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Quality of minas frescal cheese prepared from milk with different somatic cell counts

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Abstract – The objective of this work was to evaluate the effects of using bulk milk with different somatic cell counts (SCC) on the quality of minas frescal cheese. A randomized complete block design was used, with 3x5 factorial treatments, with three SCC levels (low, 125,000 cells mL^{-1}; intermediate, 437,000 cells mL^{-1}; and high, 1,053,000 cells mL^{-1}) and five storage durations. Cheese was vacuum-packed in plastic bags and analyzed after 2, 9, 16, 23 and 30 days of storage at 4°C. Somatic cell counts did not affect dry matter, fat, ash content, pH, free fatty acid concentrations and sensory parameters of minas frescal cheese. However, SCC in milk increased losses of protein in whey and decreased the cheese protein content. These changes did not affect the moisture-adjusted cheese yield and proteolysis during 30 days of storage. An interaction effect between SCC and time of storage was observed for firmness and sensory grades of cheeses. Results indicated that raw milk used to produce minas frescal cheese should not contain high SCC, in order to avoid lower acceptance of the product after 30 days of storage.

Index terms: cheese quality, proteolysis, somatic cell count.

Qualidade de queijo minas frescal preparado com leite com diferentes quantidades de células somáticas

Resumo – O objetivo deste trabalho foi avaliar o efeito do uso de leite com diferentes contagens de células somáticas (CCS), na qualidade do queijo minas frescal. Utilizou-se delineamento de blocos ao acaso, em esquema fatorial 3x5, com três níveis de CCS (baixa, 125.000 células mL^{-1}; intermediária, 437.000 células mL^{-1}; e alta, 1.053.000 células·mL^{-1}) e cinco tempos de armazenamento. Os queijos foram embalados a vácuo, em embalagens de plástico, e analisados após 2, 9, 16, 23 e 30 dias de armazenamento a 4°C. A contagem de células somáticas não afetou a matéria seca, a gordura, as cinzas, o pH, a concentração de ácidos graxos livres e os parâmetros sensoriais do minas frescal. A CCS no leite aumentou as perdas de proteína no soro e diminuiu o teor de proteína no queijo. Essas alterações não afetaram o rendimento com umidade ajustada e a proteólise durante 30 dias de armazenamento. Houve interação entre a CCS e o tempo de armazenamento, quanto à firmeza e às notas sensoriais dos queijos. O leite cru usado para a produção do queijo minas frescal não deve conter alta CCS, para evitar menor aceitação sensorial do produto após 30 dias de armazenamento.

Termos para indexação: qualidade do queijo, proteólise, contagem de células somáticas.

Introduction

Mastitis is a widespread disease that affects dairy cows and that negatively influences raw milk quality. Milk from infected cows is characterized by increased somatic cell counts (SCC) (Machado et al., 2000). The impact of mastitis to the dairy production is related to changes in milk composition, such as reduced calcium, lactose, and casein, and increases in sodium, chloride, and serum proteins (Fernandes et al., 2004). However, one of the most important adverse effects of high-SCC milk on the dairy industry is the reduced shelf life of dairy products (Santos et al., 2003). Proteolytic and lipolytic enzymes are increased in milk with high SCC (Fernandes & Oliveira, 2007), which may cause functional and sensory changes in dairy products during storage (Ma et al., 2000).

The SCC in milk can markedly influence the overall quality of cheese. Main alterations include decreases in cheese yield (Tavolaro et al., 2004), increases in fat and protein loss in whey (Andreatta et al., 2007), and inferior organoleptic properties (Auldist et al., 1996). The SCC effects on cheese manufacture have been well established for cheddar cheese (Rogers & Mitchell, 1994) and cottage cheese (Klei et al., 1998). Cheddar cheese manufactured from milk with high SCC have elevated moisture due to retarded syneresis, which leads to sensory defects in the final product. Although such effects are presumably expected to occur during manufacture of any type of cheese, very little information is available on the possible influence of high SCC in milk on the quality of other cheeses. Mazal et al. (2007) observed that prato cheese, a traditional Brazilian cheese, had high proteolysis and high moisture content when produced from milk with SCC greater than 600,000 cells mL^{-1}, although no effect was found on the cheese yield and protein recovery.

