Advances in Dairy Research
ISSN: 2329-888X

Coelho et al., Adv Dairy Res 2013, 1:2
DOI: 10.4172/2329-888X.1000105

Research Article Open Access

Somatic Cell Counts Affecting the Casein Fractions of Pasteurized Semi-Skimmed Milk during Storage

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Abstract

Mastitis is generally recognized as an important factor that influences the quality of milk and dairy products. Milk from infected cows is characterized by increased Somatic Cell Counts (SCC) associated with changes in the components and properties of raw milk. The aim of this study was to evaluate the effects of SCC in raw milk on the casein fractions of semi-skimmed pasteurized milk during storage. Raw milk were categorized in SCC groups of low (<1×10^5 cells·mL^{-1}), intermediate (≈4×10^5 cells·mL^{-1}) and high cells (>75×10^4 cells·mL^{-1}). Four replicates of semi-skimmed (1.3-1.4%) pasteurized milk within each SCC category were analyzed for composition, pH, titratable acidity and casein fractions by high performance liquid chromatography after 1, 7, 14, 21 and 28 days of storage. No effect of SCC was observed on the physical-chemical parameters and concentrations of α_s-casein, β-casein and κ-casein in pasteurized milk during storage at 5ºC. However, chromatograms of milk samples containing high SCC (>75×10^4 cells·mL^{-1}) showed increased breakdown products of α_{s1}-casein starting on the seventh day of storage. Results of this trial indicate that SCC in raw milk should not exceed 4x10^5 cells·mL^{-1}, to prevent the degradation of casein fractions in pasteurized semi-skimmed milk and the occurrence of quality defects during storage.

Keywords: Milk quality; Mastitis; SCC; Proteolysis; Casein fractions

Introduction

Mammary gland inflammation or mastitis is an extremely complex disease that results in the reduction of milk synthesized and in changes in the levels of specific components of milk, reducing its quality [1]. Mastitis determines considerable losses to dairy products industry, reducing the levels of calcium, lactose, casein and fat, besides increasing the levels of sodium, chloride and serum proteins [2]. The alterations in milk composition occur due to the inflammation that leads to a decrease in the synthesis capacity of mammary gland and in an increase in vascular permeability [3]. The main effects of high SCC in dairy products include lower yield in cheeses [4], higher lipolysis in whole milk and yoghurt [5], and higher proteolysis in pasteurized milk [6].

The proteolytic activity associated with SCC in raw milk has been extensively studied [7,8]. Approximately 80% of milk total nitrogen from bovine milk is constituted of casein. Bovine casein can be classified into four types of protein with different properties: α_{s1}-, α_{s2}-, β- and κ-casein, which accounts for 38%, 10%, 34% and 15% of total casein, respectively [9]. In raw milk, high SCC is associated with hydrolysis of α_s- and β-casein by proteases, resulting in a lower concentration of these fractions [10]. SCC-related proteases are heat-resistant [11], so increasing somatic cells (SC) levels in raw milk were associated to higher degradation of β-casein and α_s-casein in Ultra-High Temperature (UHT) milk during 4-month storage [5]. However, there is little information available on the influence of SCC on the casein fractions of pasteurized milk, especially low-fat products. Therefore, the aim of the present work was to determine the effects of SCC in milk and changes in the concentrations of casein fractions of semi-skimmed pasteurized milk during storage.

Materials and Methods

Raw milk collection and somatic cell evaluation

A sample of milk used in the present study was collected from a dairy herd located in Pirassununga-SP, Brazil. Fifteen Holstein cows were selected among animals that started the lactation period three-five months before the beginning of the experiment, and that were not submitted to antibiotic treatment seven days before milk collection. Milk from individual cows was submitted to SCC [12], to allow its distribution into three groups based on individual SCC status: cows with low SCC (<1×10^5 cells·mL^{-1}), intermediate (≈4x10^5 cells·mL^{-1}) and high SCC (>75×10^4 cells·mL^{-1}). Groups of five cows of each SCC category were milked separately two days after SCC measurement. Milk collected from cows of the same group was bulked until a minimum amount of 40 L was reached for processing. Milk was then cooled and stored in a refrigerator (4˚C) until utilization, 2-4 hours after collection. Duplicate samples of bulked raw milk of each SCC category were analyzed for composition and confirmation of SCC [12]. Raw milk samples were also analyzed for mesophilic bacteria [13] for confirmation of counts less than 1×10^4 colony forming units per mL (UFC·mL^{-1}).

