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Effects of calcium chloride substitution on the physicochemical properties of Minas Frescal cheese

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Research Article

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Abstract

The aim in this research paper was to investigate the effect of using calcium monophosphate (MCP) and MCP mixed with commercial phosphates salts, in total or partial replacement of calcium chloride ($CaCl_2$) in the manufacture of Minas Frescal cheese. Initially, model cheeses were made to perform the rheological analysis during the coagulation process. Of these, the five best treatments were chosen to carry out the production of Minas Frescal cheese, used only $CaCl_2$ and MCP, and partial replacements of MCP + polyphosphate, MCP + potassium monophosphate (MKP) and MCP. The cheeses showed no significant difference in physicochemical composition, yield and syneresis, however, the cheese with partial replacement of $CaCl_2$ by MCP + polyphosphate and MCP + MKP showed the highest hardness values, like the control. This demonstrates that it is possible to replace calcium chloride without significant changes in the physicochemical characteristics and yield of Minas Frescal cheese, and it is still possible to modulate the hardness of the cheese produced according to the type of calcium/phosphate source used. This allows the industry to replace the source of calcium in the manufacture of Minas Frescal cheese according to the desired hardness.

Minas Frescal is a fresh and soft white cheese (Codex, 1978), typically manufactured in Brazil, obtained by enzymatic coagulation of pasteurized milk with rennet and supplemented or not with specific lactic acid bacteria (Brasil, 1996, 2004). During its manufacture, heat treatment leads to a reduction of soluble calcium (Ca^{2+}), leading to a change in the salt balance of the milk (Wang and Ma, 2020). Therefore, there has long been a consensus on the need to add calcium to improve manufacturing yield of the cheese, thus replacing the calcium lost during the heat treatment, with an addition of up to 10 mM Ca^{2+} having an effect of increasing the strength of the gel. Above this, the opposite effect may occur (Lucey and Fox, 1993; Solorza and Bell, 1998; Santos *et al*., 2013). This occurs because calcium is involved in the clotting process of milk. A clear understanding of the role of calcium within this coagulation process is hampered by the complexity of forms in which calcium is present in milk. However, it is known that the effect of addition of Ca^{2+} is related to reducing the surface potential of the para-casein micelles (Ong *et al*., 2013). Ca^{2+} ions bind to the casein micelles *via* electrostatic cross linking of the phosphate moiety of the colloidal calcium phosphate, thereby neutralizing their charge and resulting in increased aggregation of the rennet micelles (Dalgleish and Law, 1983; Ong *et al*., 2013). In the cheese industry, the agent used to replace this lost calcium is $CaCl_2$.

Some studies on the influence of adding $CaCl_2$ for cheese production show an increase in the hardness of cheddar cheese (Ong *et al*., 2013) and cured Minas cheese (Santos *et al*., 2013), which leads to the formation of more homogeneous gels (Tarapata *et al*., 2020). This also improves the hardness of the milk coagulum and increases the degree of syneresis and yield (Wolfschoon-Pombo, 1997), whilst there is no change in the composition of the cheese (Santos *et al*., 2013). However, there are no studies that demonstrate the use of other sources of calcium to replace $CaCl_2$ during milk clotting (or enzymatical coagulation of milk). An alternative source of calcium may be calcium monophosphate (MCP) since, in the casein micelle, the non-protein components expressed in ions are mostly calcium (37.5%) and phosphate (50%). In addition, calcium phosphate is the main inorganic constituent of the micelle (Sleigh *et al*., 1983; Bak *et al*., 2001; Kolar *et al*., 2002). In a study carried out by Guo *et al*. (2003), the presence of calcium phosphate in a standard β-casein solution led to total protein precipitation/separation, while the presence of Ca^{2+} alone led to only minor protein separation. The authors concluded that the separation is caused by the indiscriminate co-precipitation of proteins by organic compounds compared to the selective precipitation of β-casein by calcium ions. Thus, the objective of this work was to evaluate the partial or total replacement of $CaCl_2$ by MCP and the mixture of this MCP with other types of phosphate salts, like monopotassium phosphate (MKP) and polyphosphate, in the production of Minas Frescal cheese.