Minas frescal cheese is classified as semi-fat, nonripened, and high humidity, which is obtained by enzymatic coagulation of milk with rennet, with or without starter cultures. Minas frescal cheese accounts for nearly 10% of the total cheese produced in Brazil. The potential effects of high-SCC milk on the minas frescal cheese industry are not well understood.

The aim of this work was to determine the effects of using bulk milk with different SCC on the composition, and on physicochemical and sensory characteristics of minas frescal cheese.

Materials and Methods

Milk used in the present study was collected from a dairy herd located in Pirassununga, SP, Brazil. Twenty-two Holstein cows were selected among animals that started their lactation three to five months before the beginning of the experiment. Cows were not submitted to antibiotic treatment for seven days before milk collection. Milk from individual cows was submitted to SCC according to Association of Official Analytical Chemists (2000), to allow milk distribution into three groups based on individual SCC status: cows with low SCC (less than 200,000 cells mL^{-1}), intermediate (about 400,000 cells mL^{-1}), and high SCC (more than 800,000 cells mL^{-1}). Groups of six to eight 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 100 kg was obtained for cheese processing. Milk was then cooled and stored in a refrigerator (4°C) until processing, 24 hours after collection. Duplicate samples of bulked raw milk of each SCC category were analyzed for composition and confirmation of SCC (Association of Official Analytical Chemists, 2000).

Minas frescal cheese was manufactured strictly following the procedures described by Furtado (2005). All procedures for cheese manufacturing were performed in a double-jacketed stainless steel vat (Mec Milk, Brazil). Raw milk (100 kg per SCC level) was batch-pasteurized (65°C for 30 min), and then cooled to approximately 34°C. Mesophilic and psychrotrophic bacteria were analyzed on pasteurized milk (American Public Health Association, 2004) for confirmation of milk bacterial counts. Pasteurized milk containing less than $1.0x10^2$ colony forming units per milliliter of mesophilic or psychrotrophic bacteria, with added calcium chloride solution (250 mg L^{-1}) and 40 mL of liquid rennet (chymosin and bovine pepsin, Ha-La Christian-Hansen, Brazil) was incubated at 34°C for approximately 35 min. Next, the coagulum was cut into small cubes of approximately 1 cm side and gently stirred for 5 min, followed by manual mixing for 25 min. At the end of mixing, the whey was drained and collected for analysis. All fines in the whey were added back into the vat. The curd was then removed from the vat, salted (3 g per 100 kg), and transferred to molds (250 g). Cheese was turned three times at 15-min intervals and stored at 4ºC for 18 hours. After that, the cheese was removed from the molds, and was vacuum-packed in polyethylene bags and stored at 4°C for up to 30 days, the normal shelf life of minas frescal cheese marketed in Brazil. The experiment was replicated five times, once a month, so there was a total of 15 batches of cheese, five within each SCC category. Analyses of minas frescal cheese were performed in duplicate samples after 2, 9, 16, 23 and 30 days of storage at 4ºC.

Actual cheese yield was calculated using the equation Y (%) = 100(Wf/Wi), where Y is the yield, Wf is cheese final weight, and Wi is the initial total weight (milk and ingredients). The calculated actual yield value was used for determination of the moisture-adjusted cheese yield (MACY), according to Fox et al. (2000). A value of 57% was used as the reference for cheese moisture content in minas frescal cheese (Oliveira, 1986).

Milk was submitted to pH analysis by introducing the electrode directly into the sample. Total solids, fat, total nitrogen (TN), pH 4.6 soluble nitrogen, 12% trichloroacetic acid (TCA) soluble nitrogen, and lactose were analysed according to the Association of Official Analytical Chemists (2000). Total protein was calculated by multiplying TN by 6.38. Casein nitrogen was calculated as the difference between TN and noncasein nitrogen (pH 4.6 soluble nitrogen). Whey was analyzed for total solids, fat and TN contents (Association of Official Analytical Chemists, 2000).

