Microbial and sensory changes throughout the ripening of prato cheese made from milk with different levels of somatic cells

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Microbial and Sensory Changes Throughout the Ripening of Prato Cheese Made from Milk with Different Levels of Somatic Cells

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ABSTRACT

The objective of this research was to evaluate the effects of 2 levels of raw milk somatic cell count (SCC) on the composition of Prato cheese and on the microbiological and sensory changes of Prato cheese throughout ripening. Two groups of dairy cows were selected to obtain low-SCC (<200,000 cells/mL) and high-SCC (>700,000 cells/mL) milks, which were used to manufacture 2 vats of cheese. The pasteurized milk was evaluated according to the pH, total solids, fat, total protein, lactose, standard plate count, coliforms at 45°C, and Salmonella spp. The cheese composition was evaluated 2 d after manufacture. Lactic acid bacteria, psychrotrophic bacteria, and yeast and mold counts were carried out after 3, 9, 16, 32, and 51 d of storage. Salmonella spp., Listeria monocytogenes, and coagulase-positive Staphylococcus counts were carried out after 3, 32, and 51 d of storage. A 2 × 5 factorial design with 4 replications was performed. Sensory evaluation of the cheeses from low- and high-SCC milks was carried out for overall acceptance by using a 9-point hedonic scale after 8, 22, 35, 50, and 63 d of storage. The somatic cell levels used did not affect the total protein and salt:moisture contents of the cheeses. The pH and moisture content were higher and the clotting time was longer for cheeses from high-SCC milk. Both cheeses presented the absence of Salmonella spp. and L. monocytogenes, and the coagulase-positive Staphylococcus count was below 1 × 10^2 cfu/g throughout the storage time. The lactic acid bacteria count decreased significantly during the storage time for the cheeses from both low- and high-SCC milks, but at a faster rate for the cheese from high-SCC milk. Cheeses from high-SCC milk presented lower psychrotrophic bacteria counts and higher yeast and mold counts than cheeses from low-SCC milk. Cheeses from low-SCC milk showed better overall acceptance by the consumers. The lower overall acceptance of the cheeses from high-SCC milk may be associated with texture and flavor defects, probably caused by the higher proteolysis of these cheeses.

Key words: somatic cell count, lactic acid bacteria, Prato cheese, quality

INTRODUCTION

Mastitis is an inflammatory reaction of the mammary gland caused by pathogenic bacteria. Milk from infected cows is characterized by increased SCC and changes in mammary tissue, causing physical, chemical, and microbiological changes in the milk and dairy products (Auldist and Hubble, 1998).

In infected animals, the predominant somatic cells are leukocytes, such as macrophages and neutrophils. These cells travel from the blood to the mammary gland in response to a variety of inflammatory mediators to phagocytose and kill bacterial pathogens. Macrophages appear in lower numbers than neutrophils during mastitis, but also have the function of phagocytosing bacteria and secreting substances that facilitate the migration and bactericidal activities of neutrophils (Sordillo and Streicher, 2002). The most important alterations caused by an increased SCC in milk include variations in the fat and protein contents, increased concentrations of plasmin and other enzymes, decreased lactose and TS concentrations, and increases in the pH value (Auldist and Hubble, 1998).

Higher plasmin activity in mastitic milk may be attributed to the plasminogen activators or proteolytic enzymes that occur in somatic cells. Plasmin is able to cleave β-CN and this breakdown occurs in the milk both within the udder and during storage (Saeman et al., 1988). Srinivasan and Lucey (2002) demonstrated that the plasmin hydrolysis of CN negatively affected the rheological properties of rennet-induced milk gels. This supports the hypothesis that elevated plasmin activity in mastitic milk could alter the rennet coagulation properties of milk and have negative effects on the cheese yield and texture.
Lipoprotein lipase, a glycoprotein, is relatively unstable to heat and is normally associated with the CN micelle. The level of lipoprotein lipase in milk increases as a result of mastitis and may cause fat stability problems as well as quality defects in the raw milk and dairy products (Auldist and Hubble, 1998).