Material and methods

As experimental design, this study was conducted in two steps: (I) previous evaluation of the best concentrations of total or partial replacement of $CaCl_2$ by MCP and/or Blend 1 and Blend 2, through rheological analysis of cheese models (detailed in the online Supplementary File and data reported in Supplementary Table S1) and (II) determination of physical chemical characteristics, texture and yield of Minas Frescal cheeses produced with the best treatments determined in step I.

Material

Chemical reagents of analytical grade were used for the physicochemical analyses. The following ingredients were employed for Minas Frescal cheese manufacture: raw cow's milk (Laticínio Funarbe, Viçosa, MG, Brazil); lactic acid 85% (v/v) P.A. (Dinâmica Química Contemporânea, Indaiatuba, SP, Brazil); calcium chloride 40% (w/v) (Dinâmica Química Contemporânea); rennet Maxiren XDS BF (bovine chymosin; DSM Food Specialties, Delft, the Netherlands); commercial sodium chloride (Cisne, Cabo Frio, RJ, Brazil); phosphates salts: monocalcium phosphate (MCP); a mixture of MCP with polyphosphate, named Blend 1; and a mixture of MCP with monopotassium phosphate (MKP), named Blend 2. Blends 1 and 2 contained around 14.5–15.5% calcium content.

Methods

Based on the results of step I (Supplementary File), five treatments were carried out with 50 l of raw milk and the addition of different calcium sources: (T2) control with addition of 0.24 gl^{-1} $CaCl_2$; (T4) 0.5 gl^{-1} MCP; (T9) 0.25 gl^{-1} Blend 1 + 0.12 gl^{-1} $CaCl_2$; (T10) 0.25 gl^{-1} Blend 2 + 0.12 gl^{-1} $CaCl_2$; and (T11) 0.25 gl^{-1} MCP + 0.12 gl^{-1} $CaCl_2$. The Minas Frescal cheese manufacture (online Supplementary Figure S1) was performed with the pasteurization of the milk at 65 ± 1°C for 30 min and cooled to 38 ± 1°C. Calcium sources were added according to the treatments and mixed for 2 min. In the next step, 0.16 mll^{-1} of lactic acid (10% v/v) and 0.05 mll^{-1} diluted of rennet (used according to the manufacturer's recommendations) were added and mixed for 2 min; coagulation was conducted at 38 ± 2°C for 40 min, after which the curd was cut into 1 cm cubes edge, kept at rest for 2 min, mixed slowly for 20 min and the whey partially drained (26 l; of which 500 ml were collected for further analysis). Then, sodium chloride (NaCl) was added (2.1% w/v considering the 24 l remaining in the tank), mixed for 2 min, and the rest of the whey was removed, and the curd was added to the molds. The remaining whey was drained, and 550- to 600-g cheeses were molded (2 h); the cheeses were kept in the molds until the following day at 5 ± 2°C in a ripening chamber with 85–86% relative humidity, and then they were packed in plastic bags and stored at 5 ± 2°C for 15 d. Three independent repetitions of each treatment were performed. Cheeses samples were subjected to physicochemical analyses, yield and texture profile analyses (TPA) at day 1 of storage, and syneresis analysis at day 1, 3, 5, 7, 9, 11, 13 and 15 of storage.

Physicochemical analyses

Whey and cheese samples were subjected to physicochemical analysis. Moisture, fat, protein, ash, and pH were determined for pasteurized milk and whey samples; moisture, fat in dry matter, protein, ash, pH, water activity (Aw), and syneresis were determined for cheese samples. The moisture content was determined gravimetrically by drying 5 g of samples at 105 ± 2°C until a constant mass was obtained (ISO 5534:2004). Fat content was measured by Gerber-van Gulik method (ISO 3432:2008). Protein was calculated by determination of total nitrogen by the Kjeldahl method, using a conversion factor of 6.38 (ISO 8968-1:2014). The total content of ash was determined gravimetrically by the incineration method at 550°C (IDF 27:1964). The pH of cheese samples was measured by blending 20 g of cheese with 20 ml of distilled water, whereas the pH of pasteurized milk and whey was directly determined on the pH meter (Hanna Instruments Ltd., Leighton Buzzard, UK). An AquaLab (3TE; Decagon Devices Inc., Pullman, WA, USA) was used to measure water activity; the sample cup was filled to half its depth, placed in the sample chamber and the Aw measured using the standard procedure for the instrument.

