Manufacture of probiotic Minas Frescal cheese with *Lactobacillus casei* Zhang

Aline B. Dantas,* Vitor F. Jesus,* Ramon Silva,† Carine N. Almada,‡ E. A. Esmerino,‡ Leandro P. Cappato,§ Marcia C. Silva,† Renata S. L. Raices,† Rodrigo N. Cavalcanti,‡ Celio C. Carvalho,* Anderson S. Sant’Ana,‡ Helena M. A. Bolini,‡ Monica Q. Freitas,# and Adriano G. Cruz^{†1}

*Faculdade de Tecnologia Termomecânica, Curso de Tecnologia de Alimentos, São Bernardo do Campo, 09611-900, São Paulo, Brazil

‡Faculdade de Engenharia de Alimentos (FEA), Universidade Estadual de Campinas (UNICAMP), Cidade Universitária Zeferino Vaz, Campinas, 13083-862, São Paulo, Brazil

†Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Equipe de Alimentos (PGCTA), Rua Senador Furtado, 171, Maracanã, 20270-021 Rio de Janeiro, Brazil

§Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, BR 465 km 7 Seropédica, 23890-000, Rio de Janeiro, Brazil

#Faculdade de Medicina Veterinária, Universidade Federal Fluminense (UFF), Niterói, 24230–340, Rio de Janeiro, Brazil

ABSTRACT

In this study, the addition of *Lactobacillus casei* Zhang in the manufacture of Minas Frescal cheese was investigated. Minas Frescal cheeses supplemented with probiotic bacteria (*Lactobacillus casei* Zhang) were produced by enzymatic coagulation and direct acidification and were subjected to physicochemical (pH, proteolysis, lactic acid, and acetic acid), microbiological (probiotic and lactic bacteria counts), and rheological analyses (uniaxial compression and creep test), instrumental color determination (luminosity, yellow intensity, and red intensity) and sensory acceptance test. The addition of *L. casei* Zhang resulted in low pH values and high proteolysis indexes during storage (from 5.38 to 4.94 and 0.470 to 0.702, respectively). Additionally, the cheese protocol was not a hurdle for growth of *L. casei* Zhang, as the population reached 8.16 and 9.02 log cfu/g by means of the direct acidification and enzymatic coagulation protocol, respectively, after 21 d of refrigerated storage. The rheology data showed that all samples presented a more viscous-like behavior, which rigidity tended to decrease during storage and lower luminosity values were also observed. Increased consumer acceptance was observed for the control sample produced by direct acidification (7.8), whereas the cheeses containing *L. casei* Zhang presented lower values for all sensory attributes, especially flavor and overall liking (5.37 and 4.61 for enzymatic coagulation and 5.57 and 4.72 for direct acidification, respectively). Overall, the addition of *L. casei* Zhang led to changes

in all parameters and affected negatively the sensory acceptance. The optimization of *L. casei* Zhang dosage during the manufacturing of probiotic Minas Frescal cheese should be performed.

Key words: probiotic cheese, *Lactobacillus casei* Zhang, rheology, optical analysis

INTRODUCTION

The growing demand for functional foods has driven the dairy market in Brazil and the world, in particular with respect to probiotic dairy products. The diversity of products offered is the result of efforts in research, development, and marketing, especially in the producing units (Saba et al., 2010). In this context, probiotic cheese plays an important role in the functional food industry (Granato et al., 2010), evident in the contribution of the cheese industry for the processing of healthy dairy products.

The globalization of the economy has allowed probiotic cultures of different origins to become commercially available throughout the world, resulting in probiotic food products with distinct characteristics. *Lactobacillus casei* Zhang, for example, was isolated from koumiss, a traditional fermented milk consumed in China’s Inner Mongolia Autonomous Region; it presented good performance in preliminary tests, including low acid resistance, ability to grow in de Man, Rogosa, Sharpe (MRS) agar with bile salts, antimicrobial properties (Guo et al., 2009), and preventive and ameliorative effects on induced glucose tolerance in hyperinsulinemia rats (Zhang et al., 2014a,b). Additional studies have shown good survival of this strain during cold storage of dairy foods, soy and bovine milk (Wang et al., 2009), yogurt (Wang et al., 2010a), and Cheddar cheese, which exhibited an angiotensin-converting enzyme (ACE)-

Received May 28, 2015.

Accepted September 6, 2015.

¹Corresponding author: food@globom.com

inhibitory activity (Wang et al., 2010b), suggesting this cheese may be an adequate vehicle for probiotics in the treatment of hypertension. With respect to the health benefits, *L. casei* Zhang has demonstrated antioxidative effects on hyperlipidemic rats (Zhang et al., 2010) and immunogenicity against *Cryptosporidium parvum* surface adhesion protein P23 in mice (Geriletu et al., 2011), as well as changes in the composition and diversity of the intestinal microbiota of humans and rats (Kwok et al., 2014; Zhang et al., 2014a,b).

Cheese manufacture is one of the most important activities of the dairy industry, especially in Brazil. Minas Frescal cheese is considered one of the most popular cheeses in the country, being the third most consumed cheese by all levels of population, which is attractive because of its high yield (Lollo et al., 2012, 2015). It is a fresh cheese with high moisture (46–55%) that should be consumed in the first 15 d after its manufacture, as it is highly perishable even under refrigeration (Souza et al., 2008).

Supplementation of Minas Frescal cheese with *L. casei* Zhang can be an interesting tool to provide a differentiated product to consumers presenting potential benefits to the health. This research aimed to investigate the effect of addition of *L. casei* Zhang on the physicochemical, optical, and rheological characteristics as well as the sensory acceptance of Minas Frescal cheese.

MATERIALS AND METHODS

Experimental Design

Four different cheese formulations were manufactured: cheese produced by direct acidification, without addition of starter culture, and probiotics (**CI**); cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures (**CII**); cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL (**PROB I**); and cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang at 6.8 and 8.1 log cfu/mL, respectively (**PROB II**). *Lactobacillus casei* Zhang was obtained through personal donation in direct vat-set culture, being cultivated in MRS agar at 37°C (Difco, São Paulo, Brazil).

The treatments were chosen according to all possible technologies adopted in the production of Minas Frescal cheese. Although processing using mesophilic cultures is predominant, many producing units have adopted the direct acidification process, which has a positive effect on the texture of the product and increases the

process yield (Fritzen-Freire et al., 2010b), although there is an increased risk of microbial growth due to higher moisture content of the final product (Carvalho et al., 2007).

Probiotic Cheese Processing

The cheese processing was performed in accordance with the methods described previously (Tamime, 2006), with modifications. One hundred liters of raw milk with 3.0% (wt/wt) fat (Faculdade de Tecnologia Termomecânica, São Bernardo, São Paulo, Brazil) was pasteurized at 72°C for 15 s (model pro110, Arpifrio, São Paulo, Brazil), cooled to 37°C, and equally divided into 4 portions of 25 L each. Calcium chloride (Labsynth, São Paulo, Brazil) was then added at a concentration of 0.2% (wt/vol). Lactic acid (0.75 and 0.25 mL/L, 85%, food grade) was added to formulations CI and PROB I, respectively, whereas the freeze-dried culture *Lactococcus* R-704 (Chr. Hansen) and *Lactobacillus casei* Zhang were added to the formulations CII and PROB II. Starter and probiotic cultures for direct vat inoculation were used and manual agitation was performed for about 5 min. Rennet powder (Halamix power, Chr. Hansen) was added to the all cheeses (CI, CII, PROB I, and PROB II) at 3 g/L milk, and the mixture was homogenized for 2 min.

All formulations were kept at 37°C for 30 to 40 min to coagulate. The curd was cut, the whey was removed, and the remaining curd was placed in 250-g plastic molds. Cheeses were dry salted (0.8% wt/vol NaCl, Labsynth), vacuum packaged, and stored in cold chambers at 5°C for 20 d.