Another consequence of increased SCC in milk is higher concentrations of antimicrobial substances originating from blood or secreted by somatic cells. These substances may influence the growth and metabolism of the starter bacteria used in cheese production, changing the milk coagulation and sensory characteristics of the dairy products (Okello-Uma and Marshall, 1986; Le Roux et al., 2003). These substances include Ig, lactoferrin, and BSA. During mastitis, the Ig concentration increases, enhancing phagocytosis by neutrophils and macrophages (Sordillo and Streicher, 2002). Cheese quality is influenced by the physicochemical and microbiological characteristics of the milk and by the production and ripening steps of each variety. Increases in milk SCC cause decreased curd firmness and cheese yield (Auldist and Hubble, 1998; Le Roux et al., 2003), increased cheese moisture content (Arcuri et al., 1990; Barbano et al., 1991; Mazal et al., 2007), and increased clotting time (O’Brien et al., 2001; Mazal et al., 2007).

The ripening process involves microbiological and biochemical changes to the curd, resulting in the flavor and texture characteristics of the particular variety and, in most cases, in its appearance, such as the formation of eyes and growth of molds of the individual varieties (McSweeney, 2004). Modifications of the sensory cheese characteristics caused by upstream factors are dependent on different mechanisms: protein and fat modifications; the impact of endogenous blood or milk enzymes transferred into the milk and retained in the cheese, which modify proteolysis, lipolysis, or both during ripening; and microbial ecosystem modifications (Coulon et al., 2004).

Prato is a semihard (moisture content 42 to 44%), low-scall cheese manufactured by the enzymatic coagulation of milk and ripened for 25 d (Ministério da Agricultura do Brasil, 1997). Previous studies showed that Prato cheese from high-SCC milk (>600,000 cells/mL) presented significantly greater proteolysis and higher moisture contents than Prato cheese from low-SCC milk (<200,000 cells/mL), which could negatively affect the sensory quality of this traditional Brazilian cheese (Mazal et al., 2007). The objective of this research was to evaluate the effects of 2 levels of raw milk SCC on the composition of Prato cheese and on the microbiological and sensory changes in Prato cheese throughout ripening.

MATERIALS AND METHODS

Cow Selection and Milk Collection

The milk used in this experiment was collected from Holstein cows from the University of São Paulo, Pirassununga Campus, State of São Paulo. Forty cows in an intermediate lactation stage and with no treatment with antibiotics in the last 14 d were selected. For selection, the cows were milked individually and the milk samples (50 mL) were collected at the morning milking, preserved with 8 mg of bronopol (2-bromo-2-nitropropano-1,3-diol, D&F Control Systems, Dublin, CA), and shipped at room temperature for laboratory analysis on the same day.

The milk SCC was determined by flow cytometry with a Bentley Somacount 500 instrument (Bentley Instruments Inc., Chasca, MN), and the milk composition (total protein, fat, and lactose) was analyzed by infrared spectrophotometry with a Bentley 2000 spectrophotometer (Bentley Instruments Inc.). On the basis of the individual SCC, milk yield, and protein and fat contents, 2 groups of 5 animals were separated and milked to obtain low-SCC (<200,000 cells/mL) and high-SCC milk (>700,000 cells/mL). All the selected cows were on a 2× milking regimen, averaging 160 ± 102 DIM, 2.3 ± 1.2 lactations (parity), and 23.2 kg/d of milk yield.

After milking, the raw milk was immediately cooled to 4°C and transported to the Food Technology Department Dairy Plant at the University of Campinas. A sample of milk from each batch was taken, preserved with bronopol at room temperature, and tested for SCC (AOAC, 2000; methods 17.13.01 and 978.26). Low- and high-SCC milks were heat treated at 68°C for 2 min in a batch pasteurizer (100-L capacity), cooled to 4°C, placed into sanitized cans, and stored overnight in a cooler (4°C) until further processing.

