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

Fernanda Lopes da Silva[1], Andressa Fusieger[1, 2], Maria Tereza Cratiú Moreira[1], Isabelle Carolina Oliveira[3], Ítalo Tuler Perrone[4], Naaman Francisco Nogueira Silva[3], Rodrigo Stephani[5], Antônio Fernandes de Carvalho[1]*

[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.

Short title: **Effects of calcium chloride substitution of Minas Frescal cheese**

* 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

Supplementary file

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 $CaCl_2$, twelve treatments were carried out: (T1) 0.12 gL^{-1} $CaCl_2$; (T2) 0.24 gL^{-1} $CaCl_2$; (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 2; (T9) 0.25 gL^{-1} Blend 1 + 0.12 gL^{-1} $CaCl_2$; (T10) 0.25 gL^{-1} Blend 2 + 0.12 gL^{-1} $CaCl_2$; (T11) 0.25 gL^{-1} MCP + 0.12 gL^{-1} $CaCl_2$; 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 minute and then the rennet (0.05 mLL^{-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 $CaCl_2$ 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

Gaucheron F 2005 The minerals of milk *Reprod. Nutr. Dev.* **45** 473-483.

Hussain I, Bell AE & Grandison AS 2013 Mozzarella-type curd made from buffalo, cows', and ultrafiltered cows' milk 1 Rheology and microstructure *Food and Bioprocesse Technology* **6** 1729-1740

Leite Júnior BRC, Tribst AAL & Critianini M 2014 Proteolytic and milk clotting activities of calf rennet processed by high pressure homogenization and the influence on the rheological behavior of the milk coagulation process *Innovative Food Science and Emerging Technology* **21** 44-49

On-Nom N, Grandison AS & Lewis MJ 2010 Measurement of ionic calcium, pH, and soluble divalent cations in milk at high temperature *Journal of Dairy Science* **93** 515-523.

Ong L, Dagastine RR, Kentish SE & Gras SL 2013 The effect of calcium chloride addition on the microstructure and composition of Cheedar cheese *International Dairy Journal* **33** 135-141

Sun A & Gunasekaran S 2009 Yield stress in foods: measurements and applicarions *International Journal of Food Properties* **12**(1) 70-101

Vavrusova M & Skibsted LH 2014 Calcium nutrition. Bioavailability and fortification *LWT – Food Science and Technology* **59** 1198-1204.

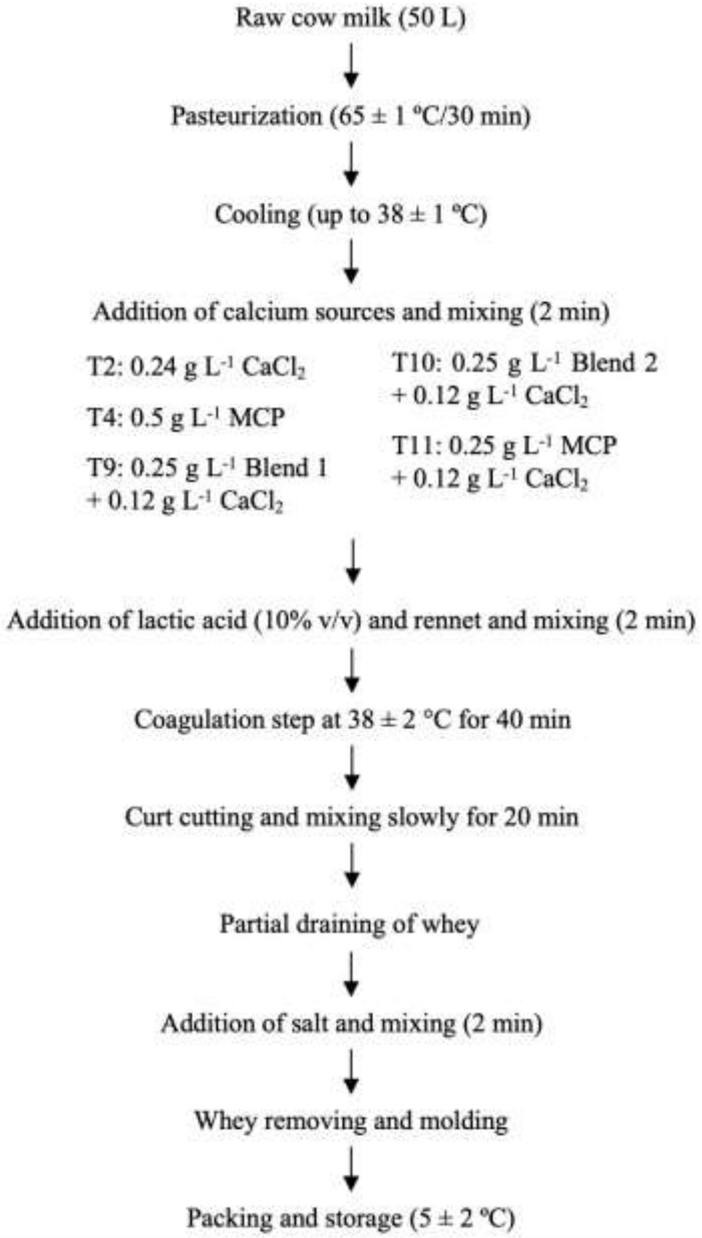
Wang Q, Holt C, Nylander T & Ma Y 2020 Salt partition, ion equilibria, and the structure, composition, and solubility of micellar calcium phosphate in bovine milk with added calcium salts *Journal of Dairy Science* **103** 9893-9905

