

Instrumental texture and sensory evaluation of fermented dairy beverages processed with reconstituted goat whey powder and a co-culture of *Streptococcus thermophilus* and *Lactobacillus casei*

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Abstract

The effects of *Lactobacillus casei* BGP93 used as adjunct culture on the physicochemical, textural and sensory characteristics of a dairy beverage processed with goat Coalho cheese whey powder and *Streptococcus thermophilus* TA-40 as starter (ST-LC beverage) were investigated in comparison to a control product (ST beverage) without *L. casei*. No significant differences were observed between the ST and ST-LC trials concerning the acidification pattern throughout the fermentation process ($P > 0.05$). Post-acidification was also not observed for both trials since their pH values were maintained stable, without significant differences during 21 days at 4 ± 1 °C. This pH stability reinforced the maintenance of firmness, consistency, cohesiveness and viscosity index without significant differences between the sampling periods throughout the whole storage in both trials, and also that no significant difference was verified between the ST and ST-LC beverages in the sensory evaluation ($P > 0.05$).

Key words: acidification, by-product upgrading, goat dairy beverages, Lactobacilli adjunct cultures, storage stability

Introduction

The goat milk is a complex food considering its nutritional composition of fatty acids, proteins and minerals. When compared to cow milk, goat milk shows higher digestibility, smaller milk fat globules and hypoallergenicity, which make it suitable for processing of cheeses, fermented milks and other dairy products (Hernández-Ledesma et al., 2011;

Costa et al., 2014). The rearing of goats gained importance worldwide over the last decade and the production of caprine dairy products experienced an important increase (Hernández-Ledesma et al., 2011; da Silveira et al., 2015). Considerable part of goat milk production is destined to cheesemaking (Tranjan et al., 2009; Hernández-Ledesma et al., 2011).

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In Brazil, the most part of goat cheese production still occurs in small- or medium-sized dairy plants and the resulting whey is often discarded without any treatment as an effluent, becoming a strong pollutant (Tranjan et al., 2009). Whey is rich in proteins, minerals, lactose, besides other constituents that turn this by-product interesting for different industry applications (Hernández-Ledesma et al., 2011). A viable form of reuse of whey showed to be the production of ready-to-drink products (Almeida et al., 2008; Tranjan et al., 2009; Pescuma et al., 2012; Buriti et al., 2014; da Silveira et al., 2015). Nonetheless the use of *in natura* whey is limited due to its perishable components (Morais et al., 2015). To overcome this limitation, the processing of whey by drying can turn it in a valuable ingredient for the production of a functional beverage through fermentation by lactic acid bacteria (Pescuma et al., 2012).

Lactic acid bacteria belonging to the *Lactobacillus casei* group, which include the *L. casei*, *L. paracasei*, and *L. rhamnosus* species, are recognized by their health promoting properties and are frequently used as probiotics in food products (Buriti and Saad, 2007). Fermented dairy products are commonly used as food vehicles to deliver such microorganisms to consumers (Costa et al., 2014). Despite that, information regarding goat whey-based beverages formulated with probiotics is still limited (da Silveira et al., 2015). The use of bacteria belonging to the *L. casei* group as a sole culture or in co-culture with other lactic acid bacteria or probiotics was reported to increase acidification in dairy products such as whey-based beverages (Almeida et al., 2008), desserts (Aragon-Alegro et al., 2007; Correa et al., 2008), cheeses (Menéndez et al., 2000; Buriti et al., 2007), and also other food products as fermented soybean beverage (Behrens, 2002) and sausages (Rubio et al., 2013). In some cases, the increased acidification affected the products' sensory features, reducing their acceptability (Behrens, 2002; Correa et al., 2008) or preference in relation to similar products with a less intense acid taste (Buriti et al., 2008). It is also known that progressive acidification in dairy products can modify their texture throughout the storage (Montanucci et al., 2012). In that manner, it is important to monitor such parameters during the storage of goat whey beverage added of a *L. casei* strain.

This study aimed to evaluate the physicochemical and instrumental texture stability of a dairy beverage processed with goat whey powder and the strain *L. casei* BGP93 as adjuvant culture with *Streptococcus thermophilus* TA-40 as starter over a storage period of 21 days under refrigerated conditions, in comparison with a control product, without *L. casei*. Due to possibility of *L. casei* also influence on sensory attributes of dairy beverages, the studied products were also investigated through sensory evaluation using a discriminative test.