Cheese samples were analyzed for moisture, fat, ash, TN, and pH (Association of Official Analytical Chemists, 2000). Total protein was calculated by multiplying TN by 6.38. The pH 4.6 soluble nitrogen and 12% TCA soluble nitrogen were determined in cheese by the macro-Kjeldahl method according to Andreatta et al. (2007). Lipolysis of cheese was estimated by measuring free fatty acids (FFA) concentration (Ma et al., 2003). Cheese firmness was determined using a TAXT2i Universal Texture Analyzer (Stable Micro Systems Ltd. Godalming, Surrey, UK), according to procedures described by Chaves et al. (1999).

Samples of minas frescal cheese were submitted to a nontrained panel of 50 individuals (Almeida et al., 1999), recruited among students and staff of the University of São Paulo. Samples were cut into portions of approximately 30 g, at approximately 10°C, without addition of any other ingredients, and submitted to the panel. Panelists were unaware of the identity of the cheese samples and were instructed to follow a hedonic scale of nine points and attribute scores to the overall quality of cheese. The descriptions used for the hedonic scale were: 9, excellent; 8, very good; 7, good; 6, fairly good; 5, indifferent; 4, fairly poor; 3, poor; 2, very poor; 1, extremely poor.

A randomized complete block design was used, with 3x5 factorial treatments with three SCC levels (low, 125,000 cells mL^{-1}; intermediate, 437,000 cells mL^{-1}; and high, 1,053,000 cells mL^{-1}), and five storage periods (2, 9, 16, 23 and 30 days after manufacture), with five replicates. The effects of the log SCC on the raw milk, whey, cheese composition and physicochemical characteristics, the free fatty acids, and the cheese yield were evaluated using ANOVA. A split plot design was used for the analysis of proteolysis, firmness and sensory grades, in which SCC was considered as the main plot, and the storage time as the subplot. Analyses were performed using SAS (SAS Institute, 2001). All statements of significance were based on 5% probability.

Results and Discussion

The average levels of somatic cells in low, intermediate and high-SCC raw milks were 125,000, 437,000, and 1,053,000 cells mL^{-1}, respectively. The low-SCC milk used in the experiment had a mean SCC value lower than 200,000 cells mL^{-1} and was representative of milk from healthy cows, whose cells originated from the natural scaling of the mammary gland epithelium (Fox et al., 2000; Fernandes et al., 2004). Milk with the highest SCC (1,053,000 cells mL^{-1}) slightly exceeded the limit for SCC in bulked raw milk as defined by Brazilian regulations (1,000,000 cells mL^{-1}).

Composition analyses of raw milk used for minas frescal cheese production had no significant differences ($p>0.05$) (Table 1). There were no differences ($p>0.05$) among SCC groups for total solids, fat, 12% TCA soluble nitrogen and pH 4.6 soluble nitrogen in whey (Table 2). Total protein was higher ($p<0.05$) in high-SCC whey, when compared with low-SCC

Table 1. Relationships between somatic cell count (SCC) and the composition of raw milk used for the manufacture of minas frescal cheese (n = 5).

Parameter	Low SCC (125,000 cells mL^{-1})	Intermediate SCC (437,000 cells mL^{-1})	High SCC (1,053,000 cells mL^{-1})	Standard error	P value
pH	6.74	6.72	6.67	0.03	ns
Total solids (%)	10.82	10.70	11.25	1.08	ns
Fat (%)	2.76	2.70	3.05	0.14	ns
Total protein (total N × 6.38)	2.69	2.76	2.88	0.06	ns
Casein (%)	2.14	1.81	1.91	0.17	ns
Nonprotein nitrogen (%)	0.14	0.15	0.15	0.01	ns
Lactose (%)	4.36	4.28	4.35	0.04	ns

[ns]Nonsignificant at 5% of probability.