Standardization and pasteurization of semi-skimmed milk

The fresh whole milk was subjected to heating in a water bath at 50ºC and transferred to creamer centrifuge (Mec Milk, Pompeia, Brazil) to partially removing cream and to standardize fat content of milk at 1.3% (w/w). The resulting cream was discharged, and the standardized semi-skimmed milk obtained was immediately submitted to pasteurization at 75˚C for 15 s in a heat exchanger plate

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Received April 29, 2013; **Accepted** July 11, 2013; **Published** July 13, 2013

Citation: Coelho VRP, Rosim RE, Petrus RR, Cruz AG, Corassin CH, et al. (2013) Somatic Cell Counts Affecting the Casein Fractions of Pasteurized Semi-Skimmed Milk during Storage. Adv Dairy Res 1: 105. doi: 10.4172/2329-888X.1000105

system (Sumá Ltd, Campinas, Brazil). Pasteurized milk was aseptically filled in white high density polyethylene (HDPE) bottles previously decontaminated by dipping in a 0.5% (v/v) ClO_2 solution during 30 min at 25˚C. Mesophile and psychrotrophic bacteria was analyzed on pasteurized milk [13] for confirmation of low counts (less than 1×10^2 UFC·mL^{-1}). The entire procedure, since the ski Final extracts were stored at 7˚C until the mming to the bottling of the final product was repeated 4 times for all milk containing different SCC, in order to obtain 4 batches of semi-skimmed pasteurized milk in each SCC category. Samples of pasteurized milk produced in each batch were properly identified and stored in a cold chamber at 5˚C for 28 days. The pasteurized semi-skimmed milk was analyzed on days 1, 7, 14, 21 and 28 after production.

Physical-chemical analysis

Duplicate samples of semi-skimmed milk were analyzed for determination of fat, total protein, total solids and non-fat total solids, pH and titratable acidity [12].

Casein fractions analysis

The concentration of milk casein fractions was determined in semi-skimmed pasteurized milk samples by reversed-phase high performance liquid chromatography (HPLC) [14]. Briefly, aliquots containing 500 µL of milk were frozen at -20˚C. A solution with 0.1 M BisTris buffer (pH 6.8), 6 M guanidine hydrochloride, 5.37 mM sodium citrate and 19.5 mM dithiothreitol (pH 7.0) was added directly to frozen aliquots in a 1/1 ratio (v/v) at room temperature. After thawing, each sample was shaken for 10 s, incubated for 1 h at room temperature, and centrifuged for 5 min at 16000 g in a microcentrifuge. The fat layer was then removed with a spatula. The remaining solubilised sample was diluted 1/3 (v/v) with a solution containing 4.5 M guanidine hydrochloride and solvent A, which consisted of acetonitrile, water, and trifluoroacetic acid in a ratio 100/900/1 (v/v/v; pH 2). The concentration of milk protein in the final diluted solution was approximately 4mg·mL^{-1}. Final extracts were stored at 7˚C until the chromatographic analysis, performed at a maximum of five days after preparation.

Separation and identification of proteins were performed in a HPLC system (Shimadzu, Japan), equipped with an UV detector at 220 nm and a Jupiter C18 column (4 µm, 4.6×150 mm) (Phenomenex, Torrance, USA). Chromatographic run was performed at room temperature using the following mobile phases: solvent A (acetonitrile: water: trifluoroacetic acid, 100:900:1) and solvent B (acetonitrile:water:trifluoroacetic acid, 900:100:1). The gradient program started with 25% solvent B, gradually increasing the proportion of solvent B immediately after sample injection [34% (4 min), 48% (11 min), 50% (13 min), 10% (17 min)] and returning to initial conditions after 2 min. The flow rate was adjusted at 1.0 mg·mL^{-1}. The quantification of casein fractions (α_{s1}, α_{s2}, β and κ) in samples was performed by measuring peak areas of samples, and plotting against the calibration curves of each casein fraction. Purified αs, β and κ casein standards (Sigma, USA) were diluted in distilled water, and aliquots of each solution were frozen at -20˚C. Casein standards were prepared in the same way as described for milk samples, at the following concentrations: αs-casein: 0.5, 1.0, 2.0 and 4.0 mg·mL^{-1}; β-casein: 0.375, 0.75, 1.50 e 3.0 mg·mL^{-1}; and κ-casein: 0.187, 0.375, 0.75 and 1.50 mg·mL^{-1}. The ratio of α_{s1} to α_{s2} casein was assumed to be 4/1 (w/w).