Prato Cheese Manufacture

The next day, 2 cheese batches were manufactured in 150-L vats with a heating-cooling jacket, stirrers, and speed control: 1) Prato cheese from low-SCC milk, and 2) Prato cheese from high-SCC milk. The same procedure was applied to each vat. The milk was heated to 35°C, followed by the addition of calcium chloride (0.025%), annatto color (0.008%), starter culture (1%) consisting of Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris (Wisby-Vishayac LC-MIX F0 2 01, Danisco Brazil Ltda., Cotia, Brazil), and rennet (calf rennet powder concentrate, Quimosina 90%, Rhodia Brazil, Paulinia, Brazil). The amount of rennet was the same for both cheeses. The clotting time was monitored and the curd was cut when it was firm. The firmness of the curd was evaluated by inserting a sanitized spatula
into the coagulum at a 45° angle, gently lifting the spatula straight up, and observing the curd as it split open. After clotting, the coagulum was cut into approximately 0.5-cm cubes and the curd plus whey were gently stirred for 20 min, maintaining the temperature at 35°C but with a gradual increase in the agitation speed. For the cooking process, 30% of the whey was removed and replaced by progressively adding hot water (80°C) to increase the temperature by 1°C every 3 min, to a temperature of 42°C. After stirring for 30 min at 42°C, all the whey was drained off, and the curd placed in 0.5-kg molds and pressed for 3 h. Finally, the cheeses were immersed for 12 h in a brine containing 20% salt, dried for 24 h at 10°C, vacuum-packaged, and stored for 51 d at 12°C.

**Sampling and Analyses**

**Milk.** After heat treatment, the low- and high-SCC milks were evaluated for pH (Digimed model DM-20, Digicron Analítica Ltda, São Paulo, Brazil) by introducing the electrode directly into the sample (25°C); TS (AOAC, 1995; methods 33.2.09/A and 925.23); fat by the Gerber method (British Standards Institution, 1989); total nitrogen (AOAC, 1995; methods 33.2.11; 991.20); and lactose by the volumetric method through determination of reducing sugars by Fehling’s solution with methylene blue indicator (Ministério da Agricultura do Brasil, 1981). Total protein was calculated by multiplying the total nitrogen by 6.38. The pasteurized milks were evaluated for the standard plate count, total and fecal coliforms, and *Salmonella* spp. according to standard methods (American Public Health Association, 2001). All the microbiological culture media used in this study were from Difco Laboratories. The standard plate count was carried out by using plate count agar, followed by incubation at 35°C for 48 h. Total and fecal coliforms were enumerated by using the most probable number (MPN) technique. Tubes were incubated at 35°C for 48 h in lauryl sulfate trypye brothe. Positive-presumptive coliform results (gas production) were confirmed in brilliant green bile (BGB) broth incubated at 35°C for 48 h. Gas production in BGB was considered as confirmation of the presence of coliforms. For fecal coliforms, tubes exhibiting gas in the BGB broth were inoculated into *Escherichia coli* broth by means of a standard loop and incubated at 45.5°C for 24 h in a water bath. Tubes showing gas production were an indication of a positive result and were reported as MPN of fecal coliforms per milliliter of milk.

*The Salmonella* spp. count was determined by pre-enrichment in lactose broth with incubation at 35°C for 18 to 24 h, followed by enrichment in tetrahionate broth and Rappaport-Vassiliadis medium, incubated at 35°C for 18 to 24 h and 42°C for 18 to 24 h, respectively. Loopfuls were transferred onto bismuth sulfite agar, Hektoen enteric agar, and xylose lysine desoxycholate citrate agar. Suspect colonies were identified on triple-sugar iron and lysine iron agars.