whey. In minas frescal cheese, no differences ($p>0.05$) were found for dry matter, fat, 12% TCA soluble nitrogen, pH 4.6 soluble nitrogen, and ash contents. Although cheese from low-SCC group had higher total protein content, when compared with intermediate and high-SCC cheeses, the difference was significant ($p<0.05$) only between low and intermediate-SCC groups. The yield and free fatty acid concentrations of cheeses were similar among treatments ($p>0.05$). The FFA content in cheeses increased slightly during storage, although the concentrations were not affected by SCC and day of storage. Similarly, the pH of cheeses was not affected by SCC and time of storage ($p>0.05$), and there was no interaction between SCC and time of storage ($p>0.05$) for FFA and pH values.

The higher protein loss in whey, during the manufacturing of minas frescal cheese from high-SCC milk, may be a consequence of the increased proteolysis of alpha-s-caseins and beta-casein to products that are more soluble in serum and are, therefore, recoverable in whey (Fox et al., 2000). In fact, a higher activity of proteolytic enzymes, such as plasmin, has been demonstrated in high-SCC milk (Cooney et al., 2000). However, the increased protein content in whey did not produce any apparent effect on the moisture-adjusted yield of cheese from the three SCC groups, even with the higher protein loss in whey, observed for the high-SCC group. These data are in disagreement with a previous report on the negative effects of milk SCC on the yield of cheddar cheese (Rogers & Mitchell, 1994).

The difference between the yield of high-SCC minas frescal cheese and those reported for high-SCC cheddar cheese may be related to the differences in the manufacturing processes of these cheeses. Cheddar is a hard cheese, with approximately 35% moisture, since curds are molded and submitted to additional whey drainage by pressing overnight at up to 200 kN m^{-2} (Fox et al., 2000). Thus, a higher loss of solids is expected to occur in whey from the pressed curd, used in cheddar cheese manufacturing, when compared with the nonpressed, high moisture minas frescal cheese. The results of the present study are consistent with those reported by Mazal et al. (2007), who did not observe any significant difference in the yield of the semi-hard prato cheese manufactured from milk containing SCC at levels from 200,000 to 600,000 cells mL^{-1}.

There was no effect of SCC and also no interaction between SCC and time of storage on casein as a percentage of true protein of minas cheese (Table 3). However, the casein content decreased ($p<0.05$) in cheese during storage, hence indicating a time effect on the proteolysis of cheeses. The reduction in casein, as a percentage of true protein of minas frescal cheese from

Table 2. Effect of the somatic cell count (SCC) on the chemical composition of minas frescal cheese and whey and on cheese yield (n = 5)[(1)].

Parameter	Low SCC (125,000 cells mL^{-1})		Intermediate SCC (437,000 cells mL^{-1})		High SCC (1,053,000 cells mL^{-1})		P value
	Mean	SE	Mean	SE	Mean	SE	
Cheese, 2 days of aging							
Dry matter (%)	36.95	1.33	37.98	1.33	39.43	1.33	ns
Fat (%)	21.12	1.25	23.00	1.25	22.75	1.25	ns
Total protein (total N × 6.38)	17.35a	0.98	14.85b	0.80	15.69ab	0.80	0.009
12% TCA soluble N (%)	0.06	0.01	0.04	0.01	0.04	0.01	ns
pH 4.6 soluble N (%)	0.30	0.04	0.29	0.04	0.22	0.04	ns
Ash (%)	4.03	0.39	3.69	0.39	3.97	0.39	ns
Free fatty acids (mM kg^{-1})	2.00	0.34	1.96	0.34	2.32	0.34	ns
pH	6.80	0.06	6.78	0.06	6.74	0.06	ns
Whey							
TS (%)	6.71	0.23	6.46	0.23	6.65	0.23	ns
Fat (%)	0.47	0.03	0.48	0.03	0.55	0.03	ns
Total protein (%)	0.81b	0.03	0.98ab	0.03	1.07a	0.03	0.02
12% TCA soluble N (%)	0.19	0.02	0.20	0.02	0.25	0.02	ns
pH 4.6 soluble N (%)	0.20	0.01	0.20	0.01	0.20	0.01	ns
Cheese yield							
MACY (kg 100 kg^{-1})	13.38	0.85	13.08	0.85	14.18	0.85	ns