Physicochemical and Microbiological Analyses

The physicochemical (pH, proteolysis, lactic acid, and acetic acid contents) and microbiological analysis (starter and probiotic counts) were carried out on the d 1, 11, and 20 after manufacture, whereas the nutrient composition (TS, protein, and fat) was determined on d 1 and 20. The manufacturing process was repeated twice and the analyses were performed in triplicate. Analytical methodologies for physicochemical and microbiological determinations were carried out in triplicate.

The pH values were determined by inserting the electrode (Digimed, Piracicaba, Brazil) directly into the samples, whereas proteolysis was determined by incubating with a reactive solution (*o*-phthalaldehyde reagent, OPA) containing SDS, sodium tetraborate decahydrate, dithiothreitol, *o*-phthalaldehyde, and ethanol, quantifying the AA and peptides liberated

by the lactic cultures from the absorbance of the OPA derivatives at 340 nm.

Water-soluble extracts of cheeses were prepared according to Meira et al. (2012), with modifications aiming to quantify lactic and acetic acids. Ten grams of cheese was suspended in 90 mL of distilled water, homogenized under gentle stirring (150 rpm) at 4°C. After centrifugation, the upper fat layer was discarded and the water extract was filtered through Whatman no. 2 paper (Sigma-Aldrich, São Paulo, Brazil). The lactic and acetic acids were quantified by HPLC using an Aminex X-87H column (300 × 7.8 mm) and a guard column containing disposable H⁺ cartridges (Bio-Rad Laboratories, Richmond, CA) at 65°C. The mobile phase was sulfuric acid (0.009 mol/L), previously prepared by diluting with ultra-pure water (Milli-Q system, Millipore Corporation, Billerica, MA), and subsequently filtering and degassing through a 0.45-mm membrane filter (Millipore, Merck, São Paulo, Brazil). The flow rate was 0.6 mL/min and a UV-VIS detector (Bio-Rad Laboratories) was used at 220 nm. Standard curves were prepared with solutions of known concentration, and the injection volume was 25 µL, injected using an automatic injector. The chromatographic peaks were integrated using the Millennium software (Scientific Equipment Source, Oshawa, Canada). Members of the *Lactobacillus casei* group, such as *L. casei* Zhang, produce acetic acid during glycolysis (Hosseini Nezhad et al., 2015); thus, it may be of interest to evaluate the production of acetic acid throughout probiotic Minas Frescal cheese storage.

The gross composition (% wt/wt of TS, fat, and protein content) were determined using traditional methods. Moisture was determined by drying 5-g aliquots of the samples at 100 to 105°C for 24 h. Fat was quantified using the Gerber butyrometer method and protein by the Kjeldahl method (AOAC International, 2002).

Regarding the microbiological analysis, the following procedure was followed (Gomes et al., 2011). First, the samples were prepared in a stomacher by adding 25 g of cheese to 225 mL of sterile 0.1% wt/vol peptone water (Oxoid, São Paulo, Brazil) from which further dilutions were prepared. *Lactococcus* R-704 counts were then performed using the pour plate technique, using M17 agar (Oxoid) with incubation at 30°C for 72 h, whereas *Lactobacillus casei* Zhang was enumerated using MRS-vancomycin agar (Oxoid) at anaerobic incubation at 37°C for 72 h (Wang et al., 2010b).

Rheological Analysis

The rheological behavior of 4 different formulations of Minas Frescal cheese (CI, CII, PROB I, and PROB

II) was evaluated at 1 and 20 d of refrigerated storage. The rheological analyses were carried out using the TA-XT21 texture analyzer (Stable Micro Systems Ltd., Surrey, UK), fitted with a 50-kg load cell, equipped with a fixed platform and an aluminum plate 35 mm in diameter. The plate and the platform were not lubricated, as it was assumed that the whey exuded during the test was sufficient to reduce the friction between the cheese surface and the plate or platform to insignificant levels. Cylindrical cheese samples (20 mm diameter, 24 mm height) were prepared, wrapped individually in polyvinyl chloride film and in water-proof plastic bags, and maintained at 10°C for at least 90 min before analyses. All rheological analyses were performed at least 5 times.

The creep test was performed according to Olivares et al. (2009) by measuring the deformation by applying a constant force of 0.75 N during 180 s. After this time, the force was removed and the sample recovery was measured for an additional 180 s. The results were expressed as the ratio between the strain measured and the initial stress applied called compliance [$J(t) = \gamma(t)/\sigma_0$]. The experimental compliance data were adjusted to a 4-component Burger model as expressed in equation 1:

$$J(t) = J_0 + J_1 \left(1 - e^{-t/\tau}\right) + \frac{t}{\eta_N}, \quad [1]$$

where $J(t)$ is the creep-recovery compliance as a function of time; J_0 is the instantaneous compliance of the Maxwell element; J_1 is the viscoelastic compliance which represents the retarded compliance related to Kelvin-Voigt element; τ is the retarded time; and η_N is the Newtonian viscosity associated with the Maxwell element. According to Olivares et al. (2009), a full mechanical characterization of the rheological parameters J_0 and J_1 can be achieved by calculating the contribution of each main compliance to the maximum deformation to which the system was submitted. The percentage deformation of J_0 and J_1 was established by equations 2 and 3, respectively. The percentage recovery of the system subjected to prior deformation (R) can be calculated by equation 4:

$$J_0^* = \left(\frac{J_0}{J_{\max}}\right) \times 100, \quad [2]$$

$$J_1^* = \left(\frac{J_1}{J_{\max}}\right) \times 100, \text{ and} \quad [3]$$

$$R = \left(\frac{J_{\max} - J_{\text{res}}}{J_{\max}} \right) \times 100, \quad [4]$$

where $J_{\max}$ corresponds to maximum compliance achieved under maximum deformation at 180 s and J_{res} is residual compliance obtained after completed recovery at 360 s.

The uniaxial compression was carried out according to Karlsson et al. (2007). The cheese samples were compressed to 80% of their height at a cross-head speed of 1 mm/s. Force-displacement data were converted to true stress (σ_t ; equation 5) and true strain (ϵ_t ; equation 6) as described by Wium and Qvist (1997):

$$\sigma_t = \frac{F(t)}{A(t)} = \frac{F(t)H(t)}{A_0H_0}, \quad [5]$$

where $F(t)$ is the applied force at any time, $A(t)$ is the cross-sectional area at any time, A_0 is the initial cross-sectional area, $H(t)$ is the sample height at any time; and H_0 is the initial sample height;

$$\epsilon_t = \left| \ln \left(\frac{H(t)}{H_0} \right) \right| = \left| \ln \left(\frac{H_0 - \Delta H}{H_0} \right) \right|, \quad [6]$$

where ΔH is deformation applied in the sample at any time.

The following parameters were calculated from the true stress-strain curve (σ_t - ϵ_t): modulus of elasticity (E), fracture stress (σ_f); fracture strain (ϵ_f); and fracture work (W_f ; Wium and Qvist, 1997; Fox et al., 2000).

Optical Analysis

The instrumental color of the cheeses was determined on 1 and 20 d of refrigerated storage. The measurements were performed in a spectrophotometer Hunter Lab model MiniScan XE (Hunter Associates Laboratory Inc., Reston, VA), with illuminant D65, 10° viewing angle, using CIELab color system, where L^* , a^* , and b^* correspond to luminosity, red intensity, and yellow intensity, respectively. The values for light and dark are represented by L (luminosity), red by $+a$, green by $-a$, yellow by $+b$, and blue by $-b$ in a Cartesian plane. The same procedure was used recently in prebiotic ice cream (Balthazar et al., 2015) and Dulce de Leche (Gaze et al., 2015a).

