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## Effects of Pasteurization and Ultra-High Temperature Processes on Proximate Composition and Fatty Acid Profile in Bovine Milk

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### ABSTRACT

The aim of this work was to evaluate the effects of pasteurization and ultra-high temperature processes on proximate and physicochemical composition, microbiological parameters and fatty acid profile in bovine milk. Raw, pasteurized and sterilized milks were collected at a dairy factory in the state of Rio Grande do Sul, Brazil. The samples were submitted to determination of moisture, protein, total fat, lactose, total solids, free-fat dry extract, urea, calcium, phosphorus, pH, acidity, density, fatty acid profile, somatic cell count and total bacterial count. Pasteurized and ultra-high temperature milks kept protein and lactose content similar to raw milk. Pasteurization and sterilization altered the composition of the milk slightly, decreasing total fat and total solids and increasing urea. These processes changed essentiality short-chain fatty acids (4:0, 6:0 and 8:0). High proportions of palmitic acid (18:0), oleic acid (18:1 n-9), stearic acid (18:0) and myristic acid (14:0) were found in all milks analyzed. The absence of large significant modifications on milk composition and fatty acid profile indicates that the processes could be applied without altering the nutritional value of the milk.

**Key words:** Pasteurization, sterilization, milk composition

### INTRODUCTION

Milk consumption provides high nutritional value, containing casein, lactose, essential fatty acids, vitamins and minerals (Claeys *et al.*, 2014). However, several controversies arise around the consumption of dairy and milk products during adulthood. Despite controversies, epidemiologic studies confirm the nutritional importance of milk in the human diet and reinforce the possible role of its consumption in preventing several chronic conditions like cardiovascular disease (CVD), some forms of cancer, obesity and diabetes (Pereira, 2014). Based on a review of epidemiological studies, there seems to be no consistent relation between a high intake of dairy products and CVD (Astrup *et al.*, 2011). Moreover, some Saturated Fatty Acids (SFA) in milk are reported to have positive effects on health; butyric acid (4:0) is a known modulator of gene function and may also play a role in cancer prevention, 8:0 and 10:0 may have a role in antiviral activities and 8:0 has been reported to delay tumor growth (Claeys *et al.*, 2014).

It is essential to keep milk conditioned at low temperatures; to maintain this high nutritional value and submit it to heat treatments such as pasteurization and Ultra-High Temperature (UHT) process. The main aims of heat treatment are to reduce the microbial population, both pathogenic and spoilage, to inactivate enzymes and to minimize chemical reactions and physical changes (Lewis and Deeth, 2009). The production of heat treated milk for human consumption covers the spectrum from pasteurization to in-container sterilization, with respect to shelf life and heat-induced changes in milk (Sakkas *et al.*, 2014).

However, some effects of heating affect quality and technological properties of milk. Sakkas *et al.* (2014) suggested that this group of effects includes degradation of lactose to organic acids and formation of lactulose, denaturation of whey proteins, destruction of vitamins and enzymes, hydrolysis of proteins and lipids and disturbance of calcium/phosphorus equilibrium. Other effects include cooked flavor and nutritional value loss due to new substances formed by the Maillard reaction, which continues during storage of heated milks (Elliott *et al.*, 2005). The effect of heat treatments on the components of milk has been described. However, the effect of processing and storage conditions on the lipid profile of milk is not fully understood and is subject of controversy, particularly for the case of fatty acids (Rodriguez-Alcala *et al.*, 2009). Little research exists on the use of pasteurization and sterilization processes, especially with regards to fatty acid profile and chemical and physical properties of milk.

The objective of this research was to determine the effects of pasteurization and ultra-high temperature processes on proximate and physicochemical composition, microbiological parameters and fatty acid profile in bovine milk.

## **MATERIALS AND METHODS**

**Chemicals:** All chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich Co. Ltd. (MO, USA).

**Milk samples:** Twelve lots of bovine milk from each different step of the processing of milk (raw, pasteurized and sterilized) were collected at a dairy factory in the region of Vale do Taquari, in the Brazilian state of Rio Grande do Sul. The same lot of raw milk was submitted to pasteurization (75°C for 15 sec) and then to ultra-high temperature process (140°C for 3 sec). The pasteurization was realized on tetra pack equipment with 30,000 L h<sup>-1</sup> and after this process the milk was maintained at 4°C. Each lot was sampled three times in order to perform analysis in triplicates. Raw, pasteurized and UHT milk samples were transferred to sterilized tubes. The tubes were immediately cooled at 4°C after sampling and transported in thermal containers.

