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Effect of the lactoperoxidase system on proteolysis and physicochemical changes in ultra high temperature milk during storage

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Indirect semi-skimmed ultra high temperature (UHT) milk was made from refrigerated milk preserved by the lactoperoxidase system (LPS). The effect of the LPS on the physicochemical and biochemical properties of UHT milk during storage period of 6 months at 30°C was assessed. The levels of soluble nitrogen at pH 4.6 and non protein nitrogen in all UHT milk samples increased during storage. However, control UHT milk, manufactured with refrigerated milk which LPS was not activated; showed the highest values (p < 0.01). Less hydrophobic amino acids concentrations, such as found in nitrogen soluble fraction, were lower in activated LPS UHT milk. Also, bacterial proteinase activities, measured using o-phthaldialdehyde (oPA) as substrate, increased during storage. They were found higher in control UHT milk than in treated milk (p < 0.01). However, a decrease of total fatty acids in UHT milk, dependent on time of storage and LPS activation, was found during UHT milk storage.

Key words: Lactoperoxidase, ultra high temperature UHT milk, proteolysis, lipolysis, storage.

INTRODUCTION

The quality of most dairy products is closely related to the microbial status of raw milk from which they are manufactured. Depending on the temperature, conditions and length of milk storage, various groups of microorganisms can undergo a period of intensive growth producing high concentrations of enzymes, particularly lipases and proteinases (Sorhaug and Stepaniak, 1997; Ben Moussa et al., 2008). Although, the psychrotropic microorganisms are destroyed by sterilization, their extracellular enzymes may remain active in sterilized products. Even low bacterial activity of lipases and/or proteinases may cause defects in products stored for

long time such as UHT milk (Valero et al., 2001).

Proteolysis in UHT milk can cause the development of bitter flavour, and leads to increase in viscosity with eventual formation of a gel during storage, which is a major factor limiting its shelf-life and market potential (Datta and Deeth, 2003). The enzymes responsible for the proteolysis are the native milk alkaline proteinase, plasmin, and heat-stable extracellular bacterial proteinase produced by psychrotrophic bacterial contaminants in raw milk (Datta and Deeth, 2003; Kelly and Foley, 1997).

Furthermore, the fat fraction of UHT milk is considered to be one of the sources of undesirable changes, including organoleptic features of product. As a result, fat milk hydrolysis and oxidation, numerous compounds are created, causing the decrease of organoleptic features of the product. Thermostable bacterial lipases are the

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primary cause of fat hydrolysis and its presence in UHT milk depends on the microbiological quality of the raw milk, as well as, on the milk heat treatment (Choi and Jeon, 1993; Matta and Punj, 1999; Rye and Schraft,1999; Chen et al., 2003).

Proteolytic and lipolytic enzymes of bacterial origin in milk depend on the growth of psychrotrophic bacteria such as *Pseudomonas* and *Aeromonas*. Sorhaug and Stepaniak (1997) reports that Gram-negative psychrotrophic bacteria found in raw milk at levels of 6.9 to 7.2 logs CFU/mL allow the formation of gel and bitter flavour development in UHT milk; although, several papers have been published on the effect of the milk refrigeration at 4°C on the physicochemical properties (Celestino et al. 1997).

For this reason, alternative solutions to inhibit psychrotrophs have been considered for example the use of lactic acid bacteria (Dacosta, 2002), activation of the lactoperoxidase (LP) system that needs the presence of hydrogen peroxide, thiocyanate and native lactoperoxidase. LP system exerts bacteriostatic and bactericidal activity mainly on gram negative bacteria (Touch et al., 2004; Seifu et al., 2005). The antibacterial action of the LP system is due to the effect of reaction products of thiocyanate oxidation, $OSCN^-$ and HOSCN, which are able to oxidize free SH– groups of various proteins that are important for the viability of pathogens, thereby, inactivating crucial enzyme and protein systems (Sermon et al., 2005). The LP system could be used as an alternative method for the preservation of raw milk, which is produced under high ambient temperature and low hygienic conditions when a cooling process is not found (CAC/GL 13, 1991, Haddadin et al., 1996).

Due to the growing interest in the use of the LP system for preservation of raw milk, LP activated milk is used for cheese manufacture (Seifu et al., 2003). However, detailed studies on UHT milk properties made from LPS activated refrigerated raw milk are lacking. The aim of this study was therefore to assess the impact of LP system activated in refrigerated raw milk on physicochemical and biochemical changes in the UHT milk, produced by indirect sterilization, during six months of storage at 30°C.

MATERIALS AND METHODS

Milk samples

Two lots of refrigerated raw milk were obtained from two collection centre situated in northern Tunisia during the spring period when the lactation curve was at its maximum. Each raw milk lot (batch 1 and batch 2) was divided in two isotherm tanks. The LP system was activated in one tank by addition of sodium thiocyanate (14 mg/L) (Fluka Chemie, Steinheim, Germany) as recommended by the Codex Alimentarius (CAC/GL 13, 1991). After mixing gently, 30 mg/L of sodium percarbonate (Fluka Chemie, Steinheim, Germany) was added as a source of hydrogen peroxide (CAC/GL 13, 1991). Then, the two milk samples were maintained at 5°C for 48 h. Stored raw milks samples (Control and activated LPS) were processed by

indirect UHT treatment in a commercial dairy plant. They were homogenized at 200 bars, and then sterilized in a plate exchanger at 137°C for 4 s and packed aseptically in Tetra Pack-type cartons of 1L. UHT milks (Control UHT milk and activated LPS UHT milk) were stored at 30°C for 6 months. This essay was performed twice (batch 1 and batch 2). UHT milk samples were analyzed after 30 days intervals during 180 days of storage using different analytical techniques, except RP-HPLC and GC-MS measurement; they were performed after 90 days interval. At each storing time, two samples of each batch were analyzed in duplicate. Results were recorded as means of duplicate analyses.

