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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.

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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-scald 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-Visbyac LC-MIX F0 2 01, Danisco Brazil Ltda., Cotia, Brazil), and rennet (calf rennet powder concentrate, Quimosina 90%, Rhodia Brazil, Paulínia, 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