**Determination proximate and physicochemical composition and microbiological parameters:** Moisture, protein, total fat, lactose, total solids, free-fat dry extract, urea, calcium and phosphorus were determined with a MilkoScan (Milkoscan FT+, FOSS, Denmark) by fourier transform infrared spectroscopy (FTIR). Analyses were done according to the procedures recommended by the manufacturer (Foss Analytical, 2008). The pH of the samples was measured using a Mettler-Toledo pH meter (Mettler-Toledo International Inc., Greifensee, Switzerland). Acidity and density were determined according to Latimer (2012). Somatic cell count and total bacterial count were analyzed by BactoScan (Bactoscan H, FOSS, Denmark) using cytometric flow. The BactoScan was operated according to the procedures recommended by the manufacturer (NCIMS., 2011). All analyzes were realized in triplicate.

**Fat extraction and fatty acid profile:** Lipid extraction followed the methodology proposed by Latimer (2012). Approximately 5 mL of milk was dissolved in 2 mL of ethanol, 4 mL of ultrapure water and 2 mL of  $\text{NH}_4\text{OH}$ . This mixture went through shaking water bath at 75°C for 10 min. The extracted fat residue was dissolved in 50 mL diethyl ether:petroleum ether (1:1, v/v). Then, the solution was evaporated to dryness in a 40°C water bath under nitrogen stream. The fat residue was dissolved in 5 mL chloroform:diethyl ether (1:1, v/v) and the solvent was evaporated under nitrogen stream. The residue was added 2 mL 7%  $\text{BF}_3$  and 1 mL toluene and heated in a 100°C oven for 45 min. After added 5 mL ultrapure water, 1 mL hexane and 0.2 g  $\text{Na}_2\text{SO}_4$ , the solution was vortex-stirred for 1 min, followed by centrifugation at 5000 rpm for 2 min. The organic upper phase was recovered and analyzed by Gas Chromatography (GC). Fatty Acid Methyl Esters (FAME) were analyzed on an Agilent GC unit (model G890N, Palo Alto, CA, USA) equipped with a flame ionization detector. Fatty acids were separated using a DB-23 fused-silica capillary column (30×0.32 mm ID×0.25 µm film thickness, Agilent, USA). Identification of peaks was accomplished by comparison of sample peak retention times with those of FAME standard mixture acquired from Supelco Inc. (Bellefonte, PA, USA). Quantification of the FAME in milk lipids was done using undecanoic acid (11:0) as an internal standard. Helium was used as the carrier gas and the injector split ratio was 1:50. After injection (1 µL), the initial column temperature of 70°C was held for 0.5 min, increased to 170°C (9°C min<sup>-1</sup>). Then, it was increased to 200°C (1°C min<sup>-1</sup>) and finally increased to 230°C (10°C min<sup>-1</sup>) and sustained for 2 min. The FAME was expressed as g/100 g of total fatty acid content, assuming a direct relationship between peak area and FAME weight. The analysis was realized in triplicate.

**Statistical analysis:** The data were analyzed using the PROC Mixed Procedure of the Statistical Analysis Systems (SAS., 2004). The statistical model considered the treatment type (raw, pasteurized and UHT) a repeated measure within the milk. Least squares means (LS means), with the option PDIFF (probability difference procedure), were determined in order to compare groups. Differences were considered significant at a value of p below 0.05.

## RESULTS AND DISCUSSION

**Proximate and physicochemical composition and microbiological parameters:** The effects of treatments on proximate and physicochemical composition and microbiological parameters in bovine milk are shown in Table 1. The proximate composition is reported on a wet matter basis.

The treatments significantly affected ( $p < 0.05$ ) the contents of moisture, total fat, total solids and urea. The difference in total fat after pasteurization and sterilization resulted from standardization by centrifugation. This is done in order to remove fat cream, complying with Brazilian legislation. The homogenization process and the thermal treatment of milk resulted in damage to the fat globule membrane (Fox and McSweeney, 1998), causing greater exposure to substances present on the membrane surface (Fox and McSweeney, 2003). This probably resulted in release of urea, allowing its quantification, which could explain the increase in urea contents found in raw, pasteurized and UHT milks, respectively.