Microbiological analyses

Total and psychrotrophic bacteria were enumerated using the pour-plate procedure as described in IDF standards (IDF, 1991 a, b). Somatic Cell Count (SCC) was determined using a Fossomatic 5000 (Foss Electric, Denmark, 8700), based on flow-cytometry technology recognizing DNA from the cells (Gonzalo et al., 1993).

Chemical analyses

The pH and lactic acidity (ºDornic) were determined according to the standard method for the examination of dairy products (Case et al., 1985).

Proteolytic measurement

Determination of proteinase activity

The proteolytic activity in milk samples was determined by the o-phthaldialdehyde (oPA) spectrophotometric method of Church et al. (1983); briefly 50 µL of UHT milk added to 2 ml of oPA and incubated at 37°C during 2 min. The α-amino groups released by hydrolysis react with oPA to form an adduct that absorbs strongly at 340 nm. Therefore, the optical density values of UHT milk at 340 nm were determined (Jenway 6305 spectrophotometer, Essex, CM6 3LB, England) and used to measure degree of proteolysis.

Measurement of nitrogen fractions

The total nitrogen (TN) contents of milk samples were determined by the reference Kjeldahl method (AOAC, 1990). The soluble nitrogen (SN) fraction was determined according to Gripon et al. (1975); milk samples were adjusted to pH 4.6 by adding 10% acetic acid with vortexing. The acidified milk was allowed to stand for 30 min at room temperature before its filtration through Whatman no. 42 filter paper. The non protein nitrogen (NPN) fraction was prepared by adding 4 ml of 60% (w/v) trichloroacetic acid solution (TCA) to 16 ml of (SN) fraction filtrate with. The mixture was allowed to stand for 1 h at room temperature before its filtration through Whatman no. 42 filter paper. The nitrogen contents were determined on aliquots of the filtrates by the Kjeldahl method (AOAC, 1990). Nitrogen quantities were determined as follows: Casein nitrogen (g/100mL of milk) (CN) = TN - SN; Proteose nitrogen (g/100 mL of milk) (PN) = SN - NPN.

Reverse phase high performance liquid chromatography (RP-HPLC)

Soluble nitrogen SN at pH 4.6 extracts was mixed with hydrochloric acid (6 M, 37%) for 24 h at 105°C. Proteolysis reaction was stopped by adding NaOH (6 M). Mixtures were filtered through a membrane

Table 1. Microbiological quality of raw milks used for UHT milks production.

Cell	Batch 1	Batch 2
Psychrotrophic bacteria count (CFU/ml)	5×10^6	6.6×10^6
Total bacteria count (CFU/ml)	7.75×10^7	5.75×10^7
Somatic cell count (ml^{-1})	4.2×10^5	8.3×10^5

with a pore size of 0.45 µM filter before its injection at RP-HPLC system of 100 µL. Separation of amino acids was performed on an Agilent 1100 Chromatograph (76337 Waldbronn, Germany) using a C_{18} reverse-phase column (250 × 4.6 mm) and a FLD injector and detector. Two solvents were used: solvent A was composed by ACN/MeOH/H_2O, while solvent B consisted of Na_2HPO_4. The solvent gradient was formed by increasing the proportion of solvent B from 10% to 90% during the first 30 min, and increasing solvent B to 100%. An aliquot (100 µL) of the filtrate was injected into the HPLC system. Excitation was at 340 nm and emission at 450 nm, respectively. The quantification of amino acids in samples was performed by measuring peak areas of samples, and plotting against the calibration curves of each amino acids (L-glycine, L-alanine, L-valine, L-proline, L-serine, L-threonine, L-aspartic acid, L-glutamine, monohydrochlorid lysine, L-asparagine, L-Histidine, L-Cystine, L-Methionine, L-glutamic acid, L-leucine, L-phenylalanine, L-tryptophan, L-isoleucine, L-tyrosine). Purified amino acids standards were obtained from Sigma Aldrich. Individual amino acids standards were prepared before RP-HPLC analysis in the same way as described by milk samples.

Determination of fatty acid composition

The lipid phase of samples was extracted by the reference method (ISO 14156, 2001) using ethanol and n-pentane as solvent. In the second step, the lipid phase was separated from non-lipid structure by washing it with sodium sulphate solution. Fatty acids were analysed as their methyl esters prepared by transesterification with 2 M methanolic potassium hydroxide. Compositions were determined of extracted milk lipid, by an Agilent gas chromatograph coupled with an Agilent 19091S-433 mass spectrometer (Agilent Technologies, 76337 Waldbronn, Germany). A capillary column HP-5MS (5% phenyl methylsiloxane) with dimension of 30 m × 0.25 mm i.d × 0.25 µM film thickness (Agilent Technologies, 76337 Waldbronn, Germany) was used for the separation of fatty acid methyl esters. The initial temperature of 90°C was maintained for 2 min raised to 270°C at the rate of 3°C/min, then temperature allowed 320°C at the rate of 10°C/min. The split ratio was 1:50, and helium was used as a carrier gas with the flow rate of 20 ml/min. The injector and detector temperatures were 280 and 260°C, respectively.

