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

Fernanda Lopes da Silva¹, Andressa Fusieger¹, ², Maria Tereza Cratiú Moreira¹, Isabelle Carolina Oliveira³, Ítalo Tuler Perrone⁴, Naaman Francisco Nogueira Silva³, Rodrigo Stephani⁵, Antônio Fernandes de Carvalho¹*

¹ Food Technology Department, Federal University of Viçosa, 36570-900 Viçosa – MG, Brazil.
² Di3A - Dipartimento di Agricoltura, Alimentazione e Ambiente, University of Catania, Catania, Italy.
³ Center for Natural Science, Federal University of São Carlos (UFSCar), 18245 000 Buri – SP, Brazil.
⁴ Pharmacy School, Federal University of Juiz de Fora, 36036-330 Juiz de Fora – MG, Brazil.
⁵ Chemistry Department, Federal University of Juiz de Fora, 36036-330 Juiz de Fora – MG, Brazil.

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* Mailing address: Antônio Fernandes de Carvalho

Food Technology Department
Federal University of
Viçosa – UFV
Campus Universitário,
Av. PH Rolfs, s/n
Campus Universitário, Viçosa
MG, 36570-900
Phone +55-31-3612-6807
E-mail: antoniofernandes@ufv.br
Material and Methods

Methods

Preparation of the gels by enzymatic coagulation

The study of gels was carried out with 50 mL of raw milk, pasteurized at 65 ± 1 ºC for 30 minutes and cooled to 38 ± 1 ºC. For step I, the previous evaluation of the best concentrations of total or partial replacement of CaCl₂, twelve treatments were carried out: (T1) 0.12 gL⁻¹ CaCl₂; (T2) 0.24 gL⁻¹ CaCl₂; (T3) 0.25 gL⁻¹ MCP; (T4) 0.50 gL⁻¹ MCP; (T5) 0.25 gL⁻¹ Blend 1; (T6) 0.50 gL⁻¹ Blend; (T7) 0.25 gL⁻¹ Blend 2; (T8) 0.50 gL⁻¹ Blend; (T9) 0.25 gL⁻¹ Blend 2; (T10) 0.25 gL⁻¹ Blend 1 + 0.12 gL⁻¹ CaCl₂; (T11) 0.25 gL⁻¹ Blend 2 + 0.12 gL⁻¹ CaCl₂; and (T12) only with raw milk. After adding the agents according to the concentration of each treatment, the milk was stirred for 3 minutes to homogenize and 0.16 mLL⁻¹ diluted lactic acid (10% v/v) was added, followed by stirring for another 2 minutes and then the rennet (0.05 mL⁻¹) was added according to the manufacturer’s recommendations, with stirring for an additional 1 minute. After the initial preparation of the cheeses model, they were immediately used for rheological analysis at 38 ± 1 ºC.

Rheological analysis of the gels

Small-amplitude oscillatory measurements were made to monitored the formation of the gels at 38 ºC using a rheometer MCR 301 (Anton Paar, Germany), equipped with thermostatic bath and a stainless steel double gap geometry. The oscillatory mode was employed at a frequency of 1 Hz and 0.1% strain, and the final G’ refers to G’ values attained after 40 minutes of oscillatory measurements. The deformation properties of gels were determined by applying a single constant shear rate (0.01 s⁻¹) up to the yielding of the gel. Yield stress (σ yield) was defined as the point when shear stress started to decrease. Yield strain (γ strain) was the strain value at the yield point.