Material and methods

Production of the spray dried goat whey powder

The goat whey powder used in this study was produced at Embrapa Goats and Sheep (Sobral, Ceará State, Brazil). The whey used was obtained during the processing of goat Coalho cheese, prepared, as described by Egito and Laguna (1999), using chymosin from *Aspergillus niger* var. *awamori* (Ha-la[®] coagulant, Chr. Hansen, Valinhos, Brazil) and a mesophilic homofermentative culture of *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* (R-704 lactic culture, Chr. Hansen). The resulting whey was dried in a mini spray drier Büchi (model B-290, Flawil, Switzerland) using an inlet temperature of 160 °C, outlet temperature of 90-92 °C, air flow of 667 L/h and a pump speed of 25 mL/min. The resulting goat whey powder was sent to the State University of Paraíba (Campina Grande, Paraíba State, Brazil) for use in the processing of the fermented goat dairy beverages.

Production of the dairy beverages

Three different batches (genuine replicates) of two pilot-scale dairy beverages-making trials were produced: ST - control, fermented with the *Streptococcus thermophilus* TA-40 starter culture, (Danisco, DuPont, Dangé-Saint-Romain, France); and ST-LC, fermented with *S. thermophilus* TA-40 plus the potentially probiotic *L. casei* BGP93 culture (Sacco, Cadorago, Italy). With this purpose, the goat whey powder was reconstituted with distilled water (20 g/100 g). The reconstituted whey was heat treated at 85 °C for 30 min and then cooled up to 45 °C for the addition of the lactic cultures - *S. thermophilus* at a concentration of 0.030 mg/L in both

ST and ST-LC trials, and *L. casei* at a concentration of 0.200 mg/L only in the ST-LC trial. At the end of the fermentation performed at 43 ± 2 °C (cca. 6 h, when the fermented reconstituted whey reached acidity of 0.6 g/100 g or higher), the sucrose syrup (at 70 ± 1 g/100 g soluble solids) was added at the proportion of 15 g/100 g of the total formulation. The beverages were stored at 4 ± 1 °C for 21 days. Viability of *L. casei* in ST-LC trial were above $7 \log$ CFU g⁻¹ during the whole storage (data not shown), which was in agreement with the minimum recommended of probiotic microorganism in food products of $6 \log$ cfu/g in order to have beneficial effects on the consumer's health (Aragon-Alegro et al., 2007).

Sampling periods

Dairy beverages from each batch were sampled for analysis after 1, 7, 14 and 21 days of storage. The reconstituted goat whey powder was sampled for titratable acidity and pH analysis during the manufacture process immediately after the addition of the starter and adjunct cultures and in 1 h intervals until the end of fermentation.

Mean composition analysis

Total solids, ash, fat and protein content of dairy beverages were determined on the 1st day of storage, in triplicate, for the three batches of each trial. In parallel, the composition of the whey powder used in the processing of beverages was also determined, in triplicate. Total solids were determined through drying 2 g samples at 70 °C under vacuum using a vacuum oven Marconi (model MA 030/12 Piracicaba, Brazil). Ash was determined gravimetrically by heating the dried samples at 550 °C. Analytical procedures for the determination of total solids and ash content of samples followed the standard methods of the Instituto Adolfo Lutz (IAL, 2008). Fat was determined according to Folch, Less, and Stanley (1957). Protein was estimated by measuring the nitrogen content of 0.2 g samples through the micro Kjeldahl method and multiplying by the conversion factor of 6.38 (AOAC International, 2003). The total carbohydrates content was obtained by difference in order to achieve 100 g/100 g of total composition (FAO, 2003).

Physicochemical determinations

Titratable acidity was determined for the three batches of reconstituted whey powder during fermentation for each trial in duplicate samples according with the sampling periods previously described using the appropriate standard methods, and the values were expressed in terms of g/100 g lactic acid (IAL, 2008). The pH values were determined for the three batches of each trial in duplicate samples according with the sampling periods previously described with a pHmeter model TEC-5 (Tecnal, Piracicaba, Brazil) using the appropriate standard method (IAL, 2008).

Textural measurements

Instrumental texture of dairy beverages during the storage was evaluated for each batch in duplicate with a TA-XTplus Texture Analyser (Stable Micro Systems, Surrey, UK), using the back extrusion test. An acrylic compression disc (35 mm diameter) was thrust into a cylindrical container (50 mm diameter \times 70 mm height), filled with samples at 4 °C up to a height of 50 mm (ca. 100 mL). The starting distance of the disc was set at 30 mm above the top of the sample surface. The disc penetrated into the sample to a depth of 30 mm at a 1 mm/s speed, and returned at 10 mm/s speed. The parameters measured consisted of firmness, consistency, cohesiveness and index of viscosity, obtained by using the Exponent[®] 2014 software, version 6.1.7.0 (Stable Micro Systems).

