LWT - Food Science and Technology 94 (2018) 57–63

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Potentially probiotic goat cheese produced with autochthonous adjunct culture of *Lactobacillus mucosae*: Microbiological, physicochemical and sensory attributes

Georgia Maciel Dias de Moraes[a,c], Karina Maria Olbrich dos Santos[b,*], Samuel Carneiro de Barcelos[c], Silmara Azevedo Lopes[c], Antônio Silvio do Egito[d]

[a] Rede Nordeste de Biotecnologia (RENORBIO), Universidade Estadual do Ceará, 60.714-903, Fortaleza, Ceará, Brazil
[b] Embrapa Agroindústria de Alimentos, 23020-470, Rio de Janeiro, Rio de Janeiro, Brazil
[c] Instituto Federal de Educação Ciência e Tecnologia – IFCE, Campus Sobral, 62040-730, Sobral, Ceará, Brazil
[d] Embrapa Caprinos e Ovinos, 62010-970, Sobral, Ceará, Brazil

ARTICLE INFO

Keywords:
Probiotic bacteria
Caprine cheese
Cheese sensory profile
Coalho cheese

ABSTRACT

A freeze-dried lactic culture was prepared with the potentially probiotic strain *Lb. mucosae* CNPC007, isolated from goat's milk, and used in the manufacture of *Coalho* goat cheese. *Lb. mucosae* viability in the powered culture was greater than 7.3 log CFU g^{-1} throughout 360 days of storage at −20 °C or 90 days at 22 °C. The goat cheese made with the autochthonous lactic culture had physical and physical-chemical characteristics compatible to those required for *Coalho* cheese, and obtained good sensory acceptability, with average scores higher than 7.0 for the attributes color, aroma, flavor, texture, and overall impression. According to the Quantitative Descriptive Analysis (QDA), the *Lb. mucosae* goat cheese showed higher homogeneity, fracturability, and a more intense yogurt flavor at 28 days of storage. By the Check All That Apply (CATA) method, the experimental cheese was characterized as white, smooth, salty, and with a yogurt and butter flavor - characteristics pointed out by consumers as ideal in *Coalho* goat cheese. *Lb. mucosae* population in the cheese was higher than 10^8 CFU g^{-1} throughout the entire studied period. Taken together, the results demonstrate the suitability of the freeze-dried culture of *Lb. mucosae* CNPC007 for the production of a novel potentially probiotic goat cheese.

1. Introduction

Incorporating probiotic lactic bacteria into dairy products has been widely used due to several health benefits attributed to these microorganisms. Some effects are the ability to inhibit intestinal pathogens and to produce enzymes for lactose digestion and deconjugation of bile salts, which contributes to a reduction in serum cholesterol levels (Vinderola et al., 2008). In addition, such bacteria can contribute to biochemical transformations that influence the taste, aroma and texture of dairy products.

Lactobacillus spp. is a special genus concerning probiotic bacteria. Strains from several lactobacilli species have been shown to exert beneficial health properties. *Lactobacillus mucosae* species was first described in 2000 (Roos, Karner, Axelsson, & Jonsson, 2000), with emphasis on the genomic proximity to *Lb. reuteri*, a species recognized for containing several strains with probiotic potential.

Properties associated with probiotic potential have been attributed to some *Lb. mucosae* strains, adhesion to intestinal mucosa, resistance to the passage through the gastrointestinal tract (GIT), and immunomodulatory capacity, as well as producing exopolysaccharides and bacteriocins (Bilková, Kinova Sepova, Bukovsky, & Bezakova, 2011; London et al., 2014).

The *Lb. mucosae* CNPC007 strain isolated from goat milk by our group brings together a set of probiotic and technological properties verified by *in vitro* tests, and is considered promising for application in functional dairy products (Moraes et al., 2017). However, in order for a microorganism to be used in the agro-industrial sector, it is necessary to transform them into a stable and easily accessible lactic culture. Adequate cultivation conditions aiming at the generation of biomass followed by lyophilization enable producing a powder culture with high a concentration of viable cells. Factors such as the cryoprotectant used, storage temperature and water activity are determinants for culture stability during the shelf-life, and vary depending on the microorganism (Capela, Hay, & Shah, 2006; Nualkaekul, Deepika, & Charalampopoulos, 2012).

Several studies have shown that cheeses are promising foods for

* Corresponding author.
E-mail address: karina.dos-santos@embrapa.br (K.M.O. dos Santos).

https://doi.org/10.1016/j.lwt.2018.04.028
Received 8 November 2017; Received in revised form 28 February 2018; Accepted 11 April 2018
Available online 11 April 2018
0023-6438/

transporting probiotic microorganisms into the human body. This is due to their fat and protein content which exert a protective effect on the probiotics in the product and during their passage through the gastrointestinal tract, contributing to probiotic survival until they reach the gut, locus of their effect on health (Dos Santos et al., 2012). *Coalho* cheese is a widely distributed product in the Brazilian Northeast and characterized by FAO (2014) as a semi-hard, white-colored cheese showing a typical opened texture with mechanical eyes, salty flavor and slightly acid. Its functional potential has been reported, associated with the presence of bioactive peptides (Fontenele, Bastos, Dos Santos, Bemquerer, & Do Egito, 2017; Silva et al., 2012). As the result of its characteristics and the relevance of its consumption in Brazil, *Coalho* cheese represents an interesting vehicle for the incorporation of probiotic bacteria.

In addition to the viability of probiotics in cheese, it is also important that the incorporation of these microorganisms do not negatively affect the product characteristics, and possibly contribute to the development of flavor and texture, which are very relevant attributes for the consumption of a product (Urala & Leahteenmeaki, 2004).

Thus, this study aimed at producing the first potentially probiotic freeze-dried culture of *Lb. mucosae* CNPC007, and its application in manufacturing *Coalho* goat cheese, characterized by its microbiological, physical, physico-chemical and sensorial parameters.