Statistical analyses

The effect of storage period, activation of lactoperoxidase system was assessed using analysis of variance (ANOVA) by the general linear model procedure of the SPSS 10.0 statistical package program.

RESULTS AND DISCUSSION

Microbiological properties of the raw milk samples are given in Table 1. Raw milks samples presented a total bacteria count higher then 10^7 CFU/ml. Moreover, psychrotrophic bacteria counts were about 5×10^6 and 6.6×10^6 CFU/ml, respectively for raw milks samples of batch 1 and batch 2. These results show the noncompliance of milk collected in the usual values required by the standards of raw milk (NT 14-141, 2007). In fact, in Tunisia, raw milk is produced, generally, in small family farms. Raw milks collected from these farms are refrigerated, but, usually, it does not meet standard quality criteria. Otherwise, it is well known that storage at 5°C does not stop the growth of bacteria, especially psychrotrophic one (Shelley and Deeth, 1986). These microorganisms produce thermoresistant proteolytic and lipolytic enzymes, which can attack milk proteins and lipids (Mc Kellar et al., 1984). In terms of somatic cells, their numbers are respectively 4.2×10^5 and 8.3×10^5 ml^{-1} in milks used respectively for production of UHT milks issued from batch 1 and batch 2. Value measured in the second batch remains away from standard (NT 14-141, 2007) requiring 5×10^5 cells/ml. The number of these cells increases in milk as a result of infection in the mammary gland. They contain neutral proteinases (Verdi and Barbano, 1991).

Changes in pH and lactic acidity are given in Figures 1 and 2. Lactic acidity increased during storage and reached 23 and 30.5°D after 6 months, respectively in controls UHT milks of batch 1 and batch 2. However, pH values of all UHT milk samples decreased during storage at 30°C; they ranged between 6.68 to 6.25 and 6.63 to 6.24, respectively for batch 1 and batch 2 samples. The acidification has been also observed by several investigators (Reddy et al., 1991; Alkanhal et al., 1994; Celestino et al., 1997; Gaucher et al., 2008). They suggest that the reduction in pH is due to a loss of positive charges on the protein molecule caused by the reaction of free ε-NH_2 groups of lysine with lactose in Maillard-type reaction. It consists in a series of different modifications leading to the formation of brown-pigmented products such as pyrazines and melanoidins, polymerized molecules such as lactuloselysine and fructoselysine, and small acid molecules. It should be noted that acidification in batch 1 is significantly lower (p<0.05) than that in batch 2. This result can be attributed to initial microbiological quality. Particularly, somatic cell number in batch 1 milk is almost half than that in batch 2 milk. These data are confirmed by extracellular proteolytic activity (Table 2). Otherwise, significant differences (p < 0.01) in pH and acidity values between
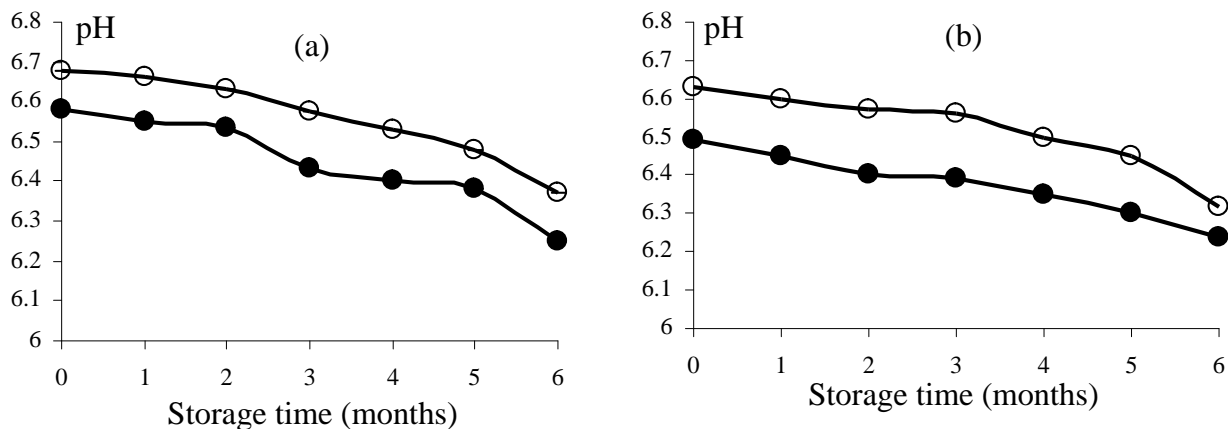
Figure 1. pH values of UHT milks processed from control and treated milk (LPS activated), stored during 6 months at 30ºC. **(a).** Batch 1. **(b).** batch 2. Standard deviations are in the range ± 0 to ± 0.014; −○−, activated LPS UHT milk; −●−, control UHT milk.
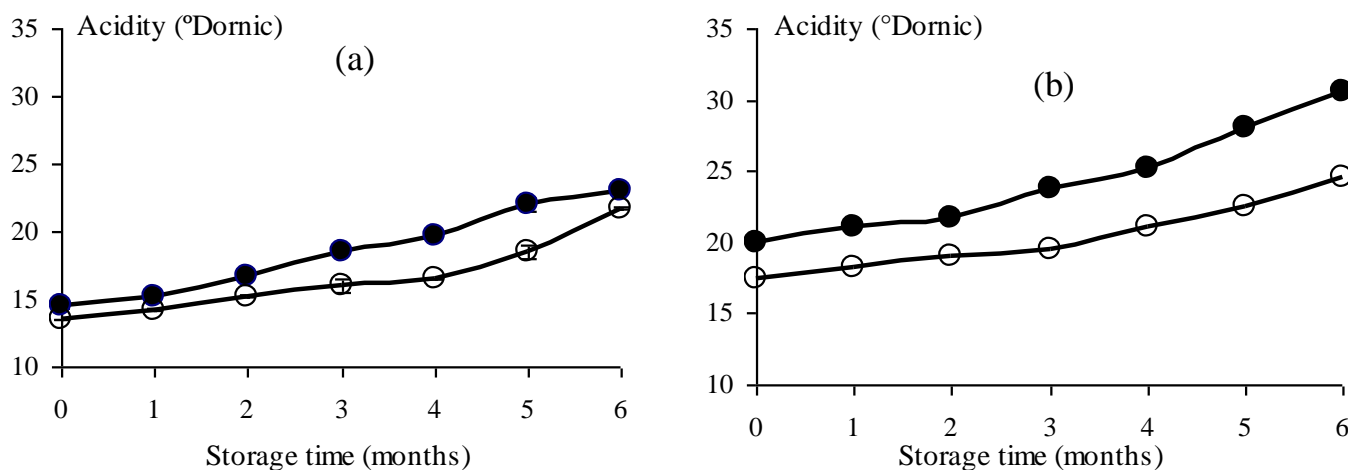

