Food Control 45 (2014) 22–28

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Rheological, physicochemical and authenticity assessment of Minas Frescal cheese

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ARTICLE INFO

Article history:
Received 27 January 2014
Received in revised form
28 March 2014
Accepted 9 April 2014
Available online 18 April 2014

Keywords:
Minas Frescal cheese
Rheology
Color
Electrophoretic profile
Authenticity

ABSTRACT

This study was carried out to assess physicochemical (color, moisture, protein and fat content) and rheological properties (fracture stress, fracture strain, degree of solidity and stress decay), as well as to assess the authenticity (using SDS-PAGE) of Minas Frescal cheese in 21 commercial samples acquired in Southern Brazil. Large differences among the samples, which tended to present a yellowish white color were observed. Moisture and protein influenced on the rheological properties and cheeses showed a tendency to being viscous and firm. SDS-PAGE revealed a wide unexpected variability in their proteins profiles and a densitometric casein/β-lactoglobulin ratio allowed to distinguish authentic cheese from adulterated ones. The profiles suggest the irregular presence of whey in the composition of these products, contrary to the enzymatic process legally required. These results emphasize the need for a more effective control, mainly in the selection of raw material, aiming to offer consumers authentic products.

1. Introduction

The Minas Frescal is a genuine Brazilian cheese and it is the country's most widely consumed cheese type (Planzer et al., 2009). It is produced mainly by small and medium-sized factories in the South and Southeast regions of Brazil. It is a fresh white cheese that has high moisture content, does not undergo maturation, is of short durability and has no preservatives (Souza, Cruz, Moura, Vieira, & Sant'Ana, 2008). This cheese is produced with pasteurized milk through enzymatic coagulation by addition of rennet and/or other appropriate clotting enzymes, whether they are complemented or not by the action of a lactic acid starter culture (Brasil, 1997). According to Brazilian regulation, Minas Frescal cheese can be presented in a cylindrical shape, weighing from 0.3 kg to 5.0 kg (Brasil, 2006). It is officially classified a semi-fat and very high moisture cheese (Brasil, 1997, 2004). In this conventional cheese-making process, the action of the clotting enzymes in milk results in the destabilization of the casein micelles. Therefore, the caseins are retained in the curd and whey proteins are expelled to the aqueous phase (whey) (Chromik, Partschefeld, Jaros, Henle, & Rohm, 2010).

The production process of Minas Frescal cheese is considered relatively simple and the use of traditional equipment and no need for maturation allow producers to have a quick investment return (Furtado, 1999). However, consumers expect cheeses to be reproducible and consistent, with appropriate composition and quality, especially among those belonging to the same category (Fox & Cogan, 2004). Characterization of cheeses is also important for the protection of traditional diversity and for contributing to baseline data for quality control. Physicochemical and rheological methods, as well as the electrophoretic profile, are used to evaluate the quality of cheese (Brighenti, Govindasamy-Lucey, Lim, Nelson, & Lucey, 2008; Foegeding & Drake, 2007; Holland, Yazdi, Titapiccolo, & Corredig, 2010; Pinho, Mendes, Alves, & Ferreira, 2004).

Consumers and inspection authorities have been increasingly concerned about the authenticity of milk and dairy products. Brazilian regulation establishes a standard of identity and quality for

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http://dx.doi.org/10.1016/j.foodcont.2014.04.012
0956-7135/

Minas Frescal cheese; however, that regulation is limited to determine physicochemical characteristics, providing only regulatory limits for moisture (>55g/100 g) and fat in dry matter (between 25.0 and 44.9 g/100 g) (Brasil, 1997). Several studies have contributed to assess the identity profile of the Minas Frescal cheese; however, they were performed on cheeses made at pilot plants (Buriti, Rocha, & Saad, 2005; Fritzen-Freire, Müller, Laurindo, Amboni, & Prudêncio, 2010; Fritzen-Freire, Müller, Laurindo, & Prudêncio, 2010; Piazzon-Gomes, Prudêncio, & Silva, 2010), having insufficient or no studies whatsoever on different brands of commercial Minas Frescal cheese. Therefore, the aim of this study was to investigate the physicochemical and rheological properties, as well as the authenticity of commercial Minas Frescal cheese produced by different factories, aiming to assess the identity and quality parameters of this product.

2. Material and methods

2.1. Minas Frescal cheese samples

A total of 21 samples of commercial Minas Frescal cheese were purchased from different producers of Southern Brazil. According to the label, all samples were made with pasteurized milk, were properly packaged, identified and inspected by sanitary authority and kept under refrigeration in the display cases at supermarkets. The collected samples were transported to the laboratory in an isothermal container and kept under refrigeration until analysis.

