*
-: not detected; +: detected

Table 2
Impact of short time storage on some quality parameters and enzyme activities of UHT milk (n=3)

Storage time (day)	Storage temperature (°C)	pH	Titrable Acidity (°D)	Fat (%)	Psychrophilic counts (CFU/ mL)	Proteolytic activity	Lipolysis (IU/mg of proteins)
0	-	6.48 [a]	14.0 [a]	15.7 [a]	<1	-	0 [a]
7	4	6.62 [a]	14.5 [a]	15.0 [a]	<1	-	0.160 [b]
	30	6.55 [a]	14.2 [a]	15.5 [a]	<1	-	0.208 [c]
14	4	6.61 [a]	16.5 [a]	15.0 [a]	<1	-	0.066 [d]
	30	6.52 [a]	15.0 [a]	15.5 [a]	<1	+	0.066 [d]

[a,b,c,d] *different letters in the same column indicate significant differences (p < 0.05)*
-: not detected; +: detected.

Fox *et al.* [18] reported similar results. In fact, the pH drop during heat treatments can be explained through three reactions: (1) thermal oxidation of lactose in organic acids (50 %), (2) the hydrolysis of organic phosphate in phosphoserines (30 %) and (3) precipitation of the tricalcium phosphate and the concurrent release of H^+ (20 %). The decrease in milk fat can be explained by the skimming step prior the pasteurization. Heat treatment had significant ($p<0.01$) effect in reducing the bacterial load. This finding is in agreement with the findings of Nagla Bourae *et al.* [16] who found that heat treatment had significant ($p<0.01$) effect on the total bacterial count. Interestingly, protease and lipase activities were detected, when milk was subjected to pasteurization [5, 6]. Pasteurization led to eliminating all relevant pathogens including vegetative microorganisms but not spores or heat-stable toxins. However, UHT sterilization led to enzyme inactivation. Chove *et al.* [8] have concluded that proteolysis can be affected by high-temperature processing. In fact, some enzymes are partially inactivated in milk pasteurization conditions (72 °C, 15 s) and inactivation rates vary depending on the bacterial species and on the strains of the same species [19, 20].

3.2. Effect of storage conditions on enzyme activities and milk quality

Table 2 showed the evolution of pH, total acidity, fat contents, psychrophilic bacterial load and enzyme activities according to storage time and temperature. No significant change in pH, titrable acidity, fat content and the psychrophilic load was observed for milks stored at 4 °C and 30 °C for 14 days. Gaucher *et al.* [21] observed similar results. Aldubhany *et al.* [22] have reported that pH was not significantly affected by the milk type and storage temperature but by storage period. However, a proteolytic activity was detected in UHT milk stored at 30 °C for 14 days. This is a concern for the Tunisian dairy industry since this temperature of 30 °C can correspond to room temperature during summertime in Tunisia. Either bacterial enzymes or naturally occurring enzymes of which plasmin is significant [13, 23-25], cause this. Plasmin has a high heat resistance and can also retain activity in UHT milk [2, 13]. Moreover, proteases from the total psychrotrophic flora of milk can survive to a heat treatment of 149 °C for 10 s [19, 25].

Lipolytic activity was also detected after storage at 4 °C and 30 °C for 14 days, with no effect of storage temperature. This is probably due to the reactivation of lipases during storage. Their presence in UHT milk

depends on the microbiological quality of the raw milk as well as on the milk heat treatment [5, 7].

3.3. Effect of storage conditions on UHT milk stability

Milk stability decreased during storage, as shown in figure 1.

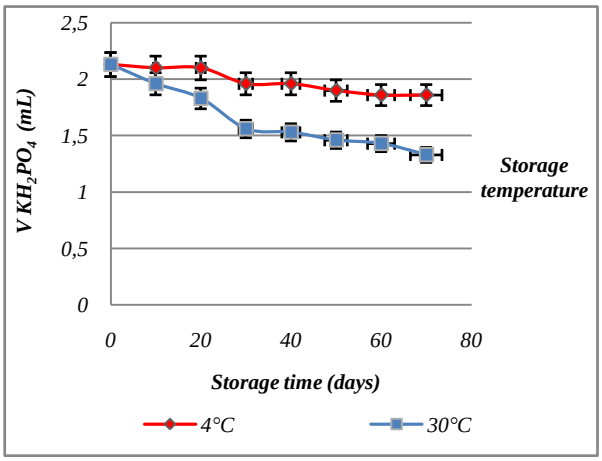

Figure 1: Effect of storage time and temperature on heat stability of UHT milk (n=3)

This decrease was more pronounced when milk was stored at 30 °C, in comparison with storage at 4 °C, indicating that storage temperature can impair milk stability. In addition, an increase in non-casein nitrogen was observed between day 0 and day 70, from 2.4 g/L to 3 g/L when milk was stored at 4 °C, and to 4 g/L when stored at 30 °C (data not shown). These results are consistent with those of Aldubhany *et al.* [22], suggesting a proteolysis during storage. Stoeckel *et al.* [3] have reported that the presence of residual proteolytic activity produced from Pseudomonas spp. during storage of raw milk, could be responsible for spoilage of UHT milk during shelf life.

