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Effect of calcium addition and pH on yield and texture of Minas cured cheese

[*Efeito da adição de cálcio e do pH sobre o rendimento e a textura de queijo-de-Minas curado*]

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ABSTRACT

Milk calcium concentration is a factor related to cheese texture, an important rheological property of cheese quality as perceived by consumers. This study aimed to evaluate the effect of different pH conditions (5.8 and 6.6) and calcium addition (0, 150, 300 ppm of $CaCl_2$), on yield and nutrient retention of the clots obtained and on the texture of *Minas* cured cheese. Clots were analyzed for wet and dry yield, percentage content and retention of protein, fat and calcium. The texture of the cheese was evaluated by instrumental and sensorial tests. No differences were observed on the wet and dry yields, or on the protein content, fat and calcium retention of clots produced in the different experimental conditions. The instrumental evaluation showed that calcium addition significantly influenced the texture of cheeses, regardless of the pH of milk clotting. The sensory panel did not find a difference in the hardness of cheeses produced at the same pH of milk clotting in function of $CaCl_2$ addition. There was no difference in the texture of *Minas* cured cheese due to the calcium addition to milk for dairy product consumers, which brings a new perspective on manufacture for cheese markers.

Keywords: cheese, pH, calcium, clotting, texture

RESUMO

Avaliou-se o efeito de diferentes condições de pH – 5,8 e 6,6 – e da adição de cálcio – 0, 150, 300ppm de $CaCl_2$ – sobre o rendimento, a retenção de nutrientes nos coágulos produzidos e a textura de queijo-de-Minas curado. Foram analisados nos coágulos os rendimentos úmido e seco, o percentual e a retenção de proteína, de gordura e de cálcio. A textura dos queijos foi avaliada por testes instrumental e sensorial. Não foram observadas diferenças nos rendimentos úmido e seco, no conteúdo percentual e na retenção de proteína, gordura e cálcio nos coágulos produzidos em diferentes condições experimentais. A análise instrumental mostrou que a adição ou não de cálcio influenciou a textura dos queijos, independentemente do pH de coagulação do leite. O painel sensorial não diferiu quanto à dureza dos queijos produzidos em um mesmo valor de pH de coagulação do leite mediante a adição de $CaCl_2$. Para os consumidores, não houve diferença na textura do queijo Minas curado em razão da adição de cálcio ao leite. Isto traz nova perspectiva na fabricação para os produtores de queijo.

Palavras-chave: queijo, pH, cálcio, coagulação, textura

INTRODUCTION

Throughout the history of cheese making, technologists always had a consensus regarding the need for additional calcium to obtain the best technological transformation of milk into cheese (Scriban, 1985; Oliveira, 1986; Lucey and Fox, 1993; Joshi *et al*., 2003). Nowadays many cheese researchers and producers believe that calcium in cheese can determine the physical-chemical and functional properties, since a reasonable amount

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of calcium was present inside casein after cheese making (Lucey *et al*., 2003; Lucey *et al*., 2005; Chevanan and Muthukumarappan, 2007; Guinee and O'Kennedy, 2009; Lee and Lee, 2009).

Calcium exists in milk in soluble (including ionic) and colloidal (including casein-bound) forms. The equilibrium between the soluble and colloidal forms is especially dependent on pH, temperature and ionic strength (Wolfschoon-Pombo, 1997). The addition of calcium increases the ionic calcium concentration in milk, and reduces the rennet clotting time, enhances the retention of this mineral in the cheeses, improves dry weight and retention rate of protein and fat (Solorza and Bell, 1998).

Milk calcium concentration is described to influence cheese texture, which is an important rheological property of cheese quality as perceived by consumers and the total calcium content of the several cheese varieties varies mostly due to differences in the manufacturing pH values (Lucey and Fox, 1993; Lee *et al*., 2005). It is known that pH has a major effect on the textural properties of cheese due to the effect on the load-bearing casein (Watkinson, 2001), as well as on colloidal calcium phosphate dissolution as a function of milk pH decreasing, disrupting the stability of casein micelles (Dalgleis and Law, 1989; Lucey *et al*., 1997). The pH variations also lead to a shift in calcium equilibrium, and are influenced by factors like composition and pre-acidification of milk, cheese manufacturing pH, acid development during aging, among others. There have been many studies showing that calcium equilibrium affects the rheological properties of cheese (Hassan *et al*., 2004; Johnson and Lucey, 2006; Lee and Lee, 2009).

