γ-Glutamyl-transferase, xanthine oxidase and total free sulfhydryls as potential markers for pasteurization treatments in dairy technology

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Summary

γ-Glutamyl-transferase (GGT), xanthine oxidase (XO) and total free sulfhydryl groups (–SH) were examined as potential markers for milk pasteurization, milk ultra-pasteurization and cream pasteurization. The activities of GGT after pasteurization of cows', ewes' and goats' milks were 7–12% of that of raw milks, while no reactivation was observed during milk storage. The activities of XO after ultra-pasteurization of cows' milk were 11–17% of that of raw milk, and after pasteurization of cows' cream were 20% of that of thermized cream. No reactivation of the enzyme was observed during storage of the products. Total free sulfhydryl in ultra-pasteurized milks were 154–190% of those in raw milks and, in pasteurized creams, were 137–150% of those in thermized creams, the values being stable during storage of the products. Present results suggest that GGT may be useful as a marker of milk pasteurization treatments, and XO and –SH groups may be useful as markers of milk ultra-pasteurization and cream pasteurization.

Keywords

pasteurization marker; pasteurization; ultra-pasteurization; milk; cream

The main purpose of heat treatment of milk and dairy products is the protection of consumer from pathogenic microorganisms. Another purpose is to prolong their shelf life by achieving the partial destruction of microorganisms. In order to obtain an optimum product quality, heat treatment should meet the minimum required temperature/time combination to limit the modifications of physicochemical, nutritional and sensory properties of the products.

Thermization of raw milk, at 60–65 °C for 15–30 s, is applied before its storage under refrigeration and the subsequent heat treatment. Low temperature long time (LTLT) pasteurization of milk is used in making dairy products such as cheeses. High temperature short time (HTST) pasteurization of milk is done at a minimum of 15 s at 71.7 °C, and the shelf-life of such pasteurized milk is 3–5, or even more days, when kept refrigerated. Ultra-pasteurization of milk, a relatively new type of processing, is done by heating for 2–5 s at 110–125 °C. The shelf-life of ultra-pasteurized milk is about 20–30 days. Cream pasteurization is performed at 75–80 °C, depending upon its fat content. Cream with 18% fat is pasteurized at lower temperatures, e.g. 75 °C during 15 s, while cream with 35% fat or more is pasteurized at higher temperatures, e.g. 80 °C for 15 s.

The detection of the inactivation of milk native enzymes is easy to perform as a routine method. Therefore, activities of enzymes such as alkaline phosphatase (ALP), lactoperoxidase (LPO), γ-glutamyl transferase (GGT) or xanthine oxidase (XO), have been studied as indication of thermal treatment of milk and milk products [1–7]. The most studied enzymatic indices of the thermal history of milk are ALP and LPO. ALP in milk is significant mainly because it is universally used as an index of pasteurization, or the addition of raw milk to pasteurized milk or milk products. Inac-

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tivation of the enzyme ensures that all the non-sporeforming pathogenic microorganisms present in milk during heat treatment are devitalized. LPO enzyme is inactivated at 75–80 °C. If the milk is overheated (> 75 °C) during pasteurization, inactivation of the enzyme will occur and give a negative lactoperoxidase (Storch) test. The test has no public health or hygiene significance, but the principle of the test is still used to identify super-pasteurized milk, i.e. milk heated at 76 °C for 15 s [1, 3]. GGT is also more heat-resistant than ALP but less heat-resistant than LPO. It was recommended for monitoring thermal processes treatments in the range of 70–80 °C, in particular at temperatures higher than 77 °C [2, 6, 8].

Thermal stability of XO is higher than LPO, GGT and ALP [1, 9]. It was been considered as a suitable indicator of milk heated in the temperature range 80–90 °C [2].

Changes in the sulfhydryl (–SH) and disulfide (–S–S–) groups of the milk proteins occur when milk is subjected to heat treatment sufficient to cause denaturation of the serum proteins. In unheated milk, these groups are masked but they become free and highly reactive when milk is heated. The evaluation of heat load in milks by measurement of total –SH groups was proposed [10–12].

There is a general pursuit to establish markers for milk ultra-pasteurization and cream pasteurization, while alternatives to alkaline phosphatase marker for milk pasteurization are of interest. Consequently, the aim of present study was to examine the inactivation of GGT and XO enzymes, and the change of –SH, as potential markers for milk pasteurization, milk ultra-pasteurization and cream pasteurization.

