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Effect of a probiotic mixed culture on texture profile and sensory performance of Minas fresh cheese in comparison with the traditional products

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SUMMARY. The effect of a mixed probiotic culture on instrumental texture, and on sensorial and related properties of Minas fresh cheese during refrigerated storage was investigated. Three cheese-making trials were prepared: T1, with the traditional type O starter culture (*Lactococcus lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris*), T2 with only lactic acid and T3, with lactic acid and the probiotic ABT culture (*Lactobacillus acidophilus* La-5 + *Bifidobacterium animalis* Bb-12 + *Streptococcus thermophilus*). Instrumental texture profile analysis and related properties were monitored during storage for up to 21 days. *Lb. acidophilus* and *B. animalis* were present in high levels throughout storage of cheeses T3, above 6 log cfu.g^{-1}, threshold required for probiotic activity, and stimulation of the La-5 growth was observed. Cheeses with added probiotic ABT culture, as well as those made adding lactic acid only, showed to be less brittle and with more favorable sensorial features, due to higher pH values. Results indicated that the use of probiotic ABT culture complementary to lactic acid for the purpose of substituting the type O (*Lc. lactis* subsp. *lactis* + *Lc. lactis* subsp. *cremoris*) culture, traditionally employed for Minas cheese production, is advantageous.
Key words: Probiotics; cheese; texture profile; *Lactobacillus*; *Bifidobacterium*.

RESUMO. Efeito de uma cultura probiótica mista sobre o perfil de textura e o desempenho sensorial de queijo Minas frescal, em comparação aos produtos tradicionais. O presente trabalho investigou o efeito de uma cultura probiótica mista sobre a textura instrumental, as características sensoriais e as propriedades relacionadas de queijo Minas frescal durante seu armazenamento refrigerado. Três variáveis de elaboração de queijo Minas frescal foram estudadas: T1, empregando-se a cultura lática mesofílica tradicional tipo O (*Lactococcus lactis* subsp. *lactis* + *L. lactis* subsp. cremoris), T2, produzido somente com ácido lático e T3, empregando-se ácido lático e a cultura probiótica ABT (*Lactobacillus acidophilus* La-5 + *Bifidobacterium animalis* Bb-12 + *Streptococcus thermophilus*). O perfil de textura instrumental e as propriedades relacionadas foram monitorados durante 21 dias de armazenamento dos queijos. As populações de *Lb. acidophilus* e de *B. animalis* estiveram elevadas durante o armazenamento do queijo T3, acima de 6 log UFC.g^{-1}, população mínima requerida para apresentar efeito probiótico, e foi observado um estímulo da multiplicação de La-5. Os queijos produzidos com a cultura probiótica ABT, assim como aqueles somente com ácido lático, apresentaram-se menos frágeis e com atributos sensoriais mais favoráveis, devido ao pH mais elevado. Os resultados indicaram ser vantajoso o emprego da cultura probiótica ABT complementarmente ao ácido lático para o propósito de substituição da cultura tipo O (*Lc. lactis* subsp. *lactis* + *Lc. lactis* subsp. *cremoris*), tradicionalmente empregada para a produção de queijo Minas frescal.
Palavras chave: Probióticos; queijo; perfil de textura; *Lactobacillus*; *Bifidobacterium*.

INTRODUCTION

Probiotics are presently considered 'live microorganisms administered in adequate amounts that positively affect the health of the host' (1,2). Such microorganisms may not necessarily be constant inhabitants of the GIT, but they should have a beneficial effect on the hostage health status (3,4). Bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* are most often used as probiotic supplements for food (5,6). Probiotic dairy foods have a high market potential. In an effort to expand the probiotic product range, a number of studies have reported on the development of several different cheese varieties harboring probiotic microorganisms. These have included Cheddar (7), Gouda (8), Cottage (9), Crescenza (10), Festivo (11), Kefalograviera (12), Argentinean fresh cheese (13) and Minas fresh cheese (14,15).