2.2. Physicochemical analysis

Samples were analyzed for moisture (g/100 g) by drying to constant weight at 105 °C (AOAC, 2005), fat (g/100 g) by Soxhlet extraction (IAL, 2005) and protein content (g/100 g) by the Kjeldahl method (N × 6.38) (AOAC, 2005). Fat in dry matter (g/100 g) was calculated by Eq. (1) as following:

$$\text{Fat in dry matter}(g/100g) = \text{Fat} \times 100/(100 - \text{Moisture}) \quad (1)$$

All analyses were carried out in triplicate and the chemicals used were of analytical grade.

2.3. Color analysis

The colorimeter Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) adjusted to operate with D65 illuminant and observation angle of 10° was used to evaluate the color of samples of Minas Frescal cheeses. The parameters L* (lightness, ranges 0–100), a* (from green (−a*) to red (+a*)), and b* (from blue (−b*) to yellow (+b*)) were measured using he CIElab color scale. The colorimeter was calibrated with a white standard plate and the measurements were performed in triplicate using the inner section of the cheeses immediately after unpacking.

2.4. Rheological properties

The rheological analyses (uniaxial compression and stress relaxation) were carried out using the TA.XT *plus* Texture Analyzer (Stable Micro Systems Ltd., texture exponent for Windows software, Surrey, UK) fitted with a 50 kg load cell and a 25 mm diameter aluminum probe. The samples were prepared by removing cylindrical pieces from the cheese (19 mm diameter; 20 mm height) which were then kept in refrigeration (5 ± 1 °C) until testing, without addition of any lubricants. All determinations were repeated five times.

2.4.1. Uniaxial compression

The cylindrical cheese samples were compressed to 50% of their height at a cross-head speed of 1 mm/s. The stress (σ) was calculated by Eq. (2), as proposed by Calzada and Peleg (1978).

$$\sigma_{(t)} = F_{(t)} \Big/ A_{(t)} \quad (2)$$

where $\sigma_{(t)}$ is the stress at time (t); $F_{(t)}$ is the force at time (t); $A_{(t)}$ is the area at time (t).

The strain (ε) was also calculated in accordance with Calzada and Peleg (1978), as shown in Eq. (3).

$$\varepsilon = \ln H_0/H_0 - \Delta_H \quad (3)$$

where H_0 is the original height; and ΔH is the change in height.

The fracture stress (σ) and fracture strain (ε) were derived in relation to the fracture point, defined as the local maximum of the stress–strain curve according to Wium and Qvist (1997).

2.4.2. Stress relaxation

The cheese samples were subjected to 10% compression for 10 min from the initial height of the sample for 60 s at a cross-head speed of 1 mm/s. The experimental results for the stress relaxation were normalized and analyzed through the empirical model proposed by Müller, Laurindo, and Yamashita (2009) and Peleg (1980), where $F(t)$ is the force at time (t) and F_0 is the initial force, as shown in Eq. (4):

$$F_{(t)} \Big/ F_0 = 1 - (c_1 \cdot t)/(c_2 + t) \quad (4)$$

The parameters c_1 and c_2 were estimated by non-linear regression using the STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA). In this model, $1 - c_1$ and c_1/c_2 provided information about the viscoelastic characteristics of the material. The value of $1 - c_1$ can be seen as a “degree of solidity”, while the ratio c_1/c_2 represents the initial rate of the stress decay. The “degree of solidity” is associated with the global behavior of the material, thus all the experimental data were considered. In order to obtain a precise estimation of the initial decay rate, the data were adjusted using the first one hundred experimental points from the relaxation curve, without dimension through the ratio $F_{(t)}/F_0$ *versus* time, as suggested by Müller et al. (2009).

2.5. Authenticity assessment

Dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as originally proposed by Laemmli (1970), was used to assess the authenticity of Minas Frescal cheese samples. Cheese samples were freeze-dried (Liotop L101 Lyophilizator, Liobrás, São Paulo, Brazil), diluted with sample buffer and heated to 95 °C for 5 min. A 15% separating gel with 4% stacking gel was used, and aliquots of 10 μL of cheese solution (4 mg/mL) were injected in each well of a vertical gel electrophoresis unit (Bio Rad Mini Protean Tetra Cell, Richmond, CA, USA). Electrophoresis separations were performed at constant voltage (200 V). The gels were stained with Coomassie Brilliant Blue R-250 (0.3% w/v) in 40% (v/v) methanol and 10% (v/v) acetic acid followed by destaining using 40% (v/v) methanol and 10% (v/v) acetic acid. A commercial milk powder (Molico®, Nestlé, Brazil) and molecular weight standards (SDS-PAGE Standards/broad range, Bio-Rad Laboratories, Richmond, CA, USA) containing proteins from 6.5 kDa (aprotinin) to 202 kDa (myosin) were loaded on separate wells. The molecular weights were estimated by comparing the relative mobility of the protein bands to standard proteins. The gels were scanned (Scan jet Hewlett Packard 5590, CA, USA), and then the standards and the proteins bands densities were processed

Table 1
Physicochemical composition and color parameters (L*, a*, b*) of the commercial Minas Frescal cheese samples.