MATERIAL AND METHODS

Experimental procedure

Raw milk samples and samples at various steps of production of milk products were obtained from Delta Dairy Tauros (Attiki, Greece). Cows' milk was produced by the Holstein-Friesian breed of cattle, ewes' milk by Chiotiko and Karagouniko sheep breeds, and goats' milk by Saanen goat breed.

In the first experimental section cows', ewes' and goats' milks were used. Laboratory-scale thermization at 63 °C for 15 s, LTLT pasteurization at 63 °C for 30 min, and HTST pasteurization at 72 °C for 15 s, were performed. Each milk sample was put in test tubes (12 × 75 mm) with closures, and 1 ml of milk was put in each test tube. In a test tube, a sensor of a thermometer was immersed in the milk through the closure in order to determine the come-up times. Test tubes were put in an oil bath. Milk samples of a temperature of 5 °C were used and come-up times were 18 s and 28 s for heating at 63 °C and 73 °C, respectively. After heat treatment, tubes were immediately immersed into an ice bath.

The average composition of cows' milk used was: non-fat dry matter 86.7 $g \cdot kg^{-1}$, fat 37.7 $g \cdot kg^{-1}$, protein 33.1 $g \cdot kg^{-1}$, lactose 46.6 $g \cdot kg^{-1}$ and acidity (expressed as milligrams of lactic acid) 1.4 $mg \cdot ml^{-1}$. The average composition of Chiotiko-ewes' milk used was: non-fat dry matter 106.5 $g \cdot kg^{-1}$, fat 73.1 $g \cdot kg^{-1}$, protein 56.4 $g \cdot kg^{-1}$, lactose 43.1 $g \cdot kg^{-1}$ and acidity 1.6 $mg \cdot ml^{-1}$. The average composition of Karagouniko-ewes' milk used was: non-fat dry matter 106.1 $g \cdot kg^{-1}$, fat 63.3 $g \cdot kg^{-1}$, protein 53.3 $g \cdot kg^{-1}$, lactose 45.8 $g \cdot kg^{-1}$ and acidity 1.6 $mg \cdot ml^{-1}$. The average composition of goats' milk used was: non-fat dry matter 86.3 $g \cdot kg^{-1}$, fat 41.9 $g \cdot kg^{-1}$, protein 35.6 $g \cdot kg^{-1}$, lactose 43.8 $g \cdot kg^{-1}$ and acidity 1.5 $mg \cdot ml^{-1}$.

In the second experimental section cows' milk was used. In this case, samples were taken from the production line of the dairy factory. The average composition of milk used was: non-fat dry matter 86.3 $g \cdot kg^{-1}$, fat 37.1 $g \cdot kg^{-1}$, protein 33.0 $g \cdot kg^{-1}$, lactose 46.3 $g \cdot kg^{-1}$ and acidity 1.4 $mg \cdot ml^{-1}$. During milk pasteurization, samples of refrigerated milk (after standardization), of milk heated at 63 °C for 15 s, and of pasteurized milk (after pasteurization heating at 72 °C for 15 s) were taken. Moreover, samples of pasteurized milk during its storage at 5 °C for up to 5 days were also taken. Full-fat milk with 35.0 $g \cdot kg^{-1}$ fat, milk with reduced fat with 15.0 $g \cdot kg^{-1}$ fat, and also skimmed milk weres used.

During production of ultra-pasteurized milk, samples of refrigerated milk (after standardization), of milk heated at 63 °C for 15 s, and of ultra-pasteurized milk (after pasteurization heating at 120 °C for 4 s) were taken. Moreover, samples of ultra-pasteurized milk during its storage at 5 °C for 7, 14 and 28 days were also taken. Full-fat milk with 35.0 $g \cdot kg^{-1}$ fat and milk with reduced fat, with 15.0 $g \cdot kg^{-1}$ fat, were used.

During production of cream, samples of cream heated at 63 °C for 15 s, and pasteurized cream (after pasteurization heating at 100 °C for 10 s) were taken. Moreover, samples of cream during its storage at 5 °C for 2–7 days were also taken. Cream with 480.0 $g \cdot kg^{-1}$ fat (total solids 534.0 $g \cdot kg^{-1}$ and acidity 1.0 $mg \cdot ml^{-1}$) and with 350.0 $g \cdot kg^{-1}$ fat (total solids 409.0 $g \cdot kg^{-1}$ and acidity 1.0 $mg \cdot ml^{-1}$) were used.