Some probiotic mixed cultures, e.g. ABT cultures (containing *Lactobacillus acidophilus*, *Bifidobacterium* and *Streptococcus thermophilus*) have been developed to bring out the preferred flavors in the products in which they are used (16,17). The introduction of cultures for direct inoculation of the cheese vat, "direct vat set" (DVS), has allowed culture producers to launch new culture blends, consisting of both thermophilic (mainly *S. thermophilus*) and mesophilic strains

(18). Additionally, strains of *Lactobacillus* spp. and of *Bifidobacterium* spp. were successfully employed as adjuncts in the production of cheese (7,14,15,19).

Minas fresh cheese is a typical Brazilian fresh cheese traditionally made using a mesophilic lactic acid starter type O culture consisting of both *Lactococcus lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*. Nowadays, Brazilian dairies tend to substitute partially or totally, the starter culture by direct acidification with lactic acid (15). Nevertheless, the absence of starter cultures might not be microbiologically safe, since only the addition of lactic cultures assures a permanent production of lactic acid and consequently fairly low pH values of the product during storage, as well as production of other antimicrobial compounds. Addition of only lactic acid results in decrease in pH, which is restricted to the manufacturing process, as it facilitates enzymatic activity over κ-casein. However, when the idea is substituting type O lactic culture by other microorganisms, particularly by probiotic bacteria, it might be advisable to associate this practice to addition of lactic acid, as most probiotic microorganisms are able to produce enough amounts of lactic acid only some hours after the beginning of the manufacturing process.

Fresh Minas cheese offers excellent conditions for survival and growth of probiotic strains, because of high water activity, pH above 5.0, low salt content, and absence of preservatives. Nevertheless, the texture and the sensorial attributes of this food product, quality parameters that obviously reflect over acceptability by consumers, may be susceptible to undesirable changes resulting from the addition of these microorganisms during cheese production. The present study aimed to verify the viability of a mixed ABT probiotic culture and the effect of its addition on instrumental texture profile, and on sensorial and related properties of Minas fresh cheese during refrigerated storage, comparing the product with cheeses manufactured following the traditional Brazilian dairy technologies.

MATERIALS AND METHODS

Minas cheese manufacture

Three pilot-scale Minas cheese-making trials, denoted T1, T2 and T3, were performed in triplicate. Cheeses T1 were manufactured with the addition of mesophilic homofermentative type O lactic culture consisting of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (R-704; Christian Hansen, Valinhos, Brazil). Cheeses T2 were manufactured through direct acidification with lactic acid (Purac Sinteses, Rio de Janeiro, Brazil; 0.25 mL L^{-1} of 85% food-grade solution) and no addition of starter cultures. Cheeses T3 were prepared through acidification with lactic acid and addition of a probiotic ABT culture (ABT-4; Chr. Hansen), composed of the probiotic microorganisms *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* Bb-12 and also of *Streptococcus thermophilus*. Minas fresh cheese was manufactured in 10 L vats from commercial pasteurized milk (CCL Paulista, São Paulo, Brazil; high temperature short time [HTST]) heated to 36-37°C, after which addition of lactic acid and/or cultures proceeded. Both cultures employed were freeze-dried commercial cultures for direct vat inoculation and they were added at 1% (w/v). Commercial rennet Estrela (85% bovine pepsin + 15% bovine chymosin, Chr. Hansen; 8 mL) and calcium chloride (2.5 g) was added to the cheese-milk in all trials. All vats were allowed to set at 36°C, until a firm curd was formed (ca. 40 min.). The gel was cut gently into cubes, allowed to drain, placed in perforated circular containers (ca. 500g capacity) and kept overnight under refrigeration for complete draining, when the product was surface-salted at 1% (w/w). The next day, cheeses were sampled for instrumental texture profile and microbiological and physico-chemical analysis of the final product, and then packaged in sealed plastic bags and stored under refrigeration (5-7°C) for up to 21 days.