**Cheese.** Two days after manufacture, the cheeses were evaluated for pH (Digimed model DM-20, Digicron Analítica Ltda) by introducing the electrode directly into the ground samples (25°C); moisture (AOAC, 1995; methods 33.2.09/A and 925.23); fat by the Gerber method (British Standards Institution, 1989); total nitrogen (AOAC, 1995; methods 33.2.11 and 991.20); and salt by the Volhard method (Richardson, 1985).

For the microbiological characterization, all the cheese samples were mechanically blended in a Stomacher (Stomacher 400, Seward, West Sussex, UK) and diluted. Microbial counts were determined after 3, 9, 16, 32, and 51 d of storage according to standard methods (American Public Health Association, 2001). Lactic acid bacteria were determined on de Man, Rogosa, and Sharpe agar incubated at 32°C for 2 d; psychrotrophic bacteria on plate count agar after incubation at 7°C for 10 d; and yeasts and molds on potato dextrose agar adjusted to pH 3.5 and incubated at 25°C for 5 d.

*Salmonella* spp., coagulase-positive *Staphylococcus*, and *Listeria monocytogenes* counts were determined after 3, 32, and 51 d of storage. *Salmonella* spp. were evaluated as described for the milk samples. Coagulase-positive *Staphylococcus* was determined on Baird-Parker agar incubated at 35°C for 48 h, and the colonies were tested for Gram staining and catalase and coagulase production. *Listeria monocytogenes* was enumerated according to Pagotto et al. (2001) as follows: enrichment in *Listeria* enrichment broth with incubation at 30°C, and after 24 and 48 h, inoculation into modified Fraser broth, followed by incubation at 35°C for 24 to 48 h. Loopfuls were then streaked onto LiCl-phenylethanol-moxalactam and Oxford agar. For identification, the colonies were tested for Gram staining, catalase detection, β-hemolysis on horse blood agar, motility after growth at 25°C on sulfide indole motility agar, and acid production from rhamnose, mannitol, and xylose. All the materials used were previously sterilized, and the microbiological procedures were carried out in a laminar flow chamber.

**Experimental Design and Statistical Analyses**

The effects of the SCC levels on the pasteurized milk and cheese composition were evaluated by ANOVA. For evaluation of the microbiological and physicochemical characteristics during storage, a 2 × 5 factorial design with 4 replications was performed. The effect of SCC at 2 levels (low and high), storage time at 5 levels (3,
Table 1. Effect of low and high SCC on the physicochemical and microbiological counts of pasteurized milk

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low SCC2</th>
<th>High SCC3</th>
<th>P-value4</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (25°C)</td>
<td>6.76</td>
<td>6.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TS, %</td>
<td>11.8</td>
<td>11.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.2</td>
<td>3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Total protein (total N × 6.38), %</td>
<td>3.13</td>
<td>3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.69</td>
<td>4.53</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Standard plate count, log cfu/mL</td>
<td>2.6</td>
<td>2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Coliforms (45°C), most probable number/mL</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Salmonella spp., cfu/25 mL</td>
<td>Absent</td>
<td>Absent</td>
<td>NS</td>
</tr>
</tbody>
</table>

1n = 4.
2Mean SCC = 86,000 cells/mL.
3Mean SCC = 785,000 cells/mL.
4P < 0.05.

9, 16, 32, and 51 d of storage), and their interaction on the evaluation of these characteristics was analyzed by ANOVA and Tukey's test. Significance was considered at P < 0.05. The statistical analyses were performed by using Statistica for Windows (version 5.5, 2000; StatSoft Inc., Tulsa, OK). A linear regression analysis was used to evaluate the effect of ripening time on the lactic acid bacteria count of the cheeses from high- and low-SCC milk.