Yield and syneresis of Minas Frescal cheese

The mass of Minas Frescal cheese (kg) was determined by weighing the samples after packaging. The yield (kgl^{-1}) was calculated based on the amount of cheese samples produced with the 50 L of milk (Fritzen-Freire *et al*., 2010). The syneresis was calculated by Equation 1, where mw (g) is the mass of the whey released from three cheeses in its package during the storage and mc (g) is the mass of each cheese in the package (Sant'Ana *et al*., 2013).

$$\text{Syneresis (\%)} = (\text{mw}/\text{mc}) \times 100 \qquad (1)$$

Texture profile analysis (TPA)

TPA was carried out using the universal testing machine (Instron – Series 3367, Canton, MA, USA, 2005). Compression measurements were performed with a cylinder 53 mm in diameter, exerting a force of up to 250 *N*, with a compression distance of 60% of the initial cube height, for a 2 mm sample. The test speed was 0.8 mm/s with two penetration cycles (Pons and Fiszman, 1996). The force exerted on the sample was automatically recorded and the hardness parameter (*N*) was automatically evaluated from the force (*N*) × time (s) curves generated during the test by the Blue Hill 2.0 software (Instron, USA, 2005). Three samples of each treatment were prepared, and at least 5 measurements were performed in each sample.

Statistical analyses

For statistical analyses, the results were compared considering the treatments by Tukey test ($P < 0.05$) to identify significant differences at 95% of confidence level, using SISVAR® software system version 5.6 (Ferreira, 2011).

Results and discussion

The average composition of the milks used to produce Minas Frescal cheese were 87.700 ± 0.30 g 100 g^{-1} of moisture content, 3.60 ± 0.1 g 100 g^{-1} of fat content, 3.03 ± 0.31 g 100 g^{-1} of protein content, 1.123 ± 0.50 g 100 g^{-1} of ash content and 6.68 ± 0.0 of pH. All values are in accordance with the Brazilian milk quality regulation (Brasil, 2018).

Regarding to the whey obtained during Minas Frescal cheese manufacture, Table 1 shows the composition. No differences were observed between the different treatments for the physicochemical parameters of whey ($P > 0.05$), the parameters of pH and total solids which are all in accordance with the Brazilian whey quality regulation (Brasil, 2020). In addition, the results are in accordance with results reported by Silva *et al.* (2013), who evaluated the effect of whey on the production of pasty dulce de leche. This study was also conducted with the production of Minas Frescal cheese to obtain the whey and the manufacture of the cheese was similar. It is important to emphasize this, since to compare the physical and chemical values of the whey, it is fundamental to observe its origin (from which type of cheese it was obtained). The amount of whey expelled in the cheese vat was not significantly different ($P > 0.05$, data not shown), suggesting that any subtle differences in the protein gel network do not have a large impact.

Table 1 also shows the evolution of physicochemical parameters and Minas Frescal cheese hardness. No differences were observed between the different treatments for the physicochemical parameters ($P > 0.05$), demonstrating the feasibility of partial or total replacement of $CaCl_2$. In addition, all cheeses produced have moisture content values within the legislation (Brasil, 1996, 2004). However, the values for fat content are above, being classified as full fat (fat dry matter 45–59.9 g 100 g^{-1}). Studies have shown that this content is quite variable, only 40% of commercial samples meet this standard, and the values found in this study are within the values reported by Magenis *et al.* (2014) with values for fat in dry matter between 23.12 and 52.47 g 100 g^{-1}. Values for protein and ash content are in line with results reported by Magenis *et al.* (2014) and Oliveira *et al.* (2014). A greater standard variation is due to the quality of the initial milk as well as the type of heat treatment used in this milk, which leads to changes in the physical and chemical values of the cheese (Cichoscki *et al.*, 2002; Guo *et al.*, 2004; Martín-González *et al.*, 2007).