Samples	Moisture (g/100 g)	Fat (g/100 g)	Fat in dry matter (g/100 g)	Protein (g/100 g)	L*	a*	b*
1	58.53 ± 0.27^{j}	15.65 ± 0.37^{i}	$37.73 \pm 0.66^{i,j}$	$19.86 \pm 0.03^{c,d,e}$	$86.59 \pm 0.22^{i,j}$	$-2.95 \pm 0.19^{g,h}$	$15.93 \pm 0.28^{g,h}$
2	60.25 ± 0.01^{h}	$9.19 \pm 0.16^{m,n}$	23.12 ± 0.38^{n}	24.95 ± 0.38^{a}	$87.94 \pm 0.14^{e,f}$	$-2.95 \pm 0.03^{g,h}$	$16.80 \pm 0.09^{e,f}$
3	68.12 ± 0.04^{d}	$14.11 \pm 0.21^{j,k}$	$44.24 \pm 0.59^{d,e,f}$	$13.46 \pm 0.33^{i,j}$	$89.02 \pm 0.19^{c,d}$	$-2.28 \pm 0.13^{b,c,d}$	19.78 ± 0.05^{c}
4	71.58 ± 0.03^{b}	10.70 ± 0.39^{l}	$37.63 \pm 1.33^{i,j}$	$13.81 \pm 0.04^{i,j}$	90.77 ± 0.17^{b}	-0.89 ± 0.04^{a}	$20.38 \pm 0.10^{b,c}$
5	59.48 ± 0.01^{i}	$15.71 \pm 0.05^{h,i}$	$38.76 \pm 0.11^{h,i}$	$20.61 \pm 0.07^{b,c}$	89.52 ± 0.36^{c}	$-2.68 \pm 0.08^{d,e,f,g}$	19.66 ± 0.33^{c}
6	74.49 ± 0.02^{a}	8.08 ± 0.33^{n}	$31.67 \pm 1.25^{l,m}$	12.71 ± 0.35^{j}	92.06 ± 0.07^{a}	$-1.99 \pm 0.05^{b,c}$	13.57 ± 0.28^{i}
7	63.33 ± 0.15^{e}	$15.24 \pm 0.48^{i,j}$	$41.44 \pm 0.99^{f,g,h}$	15.37 ± 0.12^{g}	92.38 ± 0.17^{a}	$-2.59 \pm 0.03^{d,e,f,g}$	$15.49 \pm 0.15^{g,h}$
8	68.43 ± 0.01^{d}	12.91 ± 0.39^{k}	$40.88 \pm 1.22^{g,h}$	$14.07 \pm 0.11^{h,i}$	91.01 ± 0.24^{b}	$-2.41 \pm 0.07^{c,d,e,f}$	$17.31 \pm 0.08^{d,e}$
9	57.78 ± 0.23^{k}	$16.95 \pm 0.10^{g,h}$	$40.15 \pm 0.02^{h,i}$	21.34 ± 0.61^{b}	$87.84 \pm 0.09^{e,f,g}$	$-3.22 \pm 0.08^{h,i}$	18.05 ± 0.15^{d}
10	57.84 ± 0.11^{k}	$9.80 \pm 0.13^{l,m}$	23.23 ± 0.25^{n}	25.91 ± 0.69^{a}	$87.13 \pm 0.53^{g,h,i}$	$-3.59 \pm 0.35^{i,j}$	15.21 ± 0.38^{h}
11	53.05 ± 0.04^{m}	$24.64 \pm 0.40^{a,b}$	52.47 ± 0.81^{a}	18.67 ± 0.11^{e}	86.33 ± 0.23^{j}	$-3.50 \pm 0.41^{i,j}$	$17.31 \pm 0.05^{d,e}$
12	52.46 ± 0.01^{m}	21.66 ± 0.33^{d}	$45.56 \pm 0.68^{d,e}$	$19.69 \pm 0.03^{c,d,e}$	$87.43 \pm 0.23^{f,g,h}$	$-3.55 \pm 0.12^{i,j}$	20.89 ± 0.40^{b}
13	70.26 ± 0.07^{c}	$10.38 \pm 0.01^{l,m}$	$34.91 \pm 0.04^{j,k}$	$12.81 \pm 0.04^{i,j}$	$87.68 \pm 0.18^{f,g}$	-1.92 ± 0.06^{b}	12.51 ± 0.26^{j}
14	51.13 ± 0.28^{n}	25.57 ± 0.49^{a}	$52.32 \pm 0.71^{a,b}$	$19.09 \pm 0.12^{d,e}$	$87.79 \pm 0.17^{e,f,g}$	$-2.84 \pm 0.11^{f,g,h}$	$17.36 \pm 0.20^{d,e}$
15	54.88 ± 0.24^{l}	20.36 ± 0.32^{e}	$45.13 \pm 0.49^{d,e}$	$19.24 \pm 0.23^{d,e}$	$88.43 \pm 0.34^{d,e}$	$-2.80 \pm 0.16^{e,f,g,h}$	$15.50 \pm 0.32^{g,h}$
16	61.25 ± 0.24^{g}	13.05 ± 0.14^{k}	$33.68 \pm 0.15^{k,l}$	$20.69 \pm 0.80^{b,c}$	$88.80 \pm 0.18^{c,d}$	$-2.34 \pm 0.16^{b,c,d,e,}$	13.35 ± 0.02^{i}
17	57.82 ± 0.02^{k}	18.23 ± 0.36^{f}	$43.20 \pm 0.83^{e,f,g}$	$20.05 \pm 0.01^{c,d}$	$88.85 \pm 0.29^{c,d}$	$-2.92 \pm 0.12^{g,h}$	$16.13 \pm 0.19^{f,g}$
18	62.18 ± 0.05^{f}	$17.64 \pm 0.40^{f,g}$	$46.64 \pm 0.98^{c,d}$	$15.21 \pm 0.08^{g,h}$	89.38 ± 0.17^{c}	$-3.18 \pm 0.04^{h,i}$	$16.74 \pm 0.23^{e,f}$
19	55.05 ± 0.04^{l}	$22.27 \pm 0.36^{c,d}$	$49.53 \pm 0.35^{b,c}$	16.79 ± 0.36^{f}	$87.77 \pm 0.07^{e,f,g}$	-3.78 ± 0.04^{j}	21.97 ± 0.41^{a}
20	50.07 ± 0.16^{o}	$23.44 \pm 036^{b,c}$	$46.93 \pm 0.57^{c,d}$	21.36 ± 0.13^{b}	$86.73 \pm 0.12^{h,i,j}$	$-3.20 \pm 0.09^{h,i}$	20.66 ± 0.13^{b}
21	63.03 ± 0.30^{e}	$10.92 \pm 0,20^{l}$	29.53 ± 0.30^{m}	21.81 ± 0.09^{b}	$88.98 \pm 0.29^{c,d}$	$-2.90 \pm 0.06^{g,h}$	11.59 ± 0.47^{k}