Sample collection

Cheeses from each batch were used for analysis of the final product (day 1) and after 7, 14 and 21 days of storage. For cheeses T3, portions of 25 g were collected aseptically from the centre and the surface of these cheeses, for microbiological analysis. For the instrumental texture profile analysis of cheeses T1, T2 and T3, at least 0.5 cm of the rind of the each cheese was discarded, and cheese samples were carefully collected from the centre to the outer part and the rest was grated and immediately used for physico-chemical analysis. Portions of each cheese on day 1 of storage were also collected for subsequent chemical composition analysis (moisture, ash, fat, protein and carbohydrate) of the final product.

Physico-chemical analysis of cheeses

Moisture content was determined from 5 g samples by oven drying at 70°C under vacuum (Marconi MA030112, Piracicaba, Brazil) for 24h (20). Ash was determined gravimetrically by heating the 2 g sample at 550°C, until completely ashed (20). Fat was determined through extraction of lipids with ethyl ether, using the Soxhlet device (20). Protein was estimated by measuring the N content of cheeses by the Kjeldahl method and multiplying by the conversion factor 6.38 (20). Carbohydrate content was calculated by difference to achieve 100% of total contents. The pH values of cheeses were determined on duplicate samples with a pH meter Analyser Model 300M (Analyser, São Paulo, Brasil) equipped with a penetration electrode model DME-CF (Digimed, São Paulo, Brazil). Water activity (a_w) at 25°C was determined on triplicate samples using the Novasina aw-Center Instrument equipped with a three-compartment aw box (Novasina AG, Zürich, Switzerland).

Microbiological analysis of cheeses T3

Viability of *Lactobacillus acidophilus*, *Bifidobacterium animalis* and of *Streptococcus thermophilus* were monitored during the storage period for probiotic cheeses T3. For this purpose, 25 g portions of duplicate cheese samples were blended with 225 mL of 0.1% peptone water in a Bag Mixer 400 (Interscience, St. Nom, France) and submitted to serial dilutions with the same diluent. *Lactobacillus acidophilus* was counted by pour-plating 1 mL of each dilution in modified DeMan-Rogosa-Sharpe (MRS) agar, prepared as a basal medium containing maltose, as described by the International Dairy Federation (21), after 3 days of aerobic incubation at 37°C. *Bifidobacterium animalis* was counted by pour-plating 1 ml of each dilution in modified DeMan-Rogosa-Sharpe (MRS) agar, prepared as a basal medium containing glucose, to which dichloxallin (Sigma, St. Louis, USA, 0,5mg/L), lithium chloride (Merck, Damstadt, Germany, 1g/L) and cistein hydrochloride (Merck, Damstadt, Germany, 0,5g/L) sterile solutions were added, after 3 days of anaerobic incubation (Anaerobic System Anaerogen, Oxoid Ltd. Basingstoke, UK) at 37°C, as described by Alegro (22). *Streptococcus thermophilus* was counted by pour-plating 1 mL of each dilution in M17 agar (Oxoid) with added lactose (Oxoid), followed by incubation at 37°C (23), for 48h.

Instrumental texture profile analysis (TPA)

Texture properties of cheeses were evaluated on replicated samples with a TA-XT2 Texture Analyser (Stable Micro Systems, Haslemere, England), using a two-bite compression of cylindrical samples of 2.4 cm of diameter and 3.0 cm of height by a flat aluminium plate (10 x 9 cm). The compression ratio employed was of 20% deformation from the initial height of the sample at a rate of 2 mm sec^{-1}. After being cut, the cheese samples were left at room temperature (25°C) for 20 min prior to testing. Parameters measured consisted of hardness, cohesiveness, adhesiveness, springiness, chewiness and gumminess, obtained by using the Texture Expert for Windows software version 1.20 (Stable Micro Systems, Haslemere, England).