Statistical analysis

Results from physico-chemical analyses and casein fractions of pasteurized semi-skimmed milk within each SCC category during storage were submitted to ANOVA using the SAS® General Linear Model [15]. Variable means showing significant differences between means were determined by the Turkey's Post Hoc test, probability values inferior to 0.05 were regarded as significant.

Results and Discussion

The mean SCC from batches of milk and pasteurized milk cream used in the manufacture of semi-skimmed milk were 39,000, 349, 500 and 1,297,500 SC.mL^{-1} for th mL^{-1}), intermediate e groups of low (<1×10^5 cells·mL^{-1}), intermediate (≈4×10^5 cells·mL^{-1}) and high SCC (>75×10^4 cells·mL^{-1}), respectively. The results of titratable acidity, pH and milk composition (fat, protein, total solids and not-fat milk solids)

Parameters	SCC categories[b]		
	Low	Intermediate	High
Titratable acidity (g lactic acid·mL^{-1})	1.38 ± 0.03	1.51 ± 0.04	1.54 ± 0.06
pH	6.78 ± 0.06	6.75 ± 0.04	6.77 ± 0.08
Fat (g·100 g^{-1})	1.31 ± 0.03	1.40 ± 0.04	1.43 ± 0.03
Total proteins (g·100 g^{-1})	3.26 ± 0.03	3.65 ± 0.02	3.57 ± 0.23
Total solids (g·100 g^{-1})	9.36 ± 0.10	10.40 ± 0.12	10.62 ± 0.11
Non-fat solids (g·100 g^{-1})	8.19 ± 0.15	9.15 ± 0.14	9.83 ± 0.64

[a]Results are reported as means ± standard deviation, for four batches analyzed on day 1 of storage.
[b]Mean values of SCC in each category: Low: 39,000 cells·mL^{-1}; Intermediate: 349,500 cells·mL^{-1}; High: 1,297,500 cells·mL^{-1}.

Table 1: Composition and physico-chemical results of pasteurized semi-skimmed milka.

Casein fractions (mg·mL^{-1})	SCC categories[b]			Standard Error (SE)
	Low	Intermediate	High	
α_{s1}-casein	9.95	9.34	9.47	0.17
α_{s2}-casein	2.24	2.64	2.80	0.56
β-casein	10.97	11.73	11.78	0.84
κ-casein	3.72	4.15	3.23	0.15

[a]Results are reported as means of four batches analyzed on days 1, 7, 14, 21 and 28 of storage (*n* = 20 for each SCC category).
[b]Mean values of SCC in each category: Low: 39,000 cells·mL^{-1}; Intermediate: 349,500 cells•mL-1; High: 1,297,500 cells•mL^{-1}.

Table 2: Concentrations of casein fractions in semi-skimmed pasteurized milk during storage[a].