Overall Acceptance of Prato Cheese

For the evaluation of overall acceptance, a special independent process was carried out. Fifty cheese consumers were recruited for each day of analysis. The samples were presented individually for evaluation in individual booths in a sensory panel room and evaluated after 8, 22, 35, 50, and 63 d of storage. Overall acceptance was evaluated by using a 9-point hedonic scale (Meilgaard et al., 1999), in which 1 corresponded to “Didn't like extremely,” 5 corresponded to “Neither liked nor disliked,” and 9 corresponded to “Liked extremely.”

The statistical analyses were performed by using Statistica for Windows (version 5.5, 2000; StatSoft Inc.). The effect of milk SCC (2 levels), storage time (5 levels), and their interactions on the overall acceptance of the cheeses was analyzed by ANOVA and Tukey’s test. Significance was considered at P < 0.05.

RESULTS AND DISCUSSION

Effect of Somatic Cell Levels on Milk Composition

Low-SCC raw milk presented, on average, 86,000 cells/mL (62,000; 67,000; 99,000; and 114,860 cells/mL). This result reflects milk coming from healthy cows, with the somatic cells composed mainly of cells from the natural scaling of the mammary gland epithelium (Auldist and Hubble, 1998). High-SCC raw milk presented, on average, 785,000 cells/mL (559,000; 1,041,500; 682,500; and 857,950 cells/mL). Although considered as high SCC for this study, these values complied with the limit defined by the Brazilian Legislation (1,000,000 cells/mL).

There was a significant impact of SCC on the pH of the pasteurized milks. High-SCC milk presented higher

Table 2. Effect of the SCC on clotting time and cheese composition

<table>
<thead>
<tr>
<th>Item</th>
<th>Low SCC2</th>
<th>High SCC3</th>
<th>P-value4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting time, min</td>
<td>35</td>
<td>46</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>pH (25°C)</td>
<td>5.17</td>
<td>5.24</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>44.3</td>
<td>47.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fat, %</td>
<td>29.5</td>
<td>26.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total protein (total N × 6.38), %</td>
<td>22.7</td>
<td>22.4</td>
<td>NS</td>
</tr>
<tr>
<td>Salt:moisture, %</td>
<td>3.5</td>
<td>3.55</td>
<td>NS</td>
</tr>
</tbody>
</table>

1n = 4.
2Mean SCC = 86,000 cells/mL.
3Mean SCC = 785,000 cells/mL.
4P < 0.05.
pH than low-SCC milk (Table 1). This finding is consistent with the results of previous reports (Auldist and Hubble, 1998; Klei et al., 1998). The pH increases in high-SCC milk are because during mastitis, the permeability of the mammary gland membranes increases, which permits a greater influx of blood constituents into the milk (Fox et al., 2000a).

No significant impact of SCC on the fat, TS, and total protein contents was detected under the conditions of this study. O’Brien et al. (2001) also found no significant difference in the fat and total protein contents in low- and high-SCC milks. Conversely, in a previous study (Mazal et al., 2007), working with the same herd, we found a significantly higher total protein content and lower CN content as a percentage of true protein in the high-SCC milk as compared with the low-SCC milk, reflecting the more intense proteolysis usually presented by high-SCC milk.

High-SCC milk had significantly lower lactose contents than low-SCC milk, even though the TS contents were not different. Klei et al. (1998) also observed such a result, and other authors similarly reported lower lactose contents for high-SCC milk (Auldist and Hubble, 1998). No difference in the standard plate count, coliforms, and Salmonella spp. were detected between the low- and high-SCC milks. After pasteurization, milk with low and high SCC presented standard plate counts of $3.89 \times 10^2$ and $3.54 \times 10^2$ cfu/mL, respectively, coliform counts of <0.3 MPN/mL, and the absence of Salmonella spp., indicating an adequate microbiological quality for cheese-making milk.