Experimental design and statistical analysis

The experimental treatments and levels constituted a randomized complete block design replicated three times, with repeated measures at four time points. The treatments had a factorial structure. Analysis of variance was used to determine significant differences ($P<0.05$) for every parameter between the different types of product and during storage, using the MINITAB™ Statistical Software 13.0 (Minitab Inc., State College, PE, USA). Differences between means were detected using the Tukey's test.

Sensory analysis

Comparison of samples containing ABT probiotic culture (T3) with the other cheese trials (T1 and T2) was also conducted by means of sensory evaluation, employing a Randomized Complete Block Design, using Preference-Ranking test. Sensory evaluation of the cheeses was carried out at the Department after 7 days of storage by 53 consumers (not trained panelists) of the Faculty, including teachers, students and staff, selected based on interest and Minas fresh cheese consuming habits. Samples of approximately 30 g were presented in white plastic dishes and the panel was asked to evaluate the three-digit coded samples of the three different types of cheese (T1, T2 and T3 - all of the same batch, 7 days after production) using a score from 1 (preferred sample) to 3 (less preferred sample) based on overall impression. They were also instructed to report any observations on sensory characteristics for the cheese samples (e.g. acid or bitter flavor, pasty or spongy or pasty texture, yellowish appearance). Panelists used water to clean their palates between samples. The sensory data were analyzed by the nonparametric Friedman's test, followed by the rank sum comparisons and of Kendall's concordance coefficient (24).

RESULTS

Composition and physico-chemical parameters of cheeses

Mean chemical compositions for the cheeses studied on day 1 of storage was very similar, and are presented in Table 1. Mean pH and water activity (aw) values of triplicate trials during storage under refrigeration are shown in Table 2. After 7 and 14 days of storage and when all the sampling periods are considered together, mean pH values obtained for cheeses T1 were significantly lower ($P<0.05$), due to the presence of type O lactic culture. Although ABT probiotic cheeses (T3) pH did not changed significantly during storage, these cheeses presented lower pH only on day 1 (average 5.89), probably due to the presence of *Streptococcus thermophilus*, which has the capacity of lowering the pH some time after the beginning of the manufacturing process.

After 7 days of storage, cheeses T3 mean pH values were very close to pH of cheeses manufactured with no addition of cultures (T2). A significant decrease in pH during storage was only observed for cheeses T2 after 21 days of storage ($P<0.05$) and might be attributed to lactic acid production by lactic acid bacteria from milk natural microbiota. As for T1, the constant acidifying starter culture metabolism resulted in much lower and constantly decreasing pH values.

For all cheeses studied, the a_w values were always above 0.97 during the whole storage (Table 2). When considered together, mean T1 a_w values during storage differed significantly from T2 a_w values ($P<0.05$). However, no significant differences in a_w mean values were detected either

between probiotic ABT cheeses T3 and the traditionally-made cheeses on each storage period or during the whole storage of these cheeses (P>0.05).

TABLE 1
Mean composition* of the final product of the different kinds of Minas fresh cheeses studied (T1 = type O lactic culture; T2 = lactic acid; T3 = ABT probiotic culture)

Cheeses	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
T1	64.74 (1.59)	1.75 (0.030)	15.45 (0.37)	11.22 (0.27)	6.84 (2.26)
T2	65.54 (1.86)	1.58 (0.016)	15.02 (0.46)	11.72 (0.67)	6.14 (2.74)
T3	64.24 (2.64)	2.10 (0.011)	15.37 (0.19)	11.54 (0.15)	6.75 (2.99)

* Mean values (with standard deviation in parenthesis)

TABLE 2
Mean values* of pH and water activity (Aw) of the different kinds of Minas fresh cheese studied (T1 = type O lactic culture; T2 = lactic acid; T3 = ABT probiotic culture) during storage under refrigeration