Results expressed as mean ± standard deviation ($n = 3$).
[a–o] Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the samples.

using the ImageJ image analysis software v1.46r (NIH, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/). Quantitative determination of casein and β-lactoglubulin (β-Lb) was made by peak area integration of densitometer traces and the ratio of these areas (caseins/β-Lb) was calculated. These analyses were done in triplicate.

2.6. Statistical analysis

The significance of the differences between the means of the samples was determined by one-way analysis of variance (ANOVA) followed by Tukey's test. Linear correlation from regression analysis was performed to verify the relationship between the analyses. Differences were considered statistically significant when $P < 0.05$. All statistical analyses were performed using STATISTICA 7.0 (Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Physicochemical analysis

A wide variation in the results for moisture, fat, fat in dry matter and protein contents among the different brands of commercial Minas Frescal cheese is shown in Table 1. Cichoscki, Valduga, Valduga, Tornadijo, and Fresno (2002) reported that, besides the processing technologies used, the composition of raw milk is among the major factors that determine the quality of the cheese. Guo, Park, Dixon, Gilmore, and Kindstedt (2004) and Martín-González et al. (2007) also confirm that the milk treatment prior to the cheese-making directly affects the composition of cheeses. It was possible to note that most of the cheese samples showed moisture contents higher than 55 g/100 g, as established by official

Table 2
Results of rheological parameters of uniaxial compression and relaxation of commercial Minas Frescal cheese samples.