Consumer Test

Eighty cheese consumers (60% women, 40% men, age 16–61 yr) were recruited at random from the Faculty of Thermomechanical Technology to take part in the study. The criterion for selection was the nonexistence of milk allergy. The samples were removed from the refrigerator, cut into pieces (about $1.5 \times 1.5 \times 1.5$ cm in size), and placed on white plates coded with random 3-digit numbers at room temperature (25°C) 1 h before evaluation. Consumers were instructed to evaluate the cheese with respect to the degree of liking of the appearance, aroma, flavor, texture, and overall impression using a 9-point hybrid hedonic scale (1 = disliked immensely, 9 = liked immensely). Hedonic testing has been used previously for conventional and functional dairy foods (Cruz et al., 2012; Esmerino et al., 2013; Morais et al., 2014; Gaze et al., 2015b). Participants were asked to eat a cracker and drink water between each sample. The first-order and carryover effects were balanced using a specific design and the samples were presented in a monadic form (MacFie et al., 1998). The study was approved by Human Research Ethics Committee of the University of Campinas (UNICAMP; protocol number 03135612.4.0000.5404).

Statistical Analysis

Physicochemical, microbiological, rheological, and optical results were subjected to repeated measures ANOVA at 1, 11, and 20 d using the cheese formulation as the treatment within subjects. Turkey's test was used to determine the significance differences between means at $\alpha = 0.05$. Sensory data were subjected to one-way ANOVA with sample as the fixed effect. The same procedure were also used in previous studies (Cruz et al., 2011; Shrestha et al., 2011; Hystead et al., 2013). All the statistical analyses were performed using the XLSTAT for Windows version 2013.5 (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

pH and Proteolysis

The results of pH and proteolysis of the conventional and probiotic Minas Frescal cheese are shown in Table 1. In general, the addition of *L. casei* Zhang provided lower pH and high proteolysis indexes during storage (from 5.38 to 4.94 and 0.470 to 0.702, $P < 0.05$, respectively), although lower pH and increased proteolysis values were clearly observed for the cheese produced by enzymatic coagulation (PROB II), with this change

Table 1. pH and proteolysis values¹

Cheese	Days	pH	Proteolysis ²
CI	1	6.69 ^{a,A} (0.35)	0.114 ^{c,C} (0.032)
	11	6.65 ^{a,A} (0.21)	0.124 ^{c,B} (0.034)
	20	6.67 ^{a,A} (0.23)	0.156 ^{d,A} (0.022)
CII	1	5.31 ^{b,A} (0.26)	0.310 ^{b,C} (0.017)
	11	5.19 ^{b,A} (0.35)	0.410 ^{b,B} (0.019)
	20	5.05 ^{b,B} (0.16)	0.540 ^{c,A} (0.028)
PROB I	1	5.38 ^{b,A} (0.34)	0.470 ^{a,C} (0.022)
	11	5.27 ^{b,A} (0.22)	0.584 ^{a,B} (0.015)
	20	5.02 ^{b,B} (0.23)	0.640 ^{b,A} (0.017)
PROB II	1	5.24 ^{b,A} (0.12)	0.495 ^{a,C} (0.034)
	11	5.11 ^{b,A} (0.13)	0.618 ^{a,B} (0.036)
	20	4.94 ^{c,B} (0.16)	0.702 ^{a,A} (0.029)

^{a-c}Different lowercase letters in the same column indicate a statistical difference ($P < 0.05$) among treatments (cheeses) in each day.

^{A-C}Different uppercase letters in the same column indicate a statistical difference ($P < 0.05$) along the storage days in the same cheese.

¹Values are means with standard deviations in parentheses. CI = cheese produced by direct acidification, without addition of starter culture or probiotics; CII = cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures; PROB I = cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL; and PROB II = cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang, 6.8 and 8.1 log cfu/mL, respectively.

²Proteolysis is expressed as absorbance at 340 nm.

influenced by the storage time ($P < 0.05$). On the other hand, cheeses CI and CII presented higher pH and proteolysis values [from 6.69 to 5.05 ($P < 0.05$) and 0.540 to 0.114 ($P < 0.05$), respectively]. Indeed, studies have reported that 61 genes are positively regulated during the late log phase of *L. casei* Zhang in milk, which are mainly related to carbohydrate metabolism and energy production, followed by the genes involved in nucleotide metabolism, inorganic ion transport, and AA transport and metabolism (Wang et al., 2012). This metabolic characteristic can be responsible for the high speed of glycolysis observed in cheeses added with *L. casei* Zhang.

Concerning the cheese processing, the enzymatic coagulation covers the consumption of lactose and production of lactic acid by starter cultures, whereas using the direct acidification mode the coagulation step is obtained by the addition of organic acids to milk (Tamime, 2006). It is noteworthy that, as Minas Frescal cheese is a fresh cheese and has a limited shelf life, proteolysis is carried out by the coagulant and, to a lesser extent, by plasmin, residual coagulants, and endogenous enzymes from the starter culture (Sousa et al., 2001).

Finally, it is also important to emphasize that even the cheese produced by direct acidification and with addition of *L. casei* Zhang, without *Lactococcus lactis* (PROB I) presented changes in pH and proteolysis,

suggesting that the probiotic culture has an enzymatic system with sufficient activity for the growth in milk. This finding represents an economic advantage for dairy processors, as *L. casei* Zhang would exert a double function as starter and probiotic culture. However, because our study is the first one covering *L. casei* Zhang and Minas Frescal cheese, future research is needed.

Probiotic and Starter Bacteria Count and Production of Lactic and Acetic Acid

The enumeration of starter culture and probiotics (*Lc. lactis* and *L. casei* Zhang, respectively) and the levels of organic acids (lactic acid and acetic acid) during storage are shown in Table 2. Overall, the addition of *L. casei* Zhang was beneficial to *Lc. lactis* as it improved viability of the latter culture (about 7 log cfu/g for CII vs. 7.9 to 8.8 log cfu/g for PROB II) and an effect of storage was also noted ($P > 0.05$). However, no significant decrease of *Lc. lactis* count along the storage time was observed in both cheeses (CII and PROB II), which indicates the good stability of the starter culture in cheese matrix. Regarding *L. casei* Zhang, high counts were observed along the storage time, regardless of cheese processing. In PROB II, the values ranged from 8.14 to 9.02 log cfu/g ($P < 0.05$), respectively, whereas for PROB I, the values ranged from 8.03 to 8.16 log cfu/g ($P < 0.05$), respectively, demonstrating the cheese protocol is not a hurdle for the growth of this probiotic strain. Considering a portion of 30 g of Minas Frescal cheese (one slice), the sample manufactured in the present study presents ~10 log cfu/g of *L. casei* Zhang, which is a good finding for cheese matrix.

The *L. casei* Zhang counts showed the importance of cheese as a food matrix to deliver probiotic microorganisms, as all samples reached the minimal value (~6 log cfu/g) capable of conferring therapeutic benefits on the consumer (Tripathi and Giri, 2014); this reinforced the fact that Minas Frescal cheese is a suitable vehicle to deliver probiotic bacteria. Similar results were observed for other cheeses, including Panela cheese (Escobar et al., 2012) and petit Suisse (Esmerino et al., 2013). In fact, cheese has a buffering capacity that represents a barrier along the gastrointestinal tract, and thus creates a more favorable environment for probiotic survival (Cruz et al., 2009).

With respect to the organic acids, regardless of the cheese protocol, the addition of *L. casei* Zhang led to higher lactic acid and acetic acid levels throughout the storage (ranging from 1.40 to 2.98 and 0.45 to 0.84 µg/mL, respectively; $P < 0.05$). This may impair consumer acceptance, as consumers may not appreciate acidic products, which is not expected for Minas Fres-

Table 2. Viability and metabolic activity of starter and probiotic bacteria¹

Cheese	Days	<i>Lactococcus lactis</i>	<i>Lactobacillus casei</i> Zhang	Lactic acid	Acetic acid
CII	1	7.6 ^{b,A} (0.23)	— ²	1.15 ^{b,A} (0.12)	—
	11	7.5 ^{b,A} (0.34)	—	1.61 ^{b,A} (0.09)	—
	20	7.4 ^{b,A} (0.33)	—	1.83 ^{b,A} (0.07)	—
PROB I	1	—	8.03 ^{a,A} (0.22)	1.40 ^{a,B} (0.09)	0.50 ^{a,A} (0.03)
	11	—	8.16 ^{b,A} (0.13)	1.75 ^{b,A} (0.11)	0.54 ^{a,A} (0.04)
	20	—	8.28 ^{b,A} (0.17)	2.01 ^{a,A} (0.13)	0.59 ^{a,A} (0.07)
PROB II	1	8.8 ^{a,A} (0.32)	8.14 ^{a,A} (0.18)	1.75 ^{a,B} (0.15)	0.45 ^{a,B} (0.03)
	11	8.2 ^{a,A} (0.11)	8.85 ^{b,A} (0.23)	2.12 ^{a,A} (0.06)	0.75 ^{a,A} (0.12)
	20	7.9 ^{a,A} (0.19)	9.02 ^{a,A} (0.24)	2.98 ^{a,A} (0.09)	0.84 ^{a,A} (0.10)

^{a,b}Different lowercase letters in the same column indicate a statistical difference ($P < 0.05$) among treatments (cheeses) in each day.