Analyses

Fat, lactose, protein and non-fat dry matter of milk samples were determined using Milkoscan FT120 (Foss Electric, Hillerød, Denmark). The acidity was evaluated by titration using a sodium hydroxide solution and phenolphthalein indicator [13].

Bacterial counts were determined using the pour plate technique and expressed as colony forming units per millilitre. Total bacterial counts and psychrotrophic bacterial counts were enumerated using Plate count agar with skimmed milk (Biokar Diagnostics, Beauvais, France) with incubation at 30 °C for 72 h and 21 °C for 25 h, respectively [14, 15]. Coliform bacterial counts were enumerated using Violet red bile agar (Biokar Diagnostics) with incubation at 30 °C for 24 h [16]. For mesophilic thermoduric bacterial counts enumeration, milk samples were heat-treated at 63 °C for 30 min and plated on Plate count agar with incubation at 30 °C for 72 h [17].

ALP activities, in milliunits per litre, were determined according to ISO 11816-1 [18] using the Advanced Fluorometer (Fluorophos test system, model Flm200; Advanced Instruments, Norwood, Massachusetts, USA). Enzyme activity was expressed in units. One unit per liter of the product being defined as the amount of enzyme which converts 1.0 μmol·l^{-1} of substrate per minute under the conditions of the assay.

LPO test (Storch test) was performed according to Commission Decision 91/180/EEC [19].

GGT activity was determined as described previously [20]. In a test tube, appropriately diluted milk or cream sample of 40 μl was added to 1.0 ml of GGT substrate solution containing 4.6 mmol·l^{-1} L-γ-glutamyl-*p*-nitroanilide (Sigma-Aldrich, St. Louis, Missouri, USA) and 0.1 mol·l^{-1} glycylglycine (Sigma-Aldrich) in 0.1 mol·l^{-1} Tris-HCl buffer pH 9.0 (Sigma-Aldrich). Reaction samples were incubated for 20 min at 37 °C. The reaction was stopped by the addition of 1 ml of 0.1 mol·l^{-1} glycine (Sigma-Aldrich), pH 10.5. Ethylenediaminetetraacetic acid (EDTA, 500 g·l^{-1}, Sigma-Aldrich) and Triton X-100 (500 g·l^{-1}, Merck, Darmstadt, Germany) were added (200 μl each), and the solution was mixed and incubated for 10 min at 50 °C. The absorbance was measured at 410 nm against blank, which was prepared as described above but L-γ-glutamyl-*p*-nitroanilide was not added in the substrate solution.

Enzyme activity was expressed in units. One unit was defined as the amount of the enzyme required to release 1 mmol·l^{-1} of *p*-nitroanilide under the conditions of the assay. *p*-Nitroanilide (Sigma-Aldrich) in 0.1 mol·l^{-1} Tris-HCl (Sigma-Aldrich) pH 9.0 was used to construct a standard curve.

XO activity was determined as described previously [21]. In a test tube, appropriately diluted milk or cream sample of 0.2 ml was added to 1 ml of 0.05 mol·l^{-1} phosphate buffer pH 7.5, followed by the addition of 1 ml xanthine substrate (Sigma-Aldrich; 2 mg in 100 ml distilled H_2O, prepared fresh daily) and 0.8 ml of distilled H_2O. The mixture was kept at room temperature for 5 min, the reaction was stopped by the addition of 1 ml 200 mg·ml^{-1} trichloroacetic acid (TCA), and the mixtures were centrifuged at 2 200 ×*g* for 10 min. The absorbance of supernatants was measured at 290 nm against a blank containing distilled H_2O instead of the xanthine substrate solution. XO activity was expressed in units, one unit being defined as the amount of enzyme required to give an absorbance equal to one unit of commercial xanthine oxidase (Sigma-Aldrich) under the conditions of the assay. One unit of the commercial xanthine oxidase converts 1.0 μmol·l^{-1} of xanthine to uric acid in 1 min at pH 7.5 at 25 °C, according to manufacturers. XO units were calculated from a calibration curve based on different volumes of xanthine oxidase added to milk heated at 120 °C for 30 min (i.e. without any intrinsic xanthine oxidase activity).