Sensory evaluation

The sensory evaluation of the present study was approved (CAAE: 43582715.1.0000.5187) by the Ethics Research Committee of State University of Paraíba (UEPB, Campina Grande, Paraíba State, Brazil) and was carried out at the Laboratory of Sensory Analysis of Federal University of Campina Grande, Campina Grande Campus. The purpose of this sensory analysis was to evaluate if the ST (control) and the ST-LC beverages showed or not significant differences when compared in a same storage period. Samples with 7, 14 and 21 days of storage, under refrigeration conditions (4 ± 1 °C), were evaluated through discrimination test using the triangle comparison procedure (Meilgaard et al., 1999; IAL, 2008). The samples evaluated were in agree-

ment with the Brazilian regulatory standards regarding sanitary quality (ANVISA, 2001; Brazil, 2005), since coliforms at 35 °C and at 45 °C, and also *Salmonella* spp. were not detected. On the sampling day, 30 adult consumers were recruited. The session was divided into three steps and, in each step, a volunteer analysed three samples of a same storage period (7, 14 or 21 days), in which two samples were equal and one was different. All samples were coded with three random digits, and the volunteer should identify the different sample. The samples ST (S) and ST-LC (L) were served randomly, in 20 mL disposable plastic cups, in the serving sequences SSL, LSS, SLS, SLL, LLS and LSL across the volunteers, whereby each sequence appeared in an equal number of times. Samples were maintained under refrigerated conditions prior to the tests. The consumers were also instructed to report the sensory attributes related to flavour, texture, appearance and aroma that could be related to the differences between the samples, and they were free to mention none or more than one attribute. Volunteers with poor physical conditions, as described by Meilgaard et al. (1999), were excluded from the sessions.

Statistical analysis

The results were presented as a mean \pm standard deviation. Except for the results of sensory evaluation, comparisons between ST and ST-LC dairy beverages in a same storage period were tested using an unpaired Student's *t* test with $P < 0.05$. Differences between storage periods for a same beverage trial were statistically analysed using repeated measures analysis of variance (RM-ANOVA), followed by the *post-hoc* Tukey test, with $P < 0.05$. Initially, all data were checked for normal distribution using the Shapiro-Wilk and the Kolmogorov-Smirnov tests, and also for homogeneity of variances using the Bartlett test. When these assumptions were not verified, the equivalent non-parametric tests were applied. Statistical analysis was performed using the Statistica software - version 8.0 (Statsoft Inc., Tulsa, OK, USA). The sensory evaluation data were analysed using the table for the critical number of correct responses in the triangle test, considering significance with $P < 0.05$ (Meilgaard et al., 1999; IAL, 2008).

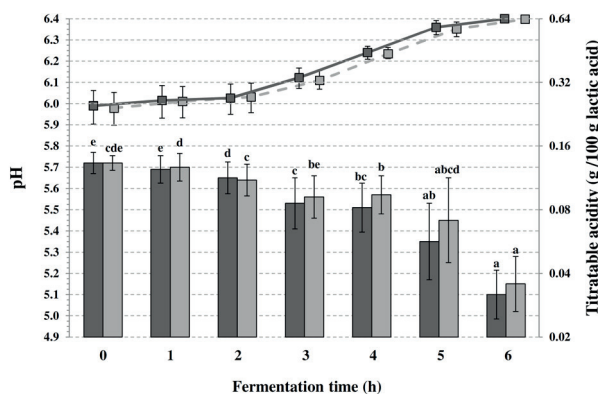
Results and discussion

Physicochemical parameters of goat whey powder and reconstituted whey used in the processing of dairy beverages

The whey powder samples showed mean composition values of 97.65 ± 0.41 g/100 g for total solids, 9.77 ± 0.77 g/100 g for ash, 7.17 ± 1.78 g/100 g for fat, 14.20 ± 0.57 g/100 g for protein and 66.51 ± 2.31 g/100 g for total carbohydrates. Similar values were found for the composition of goat whey powder in other studies: Kehagias et al. (2008) found an ash content of 8.5 g/100 g; Sanmartín et al. (2012) verified fat and protein content of 5.91 g/100 g and 12.8 g/100 g, respectively.