^{A,B}Different uppercase letters in the same column indicate a statistical difference ($P < 0.05$) along the storage days in the same cheese.

¹Values are means with standard deviations in parentheses. Microbiological analysis is expressed in log cfu/g of cheese. Lactic acid and acetic acid are expressed in µg/mL. CII = cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures; PROB I = cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL; and PROB II = cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang, 6.8 and 8.1 log cfu/mL, respectively.

²Not performed.

cal cheese. It has been claimed that some *Lactobacillus* spp. strains can metabolize citric acid present in milk into other products, such as acetic acid (Gomes and Malcata, 1999), and this was also observed in probiotic whey cheese (Madureira et al., 2013).

Gross Composition

Table 3 shows the results of the gross composition of the cheeses during the refrigerated storage. In general, an increase in TS was observed, which is related to the syneresis in the cheeses produced by enzymatic coagu-

lation and added with *L. casei* Zhang (CII and PROB II, ranging from 33.61 to 39.74 g/100 g, respectively; $P < 0.05$). This fact may be due to the continuous proteolysis caused by the starter culture and coagulant agent, which does not occur in CII manufactured by direct acidification. No significant differences were observed for protein and fat contents between cheese treatments ($P > 0.05$), suggesting that no solids loss to the whey occurred (despite syneresis). Similar results were observed in Minas Frescal cheese with added *Bifidobacterium* produced using different protocols (Fritzen-Freire et al., 2010a).

Table 3. Proximate composition of probiotic and conventional cheese¹

Cheese	Days	TS	Moisture	Protein	Fat
CI	1	33.34 ^{a,A} (1.12)	66.66 ^{a,A} (1.34)	15.32 ^{a,A} (1.30)	15.84 ^{a,A} (1.11)
	20	36.45 ^{a,B} (1.24)	63.55 ^{a,B} (1.25)	15.65 ^{a,A} (1.21)	15.90 ^{a,A} (1.23)
CII	1	33.61 ^{a,A} (1.34)	66.39 ^{a,A} (1.21)	15.39 ^{a,A} (1.19)	14.98 ^{a,A} (1.22)
	20	38.13 ^{a,B} (1.14)	61.87 ^{a,B} (1.21)	15.59 ^{a,A} (1.07)	15.62 ^{a,A} (1.22)
PROB I	1	34.05 ^{a,A} (1.12)	65.95 ^{a,A} (1.13)	15.42 ^{a,A} (1.19)	15.00 ^{a,A} (1.08)
	20	38.92 ^{a,B} (1.22)	61.08 ^{a,B} (1.19)	15.56 ^{a,A} (1.11)	15.72 ^{a,A} (1.19)
PROB II	1	34.01 ^{a,A} (1.19)	65.99 ^{a,A} (1.03)	15.26 ^{a,A} (1.07)	15.27 ^{a,A} (1.21)
	20	39.74 ^{a,B} (1.09)	60.26 ^{a,B} (1.29)	15.36 ^{a,A} (1.01)	15.77 ^{a,A} (1.15)

^{a,b}Different lowercase letters in the same column indicate a statistical difference ($P < 0.05$) among treatments (cheeses) in each day.

^{A,B}Different uppercase letters in the same column indicate a statistical difference ($P < 0.05$) along the storage days in the same cheese.

¹Values are means with standard deviations in parentheses. Gross composition is expressed in % (wt/wt). CI = cheese produced by direct acidification, without addition of starter culture or probiotics; CII = cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures; PROB I = cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL; and PROB II = cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang, 6.8 and 8.1 log cfu/mL, respectively.

Color Parameters

The color parameters of the Minas Frescal cheese samples are shown in Table 4. The L^* , a^* , and b^* values ranged from 95.74 to 93.56, 1.09 to 0.88, and 15.84 to 13.98, respectively; no significant differences were observed for a^* values among treatments ($P > 0.05$). Overall, samples showed a pale coloring with yellow tone and sub-red tone. The storage time affected the L^* , a^* , and b^* parameters, as all samples exhibited lower L^* values except for the PROB I sample, which presented higher a^* and b^* values during storage. The decrease of luminosity values during storage is probably due to the higher hydration of proteins, leading to a decrease in the number of droplets, reducing the degree of light scattering. In a solid material, such as Minas Frescal cheese, the light penetrates the upper layers and is scattered by both the fat globules (Lemay et al., 1994) and whey bags (Paulson et al., 1998). During ripening, moisture decreases leading to a decrease in the area occupied by the light scattering centers, changing the color of the cheeses. Our findings are in accordance with previous studies covering other cheeses, such as Asiago cheese (Marchesini et al., 2009), Iranian white cheese (Khosrowshahi et al., 2006), Iranian probiotic low-fat cheeses (Sabbagh et al., 2010), and low-fat cheeses (Sánchez-Macías et al., 2010).

Rheological Parameters

The experimental creep and recovery compliance of the Minas Frescal cheese at d 1 and 21 of storage are shown in Figure 1. A 4-component Burger model was used to fit the experimental creep and recovery

compliance data ($R^2 > 0.98$), and the adjusted model parameters are shown in Table 5. These parameters indicate some rheological and texture properties, in which cheese composition and processing conditions are probably the main factors of influence. Complementarily, these parameters are good indicators of texture properties and rheological behavior of cheeses throughout the shelf life as cheeses may undergo deformation during refrigerated storage.

According to Fox et al. (2000), the instantaneous compliance (J_0) is inversely related to the rigidity of a material, which has a close association with the physicochemical composition and the manufacturing process conditions. All Minas Frescal cheese samples showed higher J_0 values (i.e., as the samples exhibited a lower rigidity when compared with the other cheeses), including low-fat Minas Frescal cheese (Cunha et al., 2006), Cheddar cheese (Ma et al., 1996, 1997), and Mozzarella cheese (Olivares et al., 2009). This fact can be associated with a weak protein network structure or higher content of moisture and fat resulting in a less solid-like and more viscoelastic behavior.

Among the Minas Frescal cheese samples studied, the CI sample showed the lowest J_0 value, indicating it was the most rigid sample ($P < 0.05$), followed by PROB I, PROB II, and CII. This result demonstrates that the addition of probiotic culture decreases the rigidity, resulting in softer and less elastic cheeses (Lobato-Calleros et al., 2000). Furthermore, an increase of J_0 values (loss of rigidity) was observed throughout storage, probably due to proteolysis during shelf life. All samples, except for PROB II, showed an increase in the viscoelastic compliance parameter (J_1), which corroborates the J_0 results, indicating a loss of elasticity during

Table 4. Optical analysis of probiotic and conventional cheese¹

Cheese	Days	L^* ²	a^* ²	b^* ²
CI	1	94.1 ^{a,A} (1.7)	0.99 ^{a,A} (0.02)	15.84 ^{a,A} (1.4)
	20	91.80 ^{a,A} (1.5)	1.50 ^{a,A} (1.0)	16.90 ^{a,A} (1.1)
CII	1	95.74 ^{a,A} (1.2)	0.88 ^{a,A} (0.05)	13.98 ^{a,A} (1.3)
	20	92.64 ^{a,A} (1.3)	0.97 ^{a,A} (0.07)	16.28 ^{a,A} (1.4)
PROB I	1	93.56 ^{a,A} (1.3)	1.09 ^{a,A} (0.05)	15.00 ^{a,A} (1.1)
	20	93.92 ^{a,A} (1.4)	1.46 ^{a,A} (0.04)	15.72 ^{b,A} (1.2)
PROB II	1	95.74 ^{a,A} (1.5)	0.88 ^{a,A} (0.03)	13.98 ^{a,A} (1.3)
	20	91.86 ^{a,A} (1.4)	1.60 ^{a,A} (0.02)	18.0 ^{a,A} (1.4)

^{a,b}Different lowercase letters in the same column indicate a statistical difference ($P < 0.05$) among treatments (cheeses) in each day.