The use of different phosphates sources did not affect ($P > 0.05$) the results for cheese yield (Table 1), which basically depends on the fat and protein, that is, it varies according to the composition of the milk (Guo *et al.*, 2004). Furthermore, the results for yield agree with other studies in the literature (Sant'Ana *et al.*, 2013; Fritzen-Freire *et al.*, 2010). Likewise, the partial or total replacement of $CaCl_2$ by MCP or by its mixture with polyphosphates and MKP did not significantly affect the physicochemical composition and the pH of the cheeses according to the proportions evaluated in this study ($P > 0.05$).

On the other hand, the texture profile of the cheeses determined in term of hardness was statistically affected ($P < 0.05$, Table 1), with higher values in T2 with addition of 0.24 gl^{-1} $CaCl_2$ and T10 with 0.25 gl^{-1} of Blend 2 + 0.12 gl^{-1} of $CaCl_2$. The treatments that used the total or partial replacement of $CaCl_2$ by MCP (T4 and 11, respectively) presented the lowest values, while cheeses containing the mixture of MCP with polyphosphate or MKP (T9 and T10, respectively) demonstrated similar results to the control sample. Thus, what is observed is that the

Table 1. Evaluation of the composition and characterization of the whey and Minas Frescal cheese produced by the partial or total substitution of $CaCl_2$

	Treatment				
	T2	T4	T9	T10	T11
Whey					
Moisture content (g 100 g^{-1})	93.313 ± 0.25^{a}	93.537 ± 0.31^{a}	93.297 ± 0.172^{a}	93.350 ± 0.35^{a}	93.470 ± 0.14^{a}
Fat content (g 100 ml^{-1})	0.4 ± 0.0^{a}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}
Protein content (g 100 ml^{-1})	0.85 ± 0.06^{a}	0.80 ± 0.05^{a}	0.83 ± 0.02^{a}	0.83 ± 0.02^{a}	0.80 ± 0.05^{a}
Ash content (g 100 g^{-1})	0.577 ± 0.05^{a}	0.620 ± 0.10^{a}	0.670 ± 0.10^{a}	0.610 ± 0.09^{a}	0.567 ± 0.10^{a}
pH	6.55 ± 0.2^{a}	6.50 ± 0.1^{a}	6.52 ± 0.1^{a}	6.48 ± 0.2^{a}	6.68 ± 0.0^{a}
Minas Frescal cheese					
Moisture content (g 100 g^{-1})	59.367 ± 1.78^{a}	59.300 ± 1.79^{a}	59.690 ± 1.79^{a}	59.503 ± 2.87^{a}	59.860 ± 2.37^{a}
Fat in dry matter (g 100 g^{-1})	48.5 ± 4.2^{a}	47.2 ± 2.4^{a}	49.0 ± 0.7^{a}	51.3 ± 1.3^{a}	51.7 ± 1.7^{a}
Protein content (g 100 g^{-1})	14.67 ± 0.7^{a}	13.85 ± 1.2^{a}	13.94 ± 1.2^{a}	14.49 ± 1.4^{a}	15.25 ± 0.4^{a}
Ash content (g 100 g^{-1})	3.390 ± 0.87^{a}	3.457 ± 0.85^{a}	3.380 ± 0.31^{a}	2.880 ± 0.26^{a}	2.893 ± 0.14^{a}
pH	6.82 ± 0.1^{a}	6.71 ± 0.2^{a}	6.82 ± 0.1^{a}	6.81 ± 0.2^{a}	6.70 ± 0.1^{a}
Aw	0.989 ± 0.01^{a}	0.991 ± 0.01^{a}	0.985 ± 0.01^{a}	0.991 ± 0.01^{a}	0.990 ± 0.01^{a}
Mass of Minas cheese (kg)	8.613 ± 0.04^{a}	8.640 ± 0.11^{a}	8.390 ± 0.33^{a}	8.527 ± 0.36^{a}	8.330 ± 0.41^{a}
Yield (kgl^{-1})	17.23 ± 0.1^{a}	17.28 ± 0.2^{a}	16.78 ± 0.7^{a}	17.05 ± 0.7^{a}	16.66 ± 0.8^{a}
Hardness (N)	76.443 ± 0.358^{c}	59.026 ± $0.329^{a,b}$	65.457 ± $0.078^{b,c}$	72.983 ± $0.589^{b,c}$	46.549 ± 0.189^{a}