**Table 3. Effect of the SCC, storage time, and their interactions on lactic acid bacteria, psychrotrophic bacteria, and yeast and mold counts in Prato cheese**

<table>
<thead>
<tr>
<th>Count, log10 cfu/mL</th>
<th>Cheese from low-SCC milk²</th>
<th>Cheese from high-SCC milk³</th>
<th>P-value⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>9.12</td>
<td>0.04</td>
<td>8.71</td>
</tr>
<tr>
<td>Psychrotrophic bacteria</td>
<td>9.08</td>
<td>0.04</td>
<td>8.69</td>
</tr>
<tr>
<td>Yeasts and molds</td>
<td>3.2</td>
<td>0.1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

¹n = 4.
²Mean SCC = 86,000 cells/mL.
³Mean SCC = 785,000 cells/mL.
⁴P < 0.05.
⁵Storage time: 3, 9, 16, 32, and 51 d after manufacture.

**Effect of Somatic Cell Levels on Cheese Composition**

The SCC significantly affected the clotting time, pH, and moisture and fat contents of the cheeses (Table 2). The pH and moisture content were higher and the clotting time was longer for cheeses from high-SCC milk, whereas the fat content was lower in this cheese. No significant impact of SCC on the protein and salt:moisture was observed in this study. Other authors have also observed higher moisture content in cheeses from high-SCC milk such as Prato (Arcuri et al., 1990; Mazal et al., 2007), Cottage (Klei et al., 1998), and Cheddar (Grandisson and Ford, 1986; Politis and Ng-Kwai-Hang, 1988; Marino et al., 2005). The higher moisture content observed in this cheese could be a result of the higher pH of the high-SCC milk. An increase in milk pH delays the action of the rennet, increasing the clotting time and consequently resulting in the formation of a weaker and humid coagulum (Fox et al., 2000b). Higher moisture in the cheeses from high-SCC milk and late-lactation milk has also been associated with higher levels of proteolysis in the milk, as reported by Klei et al. (1998) and Barbano et al. (1991). However, in the present study, milk proteolysis was not evaluated.

**Effect of Somatic Cell Levels and Storage Time on Cheese Microbiological Characteristics**

Both cheeses presented the absence of Salmonella spp. and L. monocytogenes counts, and showed coagulase-positive Staphylococcus counts below $1 \times 10^2$ cfu/g during the storage time. These microbiological characteristics comply with the standards required by the Brazilian Legislation for Prato cheese (Agência Nacional de Vigilância Sanitária do Brasil, 2001).

The cheese lactic acid bacteria count was significantly affected by interactions between the milk SCC and storage time (Table 3). There was no difference between low- and high-SCC milk in the lactic acid bacteria count 3 d after cheese manufacture (Table 4 and Figure 1), indicating that the development of lactic acid bacteria was not affected by the SCC of the milk during cheese making. However, during cheese aging, the lactic acid bacteria decreased at a faster rate in the cheese from
Table 4. Effect of the SCC and storage time on lactic acid bacteria, psychrotrophic bacteria, and yeast and mold counts in Prato cheese

<table>
<thead>
<tr>
<th>Item</th>
<th>Storage time, d</th>
<th>Lactic acid bacteria, log_{10} cfu/g</th>
<th>Psychrotrophic bacteria, log_{10} cfu/g</th>
<th>Yeasts and molds, log_{10} cfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese from low-SCC milk</td>
<td>3</td>
<td>9.19 ± 0.06</td>
<td>9.11 ± 0.01</td>
<td>2.0 ± 0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>9.25 ± 0.07</td>
<td>9.14 ± 0.08</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>9.25 ± 0.07</td>
<td>9.09 ± 0.05</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>9.13 ± 0.07</td>
<td>9.1 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>8.8 ± 0.1</td>
<td>9.0 ± 0.1</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Cheese from high-SCC milk</td>
<td>3</td>
<td>9.14 ± 0.07</td>
<td>9.0 ± 0.1</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>8.92 ± 0.08</td>
<td>8.86 ± 0.08</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>8.9 ± 0.1</td>
<td>8.8 ± 0.1</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>8.6 ± 0.1</td>
<td>8.6 ± 0.2</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>7.9 ± 0.2</td>
<td>8.2 ± 0.2</td>
<td>3.7 ± 0.5</td>
</tr>
</tbody>
</table>

\(^1_n = 4.\)

\(^2\)Mean SCC = 86,000 cells/mL.