The rheological properties of cheese, which are related to the mechanical elements of texture, represent important quality aspects of the product (Foegeding, 2003). These properties are a function of cheese composition and microstructure, as well as of the physical-chemical state of its components, and the strength of the interactions between the structural elements and the macrostructure (Konstance and Holsinger, 1992; Fox *et al*., 2000).

However, some previous unpublished studies done in our laboratories have presented conflicting results with those described in literature, pointing out that calcium addition to the milk did not improve processing and cheese yield. Our previous results showed that increased milk clotting activity for different rennet was obtained under pH 5.8, temperature 45°C and without addition of chloride calcium ($CaCl_2$) to the milk. Furthermore, higher wet yield and protein, fat and calcium retention were obtained for cheese produced with microbial rennet with milk at pH 5.8 and without calcium addition, and there were no differences in the wet and dry yields for the clots or for the protein, fat and calcium retention of clots produced at pH 5.8, with or without the addition of $CaCl_2$ to milk of different brands.

Minas cured is a typical Brazilian cheese, traditionally made using a mesophilic lactic acid starter type culture consisting of both *Lactococcus lactis* ssp *cremoris* and *Lc. lactis* ssp *lactis*. This cheese is one of the most highly consumed lactic products in Brazil, showing ample acceptance in the national market.

Texture and other sensorial attributes of cheese are important quality parameters of this food product and may be susceptible to undesirable changes due to production conditions. Therefore, this study aimed to evaluate the effect of enzymic clotting of milk submitted to different pH conditions, with the addition of 0, 150, 300 ppm $CaCl_{2,}$ on yield and nutrient retention of the clots obtained and also to verify the effect of pH and calcium addition on the texture of *Minas* cured cheese.

MATERIALS E METHODS

Pasteurized milk, standardized to 3% (w/v) fat, was obtained from the market of Niterói City, in Rio de Janeiro State, Brazil, and was analyzed for pH with the potentiometric method, titratable total acidity with the volumetry of neutralization, total ash with the gravimetric method, total nitrogen with the micro-Kjeldahl method, total fat content with the Gerber method (International..., 1964; 1993; 1991), total solid content with the gravimetric method and total calcium content with the titration with EDTA (Instituto..., 2008). Clotting experiments were conducted obtaining six types of clots, according to milk pH (5.8 or 6.6, achieved by adding HCl 0.1 N), and $CaCl_2$ addition (0, 150 or 300mg.L^{-1}).

Clots were denoted T1 (pH 5.8, without $CaCl_2$ addition), T2 (pH 5.8, addition of 150 $mg.L^{-1} CaCl_2$), T3 (pH 5.8, addition of 300 $mg.L^{-1}$ $CaCl_2$), T4 (pH 6.6, without $CaCl_2$ addition), T5 (pH 6.6, addition of 150 $mg.L^{-1}$ $CaCl_2$) and T6 (pH 6.6, addition of 300 $mg.L^{-1}$ $CaCl_2$). For each clotting assay, the milk was added to recombinant chymosin (0.028 $g.L^{-1}$) (Chymogen®, Chr. Hansen Co, São Paulo, Brazil) in order to promote a clotting time of 50 minutes. Before adding the rennet, milk temperature was adjusted to 35°C. After coagulation, the obtained gel was cut and manually agitated and kept at 45°C for 10 minutes, until flakes settled to the bottom of the container. After that, the clot was separated from the whey and placed directly into cheese molds of the *Minas kind*, where it remained for seven days, kept refrigerated (12°C) and submitted to the daily turn. Thereafter, each clot was weighed, homogenized and stored in plastic pots in the freezer (-18°C) for posterior analysis. The milk clotting experiments, for each condition, were conducted in triplicate.