Total free sulfhydryls were determined as described previously [22] using Ellman's procedure and clarifying reagent. Milk samples were used directly, while cream samples were centrifuged at 2 200 ×*g* for 10 min at 5 °C and the supernatants were used. To 0.5 ml of sample, 1 ml of 8 mol·l^{-1} urea-buffered solution (0.03 mol·l^{-1} borate buffer adjusted to pH 8.5 with boric acid) (Sigma-Aldrich) and 50 μl of 5,5'-dithio-bis (2-nitrobenzoic acid), DTNB, (Sigma-Aldrich) solution (4 g·l^{-1} in 0.2 mol·l^{-1} EDTA pH 6.0 solution) were added. The mixture was shaken and kept for 5 min at room temperature. Then 0.5 ml of 0.2 mol·l^{-1} EDTA solution at pH 6.0, and 2 ml of Clarifying Reagent (Sigma-Aldrich) were added; the solution was shaken vigorously, and the tubes were placed at 37 °C for 5 min. The absorbance of the (transparent) mixture at 412 nm was read against a blank (tube) containing all reagents and 0.5 ml of distilled H_2O instead of milk. Total –SH of the samples were calculated from cysteine hydrochloride (Ferak, Berlin, Germany) standard curve and expressed as milligrams per litre. Cysteine was dissolved in water, and the same procedure was followed as for the samples.

Results were treated statistically using the one-way analysis of variance, ANOVA (SPSS 14.0 software; SPSS, Chicago, Illinois, USA), and the Bonferroni test at $p < 0.05$.

RESULTS AND DISCUSSION

Cows', ewes' and goats' milk pasteurization

Raw cows' milk from Holstein-Friesian breed of cattle, ewes' milk from Chiotiko and Karagouniko sheep breeds, and goats' milk from Saanen goat breed were heat-treated. Thermization, LTLT pasteurization and HTST pasteurization were used. In all four milks used, raw and thermized milks exhibited very high ALP activities, $> 10^4$ mU·l^{-1}, and positive results of the peroxidase test. All pasteurized milks, LTLT and HTST, exhibited low ALP activities, up to 2×10^2 mU·l^{-1}, and positive results of the peroxidase test. The above data indicate that, in all cases, milks were properly heat-treated.

The activities of GGT and XO activities of raw and treated milks are presented in Tab. 1. Raw milks exhibited similar levels of GGT activity. However, GGT activities in Karagouniko ewes' milk were considerably higher that in the other milks. In all cases, GGT activities after thermization decreased to 72–78% of those of the respective raw milks. LTLT and HTST pasteurization appeared to have similar effectiveness, decreasing GGT activities to 8–10% of those of respective raw milks. No differences were observed among four different milks examined.

XO activities in both raw ewes' milks were higher than those in cows' milk, while intermediate levels were observed in goats' milk. However, XO appeared to be more resistant to heat in both ewes' and goats' milks than in cows' milk. Remaining activities after pasteurization were about 46% in both raw ewes' milks, 42–45% in goats' milk and 29–33% in cows' milk.

Similar levels of GGT activity were observed in cows', ewes' and goats' milks. However, higher activities of GGT in the order cows' > ewes' > goats' were reported by other authors [6, 21, 23]. Thermization, LTLT pasteurization and HTST pasteurization appeared to have similar effect on GGT activities in all three milks examined. Similarly, no differences in thermal stability of GGT in cows' and goats' milk (65–90 °C for 15 s) were reported by other authors [9]. On the other hand, it was reported that the residual activities of GGT in heated (60–85 °C) cows' and ewes' milks were higher than bovine milk [6, 23]. XO activities in both raw ewes' milks were higher than those in cows' milk, while intermediate levels were observed in goats' milk. On the other hand, significantly lower XO activities in goats' than in cows' milk were reported by other authors [9].

Full-fat, reduced fat and skimmed cows' milk pasteurization

Full-fat, reduced fat and skimmed cows' milk pasteurized on industrial scale were examined. Samples of raw, thermized and pasteurized milk, and also samples of pasteurized milk during its storage for 2–5 days at 5 °C, were analysed. Milk heated at 63 °C prior to pasteurization heat treatment exhibited very high ALP activities, $> 10^4$ mU·l^{-1}, and positive results of the peroxidase test. This heating reduced the total bacterial counts to less than 50% of that of raw milk, which is about 5×10^4 CFU·ml^{-1}; psychrotrophs were reduced to about 1×10^3 CFU·ml^{-1} from about 3×10^4 CFU·ml^{-1} of raw milk; and coliforms were eliminated, while present at about 7×10^4 CFU·ml^{-1} in raw milk.