The pH values and titratable acidity of the reconstituted goat whey powder during the fermentation process is shown in Figure 1. The pH values of the reconstituted whey in both trials initiated before the incubation process close to 5.70 and after 6 h (end of the fermentation) the pH reduced significantly to about 5.10 in both trials ($P < 0.05$). The acidity values increased significantly in both trials, from about 0.245 g/100 g lactic acid at the beginning of the incubation to about 0.640 g/100 g lactic acid at the end of fermentation. ST-LC trials tend to show a slightly higher acidity throughout the fermentation process, as only values of this formulation at 4 h and 6 h differed significantly from those at

Figure 1. Changes in mean values of pH (bars) and titratable acidity (lines) of reconstituted goat milk powder of ST - control (dark grey) and ST-LC (light grey) trials during the fermentation process. Error bars represent the standard deviations



Above the bars, different lowercase letters denote significant differences between the sampling periods for a same trial ($P < 0.05$). Trials did not differ significantly among them within a same sampling period ($P > 0.05$).

0 h ($P < 0.05$). ST (control) trial showed significantly different ($P < 0.05$) values from already after 2 h of incubation in comparison to those measured at the beginning. Despite that, within a same fermentation period no significant differences for both, pH and titratable acidity, were observed between ST and ST-LC trials ($P > 0.05$). Similar pH values (close to 5.03) were verified by Buriti et al. (2014) in milk bases constituted of mixtures of sucrose, goat Coalho cheese whey and goat milk fermented by *S. thermophilus* TA-40 in co-culture with the probiotic microorganisms *Bifidobacterium animalis* ssp. *lactis* BB-12 and *Lactobacillus rhamnosus* Lr-32 after 3 h of incubation at 43 ± 2 °C. In that study, the fermentation time was probably reduced due to the addition of whole goat milk and sucrose to the fermented dairy base but also due to the presence of two adjunct cultures (*B. animalis* and *L. rhamnosus*). Similarly González-Martínez et al. (2002) reported a slower decrease in pH during fermentation as the concentration of whey powder increased

in yogurt formulations. In this study, only goat whey powder was used as a substrate for fermentations by the starter *S. thermophilus* TA-40 in ST trial and by the starter plus *L. casei* BGP93 in ST-LC trial.

Mean composition and pH of dairy beverages

The mean composition and the pH of dairy beverages are shown in Table 1. No significant differences between ST and ST-LC trials ($P > 0.05$) were verified for all the compositional parameters studied (total solids, ash, fat, protein and total carbohydrates). The proteins found were in agreement with the Brazilian regulatory standards (Brazil, 2005), which set minimum protein content at 1 g/100 g in the total dry matter for dairy beverages with other ingredients added than the dairy base (of only whey or whey plus milk). The addition of sucrose syrup to the formulation of dairy beverages reduced the values of ash, fat and protein in the dry matter in comparison to those found for whey powder, while the

Table 1. Physicochemical analysis of ST (control) and ST-LC dairy beverages (mean \pm standard deviation).

Item	Trial	
	ST	ST-LC
Total solids (g/100 g)	26.69 \pm 0.49 ^A	26.49 \pm 0.266 ^A
Ash (g/100 g)		
Whole matter	1.66 \pm 0.19 ^A	1.72 \pm 0.20 ^A
Dry matter	6.25 \pm 0.79 ^A	6.51 \pm 0.76 ^A
Fat (g/100 g)		
Whole matter	0.710 \pm 0.179 ^A	0.693 \pm 0.237 ^A
Dry matter	2.66 \pm 0.69 ^A	2.62 \pm 0.917 ^A
Protein (g/100 g)		
Whole matter	2.27 \pm 0.38 ^A	2.21 \pm 0.325 ^A
Dry matter	10.01 \pm 2.68 ^A	9.91 \pm 3.55 ^A
Total Carbohydrate (g/100 g)		
Whole matter	22.04 \pm 1.08 ^A	21.87 \pm 0.598 ^A
Dry matter	82.56 \pm 2.64 ^A	82.53 \pm 1.84 ^A
pH		
Day 1	5.00 \pm 0.07 ^{Aa}	5.04 \pm 0.11 ^{Aa}
Day 7	5.00 \pm 0.11 ^{Aa}	5.01 \pm 0.13 ^{Aa}
Day 14	4.97 \pm 0.18 ^{Aa}	4.99 \pm 0.21 ^{Aa}
Day 21	4.92 \pm 0.19 ^{Aa}	4.97 \pm 0.20 ^{Aa}

^A = In a row, trials sharing a same superscript capital letter in a same sampling period did not differ significantly regarding the parameter evaluated ($P > 0.05$)

^a = In a column, values sharing a same superscript lowercase letters for a same trial did not differ significantly during the different sampling periods ($P > 0.05$)

total carbohydrates were increased. The pH of ST and ST-LC dairy beverages remained stable throughout the whole storage of 21 days, and no significant difference was observed neither between sampling periods nor between the trials studied ($P>0.05$).