[(1)]Means followed by the same letters, within a row, are not different, according to Tukey test, at 5% of probability; SE, standard error; TCA, trichloroacetic acid; MACY, moisture-adjusted cheese yield. [ns]Nonsignificant at 5% of probability.

all SCC groups during storage, could be associated to the activity of heat-resistant, native proteinases naturally present in milk (Fox et al., 2000). In the present study, the failure of SCC in milk to affect the proteolysis of minas frescal cheese is in disagreement with previous reports that indicated that proteolysis was increased in cheddar cheese manufactured from milk with 500,000 cells mL^{-1} (Rogers & Mitchell, 1994), and in prato cheese made from milk with more than 600,000 cells mL^{-1} (Mazal et al., 2007). In fact, minas frescal is a nonripened cheese that is marketed for consumption immediately after manufacture. Therefore, relatively lower proteolysis indexes are expected to occur during its shelf life, when compared with long-ripened cheeses, such as cheddar or prato cheeses.

The cheese moisture content decreased ($p<0.05$) throughout the storage period, and no effect of SCC was observed in the cheese (Table 4). As minas frescal normally has a high moisture content, additional whey separation from the cheese inside the vacuum packaging is expected to occur during storage (Fox et al., 2000), and this could lead to the concentration of cheese solids up to 30 days.

The firmness of minas frescal increased ($p<0.05$) during 30 days of storage, but was not affected ($p>0.05$) by SCC, as shown in Table 5. Mazal et al. (2007) did not also observe any significant effect of SCC on the firmness of prato cheese. However, in the present work, there was an interaction effect between SCC and time of storage ($p<0.05$). Firmness increased during storage, and this effect was consistent with the effect of time on the moisture content during storage. Consequences of this increase in firmness should lead to lower acceptability of the product, due to consumers' expectations of soft texture, which would undoubtedly have a negative impact on the cheese marketability.

A decrease in the grades of cheeses from the three groups can be noticed during storage (Figure 1), although the overall quality was not influenced ($p>0.05$) by SCC in milk or by the storage time alone. An interaction effect ($p<0.05$) was observed between SCC and time of storage on the grades for overall acceptance of minas frescal cheese, especially during the last 15 days of storage.

Auldist et al. (1996) reported lower sensory characteristics of cheddar cheese made from milk

Table 3. Effect of the somatic cell count (SCC) on casein as a percentage of true protein of minas frescal cheese (n = 5).

Category	Time of storage (days)				
	2	9	16	23	30
	(%)				
Low SCC (125,000 cells mL^{-1})	90.7	86.3	83.3	88.7	86.3
Intermediate SCC (437,000 cells mL^{-1})	89.0	88.0	88.3	90.0	89.0
High SCC (1,053,000 cells mL^{-1})	91.7	89.0	88.0	88.3	86.3
Standard error	1.2	1.0	3.0	3.0	1.6
Source of variation	P-value				
SCC	0.5468				
Time	0.0173(1)				
SCC x time	0.4913				

(1)Polinomial effect: $y = 0.4x^2 - 2.8x + 92.5$ ($R^2 = 0.51$).

Table 4. Effect of the somatic cell count (SCC) on the moisture of minas frescal cheese (n = 5).

Category	Time of storage (days)				
	2	9	16	23	30
	(%)				
Low SCC (125,000 cells mL^{-1})	63.05	64.25	64.03	61.52	62.07
Intermediate SCC (437,000 cells mL^{-1})	62.02	61.86	60.25	60.56	60.69
High SCC (1,053,000 cells mL^{-1})	60.57	62.11	61.34	59.42	61.20
Standard error	1.45	1.01	1.62	1.29	1.31
Source of variation	P value				
SCC	0.4111				
Time	0.0041(1)				
SCC x time	0.6256				

(1)Polynomial effect: $y = 0.326x^3 - 2.978x^2 + 7.615x + 56.89$ ($R^2 = 0.988$).