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.

Figure S1:



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:

Treatment	Concentration (g L^{-1})	G' (Pa)	Yield stress (σ yield) (Pa)
T1	0.12 $CaCl_2$	86.492 ± 1.527 [c,d]	36.968 ± 1.389 [b,c]
T2	0.24 $CaCl_2$	98.423 ± 2.994 [e]	42.244 ± 3.523 [b,c]
T3	0.25 MCP	80.808 ± 1.736 [b,c]	33.672 ± 1.150 [b,c]
T4	0.50 MCP	93.874 ± 3.717 [d,e]	39.496 ± 1.114 [b]
T5	0.25 Blend 1	74.643 ± 1.184 [b]	33.167 ± 1.163 [b,c]
T6	0.50 Blend 1	87.813 ± 0.134 [c,d]	36.801 ± 0.343 [b,c]
T7	0.25 Blend 2	81.848 ± 0.216 [b,c]	34.448 ± 0.410 [b,c]
T8	0.50 Blend 2	93.098 ± 0.823 [d,e]	37.347 ± 0.532 [b,c]
T9	0.25 Blend 1 + 0.12 $CaCl_2$	93.608 ± 2.379 [d,e]	39.416 ± 0.680 [b,c]
T10	0.25 Blend 2 + 0.12 $CaCl_2$	96.927 ± 3.292 [e]	40.935 ± 1.187 [c]
T11	0.25 MCP + 0.12 $CaCl_2$	93.876 ± 1.729 [d,e]	39.582 ± 1.160 [b,c]
T12	Raw milk	62.383 ± 0.000 [a]	25.130 ± 0.000 [a]

Table S2:

	Treatment				
	T2	T4	T9	T10	T11
Gumminess	5.859 ± 0.299[c]	4.629 ± 0.377[b]	5.023 ± 0.354[b,c]	5.513 ± 0.423[b,c]	3.503 ± 0.005[a]
Chewiness	70.305 ± 3.592[b]	55.556 ± 4.519[a,b]	60.283 ± 4.254[b]	66.155 ± 5.079[b]	44.038 ± 2.773[a]
Springiness	5.299 ± 0.321[a]	4.552 ± 0.598[a]	5.370 ± 0.625[a]	5.637 ± 0.249[a]	4.363 ± 0.050[a]
Cohesiveness	0.914 ± 0.006[a]	0.923 ± 0.006[a]	0.914 ± 0.014[a]	0.913 ± 0.004[a]	0.919 ± 0.014[a]

[a-b] 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^{-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.