The use of a goat freeze-dried culture for producing goat's milk cheeses may represent an advantage depending on the natural adaptation of the bacteria to the milk of the species, in addition to providing new probiotic strains to increase the offer of this type of product in the market.

2. Material and methods

2.1. Obtaining freeze-dried cultures

Lactobacillus mucosae CNPC007 is part of the Collection of Microorganisms of the Brazilian Agricultural Research Corporation, and was cultivated according to Moraes et al. (2017). Briefly, the strain was inoculated in MRS broth and incubated at 37 °C for 24 h, followed by centrifugation at 12.000*g*/15 min at 4 °C. The pellet was washed with 10 mL of 0.85% saline solution, re-centrifuged under the same conditions, and suspended in 10 mL of skimmed goat's milk (0.5% fat), which was previously submitted to a heat treatment of 90 °C for 15 min. The inoculated milk was incubated at 37 °C for 24 h and used for bacterial multiplication in 1 L of milk, under the same conditions. The biomass generated in milk was frozen at −22 °C and lyophilized at −52 °C and 84 mmHg, until water activity < 0.2. The powdered culture was packed into polypropylene tubes and stored either at room (22 °C) or freezing temperatures (−20 °C).

2.1.1. Viability of the freeze-dried culture

In order to determine the concentration of viable cells, the lyophilized culture was rehydrated in sterile distilled water at the ratio of 1:9 w/v. Decimal dilutions were prepared in peptone water and pour plated in MRS agar (Oxoid, Basingstoke, UK) acidified at pH 5.4 with 1 M glacial acetic acid, and incubated in aerobiose at 37 °C for 48 h. The viability of *Lb. mucosae* in the freeze-dried culture was evaluated at 30, 90, 180, 270 and 360 days after the lyophilization process.

2.1.2. Water activity

The water activity of the lyophilized culture was determined in an Aqualab-3TE (Decagon devices, Pullman, USA), according to AOAC (1995). Determinations were performed in triplicate, with three repetitions.

2.2. Production of Coalho goat cheese

The cheese-making trials were conducted at the Dairy Processing Pilot Plant of Embrapa Goats and Sheep, in three repetitions, according to Egito and Laguna (1999) with modifications. After pasteurization at 65 °C for 30 min, milk temperature was adjusted to 35 °C for the addition of *Streptococcus thermophilus* TA-40 (DuPont®, Dangé, France) (0.003% w/v), and the freeze-dried culture of *L. mucosae* CNPC007 (0.2% w/v). After a rest period of 30 min, calcium chloride (0.04% w/v) and commercial Ha-la coagulant (containing protease from *Aspergillus niger* var. *awamori*, Christian Hansen, Valinhos, Brazil, 0.08% w/v) were added. After coagulation, the curd was cut into cubes and the curd was salted with NaCl (0.8%, w/v, based on the initial milk volume), maintaining contact for 8 min. The cheeses were pressed at 3.56 N using a cheese press (Brasholanda S/A, Model RP 1000, Curitiba, Brazil) for 16 h at 25 °C, kept at 4 °C for 48 h, then packed into plastic vacuum packages and stored at 4 °C for up to 28 days.

2.3. Evaluating the cheese quality

2.3.1. Microbiological quality

Serial decimal dilutions of cheese samples were prepared with 0.1% peptone water; 1 ml of each dilution were transferred to *Staphylococcus* DNase positive Petrifilm™ plates and EC Count Plates (3 M Microbiology, St. Paul, USA) for counting coliforms and *E. coli*. PetrifilmTM YM Count Plates (3 M Microbiology) plates were used to count molds and yeasts, according to the AOAC 991.14 and AOAC 997.02 methods (Knight et al., 1997). Petrifilm™ EC plates were incubated at 37 °C for 24 h, while Petrifilm™ YM count plates were incubated at 20–25 °C for 5 days. All analyzes were performed on the cheese with *Lb. mucosae* after processing (day 1) and at 7, 14 and 28 days of storage, as well as the other cheese samples used for sensory evaluation.

2.3.2. Viability of the potentially probiotic strains and the commercial culture

Lb. mucosae and *S. thermophilus* populations were determined after cheese processing (day 1) and at 14 and 28 days of storage. Decimal dilutions were prepared using APT (0.1% w/v), and 1 ml of adequate dilutions was pour plated in MRS agar (Oxoid, Basigstoke, UK) acidified at pH 5 (IDF, 1995) for *Lb. mucosae* enumeration, and in agar M17 (Oxoid) supplemented with 10% lactose solution for *S. thermophilus* counts. The plates were incubated in aerobiose at 45 °C for 48 h.

2.3.3. Physical-chemical analysis

Coalho cheese composition was determined after processing (day 1), and at 14 and 28 days of storage. Moisture, ash and fat determinations followed the standard methods of the Instituto Adolfo Lutz (IAL, 2004). Moisture and ash were determined gravimetrically, in duplicates. Fat was determined by the Gerber butyrometer method, in triplicates. Protein was estimated by measuring total nitrogen content in duplicate samples by the micro Kjeldahl method and using a conversion factor (6.38) (AOAC, 2003).

Titratable acidity and pH of the goat milk used for cheese manufacture and *Coalho* cheeses were determined in triplicate samples, and expressed as a percentage of acidity in lactic acid (IAL, 2004). Cheese pH values were measured with a FC200 penetrating electrode adapted to a Hanna® HI221 pH meter (Hanna Instruments, Woonsocket, RI, USA). The water activity of the cheeses was determined in an Aqualab apparatus (AOAC, 1995).