	Time (days)	Cheeses T1	T2	T3
pH	1	6.31^{Aa} (0.75)	7.02^{Aa} (0.15)	5.89^{Aa} (0.26)
	7	5.63^{Aa} (0.20)	6.66^{BCab} (0.28)	6.21^{ACa} (0.41)
	14	5.49^{Aa} (0.35)	6.33^{Bab} (0.17)	6.49^{Ba} (0.12)
	21	5.25^{Aa} (0.30)	6.15^{Ab} (0.47)	6.05^{Aa} (0.57)
	Mean	5.67^{A} (0.45)	6.54^{B} (0.38)	6.16^{B} (0.26)
Aw	1	0.984^{Aa} (0.003)	0.985^{Aa} (0.001)	0.983^{Aa} (0.002)
	7	0.981^{Aa} (0.004)	0.986^{Aa} (0.003)	0.984^{Aa} (0.001)
	14	0.976^{Aa} (0.008)	0.983^{Aa} (0.002)	0.984^{Aa} (0.002)
	21	0.979^{Aa} (0.009)	0.983^{Aa} (0.007)	0.981^{Aa} (0.004)
	Mean	0.980^{A} (0.003)	0.984^{B} (0.001)	0.983^{AB} (0.001)

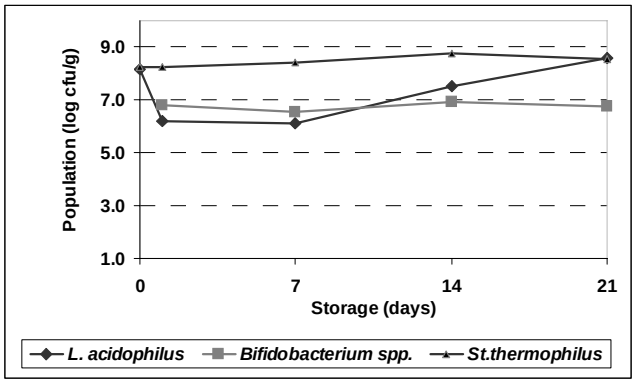
* Mean values (with standard deviation in parenthesis)
^{A,B} Within a row, different superscripts capital letters denote significant differences (P<0.05) between different trials.
^{a,b} Within a column, different lowercase superscripts letters denote significant differences (P<0.05) during storage for each parameter evaluated.

Viability of *Lactobacillus acidophilus, Bifidobacterium animalis* and *Streptococcus thermophilus* in cheeses T3

Cheese containing a probiotic culture is only considered as functional when the culture added during the manufacturing process survives maturation and does not cause damage over its composition, its texture and its sensorial features (Stanton et al.) (25). Several scientific papers propose a minimum daily dose of 10^8 - 10^9 cfu, which corresponds to 100 g of a food product containing 10^6 - 10^7 cfu g^{-1} per day (9,18,26).

In the present study, the probiotic microorganisms *Lactobacillus acidophilus* and *Bifidobacterium animalis* present in the ABT culture employed in Minas fresh cheeses T3 production maintained viable counts, respectively, always above 6.13 and 6.56 log cfu.g^{-1}, during the 21 days of storage of the product under refrigeration. The population of *Streptococcus thermophilus* remained above 8.27 log cfu.g^{-1} during storage (Figure 1). Interestingly, the viability of *L. acidophilus* increased in cheeses T3 after 7 days of storage (up to 8.61 log cfu.g^{-1} at 21 days of storage), whereas populations of *Bifidobacterium animalis* and of *Streptococcus thermophilus* remained more or less constant. A possible beneficial interaction (cooperation) between the strains present in the ABT culture employed in the present study might have occurred, which resulted in the stimulation of the La-5 *Lactobacillus acidophilus* strain growth.