Correlations of the level of stability with proteolytic activity as well as psychrophilic flora comfort this hypothesis and indicate the influence of milk proteins on stability (Table 3).

Table 3
Correlations between some quality parameters, heat stability and enzyme activities in UHT milks

Parameter		pH	Acidity	Fat	Stability	LipAc	ProAc	Psy. counts
pH	Pearson r	1	-0.016	-0.044	-0.171	-0.147	-0.005	-0.441*
	P value		0.937	0.823	0.384	0.456	0.980	0.019
	N	28	28	28	28	28	28	28
Acidity	Pearson r	-0.016	1	-0.457*	0.433*	0.099	0.145	-0.317
	P value	0.937		0.015	0.021	0.616	0.462	0.100
	N	28	28	28	28	28	28	28
Fat	Pearson r	-0.044	-0.457*	1	-0.748*	0.084	-0.456*	0.667*
	P value	0.823	0.015		0.000	0.673	0.015	0.000
	N	28	28	28	28	28	28	28
Stability	Pearson r	-0.171	0.433*	-0.748*	1	0.054	0.765*	-0.531*
	P value	0.384	0.021	0.000		0.785	0.000	0.004
	N	28	28	28	28	28	28	28
LipAc	Pearson r	-0.147	0.099	0.084	0.054	1	0.087	0.078
	P value	0.456	0.616	0.673	0.785		0.661	0.692
	N	28	28	28	28	28	28	28
ProAc	Pearson r	-0.005	0.145	-0.456*	0.765*	0.087	1	-0.326*
	P value	0.980	0.462	0.015	0.000	0.661		0.091
	N	28	28	28	28	28	28	28
Psy. counts	Pearson r	-0.441*	-0.317	0.667**	-0.531**	0.078	-0.326*	1
	P value	0.019	0.100	0.000	0.004	0.692	0.091	
	N	28	28	28	28	28	28	28

** p < 0.05 significant correlation , LipAc: lipolytic activity; ProAc: proteolytic activity; Psy: psychrophilic flora*

To cite this article: FITOUHI N., GMAR M., SBOUI N., DEBBABI H., Correlations between quality, enzymatic activities and storage stability of UHT milk in Tunisia, Nature & Technology Journal. Vol. B: Agronomic & Biological Sciences, 18 (2018) 120-125
http://www.univ-chlef.dz/revuenatec/issue-18/Article_B/Article_478.pdf

Correlations between the level of milk stability and acidity can be explained by the fact that the most important factor towards stability is pH [10, 23-25]. Therefore, following proteolysis of milk by proteases, in particular, plasmin and proteases from psychrophilic bacteria such as *Pseudomonas* and the release of amino acids and peptides will generate a change in pH (acidity systematically) therefore the stability of milk [3,7, 26].

3.4. Effect of a long time storage at 30 °C on UHT milk quality

Storage after the date of shelf life at room temperature has led to changes in color and texture (Table 4). Our results are in agreement with those of Stoeckel *et al.* [3] establishing the following order of defects: bitterness – particles – creaming – sediment – gelation in UHT milk samples containing peptidases. These changes can be explained by changes in chemical composition of gel/sediment during storage, showing a high content in solids and in particular protein (Table 4), confirming that proteolysis is one of the phenomena involved in the stability of milk. Malmgren *et al.* [10] have observed complete hydrolysis of intact β-casein, and limited lactosylation of β-lactoglobulin and κ-casein in gelled milk samples. Moreover, sediment increased with longer storage period. This result was in agreement with those of Kelly and Foley. [23] and Enright *et al.* [12].

Table 4
Visual appearance and chemical composition of sediments in UHT milk affected by long term storage at room temperature

Storage time (months)	Visual aspect		Chemical parameters			
	Color	Texture	Total solids (%)	Fat (%)	Proteins (%)	Lactose (%)
6	White -yellow	creamy	130^{a}	15^{a}	40^{a}	42^{a}
12	yellow	firm	145^{b}	14^{a}	57^{b}	40^{a}
24	brownish	very firm	170^{c}	13^{a}	68^{c}	41^{a}

^{a,b,c} *different letters in the same column indicate significant differences ($p < 0.05$)*

In conclusion, our study has confirmed that milk quality can be changed by sterilization and in a lesser extend pasteurization, as well as storage. Proteolytic and lipolytic activities could be detected in UHT milk stored for 14 days at 30 °C, which can correspond to room temperature during summer time in Tunisia. These physicochemical changes and enzymatic reactions lead to the formation of sediment in the UHT sterilized milk by a complex phenomenon that can be related to enzymatic activities, mainly from psychrophilic bacteria.