^{A,B}Different uppercase letters in the same column indicate a statistical difference ($P < 0.05$) along the storage days in the same cheese.

¹Values are means with standard deviations in parentheses. CI = cheese produced by direct acidification, without addition of starter culture or probiotics; CII = cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures; PROB I = cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL; and PROB II = cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang, 6.8 and 8.1 log cfu/mL, respectively.

² L^* , a^* , and b^* correspond to luminosity, yellow intensity, and red intensity, respectively.

storage (Ma et al., 1996). No significant difference ($P > 0.05$) was observed for retarded time (τ) among cheese formulations and storage time. Newtonian viscosity values (η_N) were generally higher for samples stored for 1 d as compared with samples stored for 21 d, indicating greater resistance to flow at the beginning of shelf life.

The percentage contribution of instantaneous compliance (J_0^* , %) to the total deformation ($J_{\max}$) tends to increase with storage time probably due to proteolysis. This biochemical effect confers less rigidity to the cheese matrix. However, for PROB I, the rate of increase of the total deformation was higher than the rate of increase of J_0 . Thus, the J_0^* behavior during storage seemed to be reversed for the cheese manufactured by direct acidification and added with *L. casei* Zhang. The percentage contribution of viscoelastic compliance (J_1^* , %) was significantly affected by cheese formulation and storage time, as lower values were observed for probiotic cheeses. Moreover, it was observed that

J_1^* generally decreases throughout refrigerated storage. For all cheese formulations, the J_1^* values were higher than J_0^* , indicating a more viscoelastic behavior, except for CII cheese that presents a higher contribution of J_0 (more solid-like sample). The values of percentage recovery of samples stored for 1 d were significantly higher for control samples, indicating that the cheese matrix can recover approximately 58 to 66% of its maximum deformation for samples CII and CI, respectively. However, this percentage recovery significantly decreased in the course of storage, which indicates clear evidence of loss of elasticity during storage. This trend was more pronounced for samples manufactured by enzymatic coagulation as compared with cheeses obtained by direct acidification.

Table 6 presents the estimated parameters calculated from uniaxial compression test of all cheese samples. The modulus of elasticity (E) indicates the material's resistance to axial deformation, which can be expressed

Figure 1. Compliance (J) curves showing the creep-recovery compliance as a function of time [$J(t)$] of the samples of Minas Frescal cheese at 1 d [CI (●), CII (◆), PROB I (■), and PROB II (▲)] and 21 d [CI (○), CII (◇), PROB I (□) and PROB II (△)] of storage time. CI = cheese produced by direct acidification, without addition of starter culture or probiotics; CII = cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures; PROB I = cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL; and PROB II = cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang, 6.8 and 8.1 log cfu/mL, respectively.

Table 5. Rheological parameters of Minas Frescal cheese at different storage times estimated from creep-recovery test.¹

Rheological parameter ²										
Cheese	Days	J_0 (10 ⁻⁴ Pa ⁻¹)	J_1 (10 ⁻⁴ Pa ⁻¹)	τ (s)	η_{Nt} (10 ⁵ Pa/s)	J_{max} (10 ⁻⁴ Pa ⁻¹)	J_{res} (10 ⁻⁴ Pa ⁻¹)	J_0^* (%)	J_1^* (%)	R (%)
CI	1	0.67 ^{cA} (0.03)	1.30 ^{dA} (0.3)	0.1 ^{aA} (0.06)	1.7 ^{cA} (0.6)	2.97 ^{dA} (1.3)	1.00 ^{dA} (1.2)	22.5 ^{cA} (1.3)	43.6 ^{bA} (2.1)	66.3 ^{bA} (1.8)
	20	1.17 ^{dB} (0.4)	1.58 ^{dB} (0.5)	0.1 ^{aA} (0.03)	11.0 ^{bA} (1.2)	4.26 ^{dB} (1.2)	2.30 ^{dB} (1.1)	27.4 ^{bB} (1.2)	37.1 ^{cB} (1.9)	46.0 ^{aB} (1.6)
CII	1	1.93 ^{aA} (0.5)	1.51 ^{cA} (0.2)	0.1 ^{aA} (0.05)	6.6 ^{aA} (1.6)	6.01 ^{bA} (1.1)	2.52 ^{cA} (1.0)	32.1 ^{aA} (1.8)	25.1 ^{cA} (1.3)	58.1 ^{dA} (1.5)
	20	3.55 ^{aA} (0.6)	3.10 ^{aB} (0.9)	0.1 ^{aA} (0.03)	5.6 ^{aB} (2.1)	9.41 ^{aB} (0.9)	9.06 ^{aB} (1.2)	37.7 ^{bB} (1.5)	32.9 ^{bB} (1.4)	3.72 ^{cB} (1.3)
PROB I	1	1.58 ^{bA} (0.3)	1.64 ^{bA} (1.1)	0.1 ^{aA} (0.04)	6.6 ^{aA} (1.8)	5.73 ^{cA} (1.7)	2.66 ^{bA} (1.4)	27.5 ^{bA} (1.6)	28.7 ^{aA} (1.4)	53.6 ^{aA} (1.1)
	20	1.71 ^{cB} (0.7)	2.17 ^{bB} (0.9)	0.1 ^{aA} (0.03)	3.6 ^{aB} (1.9)	8.25 ^{bB} (1.0)	4.13 ^{cB} (1.4)	20.7 ^{cB} (1.7)	26.4 ^{aB} (1.4)	49.9 ^{aB} (1.3)
PROB II	1	1.61 ^{bA} (1.1)	2.17 ^{aA} (1.3)	0.1 ^{aA} (0.05)	4.6 ^{aA} (1.4)	7.71 ^{aA} (1.5)	4.03 ^{aA} (1.3)	20.9 ^{dA} (1.9)	28.1 ^{aA} (1.5)	47.7 ^{cA} (1.3)
	20	1.96 ^{bB} (0.9)	1.83 ^{cB} (0.7)	0.1 ^{aA} (0.07)	3.7 ^{bA} (1.4)	8.04 ^{cB} (1.3)	7.16 ^{bB} (1.0)	24.4 ^{aB} (2.1)	22.7 ^{bB} (1.4)	10.9 ^{bB} (1.3)

^{a-d}Different lowercase letters in the same column indicate a statistical difference ($P < 0.05$) among treatments (cheeses) for each day.^{A-B}Different uppercase letters in the same column indicate a statistical difference ($P < 0.05$) along the storage days for each cheese.¹Values are means with standard deviations in parentheses. All analyses were performed in 5 replicates. CI = cheese produced by direct acidification, without addition of starter culture or probiotics; CII = cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures; PROB I = cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL; and PROB II = cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang, 6.8 and 8.1 log cfu/mL, respectively.²Creep-recovery parameters: instantaneous compliance (J_0); viscoelastic compliance (J_1); retarded time (τ); Newtonian viscosity (η_N); maximum compliance (J_{max}); residual compliance (J_{res}); percentage of instantaneous compliance (J_0^*); percentage of viscoelastic compliance (J_1^*); final percentage recovery (R).

as the rigidity of the material to an applied load (Fox et al., 2000). At d 1 of refrigerated storage, all formulations presented low E values, indicating lower rigidity when compared with low-fat Minas Frescal cheeses (Cunha et al., 2006). The sample CI showed the highest E value, thus it was the most rigid sample followed by PROB I, PROB II, and CII. The fracture stress is related to the product's toughness, whereas the fracture work is directly associated with the hardness of the tested material (Fox et al., 2000).