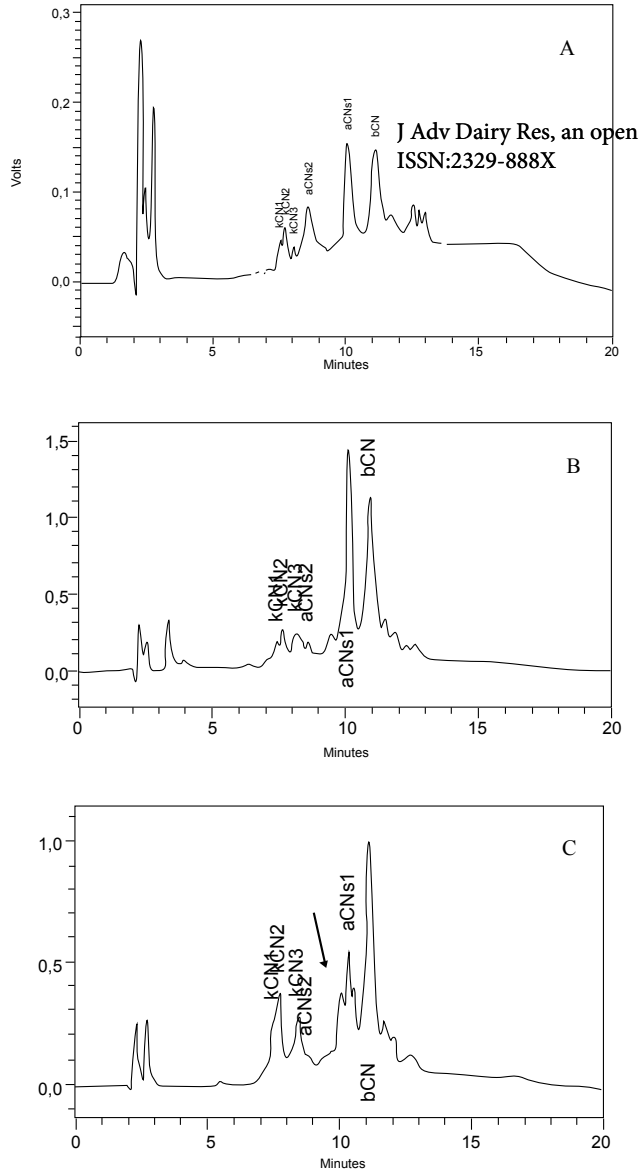

Figure 1: Chromatograms of casein standards: α_{s1}-casein (aCNs1, 3,0 mg·mL^{-1}), α_{s2}-casein (aCNs2, 1,0 mg·mL^{-1}), ß-casein (bCN, mg·mL^{-1}) and κ-casein (kCN, 1,5 mg·mL^{-1}) (A), casein fractions obtained in a semi-skimmed pasteurized milk sample containing high SCC (9x10^5 cells·mL^{-1}) on day 1 of storage (B); and casein fractions obtained in the same semi-skimmed milk as described in B, after 7 days of storage (C). The arrow indicates peaks of breakdown products of α_{s1}-casein.

are presented in Table 1. There were no significant differences (P>0.05) among samples in any of the parameters evaluated. During 28 days of storage at 5˚C, the pH and titratable acidity mean values ranged from 6.66 to 6.87 and 1.35 to 1.60 g lactic acid·mL^{-1}, respectively. These data are similar to those described previously for pasteurized milk used for manufacture of UHT milk [5]. The fat content ranged from 1.26 to 1.46 g·100g^{-1}, which is within the range recommended by the Brazilian legislation [16]. The protein content ranged from 3.11 to 3.70 g·100g^{-1} and the values were also in accordance with regulations.

Table 2 shows the concentrations of casein fractions in the pasteurized semi-skimmed milk from groups of low, intermediate and high SCC during storage. There were no effect SCC and storage of the product for 28 days under refrigeration at 5˚C on the concentrations of casein fractions of semi-skimmed milk. Our findings are in contrast with a previous researcher that demonstrated lower concentrations of β-casein in UHT milk were associated with high SCC during 120 days of storage [5], which is much longer than the period used in the present study. The SCC may be involved in the proteolytic activity, which predisposes milk to the action of proteases with subsequent enzymatic hydrolysis of α_s and β-casein [8]. It remains to be determined if the concentrations of caseins in semi-skimmed milk would modify during storage for longer periods than used in this investigation.

The absence of relationship between SCC and the concentrations of casein fractions of pasteurized semi-skimmed milk may be associated to other factors dealing with the enzymatic activity of milk and dairy products [2]. One reason may be related to the fact that this study was conducted using semi-skimmed milk even knowing that the highest concentration of hydrolytic enzymes is present in milk fat (whole milk). Another possible explanation could be related to the intrinsic characteristics of milk used in our experiment, which was from a single flock of small size. Additionally, the milk used in the current study was processed immediately after milking, which also reduced the time of action of enzymes on the protein.

The effects of mastitis on milk protein may be quantitative, but also qualitative since the absolute values of caseins not always undergo significant changes [7]. In our study, the chromatograms obtained for semi-skimmed milk samples containing high SCC presented increased fragmentation peaks for αs1-casein during storage, starting on the seventh day, as shown in Figure 1. This fact shows, qualitatively, the breaking of these casein fractions, although their respective concentrations were not altered quantitatively (P>0.05). The most important consequence of the changes in casein fractions of pasteurized milk during storage is the enzymatic hydrolysis of casein associated with SCC. Although no sensory evaluation was conducted in the present study, these changes may lead to important sensory modifications in milk, mainly the bitter taste due to peptides release, which were shown to be originated mostly from α_{s1}- and β-casein [17].

Conclusion

Results of this trial indicate that although SCC in raw milk had no effect in the percentages of casein fractions of low-fat pasteurized milk, increased breakdown products of α_{s1}-casein occurred in the milk during 28 days of storage. Therefore, it is recommended that the raw milk used for pasteurized milk production should contain somatic cells at levels as low as possible, to prevent the degradation of casein fractions and the occurrence of its related quality defects during storage.

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