Figure 2. Lactic acidity values of UHT milks processed from control and treated milk (LPS activated), stored during 6 months at 30ºC. **(a)** Batch 1. **(b),** Batch 2. −○−, ml Activated LPS UHT milk; −●−, control UHT milk. Standard deviations are in the range of ± 0 to ± 0.7.

control and activated LPS UHT milks are observed during all storage period; these differences reached about average values of 0.13 pH unit and 3ºD for acidity. Indeed, LP system, activated in refrigerated raw milk, affect growth of psychrotrophic Gram negative bacteria (Kamau et al., 1984; Wolfson and Sumner, 1993), as, it has the ability to catalyse the oxidation of thiocyanate by hydrogen peroxide with the production of the antibacterial hypothiocyanite ($OSCN^-$) and other intermediates such as, thiocyanogen (SCN_2). These compounds, which can be further oxidized to end-products that are harmless to humans, have the ability to reduce bacterial growth by damaging the cell membranes and inhibiting the synthesis of proteins such as, DNA and RNA (Seifu,

2005). Consequently both proteolytic extracellular enzymes concentration and free ε-NH$_2$ groups decreases.

Guinot-Thomas et al. (1995) reports further a greater importance of microbial proteinases on proteolysis in raw milk during refrigerated storage. These enzymes were found to be in higher levels quantity in control UHT milks than in activated LPS UHT milks, due to bacteriostatic effect of LPS in raw milk, stored at + 5°C during 48 h, on psychrotrophic Gram-negative bacteria. More, the variation of acidity between control and activated UHT milk in batch 1, obtained after 6 months of storage, is 1.25°D against 6°D for Batch 2. This result joins previous findings concerning the difference in the initial

microbiological quality and therefore the bacterial thermoresistant proteases and those of somatic cells.

Effects of storage time and activation of LPS in raw milk on the extent of extracellular proteinases activities of UHT milks are shown in Table 2. Proteolysis activities in both control and activated LPS UHT milks increased significantly during storage (p < 0.05). The highest proteolytic activity was obtained after 6 months of control UHT milk of batch 1. Therefore, OD (340 nm) value attained 7.18 when o-phthaldehyde was used as substrate. However, average final proteolysis was higher in control UHT milk samples (OD=7.09) than in activated LPS UHT milk (OD=6.96). Proteolysis in UHT milk samples may be due to plasmin and/or bacterial proteinases and/or SCC elastase and catehepsin G (Bastian and Brown, 1996; Le Roux et al., 2003), which depends on the microbiological quality of milk samples. Thus, activation of LPS in refrigerated raw milk improves its hygienic quality (Boulares et al., 2011) and reduces its concentration of extracellular bacterial proteinases, compared with control raw milk.

As regards changes in nitrogen fractions during storage period of UHT milk samples, the SN, NPN and NP increased significantly (p < 0.01) in all UHT milk samples during storage (Table 3). Indeed, the NS of control and activated LPS UHT milk samples of batch 1 and 2 increase by 87, 58 and 86, 66%, respectively, after 6 months, compared with the first day of storage. Moreover, a significant increase in NPN is found in UHT milk during storage; higher NPN values (p < 0.01) were obtained for control UHT milk samples. Thus, at the whole period of storage, NPN average value of control UHT milk (either batch 1 and batch 2) increased about 7.25 g/L compared to 5.75 g/L observed in activated LP system UHT milk. These increases, advance proteolysis during storage, could be ascribed to decomposition of the proteins by reactivated proteolytic enzymes, which may be indigenous (Driessen, 1981) or of bacterial origin (Mottar, 1981).