The major nutrients of milk (protein and lactose) were not influenced by pasteurization and UHT processing. The calcium (0.11±0.02%) and phosphorus (0.10±0.02%) concentrations in the samples did not show significant differences between the different treatments. These results agree with the concentration range found for Brazilian bovine milks described by Do-Nascimento *et al.* (2010).

Table 1: Proximate and physicochemical composition and microbiological parameters in raw, pasteurized and UHT bovine milk

Analysis	Raw	Treatments	
		Pasteurized	UHT
Moisture (g/100 g)	88.22±0.28 <sup>a</sup>	88.73±0.20 <sup>b</sup>	88.65±0.24 <sup>b</sup>
Protein (g/100 g)	3.20±0.05	3.19±0.05	3.20±0.05
Total fat (g/100 g)	3.58±0.27 <sup>a</sup>	3.07±0.22 <sup>b</sup>	3.15±0.22 <sup>b</sup>
Lactose (g/100 g)	4.28±0.07	4.25±0.06	4.27±0.09
Total solids (g/100 g)	12.06±0.25 <sup>a</sup>	11.54±0.20 <sup>b</sup>	11.56±0.21 <sup>b</sup>
Free-fat dry extract (g/100 g)	8.47±0.09	8.47±0.06	8.41±0.08
Urea (mg dL <sup>-1</sup> )	17.37±4.19 <sup>a</sup>	21.57±3.78 <sup>b</sup>	25.57±3.81 <sup>c</sup>
Calcium (g/100 g)	0.11±0.02	0.11±0.02	0.10±0.01
Phosphorus (g/100 g)	0.09±0.02	0.10±0.01	0.10±0.02
<b>Physicochemical composition</b>			
Acidity (% lactic acid)	0.22±0.08 <sup>a</sup>	0.15±0.02 <sup>b</sup>	0.15±0.01 <sup>b</sup>
pH	6.47±0.41 <sup>a</sup>	6.77±0.12 <sup>b</sup>	6.77±0.10 <sup>b</sup>
Density (g mL <sup>-1</sup> )	1.02±0.00	1.02±0.00	1.02±0.00
<b>Microbiological parameters</b>			
Somatic cell count (1000/mL)	619.79±144.78 <sup>a</sup>	365.33±92.95 <sup>b</sup>	59.08±21.80 <sup>c</sup>
Total bacterial count (1000 UFC mL <sup>-1</sup> )	6470.21±1903.57 <sup>a</sup>	1047.58±413.81 <sup>b</sup>	1031.83±739.86 <sup>b</sup>
n	12	12	12

On each line, the values with different letters are significantly different ( $p < 0.05$ ). Values are mean value±SD, n: No. of samples, UHT: Ultra high temperature

According to Table 1, the free-fat dry extract showed a reduction between raw and UHT milks. Martins *et al.* (2008) analyzed the physicochemical characteristics of UHT milk during its industrialization and observed similar results in your work.

Pasteurization and then the commercial sterilization increased the pH of milk, with consequent decrease in acidity. This increased pH can be explained by lower whey protein associating with the micelles. According to Anema and Li (2003), at pH 6.7 only about 30% of the denatured whey proteins are associated with the casein micelle surface.

Somatic cell and total bacterial counts are strongly influenced by the health of the animals and the hygiene conditions for milking. As expected, heat treatment aimed to reduce the number of pathogenic bacteria and resistant pathogenic spores and increased shelf life. In this study, we observed that the heat treatment decreased the somatic cell and total bacterial count of the milks.

**Fatty acid profile:** The effect of the treatments on the fatty acid profile of milk fat is present in Table 2.

Raw, pasteurized and UHT milks had very similar fatty acid profiles. It is suggested that the treatments during the pasteurization and commercial sterilization processes in milk little affects fatty acid profile. De Souza *et al.* (2003) also evaluated the composition and profile of raw and pasteurized milk fatty acids in mini dairies and found no significant difference ( $p > 0.05$ ) for the influence of pasteurization in raw milk.

According to Table 2, the predominant fatty acids were palmitic acid (16:0), oleic acid (18:1n-9), stearic acid (18:0) and myristic acid (14:0). Similar results were obtained in Brazilian dairy products including whole milk, described by Nunes and Torres (2010). Oleic acid was the main monounsaturated fatty acid in the samples, contributing to 25.3-26.4% of total fatty acids.