Regarding the fracture stress (σ_f), the descending sequence of toughness of the manufactured cheeses can be described with the sample CI presenting the higher σ_f value (Figure 2), followed by PROB I, CII, and PROB II, with no significant difference ($P < 0.05$) between CII and PROB II. The σ_f values found in our study were lower than the results obtained by Fritzen-Freire et al. (2010a), who studied the effect of *Bifidobacterium* Bb-12 and lactic acid on the rheological properties of Minas Frescal cheese.

The fracture strain (ϵ_f) denotes the strain required to rupture the sample and may be associated with the crumbliness of the Minas Frescal cheeses (Cunha et al., 2006). Although a significant difference ($P < 0.05$) in the fracture strain was observed with probiotics addition, ϵ_f was not significantly different ($P < 0.05$) between both probiotic cheeses (PROB I and PROB II) and the conventional cheeses (CI and CII). These results were higher than those obtained in previous studies (Fritzen-Freire et al., 2010a,b; Verruck et al., 2015). In contrast, a significant difference ($P < 0.05$) among samples was observed for fracture work (W_f). The CI cheese presented the highest W_f value, followed by PROB I, CII, and PROB II.

At 20 d of refrigerated storage, none of the samples presented enough resistance to cause a fracture point, so the uniaxial compression test was not performed. Considering these results along with the data obtained by the creep test, this phenomenon may be an indicative of loss of rigidity (not measured). As mentioned in the creep tests, this loss of elasticity may be associated with the proteolysis that cheeses undergo during storage.

Consumer Test

Table 7 shows the values of the sensory attributes of both probiotic and conventional cheeses. A higher consumer acceptance was observed for the control sample produced by direct acidification (7.83; $P < 0.05$), whereas the cheeses containing *L. casei* Zhang presented lower values for all sensory attributes. This fact may be due to one or more of the following reasons: content and profile of organic acids produced by the probiotic

Figure 2. Stress-strain curves of the samples of Minas Frescal cheese [CI (●), CII (◆), PROB I (■) and PROB II (▲)] at 1 d of storage time. CI = cheese produced by direct acidification, without addition of starter culture or probiotics; CII = cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures; PROB I = cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL; and PROB II = cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang, 6.8 and 8.1 log cfu/mL, respectively.

strain, or different texture and rheological properties compared with the conventional cheeses. These properties, alone or in combination, may have led consumers to rate negatively the Minas Frescal cheeses produced with *L. casei* Zhang.

Unlike what was observed in the current study, previous research on probiotic Minas Frescal cheese (Fritzen-Freire et al., 2010b) reported a significantly improved preference for cheeses manufactured by direct acidification when compared with the samples produced by en-

Table 6. Rheological parameters of Minas Frescal cheese at different cheese formulations estimated from uniaxial compression test^{1,2}

Cheese	Storage time (d)	E (kPa)	σ_f (kPa)	ϵ_f (-)	W_f (kJ·m ⁻³)
CI	1	10.856 ^a (1.23)	8.525 ^a (1.66)	0.785 ^a (1.66)	3.320 ^a (1.45)
CII	1	4.492 ^d (1.89)	3.675 ^c (1.98)	0.794 ^a (1.77)	1.398 ^{cb} (1.55)
PROB I	1	7.056 ^b (1.78)	4.910 ^b (1.78)	0.677 ^b (1.92)	1.593 ^b (1.67)
PROB II	1	5.035 ^c (1.67)	3.584 ^c (1.87)	0.685 ^b (1.22)	1.162 ^c (1.81)

^{a-d}Different lowercase letters in the same column indicate statistical differences ($P < 0.05$) among treatments (cheeses).

¹Values are means with standard deviations in parentheses. All analysis was performed in 5 replicates. CI = cheese produced by direct acidification, without addition of starter culture or probiotics; CII = cheese produced by enzymatic coagulation using the enzyme and starter culture, without addition of probiotic cultures; PROB I = cheese produced by direct acidification containing *L. casei* Zhang, 8.1 log cfu/mL; and PROB II = cheese produced by enzymatic coagulation, containing *Lactococcus* R-704 (Chr. Hansen, Valinhos, Brazil) and *L. casei* Zhang, 6.8 and 8.1 log cfu/mL, respectively.

²Uniaxial compression parameters: modulus of elasticity (E); fracture stress (σ_f); fracture strain (ϵ_f); fracture work (W_f).

Table 7. Sensory acceptance of probiotic and conventional cheeses¹

Cheese	Appearance	Aroma	Taste	Texture	Overall acceptance
C I	8.01 ^a (1.1)	7.32 ^a (1.5)	7.59 ^a (1.1)	7.93 ^a (1.1)	7.83 ^a (1.5)
C II	6.00 ^b (1.2)	6.41 ^b (1.7)	5.66 ^b (1.2)	5.16 ^b (1.2)	5.71 ^b (1.4)
PROB I	6.82 ^c (1.4)	6.30 ^b (0.8)	4.72 ^c (0.9)	5.21 ^b (1.1)	5.57 ^b (1.2)
PROB II	6.74 ^c (1.7)	6.29 ^b (0.9)	4.61 ^c (1.3)	5.09 ^b (1.3)	5.37 ^b (0.7)

^{a-c}Different lowercase letters in the same column indicate statistical differences ($P < 0.05$) among treatments (cheeses).

¹Values are means with standard deviations in parentheses. Mean data from 80 consumers and based on a 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely).

zymatic coagulation. The different metabolic activity of the strains may explain these results, as *L. casei* Zhang has a higher rate of organic acids production. Studies reporting the minimum level of acid (mainly acetic) where consumers should depreciate a probiotic cheese is scarce; however, previous research of probiotic Cheddar cheese with added *L. casei* 279 was considered different from the control cheese, as it had no probiotic bacteria in a triangular test and had poor acceptance scores (4.93) in a consumer test and increased vinegar and bitter scores (4.23 and 6.83, respectively) as assessed by a trained panel (Ong et al., 2007). In this sense, it is probable the poor acceptance can be linked to resulting off-flavors, which masked the liberated aroma compounds in the Minas Frescal cheese cheeses.

The success of a probiotic product is linked to the consumers' perceptions and to the product's sensory properties throughout its commercial shelf life (Drake and Drake, 2011). Indeed, besides the probiotic viability in cheese, the incorporation of probiotic bacteria should not affect the expected sensory characteristics (flavor, texture, and appearance) as compared with conventional (nonprobiotic) cheeses (Cruz et al., 2010). It is known that the effect of probiotic bacteria on flavor is mainly dependent on the species and strains added and also on the metabolic activity of the strains during cheese production and storage. The metabolites produced by probiotic strains may enhance the bitter taste and negatively affect the cheese texture, depreciating the product's taste (Karimi et al., 2012). In this sense, a technological option would use flavor-developing bacteria to improve the sensory acceptance of the cheese.

CONCLUSIONS

Cheeses supplemented with probiotic bacteria may be a suitable alternative for dairy industries aiming to diversify their products in an increasingly competitive consumer market. Even though probiotic cheeses supplemented with *L. casei* Zhang presented a less solid-like and softer cheese as compared with the con-

trol cheese produced by direct acidification, they were harder and stiffer than control cheeses manufactured by enzymatic coagulation. All cheeses showed a more viscous-like behavior, except for samples obtained by enzymatic coagulation at 21 d of storage. Furthermore, the rigidity and elasticity clearly decreased during storage probably because of the negative effect of proteolysis. In this sense, although probiotic Minas Frescal cheese has proven to be a suitable food matrix for *L. casei* Zhang growth, further studies are necessary to optimize the dosage of probiotic culture in the cheese manufacture, aiming to prevent negative effects on the intrinsic quality parameters and sensory rejection.