Tab. 1. Effect of thermization, LTLT pasteurization and HTST pasteurization of cows', ewes' and goats' milks on γ-glutamyl-transferase and xanthine oxidase activities.

	Holstein-Friesian cows' milk	Chiotiko ewes' milk	Karagouniko ewes' milk	Saanen goats' milk
γ-Glutamyl-transferase activity [U·ml^{-1}]				
Raw milk	24.5 ± 1.3^a	25.5 ± 2.5^a	27.5 ± 0.3^a	24.8 ± 0.3^a
Thermized milk	19.0 ± 1.0^b	19.8 ± 0.8^b	20.8 ± 0.3^b	17.8 ± 0.3^b
LTLT milk	2.5 ± 0.3^c	2.3 ± 0.3^c	2.3 ± 0.3^c	2.3 ± 0.3^c
HTST milk	2.0 ± 0.0^c	2.0 ± 0.3^c	2.3 ± 0.3^c	2.3 ± 0.3^c
Xanthine oxidase activity [mU·ml^{-1}]				
Raw milk	580 ± 10^a	710 ± 15^a	710 ± 10^a	645 ± 5^a
Thermized milk	445 ± 15^b	525 ± 15^b	540 ± 25^b	465 ± 20^b
LTLT milk	190 ± 15^c	325 ± 15^c	330 ± 15^c	290 ± 20^c
HTST milk	170 ± 10^c	325 ± 10^c	325 ± 10^c	270 ± 5^c

Results are expressed as means along with standard deviations; cows' milk $n = 24$, Karagouniko ewes' milk $n = 20$, Chiotiko ewes' milk $n = 20$, goats' milk $n = 30$. Means in each column and index without common superscript differ significantly.

Tab. 2. Changes of γ-glutamyl-transferase and xanthine oxidase activities during steps of pasteurization treatment and during storage at 5 °C of full-fat, reduced fat and skimmed cows' milk.

	Full fat cows' milk	Reduced fat cows' milk	Skimmed cows' milk
γ-Glutamyl-transferase activity [U·ml^{-1}]			
Raw milk	24.8 ± 1.0^{a}	20.5 ± 1.0^{a}	25.3 ± 0.3^{a}
Thermized milk	19.0 ± 1.0^{b}	16.5 ± 0.5^{b}	15.8 ± 0.3^{b}
Pasteurized milk	3.0 ± 0.8^{c}	2.0 ± 0.0^{c}	1.8 ± 0.0^{c}
Pasteurized milk kept for 2 days	3.3 ± 0.8^{c}	2.3 ± 0.0^{c}	2.0 ± 0.0^{c}
Pasteurized milk kept for 5 days	3.5 ± 0.8^{c}	3.0 ± 0.0^{c}	3.8 ± 0.5^{d}
Xanthine oxidase activity [mU·ml^{-1}]			
Raw milk	590 ± 10^{a}	610 ± 15^{a}	615 ± 15^{a}
Thermized milk	435 ± 20^{b}	420 ± 15^{b}	365 ± 15^{b}
Pasteurized milk	250 ± 30^{c}	175 ± 15^{c}	125 ± 10^{c}
Pasteurized milk kept for 2 days	235 ± 35^{c}	170 ± 20^{c}	115 ± 10^{c}
Pasteurized milk kept for 5 days	170 ± 20^{d}	135 ± 20^{d}	100 ± 25^{c}

Results are expressed as means along with standard deviations (n = 10). Means in each column and index without common superscript differ significantly.

In pasteurized milks, ALP activities were $< 5 \times 10$ mU·l^{-1} and results of peroxidase tests were positive. The same results were obtained during storage of pasteurized milk. In pasteurized milks, total bacterial counts were reduced to 2×10^3 CFU·ml^{-1}, and were stable or increased to 3×10^3 CFU·ml^{-1} up to 4×10^3 CFU·ml^{-1} during storage, while psychrotrophs and coliforms were absent. The above data demonstrate that all milks were properly pasteurized and stored.

The activities of GGT and XO during steps of pasteurization and storage of full-fat, reduced fat and skimmed cows' milk are presented in Tab. 2. Milk heating at 63 °C reduced GGT activities significantly to about 77%, 80% and 62% of those of raw full-fat, reduced fat and skimmed milk, respectively. In follow, pasteurization heating reduced them to 12%, 10% and 7% of those of raw full-fat, reduced fat and skimmed milk, respectively. During storage of pasteurized milk, GGT activities were stable or increased slightly.