2.3.4. Evaluation of the instrumental texture

The texture profile analysis (TPA) of the experimental cheeses was determined after processing (day 1), 14 and 28 days. A TA-XT.plus texture analyser (Stable Micro Systems, Haslemere, UK), in sextuplicate samples. A double compression test was employed on cylindrical samples of 2.0 cm of diameter and 2.0 cm of height by an aluminium cylinder probe set (35 mm diameter). On each sampling day, cheeses were cut, sampled and kept at 10 °C for 2 h before the analyses. The

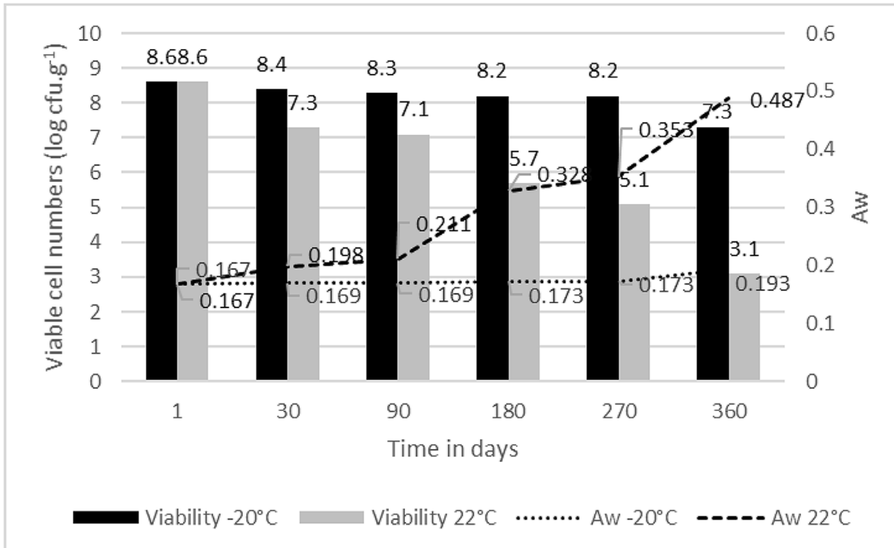

Fig. 1. Population of *Lactobacillus mucosae* CNPC007 and water activity (Aw) of the freeze-dried culture throughout 360 days at different storage temperatures.

compression distance was 10 mm from the initial height of the sample and pre-test speed: 1.0 mm/s, test speed: 2.0 mm/s, and post-test speed: 2.0 mm/s; rest of 5 s between the two cycles; trigger force 1.0 N. The measured parameters were hardness, adhesiveness, springiness, cohesiveness and chewiness, calculated according to the Bourne (2002) definitions, using the Texture Expert 1.20 for Windows (Stable Micro Systems, UK).

2.3.5. Sensory evaluation of goat Coalho cheese

Descriptive analysis and consumer acceptability test were conducted at the Laboratory of Sensory Analysis from Federal Institute of Education, Science and Technology of Ceará (IFCE), Sobral Campus, after approval by the research ethics committee of the Vale do Acaraú State University (Process n. 638.683; CAAE 25585614.4.0000.5053).

Quantitative Descriptive Analysis (QDA) and the Check-All-That-Apply (CATA) test included other goat cheese samples, in order to obtain a greater diversity of descriptive terms, and allowing a better characterization of the *Lb. mucosae* cheese.

2.3.5.1. Acceptability test. To carry out the acceptability test, samples of the potentially probiotic cheese (denoted *Lb. mucosae* cheese - LmC) at 7, 14 and 28 days of storage (LmC7, LmC14 and LmC28, respectively), and a control cheese (CC) produced under the same process conditions and without the addition of *Lb. mucosae*, were used. Overall impression, color, aroma, flavor and texture were evaluated using a structured hedonic scale of nine points (1 = I disliked it very much, 5 = I neither liked it nor disliked it 9 = I liked it a lot) (Meilgaard, Civille & Carr, 2007). The evaluation was conducted with 120 consumers, men and women, including untrained students, teachers, staff and researchers, being selected based on the interest and habits of consuming goat dairy products. The age range of the consumers was 18–45 years.

2.3.5.2. Quantitative Descriptive Analysis (QDA). Twelve assessors were selected and trained to perform the QDA; four men and eight women, aged between 18 and 38 years. The selection was based on sensorial acuity, and training was performed for each sensory attribute used, as described by NBR 14140:1998 (ABNT, 1998).

During the QDA, the panelists evaluated five samples of goat *Coalho* cheese: LmC cheese at 7 and at 28 days of maturation (LmC7 and LmC28), control cheese at 7 days of maturation (CC), a commercial cheese (ComC) purchased from a local store in the city of Sobral (CE), and a cheese produced with *Lb. rhamnosus* EM1107 culture at 7 days of maturation (LrC).

The following descriptive terms were selected to compose the analysis form: color, homogeneous, goat aroma, acid, salty, bitter, sweet, yogurt flavor, butter flavor, after taste, fracturability, and chewiness. An unstructured 9 cm scale anchored at the extremities was used to quantify each attribute.

2.3.5.3. Check-all-that-apply (CATA). One hundred twenty consumers were recruited for completing the CATA, both men and women, all being consumers of dairy products and willing to consume goat cheese. Five samples of Coalho cheese were used in the test, corresponding to the samples used for the QDA. The samples were presented monadically in disposable cups coded with three digits, at 10 °C. The panelists were asked to indicate all the terms that they considered appropriate to describe the sample from a list containing 14 words.

The CATA evaluation was performed according to the methodology described by Ares and Jaeger (2015), using terms previously obtained from the Quantitative Descriptive Analysis. The selected terms were: white, yellowy, smooth, rough, goat aroma, acid, salty, bitter, sweet, yogurt flavor, butter flavor, after-taste, brittle and chewy. In addition, the panelists were asked to select the terms necessary to describe a cheese with ideal characteristics.

2.3.6. Statistical analysis

Data were submitted to the Shapiro-Wilk and Bartlett tests in order to verify the assumptions of normality and homogeneity, respectively. Thus, we applied Analysis of Variance followed by the Tukey test for comparison of mean pairs, always considering a 5% level of significance. The statistical software used for the analyzes was SAS 9.2 (2009).

The CATA data were submitted to correspondence analysis, in which a frequency matrix was compiled by adding the individual choices for each attribute to obtain a perception map.