REFERENCES

- AOAC International. 2002. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD.
- Balthazar, C. F., H. L. A. Silva, R. M. S. Celeguini, R. Santos, G. M. Pastore, C. A. Conte Junior, M. Q. Freitas, L. C. Nogueira, M. C. Silva, and A. G. Cruz. 2015. Effect of galactooligosaccharide addition on the physical, optical, and sensory acceptance of vanilla ice cream. *J. Dairy Sci.* 98:4266–4272.
- Carvalho, J., W. W. Viotto, and A. Kuaye. 2007. The quality of Minas Frescal cheese produced by different technological processes. *Food Contr.* 18:262–267.
- Cruz, A. G., F. C. A. Buriti, C. B. H. Souza, J. A. F. Faria, and S. M. I. Saad. 2009. Probiotic cheese: Health benefits, technological and stability aspects. *Trends Food Sci. Technol.* 20:344–354.
- Cruz, A. G., R. S. Cadena, J. A. F. Faria, H. M. A. Bolini, C. Dantas, M. C. C. Ferreira, and R. Deliza. 2012. PARAFAC: Adjustment for modeling consumer study covering probiotic and conventional yogurt. *Food Res. Int.* 45:211–215.
- Cruz, A. G., R. S. Cadena, J. A. F. Faria, C. A. F. Oliveira, R. N. Cavalcanti, E. Bona, H. M. A. Bolini, and M. A. P. Silva. 2011. Consumer acceptability and purchase intent of probiotic yoghurt with added glucose oxidase using sensometrics, artificial neural networks and logistic regression. *Int. J. Dairy Technol.* 64:549–556.
- Cruz, A. G., R. S. Cadena, E. H. Walter, A. M. Mortazavian, D. Granato, J. A. Faria, and H. M. A. Bolini. 2010. Sensory analysis: Relevance for prebiotic, probiotic, and synbiotic product development. *Comp. Rev. Food Sci. Saf.* 9:358–373.
- Cunha, C., W. Viotto, and L. Viotto. 2006. Use of low concentration factor ultrafiltration retentates in reduced fat Minas Frescal cheese manufacture: Effect on composition, proteolysis, viscoelastic properties and sensory acceptance. *Int. Dairy J.* 16:215–224.
- Drake, S., and M. Drake. 2011. Application of sensory methods to development of probiotic and prebiotic foods. Pages 113–130 in *Probiotic and Prebiotic Foods: Technology, Stability and Benefits*

- to Human Health. N. P. Shah, A. G. Cruz, and J. A. F. Faria, ed. Nova Publisher, New York, NY.
- Escobar, M. C., M. L. Van Tasseli, F. Martínez-Bustos, E. Castaño-Tostado, S. L. Amaya-Llano, and M. J. Miller. 2012. Characterization of a Panela cheese with added probiotics and fava bean starch. *J. Dairy Sci.* 95:2779–2787.
- Esmerino, E. A., A. G. Cruz, E. P. R. Pereira, J. B. Rodrigues, J. A. F. Faria, and H. M. A. Bolini. 2013. The influence of sweeteners in probiotic Petit Suisse cheese in concentrations equivalent to that of sucrose. *J. Dairy Sci.* 96:5512–5521.
- Fox, P. F., T. P. Guinee, M. T. Cogan, and P. L. H. McSweeney. 2000. Cheese rheology and texture. Pages 305–340 in *Fundamentals of Cheese Science*. P. F. Fox, T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney, ed. Aspen, Gaithersburg, MD.
- Fritzen-Freire, C. B., C. M. O. Müller, J. B. Laurindo, R. M. C. Amboni, and E. S. Prudêncio. 2010b. The effect of direct acidification on the microbiological physicochemical and sensory properties of probiotic Minas Frescal cheese. *Int. J. Dairy Technol.* 63:561–568.
- Fritzen-Freire, C. B., C. M. O. Müller, J. B. Laurindo, and E. S. Prudêncio. 2010a. The influence of *Bifidobacterium* Bb-12 and lactic acid incorporation on the properties of Minas Frescal cheese. *J. Food Eng.* 96:621–627.
- Gaze, L. V., M. P. Costa, M. L. G. Monteiroa, J. A. A. Lavoratoc, C. A. Conte Júniora, R. S. L. Raicesb, A. G. Cruzb, and M. Q. Freitas. 2015a. Dulce de Leche, a typical product of Latin America: Characterization by physicochemical, optical and instrumental methods. *Food Chem.* 169:471–477.
- Gaze, L. V., B. R. Oliveira, L. L. Ferrao, D. Granato, R. N. Cavalcanti, C. A. Conte Júnior, A. G. Cruz, and M. Q. Freitas. 2015b. Preference mapping of dulce de leche commercialized in Brazilian markets. *J. Dairy Sci.* 98:1443–1454.
- Geriletu, R. Xu, H. Jia, M. A. Terkawi, X. Xuan, and H. Zhang. 2011. Immunogenicity of orally administered recombinant *Lactobacillus casei* Zhang expressing *Cryptosporidium parvum* surface adhesion protein P23 in Mice. *Curr. Microbiol.* 62:1573–1580.
- Gomes, A. A., S. P. Braga, A. G. Cruz, R. S. Cadena, P. C. B. Lollo, C. Carvalho, J. Amaya-Farfan, J. A. Faria, and H. M. Bolini. 2011. Effect of the inoculation level of *Lactobacillus acidophilus* in probiotic cheese on the physicochemical features and sensory performance compared with commercial cheeses. *J. Dairy Sci.* 94:4777–4786.
- Gomes, A.M.P., and F.X. Malcata. 1999. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends Food Sci. Technol.* 10:139–157.
- Granato, D., G. F. Branco, A. G. Cruz, J. A. F. Faria, and N. P. Shah. 2010. Probiotic dairy products as functional foods. *Comp. Rev. Food Sci. Saf.* 9:455–470.
- Guo, Z., J. Wang, L. Yan, W. Chen, X.-M. Liu, and H. P. Zhang. 2009. In vitro comparison of probiotic properties of *Lactobacillus casei* Zhang, a potential new probiotic, with selected probiotic strains. *LWT Food Sci. Technol. (Campinas)* 42:1640–1646.
- Hosseini Nezhad, M., M. A. Hussainb, and M. L. Britz. 2015. Stress responses in probiotic *Lactobacillus casei*. *Crit. Rev. Food Sci. Nutr.* 55:740–749.
- Hystead, E., F. Diez-Gonzalez, and T. C. Schoenfuss. 2013. The effect of sodium reduction with and without potassium chloride on the survival of *Listeria monocytogenes* in Cheddar cheese. *J. Dairy Sci.* 96:6172–6185.
- Karimi, R., S. Sohrabvandi, and A. Mortazavian. 2012. Sensory characteristics of probiotic cheese. *Comp. Rev. Food Sci. Safety* 11:437–452.
- Karlsson, A. O., R. Ipsen, and Y. Ardo. 2007. Influence of pH and NaCl on rheological properties of rennet-induced casein gels made from UF concentrated skim milk. *Int. Dairy J.* 17:1053–1062.
- Khosrowshahi, A., A. Madadlou, M. Ebrahim zadeh Mousavi, and Z. Emam-Djomeh. 2006. Monitoring the chemical and textural changes during ripening of Iranian White cheese made with different concentrations of starter. *J. Dairy Sci.* 89:3318–3325.
- Kwok, L. Y., L. Wang, J. Zhang, Z. Guo, and H. Zhang. 2014. A pilot study on the effect of *Lactobacillus casei* Zhang on intestinal microbiota parameters in Chinese subjects of different age. *Benef. Microbes* 5:295–304.
- Lemay, A., P. Paquin, and C. Lacroix. 1994. Influence of microfluidization of milk on Cheddar cheese composition, color, texture, and yield. *J. Dairy Sci.* 77:2870–2879.
- Lobato-Calleros, C., E. Aguirre-Mandujano, E. J. Vernon-Carter, and J. Sanchez-Garcia. 2000. Viscoelastic properties of white fresh cheese filled with sodium caseinate. *J. Texture Stud.* 31:379–390.
- Lollo, P. C. B., A. G. Cruz, P. N. Morato, C. S. Moura, L. B. Carvalho-Silva, C. A. F. Oliveira, J. A. F. Faria, and J. Amaya-Farfan. 2012. Probiotic cheese attenuates exercise-induced immune suppression in Wistar rats. *J. Dairy Sci.* 95:3549–3558.
- Lollo, P. C. B., P. N. Morato, C. S. Moura, C. N. Almada, T. L. Felicio, E. A. Esmerino, M. E. Barros, J. Amaya-Farfan, A. S. Sant'Ana, R. S. Raices, M. C. Silva, and A. G. Cruz. 2015. Hypertension parameters are attenuated by the continuous consumption of probiotic Minas cheese. *Food Res. Int.* 76:611–617.
- Ma, L., M. Drake, G. V. Barbosa-Canovas, and B. G. Swanson. 1996. Viscoelastic properties of reduced-fat and full-fat Cheddar cheeses. *J. Food Sci.* 61:821–824.
- Ma, L., M. Drake, G. V. Barbosa-Canovas, and B. G. Swanson. 1997. Rheology of full-fat and low-fat cheddar cheeses as related to type of fat mimetic. *J. Food Sci.* 62:748–752.
- MacFie, H. J., N. Bratchell, K. Greenhoff, and L. V. Vallis. 1998. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *J. Sens. Stud.* 4:129–148.
- Madureira, A. R., J. C. Soares, M. Amorim, T. Tavares, A. M. Gomes, M. M. Pintado, and F. X. Malcata. 2013. Bioactivity of probiotic whey cheese: Characterization of the content of peptides and organic acids. *J. Sci. Food Agric.* 93:1458–1465.
- Marchesini, G., S. Balzan, S. Segato, E. Novelli, and I. Andrighetto. 2009. Colour traits in the evaluation of the ripening period of Asiago cheese. *Int. J. Anim. Sci.* 9:412–413.
- Meira, S. M. M., D. J. Daroit, V. E. Helfer, A. P. F. Correa, J. Segalin, S. Cato, and A. Brandelli. 2012. Bioactive peptides in water-soluble extracts of ovine cheeses from Southern Brazil and Uruguay. *Food Res. Int.* 48:322–329.
- Morais, E. C., A. R. Morais, A. G. Cruz, and H. M. A. Bolini. 2014. Development of chocolate dairy dessert with addition of prebiotics and replacement of sucrose with different high-intensity sweeteners. *J. Dairy Sci.* 97:2600–2609.
- Olivares, M. L., S. E. Zorrilla, and A. C. Rubiolo. 2009. Rheological properties of mozzarella cheese determined by creep/recovery tests: Effect of sampling direction, test temperature and ripening time. *J. Texture Stud.* 40:300–318.
- Ong, L., A. Henrikssonb, and N. P. Shah. 2007. Chemical analysis and sensory evaluation of Cheddar cheese produced with *Lactobacillus acidophilus*, *Lb. casei*, *Lb. paracasei* or *Bifidobacterium* sp. *Int. Dairy J.* 17:937–945.
- Paulson, B. M., D. J. McMahon, and C. J. Oberg. 1998. Influence of sodium chloride on appearance, functionality, and protein arrangements in nonfat Mozzarella cheese. *J. Dairy Sci.* 81:2053–2064.
- Saba, A., M. Vassallo, R. Shepherd, P. Lampila, A. Arvola, M. Dean, M. Winkelmann, E. Claupein, and L. Lähteenmäki. 2010. Country-wise differences in perception of health-related messages in cereal-based food products. *Food Qual. Prefer.* 21:385–393.
- Sabbagh, N., H. R. Gheisari, and M. Aminlari. 2010. Monitoring the chemical and microbiological changes during ripening of Iranian probiotic low-fat white cheese. *Am. J. Anim. Vet. Sci.* 5:249–257.
- Sánchez-Macías, D., M. Fresno, I. Moreno-Indias, N. Castro, A. Morales-delaNuez, S. Álvarez, and A. Argüello. 2010. Physicochemical analysis of full-fat, reducedfat, and low-fat artisan-style goat cheese. *J. Dairy Sci.* 93:3950–3956.
- Shrestha, S., J. A. Grieder, D. J. McMahon, and B. A. Nummer. 2011. Survival of *Listeria monocytogenes* introduced as a post-aging contaminant during storage of low-salt Cheddar cheese at 4, 10, and 21°C. *J. Dairy Sci.* 94:4329–4335.
- Sousa, M. J., Y. Ardö, and P. L. H. McSweeney. 2001. Advances in the study of proteolysis during cheese ripening. *Int. Dairy J.* 11:327–345.