Milk heating at 63 °C reduced XO activities significantly to 74%, 69% and 59% of those of raw full-fat, reduced fat and skimmed milk, respectively. In follow, pasteurization heating reduced them to 42%, 27% and 20% of those of raw full-fat, reduced fat and skimmed milk, respectively. During storage of pasteurized milk, XO activities were stable or decreased slightly.

Full-fat and reduced fat cows' milk ultra-pasteurization

Full-fat and reduced fat milk ultra-pasteurized on industrial scale were examined. Samples of raw, thermized and ultra-pasteurized milk, and also samples of ultra-pasteurized milk during storage for 7, 12 and 28 days at 5 °C were analysed. Milk heated at 63 °C prior to ultra-pasteurization exhibited very high ALP activities, $> 10^4$ mU·l^{-1}, and negative results of the peroxidase test. This milk heating reduced the total bacterial counts to less than 50% of those of raw milk, that is about 5×10^4 CFU·ml^{-1} to 6×10^4 CFU·ml^{-1}; psychrotrophs were reduced to about 1×10^3 CFU·ml^{-1} from about 2×10^4 CFU·ml^{-1} to 3×10^4 CFU·ml^{-1} in raw milk, and coliforms to about 1×10^3 CFU·ml^{-1} from 7×10^4 CFU·ml^{-1} in raw milk. On the contrary, this heating had no effect on the counts of thermoduric bacteria, which remained at the same level of about 3×10^3 CFU·ml^{-1} to 4×10^3 CFU·ml^{-1}.

In ultra-pasteurized milks, ALP activities were $< 5 \times 10$ mU·l^{-1} and the peroxidase tests were negative. Total bacterial counts were reduced to 2×10^3 CFU·ml^{-1}, while psychrotrophs, coliforms and thermoduric bacteria were absent. The above data indicate that all milks were heat-treated stronger than by pasteurization. During storage of ultra-pasteurized milk, ALP activities were almost stable and results of peroxidase tests were negative. Moreover, total bacterial counts were stable or slightly increased to 3×10^3 CFU·ml^{-1}, while psychrotrophs, coliforms and thermoduric were absent. The above data show that all milks were properly stored. The activities of GGT and XO and the levels of total –SH during steps of ultra-pasteurization and storage of full-fat and reduced fat milk are presented in Tab. 3. Milk heating at

63 °C reduced GGT activity significantly to about 78% and 65% of those of raw full-fat and reduced fat milk, respectively. In follow, ultra-pasteurization heating totally inactivated GGT. During storage of ultra-pasteurized milk, GGT activities remained zero.

Milk heating at 63 °C reduced XO activities significantly to about 70% and 68% of those of raw full-fat and reduced fat milk, respectively. In follow, ultra-pasteurization heating reduced XO activities significantly to about 17% and 11% of those of raw full-fat and reduced fat milk, respectively. During storage of ultra-pasteurized milk, XO activities were stable or decreased slightly.

Milk heating increased significantly total –SH, to about 110% of the original values for both full-fat and reduced fat milks. In follow, ultra-pasteurization heating increased them significantly to about 190% and 154% of the original values for full-fat and reduced fat milk, respectively. During storage of ultra-pasteurized milks, total –SH were stable.

Cream pasteurization

Cream with 48% and also 35% fat produced on industrial scale was examined. Samples of thermized and pasteurized cream, and also samples of pasteurized cream during storage for 2–7 days at 5 °C, were analysed.

Creams heated at 63 °C exhibited very high ALP activities, $> 10^4$ mU·l^{-1}, and results of peroxidase tests were positive. The samples exhibited total bacterial counts of about 17×10^3 CFU·ml^{-1}, psychrotrophs 1×10^3 CFU·ml^{-1}, coliforms 1×10^3 CFU·ml^{-1} and thermoduric bacteria 3×10^3 CFU·ml^{-1}.