Results and Discussion

Step I: previous evaluation of the total or partial replacement of CaCl₂ in model cheese
In our first step, twelve treatments were performed in order to evaluate the total or partial replacement of CaCl$_2$, and Table S1 shows the rheological parameters of the value of the elastic modulus (G’) and yield stress (σ stress) obtained during the enzymatic milk coagulation process. Differences were observed in all parameters analyzed among the different treatments (p<0.05). Higher values of G’ and yield stress were found in treatments with twice the concentrations of each agent, being the case of T1 and T2, T3 and T4, T5 and T6, T7 and T8. In relation to the treatments with the same concentration of CaCl$_2$, but with the addition of Blend 1 (T9), or Blend 2 (T10), or MCP (T11), the highest G’ value was T10 with 0.25 gL$^{-1}$ of Blend 2 + 0.12 gL$^{-1}$ of CaCl$_2$; and when we compared these treatments with treatments only with Blend 1, Blend 2 or MCP, a lower G’ value was reported. Although treatments without CaCl$_2$ showed lower values, the incorporation of Blend 1 (T9), Blend 2 (T10) and MCP (T11) with CaCl$_2$ demonstrated the potential in increase the G’ in the gel formation when we compare to T1 with the same CaCl$_2$ concentration. Regarding yield stress, that is a known physical and rheological property defined as the minimum shear stress applied to initiate the flow process, or as the force per unit area required to break the structure (Sun & Gunasekaran, 2009) (Table 1), the same value profile was found. The values found for the G’ were considered as a means of evaluating the gel strength, since G’ is the easiest and most direct way to characterize the gel formation during the coagulation process, the increase of the storage modulus on the clotting time implicates in the formation of the gel network (Hussain et al. 2013; Leite Júnior et al. 2014). Thus, the treatments that presented the highest G’ and σ stress values were chosen to produce Minas Frescal cheese, being the following: T2 (control), T4, T9, T10 and T11.

Calcium has been added to milk as different salts such as calcium carbonate, tricalcium phosphate, calcium chloride, calcium gluconate, and calcium lactate (Vavrusova & Skibsted, 2014). However, for the production of Minas Frescal cheese, the industry has been using CaCl$_2$ in almost 100% of cases, since the easily dissolved in milk and it causes a notable decrease in pH and an increase in free calcium ion (Ca$^{2+}$) concentration. Which helps in both stages of milk clotting, since the first step requires lowering the pH for the hydrolysis of κ-casein and the second phase requires free calcium for the formation of aggregation to occur (Ong et al. 2013; Wang et al. 2020). However, it is important to evaluate other sources of calcium to replace calcium chloride in the production of Minas Frescal cheese, since several studies show that the type of calcium salt added influences
the salt balance of milk and partition of salts between casein micelles and the serum (Wang et al. 2020; Gaucheron, 2015; On-Nom et al. 2010), and consequently, can influence the final properties of cheese.

References


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Figures legends:

Figure S1: Protocol employed for the manufacture of Minas Frescal cheeses T2, T4, T9, T10 and T11. Blend 1 is a mixture of MCP with polyphosphate and Blend 2 is a mixture of MCP with MKP.
Raw cow milk (50 L)

Pasteurization (65 ± 1 °C/30 min)

Cooling (up to 38 ± 1 °C)

Addition of calcium sources and mixing (2 min)
- T2: 0.24 g L⁻¹ CaCl₂
- T4: 0.5 g L⁻¹ MCP
- T9: 0.25 g L⁻¹ Blend 1 + 0.12 g L⁻¹ CaCl₂
- T10: 0.25 g L⁻¹ Blend 2 + 0.12 g L⁻¹ CaCl₂
- T11: 0.25 g L⁻¹ MCP + 0.12 g L⁻¹ CaCl₂

Addition of lactic acid (10% v/v) and rennet and mixing (2 min)

Coagulation step at 38 ± 2 °C for 40 min

Curt cutting and mixing slowly for 20 min

Partial draining of whey

Addition of salt and mixing (2 min)

Whey removing and molding

Packing and storage (5 ± 2 °C)
Table legends:

**Table S1:** Evaluation of the rheological parameters obtained during the enzymatic milk coagulation process (n = 2).

**Table S2:** The effect of CaCl$_2$ substitution on the texture of Minas Frescal cheese (n=3).
Table S1:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (g L⁻¹)</th>
<th>G' (Pa)</th>
<th>Yield stress (σ&lt;sub&gt;yield&lt;/sub&gt;) (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.12 CaCl₂</td>
<td>86.492 ± 1.527</td>
<td>36.968 ± 1.389</td>
</tr>
<tr>
<td>T2</td>
<td>0.24 CaCl₂</td>
<td>98.423 ± 2.994</td>
<td>42.244 ± 3.523</td>
</tr>
<tr>
<td>T3</td>
<td>0.25 MCP</td>
<td>80.808 ± 1.736</td>
<td>33.672 ± 1.150</td>
</tr>
<tr>
<td>T4</td>
<td>0.50 MCP</td>
<td>93.874 ± 3.717</td>
<td>39.496 ± 1.114</td>
</tr>
<tr>
<td>T5</td>
<td>0.25 Blend 1</td>
<td>74.643 ± 1.184</td>
<td>33.167 ± 1.163</td>
</tr>
<tr>
<td>T6</td>
<td>0.50 Blend 1</td>
<td>87.813 ± 0.134</td>
<td>36.801 ± 0.343</td>
</tr>
<tr>
<td>T7</td>
<td>0.25 Blend 2</td>
<td>81.848 ± 0.216</td>
<td>34.448 ± 0.410</td>
</tr>
<tr>
<td>T8</td>
<td>0.50 Blend 2</td>
<td>93.098 ± 0.823</td>
<td>37.347 ± 0.532</td>
</tr>
<tr>
<td>T9</td>
<td>0.25 Blend 1 + 0.12 CaCl₂</td>
<td>93.608 ± 2.379</td>
<td>39.416 ± 0.680</td>
</tr>
<tr>
<td>T10</td>
<td>0.25 Blend 2 + 0.12 CaCl₂</td>
<td>96.927 ± 3.292</td>
<td>40.935 ± 1.187</td>
</tr>
<tr>
<td>T11</td>
<td>0.25 MCP + 0.12 CaCl₂</td>
<td>93.876 ± 1.729</td>
<td>39.582 ± 1.160</td>
</tr>
<tr>
<td>T12</td>
<td>Raw milk</td>
<td>62.383 ± 0.000</td>
<td>25.130 ± 0.000</td>
</tr>
</tbody>
</table>
Table S2:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T2</th>
<th>T4</th>
<th>T9</th>
<th>T10</th>
<th>T11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gumminess</td>
<td>5.859 ± 0.299&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.629 ± 0.377&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.023 ± 0.354&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>5.513 ± 0.423&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>3.503 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chewiness</td>
<td>70.305 ± 3.592&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.556 ± 4.519&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>60.283 ± 4.254&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.155 ± 5.079&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.038 ± 2.773&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Springiness</td>
<td>5.299 ± 0.321&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.552 ± 0.598&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.370 ± 0.625&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.637 ± 0.249&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.363 ± 0.050&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.914 ± 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.923 ± 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.914 ± 0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.913 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.919 ± 0.014&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Within a line, different superscript lowercase letters denote significant differences (P<0.05) among the samples.

Treatments: (T2) control with addition of 0.24 gL<sup>-1</sup> CaCl<sub>2</sub>; (T4) 0.5 gL<sup>-1</sup> MCP; (T9) 0.25 gL<sup>-1</sup> Blend 1 + 0.12 gL<sup>-1</sup> CaCl<sub>2</sub>; (T10) 0.25 gL<sup>-1</sup> Blend 2 + 0.12 gL<sup>-1</sup> CaCl<sub>2</sub>; and (T11) 0.25 gL<sup>-1</sup> MCP + 0.12 gL<sup>-1</sup> CaCl<sub>2</sub>. Blend 1 is a mixture of MCP with polyphosphate and Blend 2 is a mixture of MCP with MKP.