3. Results and discussion

3.1. Viability of Lb. mucosae CNPC 007 in the freeze-dried culture

The viable population of *Lb. mucosae* CNPC007 in the freeze-dried culture reached 8.6 log CFU/g, maintaining levels above 8.2 log CFU/g at 270 days of storage at −20 °C (Fig. 1). Meanwhile, at 22 °C, a reduction of the *Lb. mucosae* population to 7.3 log CFU/g was observed in the freeze-dried culture after 90 days of storage. A gradual increase in the water activity values (aw) of the powdered culture were observed during the storage period, being much more pronounced at 22 °C in comparison to the storage at −20 °C (Fig. 1). The decrease in the viable population of the culture throughout storage can mainly be attributed to storage temperatures and water activity, factors that directly influence the stability and survival of bacterial cells (Kurtmann, Carlsen, Skibsted, & Risbo, 2009).

The relationship between storage temperature and bacterial viability in lyophilized cultures was reported by Capela et al. (2006), who monitored *Lb. acidophilus, Lb. casei, Lb. rhamnosus* and *Bifidobacterium* spp. populations during storage at 4 °C, 21 °C and 37 °C. These authors found that cultures stored at 4 °C had their cell viability preserved for 180 days, and samples kept at 21 °C and 37 °C showed significant reductions in viability during the same storage period.

Water activity was considered the main factor that influenced the survival of *Lb. plantarum* in instant juice powders, according to Nualkaekul et al. (2012).

The cryoprotective agent choice also has a direct influence on bacterial survival in lyophilized products by providing additional protection to the cells during the freeze-drying process, considering that the sudden decrease in water activity may result in undesirable effects, such as protein denaturation (Leslie, Israeli, Lighthart, Crowe, & Crowe, 1995). Despite the cryoprotectant capacity of goat's milk being scarcely studied, tests performed by our group demonstrated the efficiency of goat's milk as a cryoprotectant in lyophilized cultures of *Lb. plantarum* and *Lb. rhamnosus* over 90 days of storage at −26 °C and 37 °C (Barcelos, Moraes, Santos & Egito, 2013).

3.2. Quality evaluation of the potentially probiotic goat cheese

3.2.1. Microbiological quality

In evaluating the microbiological quality of the experimental cheese at 7, 14 and 28 days of storage, and of the other cheese samples used in the sensory analysis, no thermotolerant coliforms, *E. coli*, molds, yeasts and *Staphylococcus (DNase positive)* were found, attesting the safety of the cheese.

3.2.2. Viability of the cultures used for cheese production

Lb. mucosae and S. *thermophilus* populations in the cheese increased significantly during the storage period at 4 °C (Table 1).

The viability of the potentially probiotic strain in experimental cheeses are in compliance with the international recommendations for probiotic foods, around 10^8 CFU g^{-1} (Champagne, Ross, Saarela, Hansen, & Charalampopoulos, 2011).

Table 1
Physicochemical and microbiological parameters of *Coalho* goat cheese produced with *Lb. mucosae* CNPC007 and *S. thermophilus* (mean ± SD) during storage at 4 °C.

Parameter	Time (days)		
	1	14	28
Viability *			
Lb. mucosae (log cfu g^{-1})	8.28 ± 0.2 [b]	8.59 ± 0.3 [ab]	8.72 ± 0.2 [a]
S. thermophilus (log cfu g^{-1})	8.84 ± 0.3 [b]	9.10 ± 0.3 [a]	9.23 ± 0.3 [a]
Physicochemical parameters **			
Titratable acidity (mg 100 g^{-1})	46.77 ± 0.5 [b]	59.00 ± 0.5 [a]	61.77 ± 0.7 [a]
pH	5.24 ± 0.01 [a]	5.26 ± 0.01 [a]	5.22 ± 0.02 [a]
Aw	0.979 ± 0.03 [a]	0.974 ± 0.05 [a]	0.974 ± 0.01 [a]

* Duplicate analysis of samples from three cheese–making batches; ** Triplicate analysis of samples from three cheese–making batches.
[a,b] Different letters in a row denote significant differences between time periods ($p < 0.05$).

3.2.3. Physico-chemical parameters

The pH values of the cheeses remained stable throughout the 28 days ($p > 0.05$), and the titratable acidity increased at 14 days ($p < 0.05$), remaining stable up to 28 days (Table 1). The cheese water activity (aw) values remained stable throughout the studied period.

The moisture content of the cheeses reduced between the first and 14th days of storage ($p < 0.05$), from 45.05% to 42.86%, remaining stable until the 28th day. The initial reduction is justified by the fact that the cheese spent 48 h under refrigeration to form the rind before being packed. Ash (3.48%), fat (26.50%) and protein (23.27%) content remained stable throughout the 28 days of storage.

Moisture and fat content of the LmC cheese corroborate with the values indicated by the FAO (2014) for *Coalho* cheese, between 42% and 48% for moisture, and between 23% and 27% for the fat content.

Based on the composition data, LmC cheese is classified by Brazilian legislation as medium moisture cheese (Brasil, 1996), usually known as semi-hard cheese.

3.2.4. Evaluation of the instrumental texture

The texture is an important attribute to be evaluated for cheeses due to its impact on sensory evaluation (Bourne, 2002). Texture properties of semi-solid or solid foods include fracturability (brittleness), hardness (or firmness), cohesiveness, springiness (elasticity), adhesiveness (or stickiness), and chewiness (Szczesniak & Bourne, 1995).

Instrumental texture values registered for the experimental cheeses are shown in Table 2. Instrumental hardness remained stable throughout the 28 days of storage ($p > 0.05$). Moisture content is an important factor that influences the hardness of cheeses by weakening the protein network (Buriti, Rocha, & Saad, 2005), yet the reduction of LmC moisture observed between the 1st and 14th days of storage did not reflect in the values of instrumental hardness. Hardness values lower to those found in this study have been reported for *Coalho* goat cheese by Queiroga et al. (2013) and were attributed to the concentration and structure of goat milk caseins.