Furthermore, a decrease in CN and an increase in NPN between the first day and after 6 months of storage are noted (Table 3). These differences between the loss in CN and the increase in NPN could be explained by the presence of other minor nitrogen compounds such as, proteose-peptones (McSweeny, 2004). They are polypeptides formed by fragments of β-casein, which increase in milk when the numbers of bacteria exceed 10^6 to 10^7 CFU/ml. However, higher difference values between CN and NPN were obtained for control UHT milk samples compared with LPS activated UHT milk samples. These findings suggest that bacterial proteinases activities in control UHT milk were higher than in activated LPS UHT milk.

The mean total amino acids present in SN fraction from UHT milk samples measured at 0, 3 and 6 months stored at 30°C, determined by the RP-HPLC method, are shown in Table 4. Amino acids concentrations increased significantly (p < 0.01) with storage period. These increases are more depicted for aspartic and glutamic acids, arginine, serine + histidine + glutamine and glycine + threonine. After 6 months of storage, they increased by 17.7% in control UHT milk of batch 1 against 14.6% inactivated LPS UHT milk compared with the first day of storage. The retention time of tryptophan was used as an indicator of hydrophobic zone (Figures 3a and 3b). The hydrophobic amino acids portion consisted of those eluted after tryptophane from 24 min. The amino acids released by bacterial proteinases were found to be less hydrophobic, since they were eluted earlier (< 24 min) in the RP-HPLC profile.

However, the amino acids produced by plasmin are more hydrophobic and eluted between 24 and 45 min (Datta and Deeth, 2003). Less hydrophobic amino acids detected in SN fraction are aspartic and glutamic acids, arginine, alanine, tyrosine, serine, histidine, glutamine, glycine and threonine. The SN extract of activated LPS UHT milk exhibited lower concentrations of hydrophobic amino acids (14.46 and 16.7 ppm, respectively in batch 1 and batch 2) than SN extract of control UHT milk (19.27 and 33.13 ppm, respectively in batch 1 and batch 2), indicating fewer activities of bacterial proteinases in activated LPS UHT milk.

From the obtained results, it can be concluded that the important bacteriostatic effect of lactoperoxidase system on Gram-negative psychrotrophic bacteria in refrigerated raw milk used to produce UHT milk is reflected on proteolysis. As for lipolysis, identification of free fatty acids of UHT milks samples by GC-MS can be investigated (Table 5). The results of fatty acid composition of analyzed UHT milks show that palmitic acid (C16: 0), oleic (C18: 1) and stearic (C18: 0) represent the dominant and their range vary from 56% in control UHT milk to 51.6% in LPS activated UHT milk. Meanwhile, the short chain fatty acids such as butyric acid (C4: 0) and caproic (C6: 0) are completely absent from our samples, contrary to Panfil-Kuncewiez et al. (2005) findings. This difference could be attributed to the composition of the fat in raw milk which is closely related to race and feed (Mahieu, 1985).

In UHT milk samples stored for 6 months at 30°C, there was a decrease in the mean levels of total fatty acids in comparison to their level in the milk samples stored at the same temperature for 3 months, as well as in initial UHT samples (Table 4). They passed from 77.9 to 41.1%, after 6 months of storage, in control UHT milk of batch 1; against 56.3 and 23.6% in control UHT milk of batch 2. In LPS activated UHT milks, these values became 72.2, 32.4, 54.4 and 16.7%, respectively. This decrease in content of total free fatty acids can be attributed to the advanced oxidation of fatty acids, including aldehydes, ketones and peroxides (Figures 4a and b). In fact, Panfil-Kuncewicz et al. (2005) found that UHT milk fat is subject to high oxidizing changes at room temperatures. Furthermore, the studies Christen et al. (1986), Celestino

Table 2. Extracellular proteolytic activities of control and activated LPS UHT milks UHT milks stored during 6 months at 30°C.

Extracellular proteolytic activity	Storage period of UHT milk (months)													
	Batch 1							Batch 2						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Activity (oPA)[a]														
Control	4.78	5.676*	6.156*	6.94*	6.985	7.07*	7.18	4.66	4.91	5.85*	6.24*	6.68*	6.98*	7.01
Activated LPS	4.52	5.425*	6.038*	6.45*.**	6.76*.**	6.91*.**	7.02	4.23*	4.34*	5.32*.**	5.76*.**	6.23*.**	6.7*.**	6.91*.**

[a]: proteolytic activity according to oPA absorbance at 340 nm; standard deviations of proteolytic activity are in the range [± 0 to ± 0.07]; *, significant differences between storage period ($p < 0.05$); **, significant differences between control and activated LPS UHT milk at the same storage ($p < 0.01$).

Table 3. Nitrogen components of control and LPS activated UHT milks, stored during 6 months at 30°C.