$^{a-b}$Within a line, different superscript lowercase letters denote significant differences ($P < 0.05$) among the samples by Tukey's test. Treatments: (T2) control with addition of 0.24 gl^{-1} $CaCl_2$; (T4) 0.5 gl^{-1} MCP; (T9) 0.25 gl^{-1} Blend 1 + 0.12 gl^{-1} $CaCl_2$; (T10) 0.25 gl^{-1} Blend 2 + 0.12 gl^{-1} $CaCl_2$; and (T11) 0.25 gl^{-1} MCP + 0.12 gl^{-1} $CaCl_2$. Blend 1 is a mixture of MCP with polyphosphate and Blend 2 is a mixture of MCP with MKP.

addition of MCP only in total or partial replacement of CaCl$_2$ did not improve cheese hardness, on the contrary, it reduced it. This behavior was different from what was expected, since, as demonstrated by Guo *et al.* (2003), the presence of monophosphate and calcium in a β-casein solution led to greater precipitation of casein and these precipitates are more resistant than precipitates formed in the presence of calcium alone. We suggest that this precipitate robustness mechanism may be different in the presence of other proteins present in milk.

Other results for the texture profile of cheeses produced with partial or total replacement of CaCl$_2$ were obtained (Table S2). For the gumminess profile, the T11 treatment, with the partial replacement by MCP, presented lower values than the control (T2). On the other hand, T9 and T10 treatments in which the CaCl$_2$ was partially replaced by the blends, presented statistically similar values ($P < 0.05$) to the control. The same behavior was verified for the chewiness profile. The gumminess and chewiness of the cheeses also followed a similar trend to the hardness (Table 1). No significant trend ($P > 0.05$) was observed in cohesiveness and springiness of the samples. Similar results were found by Ong *et al.* (2013) when verifying the effect to different concentrations of CaCl$_2$ on the production of Cheedar cheese. The results obtained for the texture profiles of the cheeses show that the replacement only by MCP of CaCl$_2$, either partially or totally, does not produce cheeses with texture similar to the control, requiring the addition of another source of phosphate for the cheeses to resemble the cheese control. This may also demonstrate the effect of phosphate on the texture of the final product, and not just calcium as observed in other studies (Ong *et al.*, 2013, 2015).

The values obtained for syneresis over the 15 d were also not significantly different ($P > 0.05$, Table 2). However, there was an increase in syneresis with storage time. Sant'Ana *et al.* (2013) also monitored the syneresis of Minas Frescal cheeses produced with milk from different sources, and the authors observed an increase in syneresis over the 21-day storage period, accompanied by a decrease in pH during the same period. The authors pointed out that the increase in syneresis was due to the increase in hydrogen ions and the acidification of the medium, which led to a reduction in repulsive forces of the casein micelles and, consequently, greater aggregation and expulsion of whey from the cheese mass. Another observation is that the T11 that presented the lowest hardness value was the treatment that also presented the highest levels of syneresis from day 1 (Table 2).

Overall, the replacement of CaCl$_2$ by MCP or MCP mixed with polyphosphate or MKP did not affect the general properties of Minas Frescal cheese, indicating that is a sufficient amount of Ca is still present in the milk. Thus, as noted by Ong *et al.* (2013) for the production of cheddar cheese in which various concentrations of CaCl$_2$ addition did not change characteristics such as final cheese composition or yield and there were small variations in cheese hardness as the CaCl$_2$ concentration increased, from 50 mgl^{-1} we also noted an increase in hardness.