Samples	Fracture stress (σ) (kPa)	Fracture strain (ε) (−)	Degree of solidity ($1 - c_1$) (−)	Stress decay rate (c_1/c_2) (s^{-1})
1	$86.99 \pm 2.48^{c,d}$	0.69 ± 0.00^{a}	0.416 ± 0.017^{c}	$0.663 \pm 0.033^{d,e,f}$
2	140.01 ± 2.56^{a}	0.69 ± 0.00^{a}	0.415 ± 0.010^{c}	$0.697 \pm 0.047^{d,e}$
3	$66.94 \pm 4.37^{f,g}$	$0.53 \pm 0.01^{c,d}$	$0.503 \pm 0.013^{a,b}$	$0.888 \pm 0.024^{a,b}$
4	$65.47 \pm 2.55^{f,g}$	0.57 ± 0.05^{a}	0.509 ± 0.007^{a}	$0.693 \pm 0.037^{d,e}$
5	$89.55 \pm 7.59^{c,d}$	0.67 ± 0.02^{a}	$0.493 \pm 0.009^{a,b}$	$0.871 \pm 0.040^{a,b,c}$
6	$69.82 \pm 3,76^{f,g}$	$0.57 \pm 0.04^{b,c}$	0.507 ± 0.016^{a}	$0.723 \pm 0.055^{d,e}$
7	96.62 ± 5.50^{c}	0.51 ± 0.05^{d}	0.517 ± 0.008^{a}	$0.760 \pm 0.026^{b,c,d,e}$
8	48.06 ± 3.20^{i}	$0.57 \pm 0.04^{b,c}$	$0.495 \pm 0.016^{a,b}$	$0.701 \pm 0.085^{d,e}$
9	$86.21 \pm 2.65^{c,d,e}$	0.69 ± 0.00^{a}	$0.392 \pm 0.022^{c,d}$	$0.728 \pm 0.040^{d,e}$
10	135.26 ± 6.91^{a}	0.69 ± 0.00^{a}	$0.384 \pm 0.008^{c,d,e}$	$0.682 \pm 0.053^{d,e}$
11	119.38 ± 6.25^{b}	0.69 ± 0.00^{a}	0.470 ± 0.014^{b}	0.937 ± 0.017^{a}
12	$86.99 \pm 5.76^{c,d}$	0.69 ± 0.00^{a}	$0.332 \pm 0.010^{f,g}$	$0.878 \pm 0.082^{a,b,c}$
13	$82.00 \pm 2.57^{d,e}$	$0.54 \pm 0.02^{b,c,d}$	0.507 ± 0.011^{a}	$0.715 \pm 0.044^{d,e}$
14	$61.04 \pm 3.32^{g,h}$	0.69 ± 0.00^{a}	0.336 ± 0.008^{f}	$0.863 \pm 0.046^{a,b,c}$
15	$51.77 \pm 1.92^{h,i}$	0.69 ± 0.00^{a}	0.334 ± 0.008^{f}	$0.771 \pm 0.086^{b,c,d}$
16	42.94 ± 0.12^{i}	0.69 ± 0.00^{a}	0.297 ± 0.006^{g}	$0.875 \pm 0.022^{a,b,c}$
17	$75.26 \pm 1.99^{e,f}$	0.69 ± 0.00^{a}	$0.364 \pm 0.014^{d,e,f}$	$0.718 \pm 0.047^{d,e}$
18	$67.60 \pm 2.18^{f,g}$	0.59 ± 0.03^{b}	$0.494 \pm 0.015^{a,b}$	$0.755 \pm 0.056^{c,d,e}$
19	$66.93 \pm 4.46^{f,g}$	0.69 ± 0.00^{a}	$0.376 \pm 0.015^{d,e}$	0.534 ± 0.032^{f}
20	123.74 ± 6.84^{b}	0.69 ± 0.00^{a}	$0.355 \pm 0.018^{e,f}$	$0.761 \pm 0.053^{b,c,d,e}$
21	42.52 ± 1.78^{i}	0.69 ± 0.00^{a}	$0.376 \pm 0.015^{d,e}$	$0.640 \pm 0.032^{e,f}$

Results expressed as mean ± standard deviation ($n = 3$).
[a–i] Within a column, different superscript lowercase letters denote significant differences ($P < 0.05$) among the samples.