Nitrogen components (g/100 mL of milk)	Storage period of UHT milk (months)													
	Batch 1							Batch 2						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Total nitrogen														
Control	4.84	4.81*	4,75*	4.68*	4,52*	4,48*	4,46*	4.79	4.7*	4.62*	4.68*	4.54*	4.46*	4.4*
Activated LPS	4.89**	4.84*.**	4.81*.**	4.74*.**	4.69*.**	4.65*.**	4.59*.**	4.84**	4.79*.**	4.69*.**	4.65*.**	4.59*.**	4.51*.**	4.5*.**
Soluble nitrogen														
Control	0.79	1.02*	1.11	1.23*	1.29*	1.32*	1.48*	0.82	0.92*	1.01*	1.09*	1.15*	1.28*	1.53*
Activated LPS	0.76**	0.85*.**	0.93*.**	0.96*.**	1.02*.**	1.14*.**	1.20*.**	0.74**	0.79*.**	0.85*.**	0.9*.**	1.02*.**	1.11*.**	1.23*.**
Non proteic nitrogen														
Control	0.32	0.43*	0.53*	0,77*	0.86*	1.05*	1.1*	0.35	0.48*	0.55*	0.61*	0.68*	0.82*	1.02*
Activated LPS	0.3**	0.36*.**	0.50*.**	0.68*.**	0.74*.**	0.87*.**	0.9**	0.32**	0.43*.**	0.48*.**	0.53*.**	0.59*.**	0.7*.**	0.87*.**
Caseic N														
Control	4.05	3.79	3.64	3.45	3.23	3.16*	2.97*	3.98	3.78	3.61	3.59	3.39*	3.17	2.87*
Activated LPS	4.13**	4*.**	3.88*.**	3.78**	3.67**	3.51**	3.38*.**	4.1**	3.84*.**	3.75**	3.67**	3.57**	3.4*.**	3.27*.**
Proteose nitrogen														
Control	0.468	0.588*	0.576*	0.454*	0.425*	0.274*	0.382*	0.465	0.441*	0.466*	0.477*	0.467*	0.460*	0.531*
Activated LPS	0.458**	0.484*.**	0.431*.**	0.279*.**	0.282*.**	0.271*.**	0.303*.**	0.42**	0.356*.**	0.366*.**	0.366*.**	0.428*.**	0.408*.**	0.358*.**

Standard deviations are in the range [± 0 to ± 0.1]; *: significant differences between storage period ($p < 0.05$); **: significant differences between control and activated LPS UHT milk for the same stage of storage time ($p < 0.01$).

Table 4. Amino acids concentrations of soluble nitrogen determined by RP-HPLC of control and activated LPS UHT milks stored during 6 months at 30°C.

Amino acids concentration (ppm)	Storage period of UHT milk (months)											
	Batch 1						Batch 2					
	0		3		6		0		3		6	
	Control	Activated LPS	Control	Activated LPS	Control	Activated LPS	Control	Activated LPS	Control	Activated LPS	Control	Activated LPS
Aspartic acid	2.36	1.89**	1.55	1.24*.**	3.57*	2.72*.**	7.50	4.40*.**	8.51*	6.62*.**	20.51*	7.91*.**
Glutamic acid	3.24	2.47**	3.54*	2.83*.**	4.27*	2.95*.**	7.72	4.60*.**	12.53*	10.26*.**	32.61*	10.86*.**
Serine + Histidine + Glutamine	1.46	0.84**	3.61*	2.88*.**	2.63*	1.66**	3.16	1.16*.**	4.92*	2.00*.**	17.80*	1.77*.**
Arginine	0.34	0.14**	0.66*	0.53*.**	0.57*	0.35**	1.22	0.49*.**	1.00	0.92*.**	3.41*	1.13*.**
Glycine + Threonine	2.42	1.13**	3.99*	2.79*.**	3.10*	2.35**	2.72	2.10*.**	2.66	0.20**	7.3*	1.48*.**
Alanine	4.87	3.87**	3.13	2.19**	2.43	1.92**	6.68	1.46*.**	1.62	0.97*.**	1.56	0.98*.**
Valine + Methionine	0.63	0.44**	1.12*	0.78*.**	1.02	0.68**	1.23	0.50*.**	2.49*	1.41*.**	5.92*	1.57*.**
Tyrosine	4.56	4.12**	4.93*	3.70**	4.95	4.56**	4.13	2.49*.**	6.87*	6.82*.**	6.04	0.99*.**
Lysine	0.62	0.59**	0.35	0.26**	0.23	0.12**	0.34	0.001*.**	0.105	0.15*	1.52*	0.105*.**
Phenylalanine	1.05	0.10**	2.53*	1.89*.**	2.08	1.19**	2.28	1.68*.**	2.66*	2.04*.**	5.16*	2.07*.**
Isoleucine	1.11	0.96**	1.02	0.79**	0.99	0.82**	1.72	1.09*.**	3.11*	1.91*.**	5.6*	2.07*.**
Leucine	2.08	1.79**	2.52*	1.96*.**	2.35	1.68**	3.69	2.01*.**	5.18*	3.09*.**	12.08*	2.93**

Standard deviations are in the range [± 0 to ± 0.09]; *, significant differences between storage period ($p < 0.05$); **, significant differences between control and activated LPS UHT milk at the same storage time ($p < 0.01$).

Table 5. Fatty acids composition (percentage) of control and activated LPS UHT milks stored during 6 months at 30ºC.