A significant decrease of the instrumental values of springiness ($p < 0.05$) of the cheeses was observed between the 1st and 28th days of storage. Decrease of springiness in cheeses are attributed to proteolytic changes in the aggregates of proteins (Szczesniak, 1997), which may be caused by the increase in acidification that occurs during ripening. In fact, a significant increase in the titratable acidity of LmC cheese was observed in the present study at 14 days of storage ($p < 0.05$).

The cohesiveness and chewiness of LmC cheese remained stable until the 14th day of storage ($p > 0.05$); however, a significant reduction of these values was observed between the 14th and 28th days of storage ($p < 0.05$). The reduction in cohesiveness may be related to the increase in titratable acidity observed in the same period. Buriti et al. (2005) pointed out that changes in cheese titratable acidity resulting from the production of lactic acid and other organic acids have a direct influence on the cheese texture, making them more fragile. The lower chewiness reflect the change in cheese hardness, cohesiveness and springiness, because instrumental chewiness are the product of these three parameters. These changes would imply a lower resistance of the cheese to disintegration, since cohesiveness is related to the effort required to overcome the internal bonding of the material, and chewiness is defined as the energy required in chewing to properly reduce the solid food to be swallowed (Bourne, 2002).

3.2.5. Sensory evaluation of caprine Coalho cheeses

3.2.5.1. Acceptability test. LmC cheese at 28 days of storage (LmC28) obtained significantly higher average scores for aroma, flavor and overall impression ($p < 0.05$) compared to the other cheeses (Fig. 2). This result can be attributed to the presence of the *Lb. mucosae* CNPC007, which produces diacetyl (2,3-butanedione) (Moraes et al., 2017), a compound responsible for developing flavor, aroma and the characteristic odor of cheeses throughout the storage period (Varnam &

Table 2
Instrumental texture of cheese produced with *Lactobacillus mucosae* CNPC007 and *Streptococcus thermophilus* during storage at 4 °C.

Time (days)	Instrumental parameter*				
	Hardness (N min^{-1})	Adhesiveness (g s^{-1})	Springiness	Cohesiveness	Chewiness
1	66.37 ± 4.32 [a]	−31.37.1 ± 7.26 [b]	0.90 ± 0.07 [a]	0.56 ± 0.01 [a]	0.38 ± 0.05 [a]
14	62.45 ± 2.75 [a]	−54.017 ± 3.33 [c]	0.82 ± 0.02 [b]	0.59 ± 0.03 [a]	0.32 ± 0.03 [a]
28	61.70 ± 2.98 [a]	−21.427 ± 4.29 [a]	0.74 ± 0.03 [c]	0.47 ± 0.01 [b]	0.25 ± 0.01 [b]

* Sample analyzed in sextuplicates.
[a,b,c] Different letters in a column denote significant differences between time periods ($p < 0.05$).

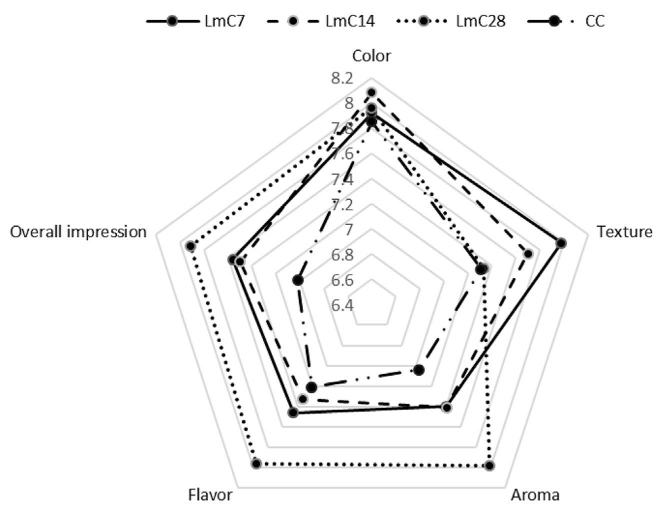

Fig. 2. Mean scores of sensory acceptability of control cheese (CC) and cheese produced with *Lb. mucosae* CNPC 007 and *S. thermophilus* during storage. LmC7 = cheese with *Lb. mucosae* CNPC007 and *S. termophilus* at 7 days of storage; LmC14 = cheese with *Lb. mucosae* CNPC007 and *S. termophilus* at 14 days of storage; LmC28 = cheese with *Lb. mucosae* CNPC007 and *S. termophilus* at 28 days of storage.

Sutherland, 1994).

Regarding texture, average acceptability scores of LmC cheese decreased over the 28 days of storage, probably due to the local habit of consuming *Coalho* cheeses in the first week of manufacture, when it presents greater adhesiveness and springiness (elasticity), as seen in Table 2. However, the lower scores attributed to the cheese texture did not influence the mean overall acceptability of the product, which was significantly higher after 28 days of storage compared to 7 days ($p < 0.05$).

According to the results, the cheese produced with *Lb. mucosae* and *S. thermophilus* in co-culture were well-accepted by the consumers, obtaining higher scores than the control cheese (CC) regarding the overall acceptability and individual attributes, with average scores between seven and eight, corresponding in the hedonic scale respectively to “I moderately liked it” and “I liked it a lot”.

3.2.5.2. Quantitative Descriptive Analysis (QDA) of the cheeses. In the QDA, the cheese with *Lb. mucosae* CNPC007 was characterized by trained panelists as a slightly acidic, salty cheese with a yogurt flavor; characteristics that intensified at 28 days of maturation. Moreover, LmC28 was described as a visually homogeneous cheese of greater fracturability and chewability upon being compressed during mastication, which differed significantly from the other evaluated cheeses ($p < 0.05$). Greater chewing indicates that a greater number of chews were required for the product to be disintegrated, characterizing the sample as a “chewy cheese”. For Karimi, Sohrabvandi, and Mortazavian (2012), the presence of probiotics in cheeses can directly affect the texture, since proteolysis involving casein may occur during ripening, in addition to a greater acidification of the cheese.