Pasteurized creams exhibited ALP activities close to zero and results of peroxidase tests were negative. The samples exhibited about 2×10^3 CFU·ml^{-1} total bacterial counts, while psychrotrophs, coliforms and thermoduric bacteria were absent. The above data indicate that all creams were successfully pasteurized. During storage of pasteurized creams for 2–7 days, ALP activities remained close to zero and results of per-

Tab. 3. Changes of γ-glutamyl-transferase and xanthine oxidase activities and of total sulfhydryls during steps of ultra-pasteurization treatment and during storage at 5 °C of full-fat and reduced fat cows' milk.

	Full fat cows' milk	Reduced fat cows' milk
γ-Glutamyl-transferase activity [U·ml^{-1}]		
Raw milk	24.5 ± 1.0^{a}	25.3 ± 0.8^{a}
Thermized milk	19.0 ± 1.3^{b}	16.5 ± 0.3^{b}
Ultra-pasteurized milk	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}
Ultra-pasteurized milk kept for 7 days	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}
Ultra-pasteurized milk kept for 14 days	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}
Ultra-pasteurized milk kept for 28 days	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}
Xanthine oxidase activity [mU·ml^{-1}]		
Raw milk	590 ± 20^{a}	620 ± 25^{a}
Thermized milk	415 ± 35^{b}	415 ± 15^{b}
Ultra-pasteurized milk	100 ± 20^{c}	65 ± 5^{c}
Ultra-pasteurized milk kept for 7 days	85 ± 20^{cd}	55 ± 5^{cd}
Ultra-pasteurized milk kept for 14 days	75 ± 10^{cd}	55 ± 5^{cd}
Ultra-pasteurized milk kept for 28 days	65 ± 10^{d}	50 ± 5^{d}
Total sulfhydryls [mg·l^{-1}]		
Raw milk	38.5 ± 1.4^{a}	36.9 ± 1.0^{a}
Thermized milk	42.6 ± 1.8^{b}	40.8 ± 1.6^{b}
Ultra-pasteurized milk	73.0 ± 4.9^{c}	56.8 ± 3.2^{c}
Ultra-pasteurized milk kept for 7 days	72.0 ± 4.9^{c}	53.9 ± 2.1^{c}
Ultra-pasteurized milk kept for 14 days	71.3 ± 4.9^{c}	53.4 ± 2.0^{c}
Ultra-pasteurized milk kept for 28 days	71.1 ± 5.0^{c}	52.1 ± 1.6^{c}

Total sulfhydryls are expressed as milligrams of cysteine per litre. Results are expressed as means along with standard deviations (n = 10). Means in each column and index without common superscript differ significantly.

Tab. 4. Changes of γ-glutamyl-transferase and xanthine oxidase activities, and of total sulfhydryls, during steps of pasteurization treatment and storage at 5 °C of cow cream with 48% and 35% fat.

	Cream with 48% fat	Cream with 35% fat
γ-Glutamyl-transferase activity [U·ml^{-1}]		
Thermized cream	20.5 ± 0.8^{a}	19.0 ± 1.3^{a}
Pasteurized cream	1.0 ± 0.3^{b}	1.0 ± 0.3^{b}
Pasteurized cream kept for 2 days	1.0 ± 0.3^{b}	1.0 ± 0.3^{b}
Pasteurized cream kept for 7 days	1.0 ± 0.3^{b}	1.0 ± 0.3^{b}
Xanthine oxidase activity [mU·ml^{-1}]		
Thermized cream	230 ± 40^{a}	170 ± 10^{a}
Pasteurized cream	50 ± 5^{b}	35 ± 5^{b}
Pasteurized cream kept for 2 days	30 ± 5^{c}	20 ± 5^{c}
Pasteurized cream kept for 7 days	25 ± 5^{c}	20 ± 5^{c}
Total sulfhydryls [mg·l^{-1}]		
Thermized cream	55.7 ± 4.7^{a}	45.1 ± 2.7^{a}
Pasteurized cream	76.1 ± 6.6^{b}	67.7 ± 3.3^{b}
Pasteurized cream kept for 2 days	72.9 ± 3.0^{b}	66.7 ± 1.7^{b}
Pasteurized cream kept for 7 days	69.6 ± 3.4^{b}	63.0 ± 2.8^{b}

Total sulfhydryls are expressed as micrograms of cysteine per litre. Results are expressed as means along with standard deviations (n = 10). Means in each column and index without common superscript differ significantly.

oxidase tests were negative. Total bacterial counts were stable, while psychrotrophs, coliforms and thermoduric bacteria were absent. The above data show that all pasteurized creams were properly stored.