Storage period (months)			Fatty acids (%)										
			$C_{8:0}$	$C_{10:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{22:0}$
Batch 1	0	Control	0.85	2.37	0.8	1.36	29.84	1.74	0.83	11.91	22.53	2.45	3.29
		Activated LPS	0.77	2.07	0.64	1.13	29.81	1.5	0.68	9.94	20.17	2.61	2.85
	3	Control	0.82	2.22	0.68	1.24	25.88	1.72	0.57	10.92	20.13	0.78	3.05
		Activated LPS	0.65	1.75	0.54	0.94	26.89	1.28	0.46	8.87	17.88	2.35	2.4
	6	Control	0.44	1.17	0.34	0.62	17.1	0.8	0.37	5.7	11.38	1.65	1.57
		Activated LPS	0.36	0.94	nd	0.50	14.05	0.67	0.31	4.67	9.35	1.27	0.3
Batch 2	0	Control	0.65	1.75	0.55	1.38	22.18	1.31	0.64	9.6	16.27	1.92	2.38
		Activated LPS	0.67	1.83	0.57	1.10	20.9	1.29	0.41	8.86	13.55	1.64	2.52
	3	Control	0.53	1.4	0.4	0.81	21.25	1.00	0.37	7.79	14.72	0.53	1.88
		Activated LPS	0.39	1.04	nd	0.61	15.01	0.71	0.31	5.61	9.9	1.39	1.39
	6	Control	0.26	0.66	0.18	0.09	9.74	0.45	0.23	3.52	6.67	0.92	0.89
		Activated LPS	0.17	0.43	0.12	0.28	7.07	0.28	0.15	2.62	4.92	0.17	0.58

$C_{8:0}$, Caprylic acid; $C_{10:0}$, capric acid; $C_{14:0}$, myristic acid; $C_{15:0}$, pentadecanoic acid; $C_{16:0}$, palmitic acid; $C_{16:1}$, palmitoleic acid; $C_{17:0}$, heptadecanoic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{22:0}$, docosanoic acid; nd, not determined.
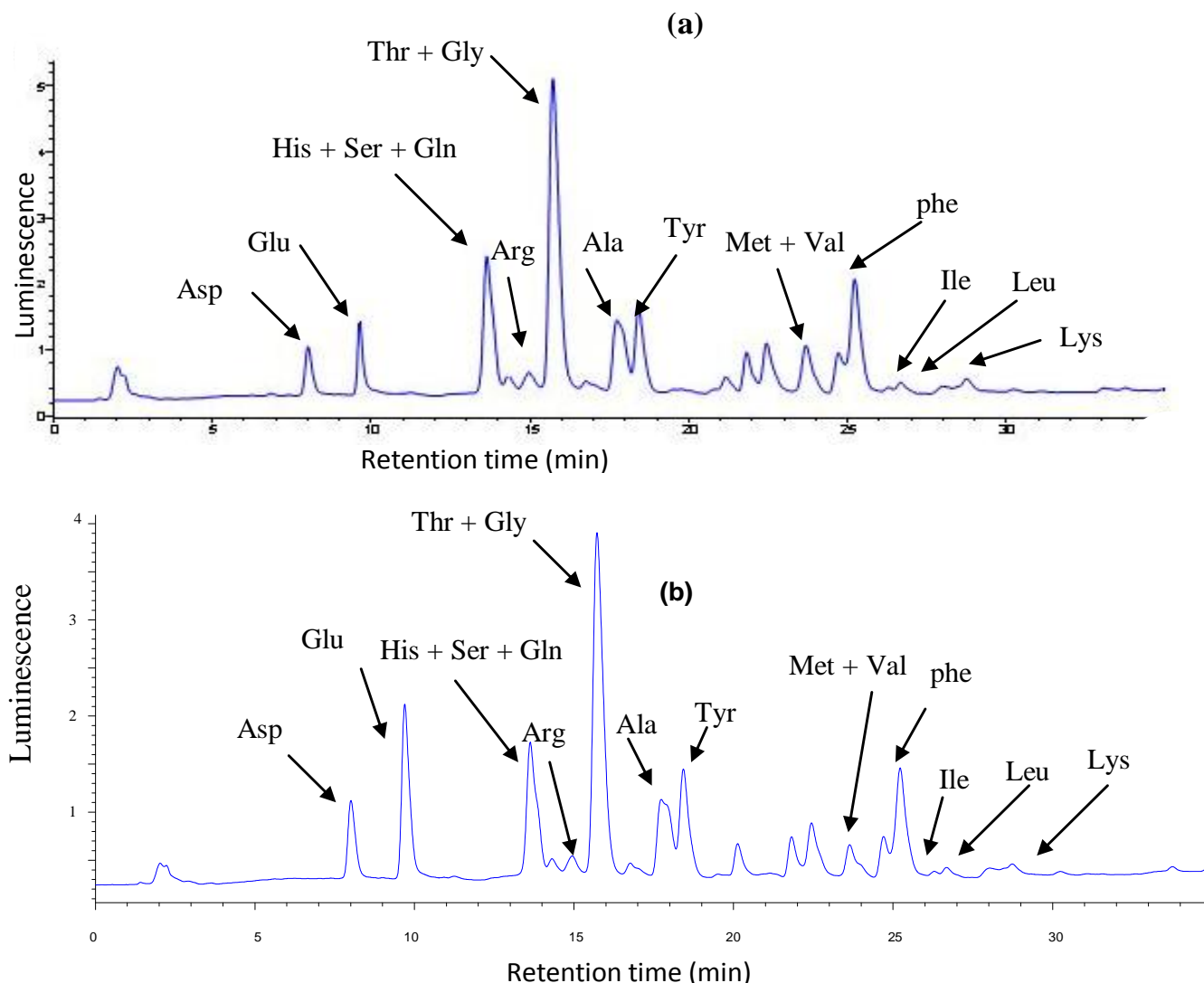
Figure 3. Chromatographic profiles of amino-acids, obtained from soluble nitrogen of UHT milk batch 1 stored during 6 months at 30°C. (a). Control UHT milk. (b). activated LPS UHT milk.

et al. (1997) and AlKanhal et al. (1994) prove that free fatty acids increase during storage of UHT milk. The majority of authors confirm that the basic reason for this growth can be found in the low microbiological quality of raw milk and its contamination with lipolytic psychrotrophic bacteria.