Short-chain fatty acids such as butyric acid (4:0), caproic acid (6:0) and caprylic acid (8:0) were affected in pasteurized and UHT milks, but there were no differences between treatments. These results could indicate how pasteurization and commercial sterilization processes can decrease butyric, caproic and caprylic acid concentrations. Herzallah *et al.* (2005), who evaluated the influence of the low temperature pasteurization process on the lipid profile of milk, did not find significant differences in SFA. In this study, the SFA (4:0, 6:0, 8:0 and 20:0) were affected by

Table 2: Fatty acid profiles of total lipids in raw, pasteurized and UHT bovine milk

Fatty acid	Treatments		
	Raw	Pasteurized	UHT
4:0	3.53±0.95 <sup>a</sup>	2.87±0.46 <sup>b</sup>	2.86±0.40 <sup>b</sup>
6:0	2.46±0.39 <sup>a</sup>	2.18±0.26 <sup>b</sup>	2.17±0.26 <sup>b</sup>
8:0	1.46±0.16 <sup>a</sup>	1.33±0.12 <sup>b</sup>	1.34±0.15 <sup>b</sup>
10:0	3.05±0.22	2.89±0.02	2.91±0.29
12:0	3.51±0.19	3.32±0.19	3.38±0.30
13:0	0.08±0.04	0.08±0.03	0.09±0.03
14:0	11.42±0.44	11.26±0.40	11.28±0.50
14:1 n-9	0.98±0.07	0.95±0.05	0.96±0.05
15:0	1.15±0.04	1.16±0.06	1.15±0.06
15:1	0.03±0.08	0.05±0.01	0.05±0.11
16:0	30.23±1.39	29.82±0.14	29.73±0.80
16:1 n-9	1.23±0.43 <sup>a</sup>	1.31±0.34 <sup>a</sup>	1.44±0.08 <sup>b</sup>
17:0	0.43±0.26	0.60±0.06	0.47±0.22
17:1	0.21±0.16	0.18±0.17	0.18±0.16
18:0	12.54±0.84	13.05±0.08	13.08±0.70
18:1 n-9	25.31±1.38 <sup>a</sup>	26.28±1.16 <sup>ab</sup>	26.42±1.27 <sup>b</sup>
18:2 n-6	1.79±0.22	1.74±0.50	1.83±0.20
18:3 n-3	0.55±0.05	0.58±0.07	0.55±0.04
18:3 n-6	0.05±0.07	0.05±0.03	0.04±0.00
20:0	0.13±0.01 <sup>a</sup>	0.15±0.00 <sup>b</sup>	0.14±0.00 <sup>c</sup>
20:1	0.02±0.00	ND	ND
SFA	70.01±1.79 <sup>a</sup>	68.73±1.37 <sup>b</sup>	68.62±1.66 <sup>b</sup>
MUFA	27.79±1.54 <sup>a</sup>	28.79±1.12 <sup>b</sup>	29.06±1.43 <sup>b</sup>
PUFA	2.48±0.32	2.62±0.72	2.46±0.23
n-3	0.55±0.05	0.58±0.07	0.55±0.04
n-6	1.93±0.32	2.04±0.68	1.90±0.23
n-6/n-3	3.51±0.75	3.47±1.09	3.49±0.54
PUFA/SFA	0.04±0.01	0.04±0.01	0.04±0.00
n	12	12	12

On each line, the values with different letters are significantly different ( $p < 0.05$ ). Values are mean value±SD (% total fatty acids). ND: Not detected, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, n: No. of samples, UHT: Ultra high temperature

treatments ( $p < 0.05$ ). Costa *et al.* (2011) reported there was no significant difference between raw and pasteurized milk for all SFA analyzed.

Oleic acid (18:1 n-9) was found in higher levels after UHT treatment than in raw milk. Jenkins (1994) reported that diets based on concentrates are expected to increase the absorption of oleic acid and linoleic acid (18:2 n-6), which are major fatty acids in cereal grains and subsequently transferred into milk. On the contrary, it is well known that fresh pasture contains a high percentage of polyunsaturated fatty acids (PUFA), with  $\alpha$ -linolenic acid being the predominant n-3 fatty acid in fresh grass (Elgersma *et al.*, 2006) and all values present in milk in this study are close to those previously found for milk based on grass feeding the cows (Capuano *et al.*, 2014).