At 28 days of storage, LmC28 was described with a lower intensity of goat aroma, differing statistically from the other analyzed samples (Fig. 3). This result can be justified because *Lb. mucosae* CNPC007 is a producer of diacetyl, a metabolic compound generated during cheese ripening, which may have masked the goat aroma. According to Fox et al. (2000), the presence of diacetyl may provide the product with a creamy, buttery flavor. In addition, as the strain exhibit high proteolytic activity (Moraes et al., 2017), aroma alteration may also be related to the proteolysis that may occur in the cheese ripening process, as highlighted by Delgado, González-Crespo, Cava, and Ramírez (2011). For Karimi et al. (2012), the aroma is a very complex attribute in cheeses, since in addition to involving proteolysis, it may be associated with compounds resulting from lipolysis and glycolysis.

Regarding sweetness, cheese samples showed no significant difference between them. However, the attributes yellowy color, goat aroma, acid taste, salty taste, bitter taste, butter flavor and after-taste were observed in higher intensity in the ComC cheese when compared to the other samples ($p < 0.05$).

The impact of added probiotic cultures on the sensory attributes of cheese depends on the bacterial strain, since each strain has specific metabolic activity during the production and storage of cheese (Karimi et al., 2012). Thus, we can infer that the addition of *Lb. mucosae* CNPC007 had a positive impact on the production of *Coalho* goat cheese, as it provided desirable modifications of the sensory characteristics of the product.

3.2.5.3. Check-all-that-apply (CATA) of the cheeses. Based on CATA methodology, the most important characteristics or “drivers of liking” which justify the choice of the ideal goat cheese were linked to five terms selected by the consumers among the list of 14 terms: white, smooth, salty, butter flavor and chewy (Table 3).

Correspondence analysis (CA) of the CATA data (Fig. 4) showed that the first and second dimensions explain 92.62% of the experiment variation, and the five cheese samples were positioned into three different groups. LmC7 and LmC28 samples were associated to the terms salty taste, yogurt flavor, butter flavor and chewy, being grouped in the same dimension and proximity to the descriptors of the “ideal” goat cheese. It is worth pointing out that according to the QDA method, LmC28 was indicated as the saltiest cheese and having the most intense yogurt flavor, showing that the characterization carried out by trained panelists can also be perceived by potential consumers of the product.

The commercial cheese (ComC) was associated with the terms yellowy, goat aroma and after-taste. These attributes were also identified as being present in ComC by the trained assessors who performed the QDA. On the other hand, CC and LrC cheeses were grouped in the same dimension and were characterized by the smooth texture and acid taste.

In the CATA analysis, untrained consumers recognized many attributes pointed out by trained panelists in the QDA, reinforcing the sensory perception of these features in the evaluated cheeses. The high correlation between the sensory profile generated in the CATA by untrained tasters and the profiles generated by trained tasters were also pointed out by Ares and Jaeger (2015).

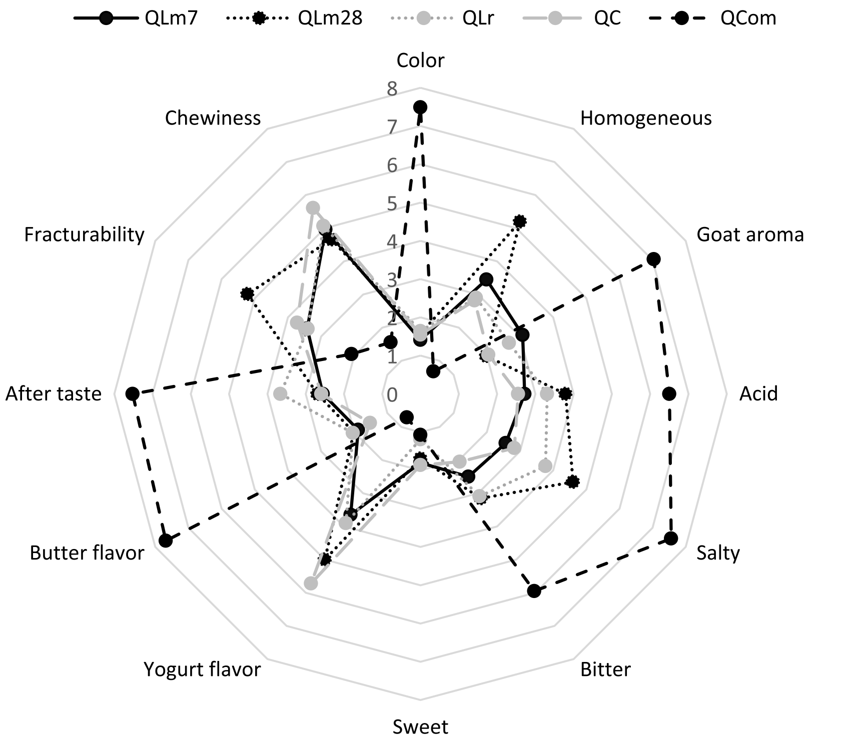

Fig. 3. Sensory attributes intensity of the goat cheeses in the Quantitative Descriptive Analysis.
LmC7 = cheese with *Lb. mucosae* CNPC007 and *S. termophilus* at 7 days of storage; LmC28 = cheese with *Lb. mucosae* CNPC007 and *S. termophilus* at 28 days of storage; LrC = cheese with *Lb. rhamnosus* and *S. termophilus* at 7 days of storage; CC = control cheese with *S. termophilus* at 7 days of storage; ComC = commercial *Coalho* goat cheese purchased from local store.

Table 3
Frequencies for each attribute and evaluated cheese in the Check-All-That-Apply (CATA) question.