\(^3\)Mean SCC = 785,000 cells/mL.

high-SCC milk (Figure 1). Antimicrobial substances secreted by leukocytes or originating from blood in the high-SCC raw milk could not be completely eliminated after pasteurization, as observed by Marino et al. (2005). Thus, antimicrobial substances would still be active in the cheeses from high-SCC milk in this experiment, causing a faster death rate of lactic acid bacteria in this product.

The psychrotrophic bacteria and yeast and mold counts in the cheeses were significantly affected by the milk SCC and storage time (Table 3). The cheeses from low-SCC milk presented higher psychrotrophic bacteria counts and lower yeast and mold counts than cheeses from high-SCC milk. The psychrotrophic bacteria counts decreased significantly during storage, whereas the yeast and mold counts increased (Table 4). Psychrotrophic bacteria originate from milk contamination from the milking equipment, water, ground, and air, and they produce lipolytic and proteolytic thermoresistant enzymes, which may influence the quality of the dairy products. In cheeses, their presence is attributed to contamination during processing and may produce quality defects such as bitter flavors (Sørhaug and Step-aniak, 1997). Yeasts and molds, having both proteolytic and lipolytic activity, may contribute to flavor development during ripening; however, they may also cause deterioration of the product (Fox et al., 2000c).

**Overall Acceptance of Prato Cheeses**

The overall acceptance of the Prato cheeses was significantly affected by milk SCC ($P = 0.015$), storage time ($P < 0.0001$), and the interaction of these 2 factors ($P < 0.0001$). Cheeses from low-SCC milk presented higher mean overall acceptance ($6.7 ± 0.1$) than cheeses from high-SCC milk ($6.3 ± 0.1$). Figure 2 shows the overall acceptance of cheeses from low- and high-SCC milks during the storage time. Cheeses from low- and high-SCC milks differed at the beginning of aging (8 d) and at the end of a typical shelf life (~60 d). The sensory quality of cheese depends on a number of factors linked to both the cheese-making technology and the chemical and microbiological characteristics of the raw milk used (Coulon et al., 2004). Milks with high SCC are classically associated with lower overall appreciation and with texture and flavor defects in the cheeses (Auldist and Hubble, 1998).

Proteolysis contributes to softening of the cheese texture during ripening and has a direct influence on flavor through the production of short peptides and AA, some
of which are often bitter (McSweeney, 2004). In this study, the proteolysis and other sensory descriptors, such as texture and bitter taste, were not evaluated. However, using the same levels of somatic cells as in the present study, Mazal et al. (2007) showed that Prato cheese from high-SCC milk presented higher proteolysis than Prato cheese from low-SCC milk. Thus, the lower overall acceptance of the cheeses from high-SCC milk may be associated with texture and flavor defects of these cheeses.

CONCLUSIONS

The level of somatic cells affected the lactic acid bacteria, psychrotrophic bacteria, and yeast and mold counts, the overall acceptance, and the composition of the Prato cheese evaluated in the present study. The clotting time at which the lactic acid bacteria died during ripening of the cheeses from high-SCC milk may have been the result of the presence of antimicrobial substances that were not completely eliminated after milk pasteurization, and would therefore still be active in the cheese. Cheeses from high-SCC milk showed lower overall acceptance by consumers. The higher levels of proteolysis and moisture content usually presented by cheeses from high-SCC milk are associated with texture and flavor defects that may be responsible for the lower overall acceptance of this cheese. The results showed that even when working with the somatic cell levels allowed by Brazilian law, the overall acceptance of Prato cheese from high-SCC milk was impaired.

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