Moreover, at each storage time, total fatty acids content of activated LPS UHT milks were lower than those obtained in control UHT milk samples. These findings suggest that LPS activated in raw milk processed reduce bacterial lipolysis of UHT milk.

Conclusion

The results of the current study indicate that the combination of refrigeration and activation of the LP system in raw milk decrease proteolysis and lipolysis of UHT milk caused by thermoresistant proteinases and lipases of Gram negative psychrotrophs bacteria. As control UHT milk show high levels of proteolysis and lipolysis during storage for 6 months at 30°C, it results to bitterness, gelation and rancidity which could reduce the shelf life of the UHT milk. Thus, activation of LP system in refrigerated raw milk would improve dairy products quality.
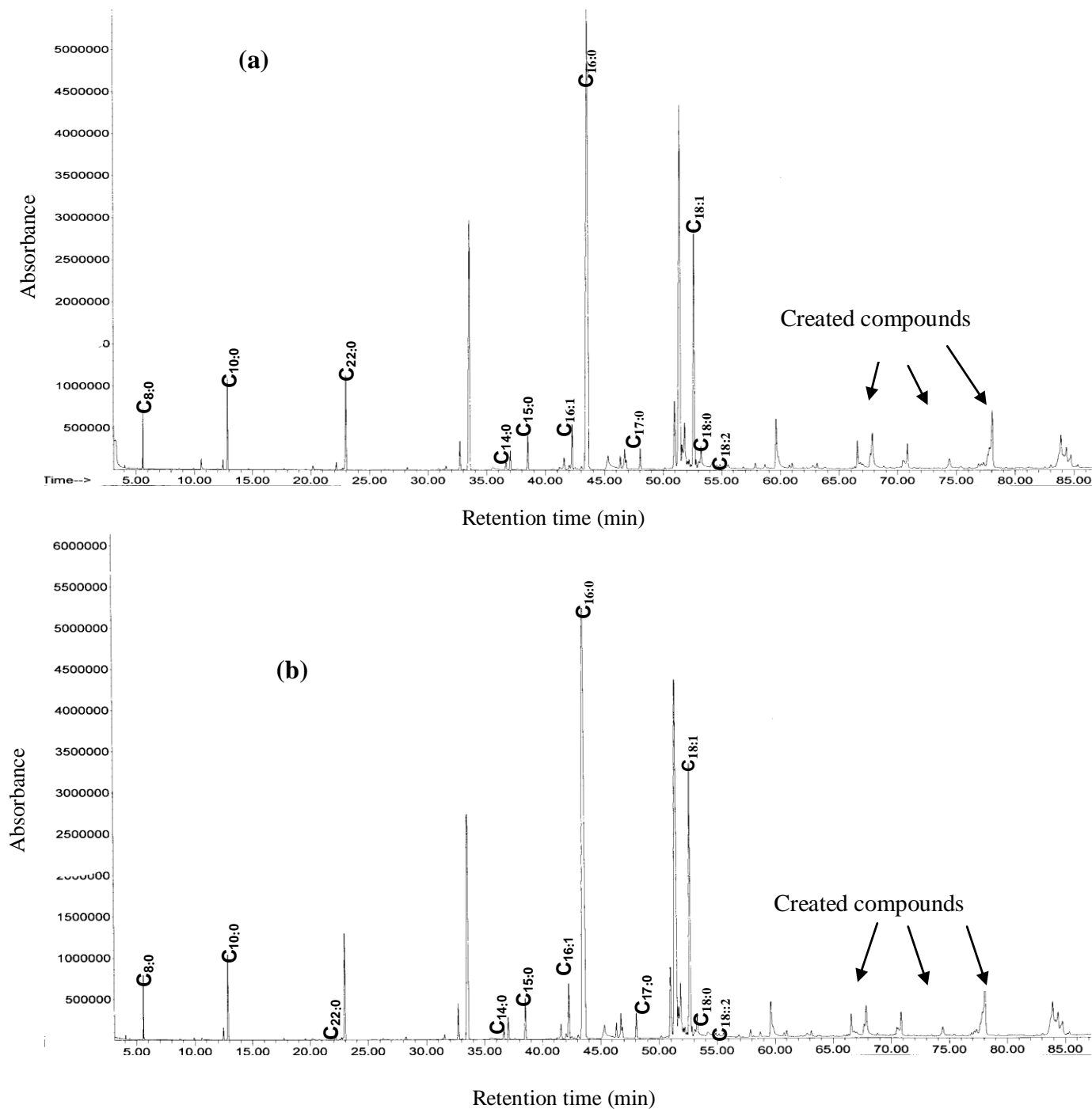
ACKNOWLEDGEMENTS

Figure 4. Chromatographic profile of fatty acids of UHT milk (batch 1) stored during 6 months at 30°C. **(a).** Control UHT milk. **(b).** activated LPS UHT milk.

production group of factory plant Générale Industrielle des Produits Alimentaires» (Tunisia).

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