Descriptive term	LmC7	LmC28	LrC	CC	ComC	Ideal Cheese
White*	99	100	99	104	10	102
Yellowy	11	12	12	11	98	15
Smooth*	78	78	73	79	54	83
Rough	13	11	13	9	16	8
Goat aroma	16	8	33	31	46	9
Acid	6	11	10	10	8	10
Salty*	39	80	48	38	65	88
Bitter	15	6	16	10	16	3
Sweet**	12	10	13	17	19	14
Yogurt flavor	18	13	9	10	5	35
Butter flavor*	60	86	25	25	53	92
After-taste	29	27	21	21	44	22
Brittle**	11	10	10	10	5	10
Chewy*	78	56	37	37	21	98

* Attributes considered ideal for *Coalho* cheese by the panelists.
** Indicates that there were no significant differences between the samples ($p > 0.05$).
The ideal product was not included in the Cochran's Q-test.
LmC7 = cheese with *L. mucosae* CNPC007 and *S. termophilus* at 7 days of storage; LmC28 = cheese with *L. mucosae* CNPC007 and *S. termophilus* at 28 days of storage; LrC = cheese with *Lb. rhamnosus* and *S. termophilus;* CC = control cheese; ComC = commercial cheese.

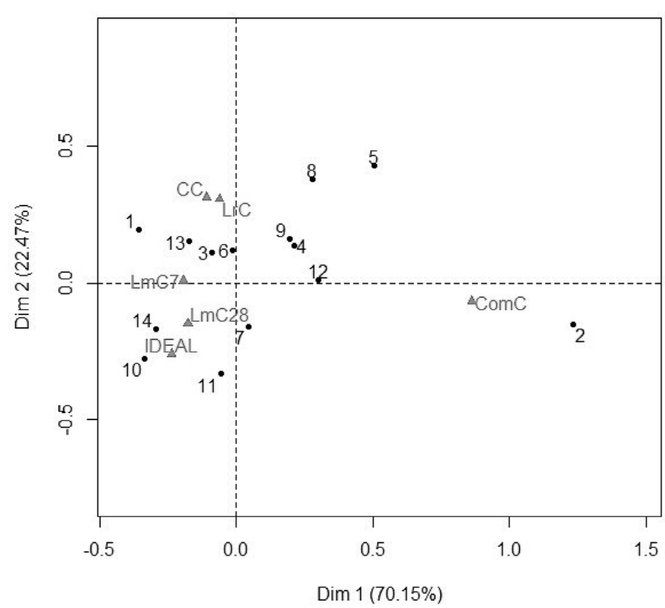

Fig. 4. Correspondence analysis of the Check-All-That-Apply (CATA) results
1: White; 2: Yellowy; 3: Smooth; 4: Rough; 5: Goat aroma; 6: Acid; 7: Salty; 8: Bitter; 9: Sweet; 10: Yogurt flavor; 11: Butter flavor; 12: After-taste; 13: Brittle; 14: Chewy.
LmC7 = cheese with *Lb. mucosae* CNPC007 and *S. termophilus* at 7 days of storage; LmC28 = cheese with *Lb. mucosae* CNPC007 and *S. termophilus* at 28 days of storage; LrC = cheese with *Lb. rhamnosus* and *S. termophilus* at 7 days of storage; CC = control cheese with *S. termophilus* at 7 days of storage; ComC = commercial *Coalho* goat cheese purchased from local store.

4. Conclusions

The potentially probiotic strain *Lb. mucosae* CNPC007 showed good resistance to freeze-drying conditions, using goat milk as a cultivation media and cryoprotectant. The freeze-dried culture exhibited good stability throughout storage, remaining with a high concentration of viable cells over 360 days of frozen storage or 90 days at room temperature. The powdered culture, the first produced with a strain of the *Lb. mucosae*, demonstrated technological aptitude for producing *Coalho* goat cheese in co-culture with *S. thermophilus*. The population of *Lb. mucosae* remained viable in the cheese at a concentration above 10^8 CFU g^{-1} over 28 days of refrigerated storage, being compatible to the requirements for probiotic products.

In the sensory evaluation, the cheese produced with *Lb. mucosae* CNPC007 showed an increase in the acceptability scores for aroma, flavor and overall impression after ripening. Moreover, CATA data pointed out that the novel cheese presented the closest characteristics to an ideal goat cheese as considered by the consumers, thus reinforcing the strain's potential for application in cheese production.

Acknowledgements

The authors would like to thank Brazilian Agricultural Research Corporation for financial support (grant number 02.11.07.020.00.05), José dos Santos Tabosa for technical assistance, and Adriano R. Lima for statistical analysis.

References

ABNT (1998). *NBR 14140: Food and beverages - sensory analysis - quantitative descriptive analysis (QDA)*. Rio de Janeiro: ABNT.

AOAC (1995). *Official methods of analysis of the association of official analytical chemists* (16th ed.). Washington, DC: AOAC.

AOAC (2003). *Association of official analytical chemists. Official methods of analysis of AOAC international* (16th ed.). Gaithersburg: AOAC.

Ares, G., & Jaeger, S. R. (2015). Check-all-that-apply (CATA) questions with consumers in practice: Experimental considerations and impact on outcome. In J. Delarue, B. Lawlor, & M. Rogeaux (Eds.). *Rapid sensory profiling techniques and related methods* (pp. 227–245). Elsevier.

Barcelos, S. C., Moraes, G. M. D., Santos, K. M. O., & do Egito, A. S. (2013). Viability of Lactobacillus rhamnosus 1107 and Lactobacillus plantarum 270, freeze-dried after fermentation in goat milk. *International congress of gastronomy and food science*.

Bilková, A., Kinova Sepova, H., Bukovsky, M., & Bezakova, L. (2011). Antibacterial potential of lactobacilli isolated from a lamb. *Veterinary Medicina, 56*, 319–324.

Bourne, M. C. (2002). *Food textures and viscosity: Concept and measurement.* NewYork: Academic Press.

Brazil Ordinance nº 146, March 7th (1996). *Approves the technical regulation of identity and quality of dairy products - technical Regulation of Identity and Quality of Cheeses.* Brasilia, DF.

Buriti, F. C. A., Rocha, J. S., & Saad, S. M. I. (2005). Incorporation of *Lactobacillus acidophilus* in Minas fresh cheese and its implications for textural and sensorial properties during storage. *International Dairy Journal, 15*, 1279–1288.