Each clot produced was analyzed for total nitrogen, fat, total solid, and Ca contents with the same methods used for milk analysis (International..., 1993; 1991; Instituto..., 2008). Wet and dry yields for the clots were also calculated and expressed in g of $clot.L^{-1}$ of milk, calculations of protein, fat and calcium retention were performed for each clot.

Six *Minas* cured cheeses, denoted C1, C2, C3, C4, C5 and C6, were produced and submitted to sensorial and instrumental texture analysis. Cheeses denoted C1, C2 and C3 were obtained from 10 liters of standardized pasteurized milk each, which were heated to 35°C and added of 0.001% (w/v) mesophilic homofermentative starter culture containing *Lactococcus lactis* ssp *cremoris* and *Lc. lactis* ssp *lactis* (R704, Chr. Hansen, Co., Brazil), and after 4 hours of fermentation, the milk pH value reached 5.8. The other group of cheeses (C4, C5 and C6) did not undergo fermentation. Subsequently, for both groups, milk was added at 0, (C1 and C4), 150 (C2 and C5), 300 (C3 and C6) $mg.L^{-1}$ $CaCl_2$. Rennet consisting of recombinant chymosin was diluted in distilled water and added to the milk. Temperature was held at 35°C and the coagulation process lasted about 40 minutes. The curd was cut into 20-30 mm cubes, drained, pre-pressed at 13kg for 20 minutes, then molded into pieces of about 1,000g and then pressed on one side in a vertical mechanic press at 13kg for 30 minutes, followed by 90 minutes on the other side. Cheeses were salted by immersion in brine solution (20% (w/v) sodium chloride) for 24 hours. Cheeses were ripened in a chamber at 12°C and 85% relative humidity, where they were turned over for 10 days, then packaged in polyethylene plastic film and held at the ripening chamber for another 20 days (Furtado and Lourenço-Neto, 1994).

The texture profile analysis of the cheeses was determined in terms of hardness. Texture properties of cheeses were evaluated with TA-HDi Texture Analyzer (Stable Micro Systems, Surrey, UK) with a 50kg load cell, using a uniaxial compression of cube samples of $2cm^3$. The compression ratio employed was of 20% deformation from the initial height of the sample at a rate of $2mm\ s^{-1}$. After being cut, the cheese samples were left at room temperature (25°C) for 20 minutes prior to testing. The analysis was carried out ten times for each cheese in each treatment. Parameters measured were obtained by using the Texture Expert for Windows software version 1.20.

The effects of the different treatments on the cured *Minas* cheese texture were performed by comparison of samples using the Ordering Test, according to the methodology described by Dutcosky (1996) and Meilgaard *et al*. (1991).

Sensory assessments of *Minas* cured cheese samples were carried out by 49 untrained panelists. All of them reported to be dairy product consumers and were familiar with this type of cheese, commonly produced in Brazil. Six cube samples ($2cm^3$) were presented at room temperature (25°C) in white cups, along with the sensory evaluation form. The samples were identified using random three-digit codes in such a manner that the panelists would not know how to identify the treatment. After tasting the samples, panelists were instructed to arrange the samples in decreasing order of hardness. Water was provided to neutralize the taste between the different cheese sample assessments. The results were processed using the Table of Newell and MacFarlane, the significance level of 5% (Dutcosky, 1996).

Analysis of variance for the results obtained for physicochemical analysis, retention calculations and texture was made within each group of clot or cheese (with or without $CaCl_2$ addition), followed by the Turkey test for average comparison, using the Graph Pad Prism version 5.0 software at 5% significance.

RESULTS AND DISCUSSION

The milk used in this study showed satisfactory quality. The results of analysis carried out in milk in the clotting experiments are presented in Table 1. These results are important for assessing the retention of major milk nutrients in the clots.

Table 1. Compositional and physicochemical analyses of milk used in clotting assays

Milk	pH	TTA	TSC	TA	TPC	TFC	TCC
		% (w/v)	% (w/w)	% (w/v)	% (w/v)	% (w/v)	% (w/w)
Mean*	6.55	0.15	88.25	0.68	3.97	2.8	0.11
SD	±0.05	±0.46	±0.06	±0.01	±0.22	±0.16	±0.02

*Mean±SD: means 3 replicate analyses.
TTA = titratable total acidity (% g lactic acid); TSC = total solid content (moisture); TA = total ash; TPC = total protein content; TFC = total fat content; TCC = calcium content.