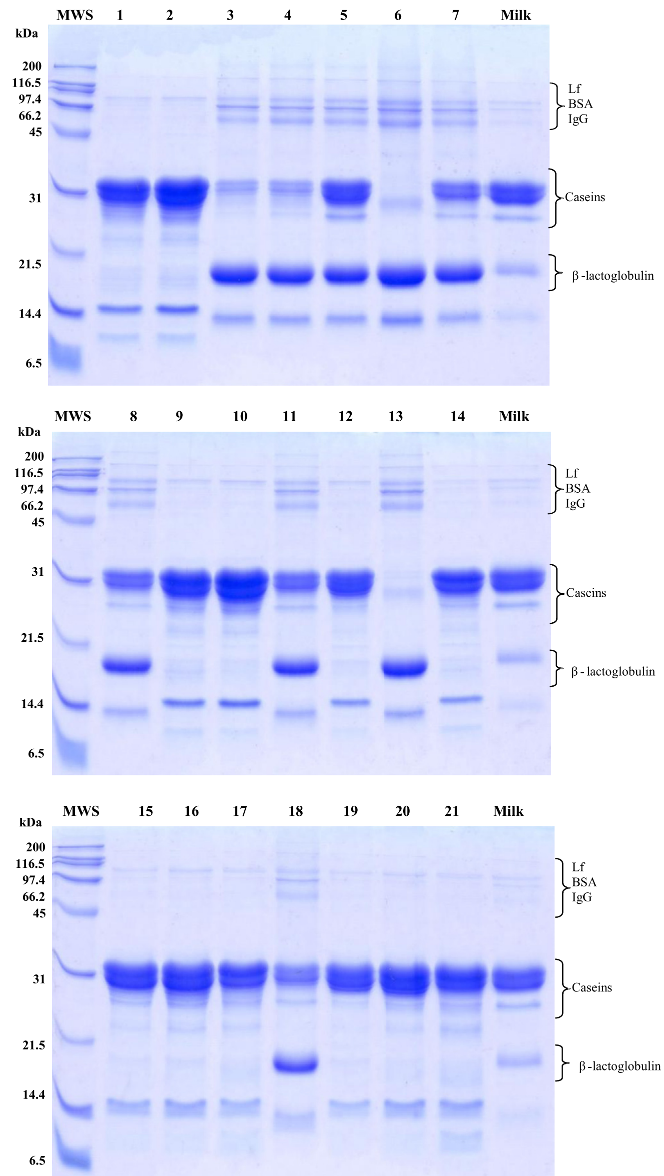

Fig. 1. SDS–PAGE electropherograms of proteins from molecular weight standards (MWS), commercial Minas Frescal cheese samples (1 up to 21) and milk. Lf: lactoferrin, BSA: Bovine Serum Albumin and IgG: Immunoglobulin G.

regulation in Brazil (Brasil, 2004). Regarding fat content, Cruz and Gomes (2001) also noted similar variation in commercial Minas Frescal cheese from different brands. However, the fat content determined in the present study for some samples was lower than that verified by Fritzen-Freire, Müller, Laurindo, et al. (2010) and Piazzon-Gomes et al. (2010), who obtained values for Minas Frescal cheese equal to 18.1 g/100 g and between 15.8 and 16.2 g/100 g, respectively. In this study, some samples showed contents of fat in dry matter higher or lower than those established by Brazilian official regulation, i.e., between 25.0 and 44.9 g/100 g. These inconsistencies could be correlated ($P < 0.05$) with the moisture content ($R = -0.457$) and strongly correlated ($P < 0.05$) with the fat content ($R = 0.886$). In relation to protein content, it was noted that their values were similar or higher than those determined by Buriti et al. (2005) (11.22–11.86 g/100 g), Fritzen-Freire, Müller, Laurindo, et al. (2010) (13.44 g/100 g) and Piazzon-Gomes et al. (2010) (14.98 g/100 g). A correlation ($P < 0.05$) was also noted between the contents of moisture and protein ($R = -0.649$). Guinee, O'Kennedy, and Kelly (2006) noted that the level of cheese moisture decreased significantly upon increasing protein level. Since those authors used one definite manufacturing process and only varied the protein level, it's difficult to compare these conditions with the wide differences in manufacture and composition of the commercial samples assessed in this work. Due to the low correlation coefficients found in this study, along with the wide variation in physicochemical results, it can be concluded that processing parameters shall be quite different for the evaluated products.

3.2. Color analysis

Color parameters are extremely important in food science, because they are directly responsible for product appeal and consumer acceptability. However, data referring to Minas Frescal commercial samples L*a*b* color assessment is inexistent. In the present work, it was possible to note relevant differences in the L*, a* and b* parameters among the samples (Table 1). L* parameter indicates lightness and the capacity of an object to either reflect or transmit light. The present results for lightness were lower than those obtained by Fritzen-Freire, Müller, Laurindo, Amboni, et al. (2010) in Minas Frescal cheeses made with probiotics and direct acidification.

All the samples showed a low a* value, indicating a tendency to the green color. These results occurred probably because of the presence of riboflavin, as cited by Mestdagh, Kerkaert, Cucu, and De Meulenaer (2011). In relation to the b* parameter, all the cheese samples showed positive values, indicating a tendency towards yellow, Finally, it was possible to verify in this work that the cheeses showed a yellowish white color, which mostly contributed to the color characteristics of the cheeses, due to the predominance of yellow rather than green. Unfortunately, correlation coefficients between L*, a* and b* and cheeses' chemical compositional were very low. Since Brazilian regulation does not make any mention about instrumental color parameters, a lack of standardization in Minas Frescal processing seems to be evident, and additional research focusing on instrumental color assessment shall be encouraged to obtain clear data.