Capela, P., Hay, T. K. C., & Shah, N. P. (2006). Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yogurt and freeze dried yogurt. *Food Research International, 39*, 203–211.

Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. F., & Charalampopoulos, D. (2011). Recommendations of the viability assessment of probiotics as concentrated cultures and in food matrices. *International Journal of Food Microbiology, 149*, 185–193.

Delgado, F. J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Effect of high-pressure treatment on the volatile profile of a mature raw goat milk cheese with paprika on rind. *Innovative Food Science & Emerging Technologies, 12*, 98–103.

Dos Santos, K. M. O., Bomfim, M. A. D., Vieira, A. D. S., Benevides, S. D., Saad, S. M. I., Buriti, F. C. A., et al. (2012). Probiotic caprine Coalho cheese naturally enriched in conjugated linoleic acid as a vehicle for Lactobacillus acidophilus and beneficial fatty acids. *International Dairy Journal, 24*, 107–112.

Egito, A. S., & Laguna, L. E. (1999). *Manufacture of coalho goat's cheese.* Sobral: Embrapa Goats and Sheep 15 pp. (Technical Communicate n.16).

FAO (2014). *Food and agriculture organization. Coalho cheese.* http://www.fao.org/3/a-au133e.pdf, Accessed date: 22 August 2016.

Fontenele, M. A., Bastos, M. S. R., Dos Santos, K. M. O., Bemquerer, M. P., & Do Egito, A. S. (2017). Peptide profile of Coalho cheese: A contribution for protected designation of origin (PDO). *Food Chemistry, 219*, 382–390.

Fox, P., et al. (2000). *Fundamentals of cheese science.* Gaithersburg, MD: Aspen Publishers Inc.

IAL (2004). *Physical-chemical methods for food analysis* (4th ed.). São Paulo: IAL.

IDF (1995). *Fermented and non-fermented milk products. detection and enumeration of Lactobacillus acidophilus. Culture media.* Brussels: International Dairy Federation.

Karimi, R., Sohrabvandi, S., & Mortazavian, A. M. (2012). Sensory characteristics of probiotic cheese. *Comprehensive Reviews in Food Science and Food Safety, 11*, 437–452.

Knight, M. T., Newman, M. C., Benzinger, M. J., Jr., Neufang, K. L., Agi, J. R., McAllister, J. S., et al. (1997). Comparison of the Petrifilm dry rehydratable film and conventional culture method - enumeration of yeasts and molds in Foods: Collaborative study. *Journal Association Official Analytical Chemistry, 80*, 806–823.

Kurtmann, L., Carlsen, C. U., Skibsted, L. H., & Risbo, J. (2009). Water activity-temperature state diagrams of freeze-dried *Lactobacillus acidophilus* (La-5): Influence of physical state on bacterial survival during storage. *Biotechnology Progress, 25*, 265–270.

Leslie, S. B., Israeli, E., Lighthart, B., Crowe, J. H., & Crowe, L. M. (1995). Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Applied and Environmental Microbiology, 61*, 3592–3597.

London, L., Price, N. P. J., Ryan, P., Wang, L., Auty, M. A. E., Fitzgerald, G. F., et al. (2014). Characterization of a bovine isolate *Lactobacillus mucosae* DPC 6426 which produces an exopolysaccharide composed predominantly of mannose residues. *Journal of Applied Microbiology, 117*, 509–517.

Meilgaard, M. C., Civille, G., & Carr, B. T. (2007). *Sensory evaluation techniques* (4th ed.). FL: Boca Raton: CRCPress.

Moraes, G. M. D., Abreu, l. R., Egito, A. S., Salles, H. O., Silva, L. M. F., & Nero, L. A. (2017). Functional properties of *Lactobacillus mucosae* strains isolated from Brazilian goat milk. *Probiotics & Antimicrobial Proteins, 9*, 235–245.

Nualkaekul, S., Deepika, G., & Charalampopoulos, D. (2012). Survival of freeze dried *Lactobacillus plantarum* in instant fruit powders and reconstituted fruit juices. *Food Research International, 48*, 627–633.

Queiroga, R. C. R. E., Santos, B. M., Gomes, A. M. P., Monteiro, M. J., Teixeira, S. M., Souza, E. L., et al. (2013). Nutritional, textural and sensory properties of Coalho cheese made of goats', cows' milk and their mixture. *LWT - Food Science and Technology, 50*, 538–544.

Roos, S., Karner, F., Axelsson, L., & Jonsson, H. (2000). *Lactobacillus mucosae* sp. nov., a new species with in vitro mucus-binding activity isolated from pig intestine. *International Journal of Systematic and Evolutionary Microbiology, 50*, 251–258.

SAS Institute (2009). *SAS OnlineDoc. 9.2.* Cary, NC, USA: SAS Institute Inc.

Silva, R. A., Lima, M. S. F., Viana, J. B. M., Bezerra, V. S., Pimentel, M. C. B., Porto, A. L. F., et al. (2012). Can artisanal "Coalho" cheese from Northeastern Brazil be used as a functional food? *Food Chemistry, 135*, 1533–1538.

Szczesniak, A. S. (1997). Effect of storage on texture. *Food storage stability*. New York: CRC Press.

Szczesniak, A. S., & Bourne, M. C. (1995). Letters. *Journal of Food Science, 60*, 7.

Urala, N., & Leahteenmeaki, L. (2004). Attitudes behind consumers' willingness to use functional foods. *Food Quality and Preference, 15*, 793–803.

Varnam, A. H., & Sutherland, J. P. (1994). *Milk and milk products.* Springer US (Chapter 8).

Vinderola, G., Capellini, B., Villareal, F., Suarez, V., Quiberoni, A., & Reinheimer, J. (2008). Usefulness of a set of simple in vitro test for the screening and identification of probiotic candidate strains for dairy use. *LWT - Food Science and Technology, 41*, 1678–1688.