The results for clot yield are shown in Figure 1. The results show no significant difference in yield for wet or dry basis, and clots obtained at different pH values of milk clotting, regardless of the condition of adding $CaCl_2$. The cheese yield is described as recovery cheese nutrients previously contained in milk (Solorza and Bell, 1998), so the yield will be related to retention, especially of protein, fat and calcium in the clot associated with the moisture retention. The moisture content of the cheese was not significantly affected by the addition of calcium in different concentrations regardless of the pH of milk clotting. Different results were found for cheddar cheese by Guinee and O'Kennedy (2009) and Upreti and Metzger (2006), in which cheeses with low calcium had higher moisture compared to those with high calcium. A similar influence of calcium content on cheese moisture was observed by other researchers; they attributed the lower moisture in high calcium cheese to reduced hydration of the cheese para-casein matrix compared with low calcium cheese (Guinee *et al*., 2002; Pastorino *et al*., 2003a; Joshi *et al*., 2004).

The results for protein, fat and calcium in the clots produced at pH 5.8 and pH 6.6 are presented in Figure 2 and 3, respectively. The results demonstrate that there was no difference in the content and protein and fat retention of clots obtained for both pH values used for milk coagulation due to the addition of 150 and 300 ppm or not $CaCl_2$. These results are similar to those described by Chevanan and Muthukumarappan (2007) who found no significant difference in protein content of Cheddar cheeses produced with different concentration of calcium. Furthermore, it partially agree with the results obtained by Upreti and Metzger (2006) in which the protein and fat content of cheddar cheeses was significantly affected by a variation in the concentration of calcium in cheese making, however these differences in protein and fat of cheeses were attributed to differences in their moisture content. This is supported by the fact that protein and fat content on a dry basis was not affected by different levels of calcium. Solorza and Bell (1998) found that protein retention in mozzarella cheeses was not higher by adding $CaCl_2$ to the reconstituted skim milk with 15% (w/v) total solids. In the case of fat retention, this result was repeated for whole milk. This study demonstrates that several factors are related to the retention of milk nutrients in cheese, and that the role of calcium may be related to the presence of other constituents.

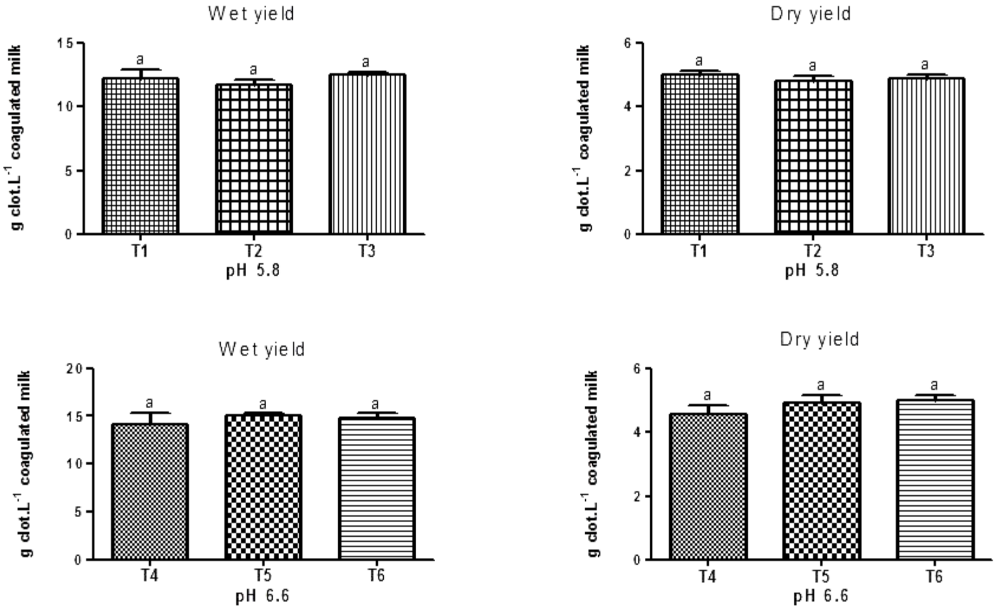

Figure 1. Comparison of wet and dry yields of clots produced in different conditions of calcium additin at each pH of milk clotting.
*Column with different lowercase letters differ significantly (P<0.05).