3.3. Rheological properties

The rheological properties of cheese are important as a means of determining its body and texture for quality and identity, as well as a means of studying its structure according to its composition and the processing techniques used (Konstance & Holsinger, 1992). In the uniaxial compression tests, a constant rate of compression is applied to the material at large deformations to determine fracture properties (Pappa, Kandarakis, & Mallatou, 2007). In stress relaxation tests, a constant strain is applied and the stress required to maintain the deformation is measured as a function of time (Del Nobile, Chillo, Mentana, & Baiano, 2007). The rheological data of uniaxial compression (fracture stress (σ) and fracture strain (ε)), as well as the stress relaxation data (degree of solidity ($1 - c_1$) and the stress decay (c_1/c_2)), are shown in Table 2. For each parameter evaluated, it was possible to note a large variation between the samples.

Fracture stress (σ) is a point of maximum stress, where the material shows macroscopic failure (Watkinson et al., 1997) and a high numerical value indicates toughness (Juan, Trujillo, Guamis, Buffa, & Ferragut, 2007). Based on the data presented in this work, it can be concluded that the higher numerical values indicate a harder and less breakable material, requiring more strength to fracture it. O'Callaghan and Guinee (2004) confirm that this parameter is related with the strength of cheese matrix,

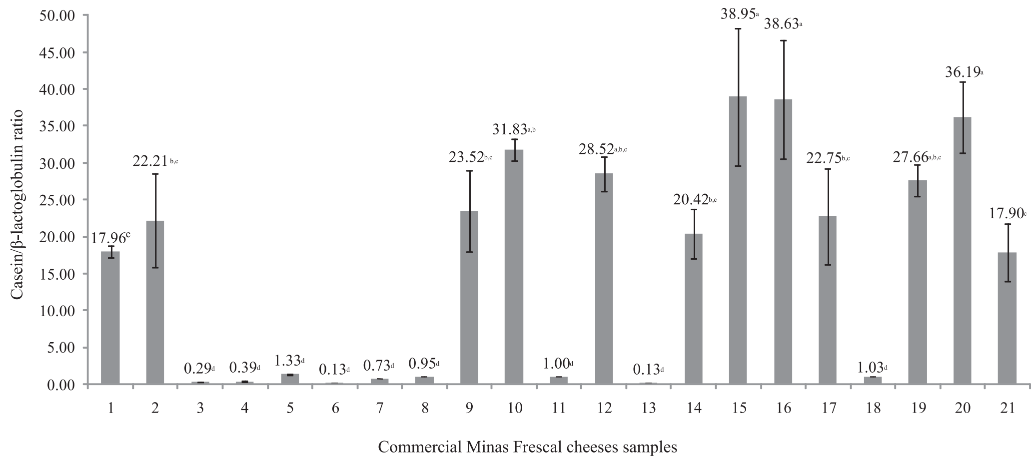

Fig. 2. Results of the average ± standard deviation of the casein/β-lactoglobulin ratio from densitometry analysis ($n = 3$). a–d Different superscript lowercase letters denote significant differences ($P < 0.05$) among the samples.

while Fox Guinee, Cogan, and Mcsweeney (2000) state that this behavior is directly related with cheeses that have greater hardness.

The fracture strain (ε) is the point which describes the deformability of cheese (Juan et al., 2007). The ε values indicated correlations with protein ($R = 0.831$) and moisture ($R = -0.791$) contents. The cheese samples with lower moisture and higher protein contents showed resistance to fracture and these results are in agreement with those noted in the σ parameter. Creamer and Olson (1982) reported that the fracture strain in cheese is related to the loss of elastic structural elements when the water content available for the solvation of protein is reduced. According to Juan et al. (2007) and O'Callaghan and Guinee (2004), higher numerical values to ε indicate greater deformability, since the ε parameter measures the deformation that occurs before the cheese ruptures. The higher numerical values obtained in this study, when compared with those obtained by Fritzen-Freire, Müller, Laurindo, et al. (2010), indicate less deformable cheeses. The deformability may be related to the chemical structures of cheese components and also by small variations in the processing technology (Ferrandini, López, Castillo, & Laencina, 2011). Such variations may occur especially when cheese is produced by different manufacturers, as observed in this work.

Lucey, Johnson, and Horne (2003) reported that cheese is a viscoelastic material because, during (and after) deformation, part of the mechanical energy supplied is stored in the material (elastic part) and part is dissipated (viscous part). According to the model proposed by Peleg (1980), when $t \to \infty$, $F_{(t)}/F_0 \to 1 - c_1$. Thus, if the stress of the specimen fully relaxes, then, $c_1 = 1$ and $1 - c_1 = 0$. When $(1 - c_1)$ reaches 1, the material behaves as an elastic solid; and when $(1 - c_1)$ reaches 0, the material behavior is controlled by the viscous component (Müller et al., 2009). Therefore, from the data obtained for the 'degree of solidity', it was possible to verify that all the analyzed samples were characterized by a tendency to be more viscous than elastic. Moreover, a moderate degree of correlation ($P < 0.05$) with the moisture ($R = 0.710$) and protein ($R = -0.661$) contents could also be verified. According to Fox et al. (2000), the whey present among the casein micelles acts as lubricant, and thus the cheese samples with higher moisture and lower protein contents result in a net with more viscous characteristics.