FIGURE 1
Viability of *Lactobacillus acidophilus*, *Bifidobacterium animalis* and *Streptococcus thermophilus* in Minas fresh cheese T3 (supplemented with ABT probiotic culture) during storage under refrigeration



Textural properties of cheeses during storage

Evolution of texture properties of cheeses during storage are shown in Table 3. In the present TPA study, all cheeses showed stability during storage, maintaining constant values of hardness, cohesiveness, adhesiveness, chewiness and gumminess, with no significant changes during storage (P>0.05). Hardness, chewiness and gumminess of cheeses manufactured with the addition of type O lactic culture (T1) were significantly lower, when compared to cheeses T2 and T3 in the 21st day of storage (P<0.05). The lower cheeses T1 hardness values by the end of storage came along with decreased pH, particularly after 7 days of storage (Table 2). On the other hand, for cheeses T2 and T3, the significantly higher pH values obtained during some sampling periods came along with increments in hardness values by the end of the storage period (Tables 2 and 3). Considering all mean values for each cheese, during the whole storage period, T1 was significantly different from cheeses T2 and T3 in relation to hardness, cohesiveness, chewiness and gumminess, and only from T3 in relation to adhesiveness.

TABLE 3
Texture profile analysis (TPA) of the different kinds of Minas fresh cheese studied (T1 = type O lactic culture; T2 = lactic acid; T3 = ABT probiotic culture) during storage under refrigeration

Cheeses	Time (days)	Hardness* (N)		Cohesiveness*		Adhesiveness* (N*s)		Springiness*		Chewiness* (N)		Gumminess* (N)	
T1	1	2.51^{Aa}	(0.39)	0.72^{Aa}	(0.02)	-0.09^{Aa}	(0.02)	0.88^{Aa}	(0.04)	1.60^{Aa}	(0.35)	1.81^{Aa}	(0.33)
	7	2.75^{Aa}	(0.57)	0.74^{Aa}	(0.02)	-0.07^{Aa}	(0.04)	0.89^{Aa}	(0.01)	1.82^{Aa}	(0.44)	2.05^{Aa}	(0.48)
	14	1.84^{Aa}	(1.15)	0.70^{Aa}	(0.05)	-0.09^{Aa}	(0.04)	0.90^{Aa}	(0.02)	1.21^{Aa}	(0.84)	1.34^{Aa}	(0.93)
	21	1.80^{Aa}	(0.61)	0.68^{Aa}	(0.08)	-0.09^{Aa}	(0.01)	0.90^{Aa}	(0.02)	1.13^{Aa}	(0.49)	1.26^{Aa}	(0.55)
	Mean	2.23^{A}	(0.48)	0.71^{A}	(0.02)	-0.09^{A}	(0.01)	0.89^{A}	(0.01)	1.44^{A}	(0.33)	1.61^{A}	(0.38)
T2	1	2.49^{Aa}	(0.24)	0.73^{Aa}	(0.06)	-0.12^{Aa}	(0.05)	0.90^{Aa}	(0.02)	1.64^{Aa}	(0.25)	1.82^{Aa}	(0.32)
	7	2.48^{Aa}	(0.44)	0.76^{Aa}	(0.01)	-0.10^{ABa}	(0.02)	0.91^{Aa}	(0.01)	1.71^{Aa}	(0.30)	1.88^{Aa}	(0.36)
	14	3.94^{Aa}	(0.73)	0.78^{Aa}	(0.00)	-0.10^{Aa}	(0.04)	0.90^{Aa}	(0.00)	2.74^{Aa}	(0.48)	3.05^{Aa}	(0.55)
	21	3.94^{Ba}	(0.35)	0.79^{Aa}	(0.02)	-0.14^{Aa}	(0.00)	0.90^{Aa}	(0.01)	2.80^{Ba}	(0.21)	3.12^{Ba}	(0.20)
	Mean	3.21^{B}	(0.84)	0.76^{B}	(0.03)	-0.11^{AB}	(0.02)	0.90^{A}	(0.00)	2.22^{B}	(0.64)	2.47^{B}	(0.72)
T3	1	2.71^{Aa}	(0.20)	0.75^{Aa}	(0.01)	-0.12^{Aa}	(0.05)	0.89^{Aa}	(0.02)	1.81^{Aa}	(0.17)	2.02^{Aa}	(0.16)
	7	3.58^{Aa}	(0.79)	0.77^{Aa}	(0.03)	-0.16^{Ba}	(0.04)	0.89^{Aa}	(0.01)	2.46^{Aa}	(0.65)	2.78^{Aa}	(0.72)
	14	3.11^{Aa}	(0.33)	0.78^{Aa}	(0.02)	-0.12^{Aa}	(0.03)	0.89^{Aa}	(0.01)	2.16^{Aa}	(0.19)	2.43^{Aa}	(0.22)
	21	3.16^{Ba}	(0.35)	0.78^{Aa}	(0.04)	-0.13^{Aa}	(0.02)	0.89^{Aa}	(0.01)	2.19^{Ba}	(0.12)	2.47^{Ba}	(0.17)
	Mean	3.14^{B}	(0.35)	0.77^{B}	(0.02)	-0.13^{B}	(0.02)	0.89^{A}	(0.00)	2.16^{B}	(0.27)	2.43^{B}	(0.31)