This study found that odd-numbered fatty acids (13:0, 15:0 and 17:0), representing 2% of total fatty acids did not present differences after pasteurization and commercial sterilization processes. Odd-fatty acids in milk and dairy of cows could be reflecting rumen function (e.g., ruminal fermentation pattern, including methane, duodenal flow of microbial protein and acidosis) (Fievez *et al.*, 2012). Endogenous chain elongation in the mammary gland using propionyl-CoA as a precursor (Dodds *et al.*, 1981; Massart-Leen *et al.*, 1983) explains the occurrence of C5:0, C7:0, C9:0 and C11:0 in milk and adds to odd-chain fatty acids transferred from the duodenum (C13:0, C15:0 and C17:0).

According to Table 2, lauric acid (12:0) did not present differences after pasteurization and UHT processing in this study. Lauric acid may have antiviral and antibacterial functions and might act as an anti caries and anti-plaque agent (Claeys *et al.*, 2014).

Regarding partial sums of fatty acids (Table 2), the UHT milk affected significantly the SFA and monounsaturated fatty acids (MUFA). The UHT milk, compared with the raw, had higher relative proportions of MUFA ( $p < 0.05$ ), but lower percentages ( $p < 0.05$ ) of SFA. The PUFA, n-3 PUFA and n-6 PUFA did not present differences between treatments. As fatness increased, increments in the content of SFA were larger than those of PUFA (German and Dillard, 2006). Raw milk had higher amounts of total fat and consequently higher SFA levels.

There was no observed difference between treatments ( $p > 0.05$ ) for the PUFA/SFA and n-6/n-3 ratio. According to current nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45 and, for the PUFA, the n-6/n-3 ratio should not exceed 4.0 (British Department of Health, 1994). This last index has been the subject of some debate. Stanley *et al.* (2007) have proposed that it is more important to evaluate the total amounts of dietary PUFA than their respective ratio. Moreover, Brennan *et al.* (2003) proposed that the status of n-3 PUFA could be improved by increasing dietary intake of n-3 PUFA or by reducing the intake of n-6 PUFA and that combining both strategies would be most effective. In the present study, the PUFA/SFA and n-6/n-3 ratios were within the recommended guidelines for the human diet, despite the PUFA/SFA being much below 0.45. The PUFA/SFA reflected the fact that SFA and PUFA are more abundant in the triacylglycerol and phospholipid fractions (Dewhurst *et al.*, 2006). This is also consistent with the reports of Pereira (2014); the fat fraction of milk provides 98% triacylglycerol and approximately 1% phospholipids.

The results of this study indicate that pasteurization and UHT processing does not substantially affect the fatty acid profile of milk. Our results agree with those published by Claeys *et al.* (2014). These authors suggested that thermal degradation of milk lipids is generally not observed, because the temperature required for non-oxidative decomposition of fatty acids ( $> 200^\circ\text{C}$ ) is well outside the range in which milk products are heated, similar to what occurred in this research when raw milk was submitted to ultra-high temperature ( $140^\circ\text{C}$  for 3 sec). It can thus be presumed that heating has a minor effect on the nutritional value of milk fat, which explains the small variations found in the fatty acid profiles of the milk analyzed. Moreover, changes observed in the fatty acid content of milk after heating appeared to be less relevant than the well-known fact that ruminant milk lipid composition is affected by genetic (e.g., breed) and environmental factors (e.g., diet and management), as proposed by Caroli *et al.* (2009).

## CONCLUSION

Pasteurized and ultra-high temperature milks kept protein and lactose content similar to raw milk. Pasteurization and ultra-high temperature processes altered the composition of the milk slightly, decreasing total fat and total solids and increasing urea. These processes changed essentiality short-chain fatty acids (4:0, 6:0 and 8:0). High proportions of palmitic acid (18:0), oleic acid (18:1 n-9), stearic acid (18:0) and myristic acid (14:0) were found in all milks analyzed. Pasteurization and ultra-high temperature processes does not substantially change the proximate composition and fatty acids profile in raw bovine milk, regarding its potential nutritive properties and consequent benefits for human health.

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