An accurate estimation of c_2 is important to determine the rate of stress decay (c_1/c_2). The values of c_1/c_2 for the investigated samples were higher ($P < 0.05$) than those obtained by Fritzen-Freire, Müller, Laurindo, et al. (2010) in Minas Frescal cheese. In the present study, all samples showed low mechanical resistance and, therefore, had less elasticity.

3.4. Authenticity assessment

Fig. 1 shows the electrophoretic profiles of the studied samples. The identification of proteins may be performed by comparing the electrophoretic mobility of the milk proteins and the molecular weight standard used, as well as by comparing electropherograms from previous studies (Hernández & Harte, 2009; Holland et al., 2010; Jovanovic, Barac, Macej, Vucic, & Lacnjevac, 2007). In the present study, a good resolution was obtained, with a clear separation of proteins by SDS-PAGE. Although samples were all labeled as being the same product, electropherograms revealed a wide unexpected variability in their proteins profile, with both caseins (CN) and β-lactoglobulin (β-Lb) being present in some samples. Thus, as a way to assess which proteins prevail, a CN/β-Lb ratio was established from the densitometric analysis of the respective bands. These calculations allowed to divide samples into two significantly different groups ($P < 0.05$): the first ($n = 12$), wherein the CN/β-Lb ratio is always greater than 17.9, and the second ($n = 9$), wherein this ratio is close to zero (Fig. 2). In the first group, bands corresponding to caseins appear intensively stained, as expected in authentic cheeses. On the other hand, it can be seen, in the second group, that β-Lb is predominant in their electropherograms, while caseins appear in low concentration. Moreover, bands corresponding to lactoferrin (Lf), bovine serum albumin (BSA) and immunoglobulin G (IgG) appear quite stained in this group, while in the other samples they are practically inexistent. These facts suggest the presence of whey in the composition of these products, contrary to the enzymatic process required for manufacturing of Minas cheese.

The CN/β-Lb ratios showed a strong correlation ($P < 0.05$) with the degree of solidity $(1 - c_1)$ ($R = -0.942$), confirming the tendency to being more viscous shown in the cheese samples with higher casein content. A viscous behavior was also noted by Dahbi, Alexander, Trappe, Dhont, and Schurtenberger (2010) for concentrated industrial suspension with high casein content.

A correlation ($P < 0.05$) was also verified between the CN/β-Lb ratio and fracture strain values ($R = 0.767$). Therefore, higher casein content could be responsible for greater cheese deformability. As suggested by Fox et al. (2000), the concentration of casein in the cheese matrix increases, the intra- and inter-strand linkages become more numerous, and the matrix shows a decrease in the viscous component, resulting in an easily deformable structure.

Several reports demonstrate great variation in sanitary and sensorial quality in commercial samples of Minas Frescal cheese (Nogueira, Lubachevsky, & Rankin, 2005; Rocha, Buriti, & Saad, 2006; Souza et al., 2008; Visotto, Oliveira, Prado, & Bergamini., 2011). According to Brighenti et al. (2008), variations can be expected due to differences in the chemical composition of the raw material or in some manufacturing parameters adopted by the plants. Although these variations have been reported, this is the first study that focuses on authenticity and rheological aspects of commercial samples of Minas Frescal cheese. Furthermore, the correlations between the parameters evaluated in this study indicate that there is a lack of standardization among manufacturers, probably due to the use of unallowed raw material, which may severely compromise the identity of this traditional product.

4. Conclusions

The results from the physicochemical, color, rheological and electrophoretic profile showed that the commercial Minas Frescal cheeses were different, indicating heterogeneity among the different brands available on the market. A greater tendency toward a yellowish white color was noted in the cheese samples. Among the evaluated parameters, the moisture and protein contents affected the rheological properties of the cheeses, which showed a tendency to a matrix with more viscous and firmer characteristics. The SDS-PAGE analysis showed a wide unexpected variation in the type of proteins, with casein bands prevailing in some samples, while β-Lb prevailed in others. This wide variation was confirmed in the results of the CN/β-Lb ratio and it was noted that authentic Minas Frescal samples presented higher CN/β-Lb ratios and showed a decrease in the degree of lightness, a viscous behavior, and a greater deformability. These results emphasize the need for a more effective control, mainly in the selection of raw material, aiming to offer consumers authentic products.

Acknowledgments

The authors acknowledge financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) and Ministério da Agricultura, Pecuária e Abastecimento (MAPA).

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