* Mean values (with standard deviation in parenthesis)
^{A,B} Within a column, different superscripts capital letters denote significant differences ($P<0.05$) between different trials for the same day of storage.
^{a,b} Within a column, different superscripts lowercase letters denote significant differences ($P<0.05$) during storage for each trial.

As shown in Table 3, springiness was the only parameter for which no significant differences between cheeses T1 and the other cheeses were detected, due to very similar mean values obtained for samples of the three different kinds of cheeses studied and also during storage of each kind of cheese (always between 0.88 and 0.91). No significant differences were detected between probiotic ABT cheeses T3 and their controls cheeses T2 in any of the texture parameter evaluated ($P>0.05$).

Even though without significant differences during storage, cheeses supplemented with the type O starter culture (cheeses T1) revealed a slight tendency in loosing hardness, chewiness and gumminess after 7 days of storage, whereas non culture supplemented cheeses (T2 cheeses) revealed a slight tendency in increasing hardness, chewiness and gumminess after 7 days of storage. None of these behaviors was observed for probiotic ABT cheeses T3, which showed more constant hardness, chewiness and gumminess mean values, particularly after 7 days of storage, period that was probably required for the stability of cheese components and time that is usually needed for the cheese to reach the consumers' home.

Probiotic ABT cheeses T3 behaved very similarly to their controls – cheeses T2, in terms of textural and physico-chemical parameters during refrigerated storage. Therefore, differences in pH values along storage observed for the three types of cheeses studied, and consequently the evolution of the texture parameters, might be attributable to the presence or not of the type O starter culture, rather than the use of ABT culture as a starter adjunct.

Sensory evaluation of cheeses

Significant differences were detected between cheeses T1 and the other two types of cheeses studied as a result of sensory evaluation ($P<0.05$), due to preference of consumers for cheeses T2 (total score 95; 21 scores 1 and 10 scores 3) and T3 (total score 92; 21 scores 1 and 7 scores 3), particularly because of an excessively acid and slightly bitter tastes attributed to cheeses T1 (total score 131; 11 scores 1 and 36 scores 3). Therefore, as well as it was observed for differences in physico-chemical and texture parameters between the three types of cheeses studied during storage, difference in sensorial characteristics might be attributed to the presence or not of the type O starter culture, rather than the use of ABT probiotic culture as a starter adjunct.

DISCUSSION

Differences in pH may help to explain different rheological properties showed by some cheeses during compression. The maximum level of casein hydration occurs in pH values around 5.2 (27). In the present study, cheeses containing type O lactic culture (cheeses T1) revealed decreasing pH values during storage, more and more close to 5.2, reaching 5.25 after 21 days, whereas cheeses T2 and T3 presented much higher pH

values during the whole storage period (Table 2). So, higher casein hydration degree during storage, particularly after a longer storage period, might have contributed for the decrease in hardness observed for cheeses T1 during storage (Tables 2 and 3). This may happen, since the presence of more water in the protein matrix turns it less elastic and more susceptible to fracture upon the application of a stress to the cheese. This fact is attributable to the direct effect of water as plasticizer, and indirectly, by means of decrease in the concentration of casein in the cheese matrix, which displays lower elasticity and becomes easier to deform, as intra and inter-strand linkages become less numerous (27).