The activities of GGT and XO and the levels of total –SH during steps of pasteurization and storage of creams with 48% and also 35% fat are presented in Tab. 4.

Heated creams exhibited significant GGT activities, which were reduced to close to zero in pasteurized creams. The levels of GGT activities remained close to zero during storage. Heated creams exhibited significant XO activities, which were reduced to 22% and 20% by pasteurization of creams with 48% and 35% fat, respectively. During storage of pasteurized creams, XO activities exhibited a slight decrease.

Heated creams exhibited significant total –SH, which were increased to 137% and 150% by pasteurization for creams with 48% and 35% fat, respectively. During storage of pasteurized creams, total –SH were stable.

In pasteurized milks, GGT activities were about 10% of those observed in raw milk, and they remained stable during storage of milk for up to 5 days. These results indicate that monitoring of GGT activities may be a useful indication of proper milk pasteurization. On the other hand, GGT was completely inactivated by treating of milk at 120 °C for 4 s, and of cream at 100 °C for 10 s, indicating that the enzyme is not suitable as indicator of such heat treatments.

All results on GGT thermostability are consistent with other reports. In milk, GGT was completely inactivated at 80 °C during 15 s and it was proposed as a suitable indicator at temperatures > 77 °C, while in cream with 40% fat, the enzyme was completely inactivated at 90 °C during 15 s [8, 9]. Another GGT inactivation study showed that, in milk with 3.5% fat and 0% fat, as well as in cream with 45% fat, thermal inactivation of the enzyme followed first order kinetics at temperatures in the range from 60 °C to 85 °C [24]. No reactivation of GGT activities was found in milk and cream [20, 23, 25]. ALP is generally used as an indicator of proper milk pasteurization. However, it was suggested that the enzyme is not fully appropriate for this purpose for some reasons, such as the fact that it is inactivated by heat treatments less severe than HTST conditions [3]. Moreover, recent report indicated that *Mycobacterium avium* subsp. *paratuberculosis*, an emerging pathogen in raw milk, is not always destroyed when milk is heated at 72 °C for 16 s [26, 27]. Therefore, there is a need to reconsider the heat treatment (time-temperature combination) to ensure devitalization of *M. paratuberculosis*, and accordingly search for other enzymes as indicators of proper pasteurization. The above data indicate that GGT may be

a suitable alternative to ALP as an indicator of proper milk pasteurization.

In pasteurized milks, XO activities were 20–42% of those in raw milks indicating its limited usefulness as index of pasteurization. XO activities were decreased significantly during treatment of milk at 120 °C for 4 s, and of cream at 100 °C for 10 s. Ultra-pasteurized milks exhibited about 15% of XO activities of raw milk, while pasteurized cream exhibited about 20% of the XO activities of heated cream. No reactivation of XO activities was observed during storage of ultra-pasteurized milks and pasteurized creams. The above results indicate that monitoring of XO activity may be useful as an indicator of ultra-pasteurization of milk with 3.5% and 1.5% fat, at 120 °C for 4 s, as well as of pasteurization of cream with 35–48% fat, at 100 °C for 10 s. It was been reported that XO was completely inactivated in milk at 90°C during 15 s, while it was not inactivated at 80 °C during 120 s [1, 9]. XO was considered a suitable indicator of milk heating in the temperature range of 80–90 °C [2]. Present results indicate that XO may be a useful indicator of treatments at 100 °C and at higher temperatures.

Total –SH increased significantly by milk ultra-pasteurization and cream pasteurization, while they were stable during storage of the products at 5 °C. A similar marked increase of total –SH was observed by others at heating temperatures higher than 70 °C during 15 min, and little change of free (and total –SH) during storage of heated milk at 4 °C was observed by other authors [28]. Other authors reported that formation of total –SH during heating of goats' milk at 75–95 °C varied depending on heating time [29]. On the other hand, it was described that heat treatments decrease free –SH groups, as a result of the exposure of masked –SH groups, which is followed by their oxidation to –S–S– groups [12]. The above results suggest that monitoring of total –SH may be useful as an indicator of heat treatments at 100 °C and at higher temperatures. This may be useful as a marker of proper milk ultra-pasteurization and cream pasteurization.

CONCLUSIONS

Present results suggest that monitoring of GGT activities is a useful marker for milk LTLT and HTST pasteurization, and monitoring of XO activities and total free sulfhydryl groups are useful as markers of milk ultra-pasteurization and cream pasteurization.

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