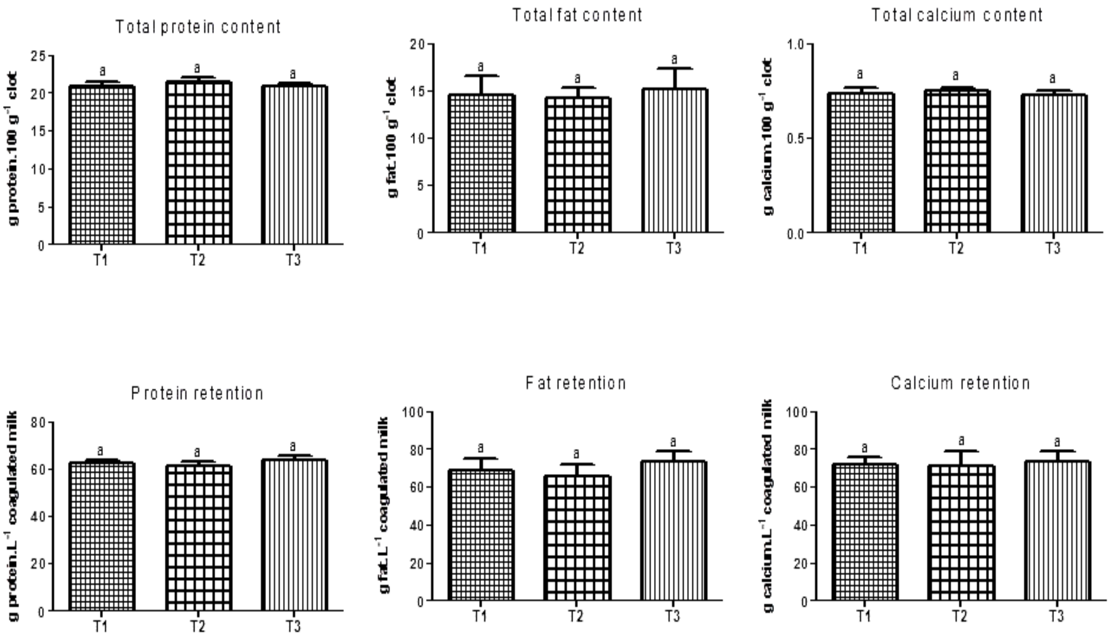

Figure 2. Comparison of protein, fat and calcium content and retention of same nutrients in clots produced in different conditions of calcium addition at pH 5.8 of milk clotting.