Table 5. Effect of somatic cell count (SCC) on the firmness of minas frescal cheese (n = 5).

Category	Time of storage (days)				
	2	9	16	23	30
	(g)				
Low SCC (125,000 cells mL^{-1})	1,405	1,606	1,939	1,919	2,096
Intermediate SCC (437,000 cells mL^{-1})	1,425	1,607	1,729	1,719	2,151
High SCC (1,053,000 cells mL^{-1})	1,390	1,507	1,396	1,673	1,511
Standard error	98	145	125	126	97
Source of variation	P value				
SCC	0.0730				
Time	<0.0001(1)				
SCC x time	0.0047(2)				

(1)Linear effect: $y = 122x + 1,305$ ($R^2 = 0.99$). (2)Linear effect: 125,000 cells mL^{-1}: $y = 169x + 1,285$ ($R^2 = 0.91$); 437,000 cells mL^{-1}: $y = 156x + 1,257$ ($R^2 = 0.86$); 1,053,000 cells mL^{-1}: $y = 40x + 1,373$ ($R^2 = 0.31$).

with SCC above 1,500,000 cells mL^{-1}, a level that is much higher than the ones used in the present experiment. A possible explanation for the difference between this report and data obtained in the present study may be the different manufacturing methods. Cheddar cheese is usually ripened from three or four months to two years (Fox et al., 2000), and this period would allow the action of proteolytic enzymes present in high-SCC milk, leading to a greater sensory modification in cheddar, compared to minas frescal cheese.

There was interaction effect between SCC and time of storage on the grades for overall acceptance of minas frescal cheese, especially evident during the last 15 days of storage. This effect was consistent with the interaction effect of time and SCC on the firmness of minas frescal cheese during storage. However, along with the lower grades for the high-SCC cheese, panelists reported low sensory descriptors only after days 23 and 30 of storage, describing the taste as bitter or astringent.

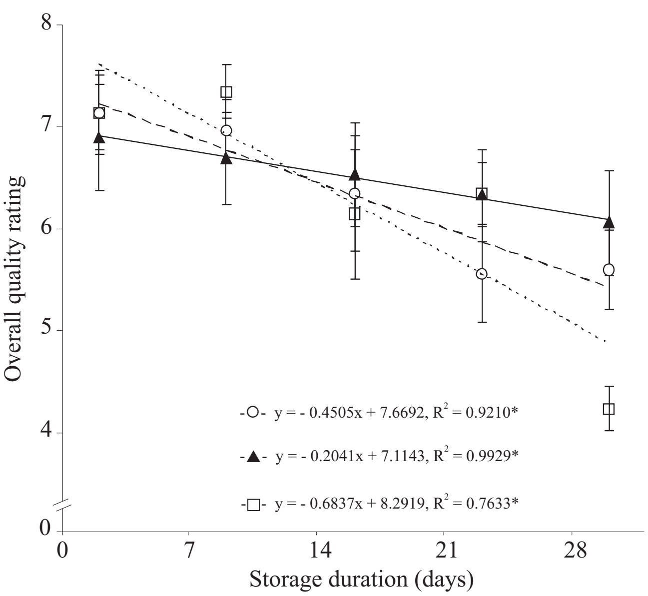

Figure 1. Interaction between low (○ - mean SCC = 125,000 cells mL^{-1}), intermediate (▲ - mean SCC = 437,000 cells mL^{-1}) and high (□ - mean SCC = 1,053,000 cells mL^{-1}) SCC in milk, and days of storage for the overall acceptance of minas frescal cheese, during 30 days of storage at 4°C. Results are mean grades of individual scores obtained from 50 panelists. The equation for time of storage alone is $y = -0.4489x + 7.707$, $R^2 = 0.9413$. *$p<0.05$.

Conclusion

Bulked milk used to produce minas frescal cheeses should not contain high SCC, in order to avoid lower acceptability of the product after 30 days of storage.

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