Table 3 summarizes the results obtained in this study, which facilitates the industry's understanding of the possibilities of replacing CaCl$_2$ in Minas Frescal cheese according to the characteristics evaluated. This table was built by comparing the control treatment (T2) with the other treatments, showing which characteristics are the same or different. When different, if they are better (>) or worse (<) than the control, according to the results obtained in this study, and in order to summarize these observations. The modulation of cheese hardness is possible according to the industrial objective, without changing the physicochemical characteristics, yield and syneresis of the cheese.

In conclusion, this study shows that it is possible for the industry to manufacture Minas Frescal cheese using other sources of

Table 3. Modulation of Minas Frescal cheese samples produced by the partial or total substitution of CaCl$_2$ by MCP and its blends and compared with the control treatment (T2)

Treatment	T4	T9	T10	T11
Physicochemical	=	=	=	=
Syneresis	=	=	=	=
Yield	=	=	=	=
Hardness	<	=	=	<

Compared to the control treatment (T2): better (>); worse (<); or the same (=). Treatments: (T2) control with addition of 0.24 gl^{-1} CaCl$_2$; (T4) 0.5 gl^{-1} MCP; (T9) 0.25 gl^{-1} Blend 1 + 0.12 gl^{-1} CaCl$_2$; (T10) 0.25 gl^{-1} Blend 2 + 0.12 gl^{-1} CaCl$_2$; and (T11) 0.25 gl^{-1} MCP + 0.12 gl^{-1} CaCl$_2$. Blend 1 is a mixture of MCP with polyphosphate and Blend 2 is a mixture of MCP with MKP.

Table 2. Evaluation of the syneresis of the Minas Frescal cheese produced by the partial or total substitution of CaCl$_2$

	Syneresis (%)							
Treatment	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
T2	0.206 ± 0.218^a	1.012 ± 0.806^a	1.894 ± 1.763^a	2.560 ± 1.570^a	3.234 ± 1.748^a	4.164 ± 1.446^a	4.550 ± 1.574^a	5.273 ± 0.925^a
T4	0.433 ± 0.162^a	1.179 ± 0.548^a	1.870 ± 1.090^a	2.760 ± 0.699^a	3.249 ± 0.899^a	3.793 ± 0.907^a	4.114 ± 1.282^a	4.498 ± 1.069^a
T9	0.463 ± 0.477^a	1.435 ± 1.063^a	2.259 ± 1.772^a	2.935 ± 1.543^a	3.336 ± 1.876^a	4.146 ± 1.849^a	4.298 ± 1.876^a	4.706 ± 1.834^a
T10	0.394 ± 0.525^a	1.226 ± 1.054^a	1.988 ± 1.415^a	3.081 ± 0.955^a	3.759 ± 1.247^a	4.893 ± 1.251^a	5.215 ± 1.648^a	5.760 ± 1.294^a
T11	0.975 ± 0.985^a	3.066 ± 3.788^a	4.502 ± 5.364^a	4.802 ± 5.159^a	5.164 ± 5.153^a	5.634 ± 4.830^a	5.897 ± 4.928^a	6.201 ± 5.126^a

$^{a-b}$Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the samples by Tukey's test. Treatments: (T2) control with addition of 0.24 gl^{-1} CaCl$_2$; (T4) 0.5 gl^{-1} MCP; (T9) 0.25 gl^{-1} Blend 1 + 0.12 gl^{-1} CaCl$_2$; (T10) 0.25 gl^{-1} Blend 2 + 0.12 gl^{-1} CaCl$_2$; and (T11) 0.25 gl^{-1} MCP + 0.12 gl^{-1} CaCl$_2$. Blend 1 is a mixture of MCP with polyphosphate and Blend 2 is a mixture of MCP with MKP.

calcium, such as MCP, without changing its physical chemical characteristics and yield. It is also possible to modulate the hardness of the cheese according to the proportion of MCP or the blends used in this study, without any change in syneresis during storage. Cheeses produced with Blend 2 and $CaCl_2$ (0.25 gl^{-1} of Blend 2 + 0.12 gl^{-1} of $CaCl_2$) showed higher values for hardness, however, further studies are needed to identify which characteristics this cheese presents differently from other, such as soluble calcium content.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029923000316

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