Behavior of cheeses T1 texture profile during storage, except in the case of springiness, and differently from what was observed for cheeses T2 and T3, revealed similar trends to those described in literature for Mozzarella and other low moisture cheeses. In these cheeses, hardness, gumminess, springiness and chewiness tend to decrease during storage (28).

In this study, texture profile of Minas fresh cheeses manufactured with the addition of a probiotic ABT culture revealed to have a greater stability in relation to the different texture parameters evaluated, during refrigerated storage for up to 21 days, when compared to texture profile of Minas fresh cheeses processed according to the traditional dairy technologies, involving addition of the type O lactic culture. The behavior of the texture profile during storage of cheeses manufactured with the addition of the type O lactic culture was closer to what is described in the literature. In spite of that, cheeses supplemented with the probiotic ABT culture, as well as those made adding lactic acid only, showed to be less brittle and with more favorable sensorial features (particularly expressed by means of higher pH values) for acceptance by consumers.

As far as the two microorganisms present in the type O lactic culture employed in the production of cheeses T1 in the present study are concerned, *Lc. lactis* subsp. *lactis* is indeed associated with the promotion of undesirable flavors, being less preferred, whereas *Lc. lactis* subsp. c*remoris* are traditionally considered as major starter culture for the production of cheese (29). Though different types of cheese are traditionally manufactured with starter cultures composed of several *Lactococccus* spp., particularly *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. c*remoris*, substituting these cultures by probiotic cultures seems to be reasonable.

In the present study, the use of a probiotic ABT culture, composed of the probiotic microorganisms *L. acidophilus* and *B. animalis* and also of *S. thermophilus* added as a mixed culture, demonstrated to be beneficial as a co-adjuvant for the manufacture of Minas fresh cheese employing acidification with lactic acid. The use of probiotic ABT culture for the purpose of substituting the type O (*Lc. lactis* subsp. *lactis* + *Lc. lactis* subsp. c*remoris*) culture, traditionally employed for Minas cheese production, was advantageous, as the probiotic ABT Minas cheese revealed a better texture behavior during storage and also sensorial preference by consumers.

Indeed, the use of combined cultures of bifidobacteria and *L. acidophilus* or other lactic bacteria, particularly in association with *Streptococcus thermophilus* has been reported as advantageous, due to absence of certain sensory and texture defects and improvement of nutritional value of 'bifidus" products, besides increased growth rates and reduction of fermentation times (30).

CONCLUSIONS

Minas cheeses manufactured with the probiotic ABT culture, added as an adjunct starter mixed culture composed of the probiotic microorganisms *Lactobacillus acidophilus* and *Bifidobacterium animalis* and also of *Streptococcus thermophilus*, in the manufacturing process involving direct acidification with lactic acid (T3), were the ones to exhibit a more constant behavior during storage in terms of texture, when compared with Minas cheeses manufactured according to the traditional and alternative procedures employed by Brazilian dairies – addition of type O starter culture (T1) and no addition of cultures, only of lactic acid (T2), respectively. No significant differences were observed between texture profiles of cheeses T2 and T3 during storage ($P>0.05$), which showed to be less brittle and with more favorable sensorial features than T1, due to higher pH values. *L. acidophilus* and *B. animalis* were present in high levels throughout storage of cheeses T3, and stimulation of the La-5 growth was observed. Results indicated that the use of probiotic ABT culture complementary to lactic acid for the purpose of substituting the type O (*Lc. lactis* subsp. *lactis* + *Lc. lactis* subsp. c*remoris*) culture, traditionally employed for Minas cheese production, is advantageous. Further studies should be directed towards testing the Minas fresh cheese containing probiotic ABT culture potential as a functional food.

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