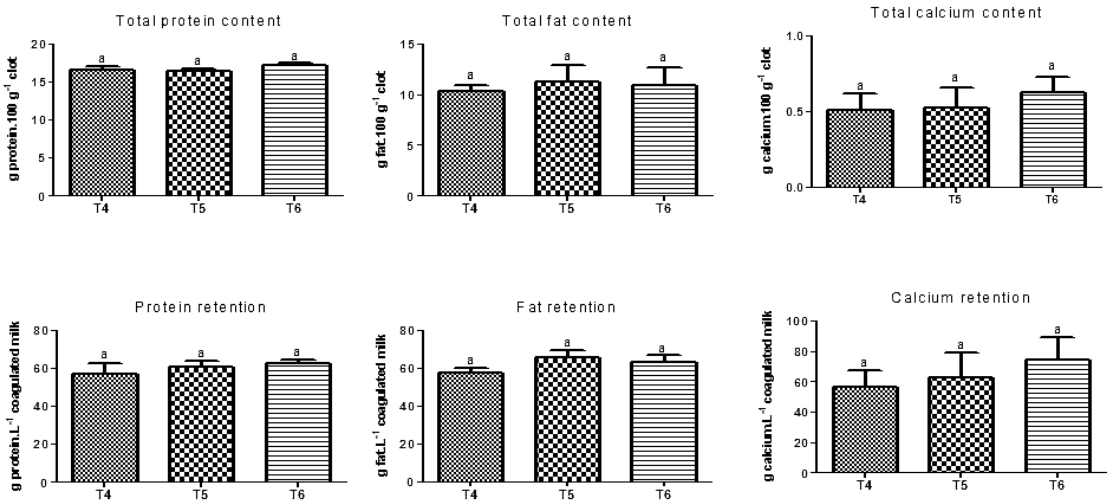

Figure 3. Comparison of protein, fat and calcium content and retention of same nutrients in clots produced in different conditions of calcium addition at pH 6.6 of milk clotting.

Assuming, as described in the literature, that the addition of calcium helps form a network structure during milk coagulation and provides links within and between casein micelles (Joshi *et al.*, 2003), this function could contribute to a strengthening of higher coagulated protein network, which would promote greater inclusion of fat globules in the clot mass. However, this did not happen to the pH of milk clotting 5.8 and 6.6, since there was no significant difference in the retention of fat clots due to the addition or absence of $CaCl_2$. Considering the results for calcium, they show no significant difference in content and calcium retention of clots obtained at different pH values of milk clotting, regardless of the condition of adding $CaCl_2$. These results are conflicting with those described by Upetri and Metzger (2006) who demonstrated higher calcium content in cheeses with high calcium concentrations compared with those produced with lower amounts of calcium. According to the literature, the decrease in milk pH causes the solubilization of protein-bound calcium that is subsequently lost in whey during cheese making, leading to lower calcium content in the final cheese. A decrease in pH not only causes solubilization of calcium, but can also influence protein-protein interaction, due to a decrease in casein charge when the pH is lowered toward the isoelectric point (Pastorino, 2003b; Upetri and Metzger, 2006). This approach to alter the level of calcium in cheese has been used by other researchers. However, this did not happen with the clots produced with different calcium concentrations at pH 5.8, showing no significant difference between the various clots in the content and calcium retention.

Fig. 4 presents the results of compressive force evaluation of cheeses obtained in different experimental conditions for pH of milk clotting and calcium concentration. The results show that calcium addition significantly influences the texture of cheeses, regardless of the pH of milk clotting. Cheeses C1 and C4, obtained without calcium addition, presented main values of compressive force 1247.14 g and 610.76g for pH clotting 5.8 and 6.6, respectively. These values are lower than those measured in cheeses added of 150 and 300 ppm $CaCl_2$. Cheeses C2 and C3 obtained at pH 5.8 presented main value 2049.32g and 1828.28g, respectively, while cheeses C5 and C6 obtained at pH 6.6 presented 928.68g and 975.75g of compressive force, respectively. The addition of calcium influenced the texture, increasing the hardness of cheeses in both clotting pH, but this increase was significant for the condition of addition or not of $CaCl_2$, there was no significant difference in hardness of cheeses added of different calcium concentrations and this fact can be explained by the occurrence of saturation of the binding sites calcium-casein.

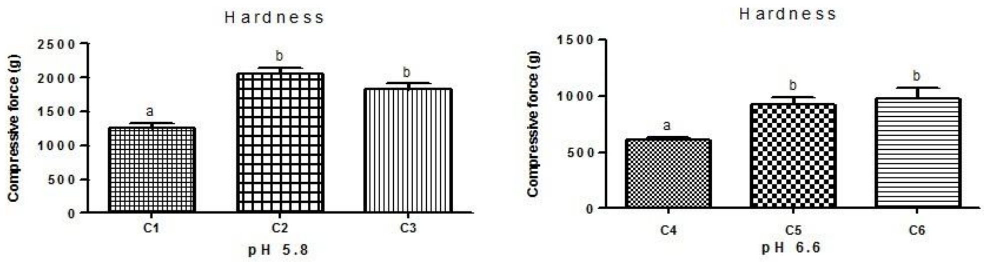

Figure 4. Comparison of compressive force of cheeses produced in different conditions of calcium addition at each pH of milk clotting.
*Column with different lowercase letters differ significantly (P<0.05).