- Souza, T. B., A. G. Cruz, M. R. L. Moura, A. C. M. Vieira, and A. S. Sant'Ana. 2008. Microscopic quality indicators of Minas frescal cheese. *Food Contr.* 19:71–75.
- Tamime, A. Y. 2006. *Brined Cheeses*. Wiley, Oxford, UK.
- Tripathi, M. K., and S. K. Giri. 2014. Probiotic functional foods: Survival of probiotics during processing and storage. *J. Funct. Foods* 9:225–241.
- Verruck, S., E. S. Prudêncio, C. M. O. Muller, C. F. Fritzen-Freire, and R. D. M. C. Amboni. 2015. Influence of *Bifidobacterium* Bb-12 on the physicochemical and rheological properties of buffalo Minas Frescal cheese during cold storage. *J. Food Eng.* 151:4–42.
- Wang, J., Z. Guo, Q. Zhang, L. Yan, W. Chen, X.-M. Liu, and H.-P. Zhang. 2009. Fermentation characteristics and transit tolerance of probiotic *Lactobacillus casei* Zhang in soymilk and bovine milk during storage. *J. Dairy Sci.* 92:2468–2476.
- Wang, J., Z. Guo, Q. Zhang, L. Yan, Y. Chen, X. Chen, X.-M. Liu, W. Chen, and H.-P. Zhang. 2010a. Effect of probiotic *Lactobacillus casei* Zhang on fermentation characteristics of set yogurt. *Int. J. Dairy Technol.* 63:105–112.
- Wang, J., W. Zhang, Z. Zhang, A. Wei, Q. Bao, Y. Zhang, T. Sun, A. Postmikko, H. Meng, and H. Zhang. 2012. Gene expression profile of probiotic *Lactobacillus casei* Zhang during the late stage of milk fermentation. *Food Contr.* 25:321–327.
- Wang, J. H. K., C. Dong, Y. F. Chen, L. M. Cui, and H. Zhang. 2010b. A new probiotic Cheddar cheese with high ACE-inhibitory activity and γ -aminobutyric acid content produced with koumiss-derived *Lactobacillus casei* Zhang. *Food Technol. Biotechnol.* 48:62–70.
- Wium, H., and K. B. Qvist. 1997. Rheological properties of UF-feta cheese determined by uniaxial compression and dynamic testing. *J. Texture Stud.* 28:435–454.
- Zhang, J., L. Wang, Z. Guo, Z. Sun, Q. Gesudu, L. Kwok, Menghe-bilige, and H. Zhang. 2014a. 454 pyrosequencing reveals changes in the faecal microbiota of adults consuming *Lactobacillus casei* Zhang. *FEMS Microbiol. Ecol.* 88:612–622.
- Zhang, Y., R. Du, L. Wang, and H. Zhang. 2010. The antioxidative effects of probiotic *Lactobacillus casei* Zhang on the hyperlipidemic rats. *Eur. Food Res. Technol.* 231:151–158.
- Zhang, Y., L. Wang, J. Zhang, Y. Li, Q. He, H. Li, X. Guo, J. Guo, and H. Zhang. 2014b. Probiotic *Lactobacillus casei* Zhang ameliorates high-fructose-induced impaired glucose tolerance in hyperinsulinemia rats. *Eur. J. Nutr.* 53:221–232.