Acknowledgments

The authors want to thank the director and the team of "Centrale laitière de Mahdia" (Tunisia) for milk sampling and treatments.

References

[1] Louhichi R., (2013) Available on Internet: http://www.pole-competitivite-bizerte.com.tn/image.php?id=106
[2] Chavan R. S., Khedkar C. D., Jana A. H.,Comprehensive Reviews in Food Science and Food Safety, 10 (2011) 251–268
[3] Stoeckel M., Lidolt M., Achberger V., Glück C., Krewinkel M., Stressler T., Hinrichs J., International Dairy Journal, 59 (2016) 20-28.
[4] Ul Haq I., Khaskheli M., Salman M., Marri M.Y., Marri M. A., Lochi G.M., Habib F., African Journal of Food Science 8 (2014) 75-79.
[5] Xin L., Zhang L., Meng Z., Di W., Han X., Yi H., Cui Y., Journal of Food Processing and Preservation, (2017) DOI: 10.1111/jfpp.13289
[6] Chove L.M., Abdulsudi I.Z., Lewis M.J., African Journal of Food Science, 7 (2013) 232-237.
[7] Machado S.G, Baglinière F., Marchand S., Van Coillie E., Vanetti M.C., De Block J., Heyndrickx M., Frontiers in Microbiology, 8 (2017) 8
[8] Datta N., Deeth H.C., Age gelation of UHT milk—a review. Food and Bioproducts processing, 79 (2001) 197-210.
[9] Crudden A., Afoufa-Bastien D., Fox P.F., Brisson G., Kelly A.L., International dairy journal, 15 (2005) 1017-1025
[10] Malmgren B., Ardö Y., Langton M., Altskär A., Bremer M.G., Dejmek P.M., Paulsson M., International Dairy Journal, 71 (2017) 60-75.
[11] Siddique F., Riffat S., Arshad M., Farooq U., Iftikhar K., Kharal S., International Journal of Food and Allied Sciences, 2 (2016) 52-57.
[12] Enright E., Bland A.P., Needs E.C., Kelly A.L., International Dairy Journal, 9 (1999) 581-591.
[13] Rauh V.M., Johansen L.B., Ipsen R., Paulsson M., Larsen L.B., Hammershøj M., Plasmin activity in UHT milk: relationship between proteolysis, age gelation, and bitterness, Journal of agricultural and food chemistry, 62 (2014) 6852-6860.
[14] AOAC INTERNATIONAL (2010). Official Methods of Analysis, 18th Edition, Association of Official Analytical Chemists, Washington DC, 2010
[15] Ramsdell G.A., Johnson Jr W.M.T., Evans F.R., Journal of Dairy Science, 14 (1931) 93–106.
[16] Nagla B., Hassan A., Osman M., Abdallah M., Azim A., Nour M., Pakistan Journal of Nutrition, 8 (2009) 1845-1848.

[17] Parry R.M., Chandan R.C., Shahani K.M., Journal of Dairy Science, 49 (1966) 356-360.
[18] Fox P.F., Uniacke-Lowe T., McSweeney P.L.H., O'Mahony A.J , In: Dairy Chemistry and Biochemistry, Springer International Publishing, Basel, 2015, pp. 345-375.
[19] Fox P.F., Uniacke-Lowe T., McSweeney P.L.H., J O'Mahony A., In: Dairy Chemistry and Biochemistry, Springer International Publishing, Basel, 2015, pp. 377-414.
[20] Sørhaug T., Stepaniak L., Trends in Food Science & Technology, 8 (1997) 35-41.
[21] Gaucher I., Mollé D., Gagnaire V., Gaucheron F.. Food, Hydrocolloids, 22 (2008) 130-143.
[22] Aldubhany T.A.W., Gouda E., Khattab A., Dabour N., Alexandria Science Exchange, 35 (2014) 108.
[23] Kelly A.L., Foley J., International Dairy Journal, 7 (1997, 411-420.
[24] Jansson T., Jensen H.B., Sundekilde U.K., Clausen M.R., Eggers N., Larsen L.B., Bertram H.C., Journal of agricultural and food chemistry, 62 (2014) 11270-11278.
[25] Gebre-Egziabher A., Humbert E.S., Blankenagel G., Journal of Food Protection, 43 (1980) 197-200.
[26] Datta N., Deeth H.C., LWT-Food Science and Technology, 36 (2003), 173-182.

To cite this article: FITOUHI N., GMAR M., SBOUI N., DEBBABI H., Correlations between quality, enzymatic activities and storage stability of UHT milk in Tunisia, Nature & Technology Journal. Vol. B: Agronomic & Biological Sciences, 18 (2018) 120-125
http://www.univ-chlef.dz/revuenatec/issue-18/Article_B/Article_478.pdf