Although these results are repeated for two pH used in the study (5.8 and 6.6), the literature describes that a reduction in pH of milk clotting alters the concentration of calcium in cheese and may have some secondary effects on cheese characteristics (Upetri and Metzger, 2006). An increase in pH results in decreased firmness/hardness, increased meltability in more continuous microstructure, while low pH values may produce a firm cheese, less fluid in melting (Monteiro *et al*., 2009). Therefore, any observed differences in cheese characteristics may not be exclusively due to differences in calcium content, but may be related to other factors (Upetri and Metzger, 2006). The results are concordant with statements in literature that affirm that calcium concentration influences rheological properties of cheeses, including hardness (Hassan, 2004). As evidenced in a study, the hardness of cheddar cheese prepared with low calcium was always lower than that prepared with high levels of calcium and there was an inverse correlation between hardness and meltability at any time during ripening (Chevanan and Muthukumarappan, 2007). Results of another study indicated that low levels of calcium in natural cheeses produce processed cheeses that are softer and more brittle, and they become fluid upon heating, which acquire this fluency in the shortest time at lower temperature, but are less stable upon heating at high temperatures for a longer period of time (Guinee and O'Kennedy, 2009).

The sensorial evaluation of cheeses C1, C2, C3, C4, C5 and C6 regarding the hardness parameter of the texture showed that, in spite of panelists (consumers) indicating C1 as the hardest cheese produced and C6 as the less hard *Minas* cured cheese produced, there was no significant difference between cheeses produced at the same pH of milk clotting regarding the addition of 150 or 300 ppm of $CaCl_2$ or not. These results disagree with the results obtained by Hassan *et al*. (2004), who affirms that the composition of minerals, especially calcium concentration, is a parameter that influences the texture and functional properties of cheese, including the hardness/elasticity and firmness. Also, they disagree with other literature results discussed previously (Upetri and Metzger, 2006; Chevanan and Muthukumarappan, 2007; Guinee and O'Kennedy, 2009; Monteiro *et al*., 2009).

These results also differed, partly, from those results obtained in the instrumental analysis of texture. As discussed above, the instrumental analysis led to observe a difference in hardness between cheeses produced with addition or not of $CaCl_2$ at each pH of milk clotting, suggesting a higher sensitivity of the instrumental method. On the other hand, among the cheeses produced with added $CaCl_2$, instrumental analysis was not able to point to a significant difference in hardness between cheeses in both pH of milk clotting, as occurred in the sensory analysis.

Although the test used for instrumental evaluation is widely used to assess the texture of cheeses, it is especially important in the determination of the mechanical properties (Fox *et al*., 2000) and is relevant for a comparison with sensory texture attributes evaluated during mastication (Drake *et al*., 1999; Breuil and Meullenet, 2001). It’s the consumer’s perception that establishes the overall product/cheese quality and its acceptability. In this work, untrained panelists represented the final consumer for the dairy industry. Among the panelists who participated in sensory analysis, 99% reported a regular consumption of cheese in the questionnaire, a fact that strengthens the results obtained with the panelists.

CONCLUSIONS

The results of this study demonstrated that the addition of calcium at different concentrations did not influence wet and dry yield and protein, fat and calcium contents regardless of the pH of cheeses which were produced. The same was true for the retention component of milk, protein, fat and calcium in cheeses. Regarding texture, the instrumental evaluation showed that the addition of calcium increases the hardness of cheeses regardless of pH of milk clotting, however, this change in the hardness of cheeses made with different concentrations of calcium was not evident in the sensory analysis, indicating that consumers feel no difference in the texture of cheeses due to the calcium addition to milk. The modifications in cheese making protocols used in this study produced *Minas* cured cheeses with the desired differences in calcium concentration and pH of milk clotting. All the modifications used in this study for varying the levels of experimental factors were chosen with the anticipation that cheese makers could follow these modifications in cheese-making protocols to use the best conclusions of this study.

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