The development of acidity and it remaining at stable levels are among the key points to ensure the quality of dairy products such as yogurts and fermented dairy beverages (Tamime and Robinson, 2007). The progressive fermentation of lactose due to active metabolism of the added cultures can result in post acidification reducing the pH (Lobato Calleros et al., 2014), which can in turn result in textural changes (Montanuci et al., 2012) and syneresis (Lucey, 2004). Such changes affect negatively the products' quality and reduce its shelf life (Zhao et al., 2006). In the specific case of probiotic fermented products, the post-acidification can also result in a decreased viability of probiotic bacteria (Dave and Shah, 1998; Zacarchenco and

Massaguer-Roig, 2004). Consequently, these factors are of considerable attention when working with different strains combined in a cultured dairy base (Tamime et al., 2006). The results of the present study showed, therefore, that *S. thermophilus* TA-40 in co-culture with *L. casei* BGP93 did not result in post-acidification in the goat dairy beverages during storage.

Textural parameters and sensory evaluation of dairy beverages

The instrumental texture parameters of the dairy beverages during storage are shown in Table 2. Significant differences were not verified for firmness, consistency, cohesiveness, and viscosity index of the dairy beverages studied, either between trials or between the storage periods ($P>0.05$). This texture stability of the studied dairy beverages was probably a result of maintaining constant pH values during the 21 days of storage. The instrumental

Table 2. Texture parameters (mean \pm standard deviation) obtained for ST (control) and ST-LC dairy beverages the after 1, 7, 14 and 21 days of storage at 4 ± 1 °C

Trial	Storage (days)	Firmness (mN)	Consistency (N \times s)	Cohesiveness (mN)	Viscosity index (mN \times s)
ST	1	132.68 \pm 3.11 ^{Aa}	2.70 \pm 0.02 ^{Aa}	82.60 \pm 5.90 ^{Aa}	72.94 \pm 10.52 ^{Aa}
	7	133.41 \pm 3.78 ^{Aa}	2.73 \pm 0.06 ^{Aa}	78.58 \pm 5.03 ^{Aa}	74.81 \pm 9.89 ^{Aa}
	14	134.69 \pm 4.46 ^{Aa}	2.76 \pm 0.04 ^{Aa}	75.67 \pm 3.18 ^{Aa}	66.66 \pm 6.95 ^{Aa}
	21	131.96 \pm 0.56 ^{Aa}	2.74 \pm 0.03 ^{Aa}	79.13 \pm 5.25 ^{Aa}	71.62 \pm 3.10 ^{Aa}
ST-LC	1	134.85 \pm 5.15 ^{Aa}	2.74 \pm 0.08 ^{Aa}	77.31 \pm 6.08 ^{Aa}	64.14 \pm 12.93 ^{Aa}
	7	133.43 \pm 5.78 ^{Aa}	2.75 \pm 0.09 ^{Aa}	75.11 \pm 4.64 ^{Aa}	64.41 \pm 7.02 ^{Aa}
	14	133.96 \pm 3.90 ^{Aa}	2.73 \pm 0.04 ^{Aa}	79.32 \pm 5.57 ^{Aa}	63.02 \pm 6.92 ^{Aa}
	21	133.60 \pm 3.88 ^{Aa}	2.72 \pm 0.04 ^{Aa}	76.58 \pm 4.92 ^{Aa}	63.40 \pm 6.14 ^{Aa}

^A = In a column, trials sharing a same superscript capital letter in a same sampling period did not differ significantly regarding the parameter evaluated ($P > 0.05$).

^a = In a column, values sharing a same superscript lowercase letter for a same trial did not differ significantly during the different sampling periods regarding the parameter evaluated ($P > 0.05$)

Table 3. Distribution of the consumer's judgements ($n=30$) in the discrimination test using the triangle comparison procedure for ST (control) and ST-LC beverages analysed after 7, 14 and 21 days of storage

Responses	Time (days)		
	7	14	21
Correct	14 ^A	9 ^A	10 ^A
Wrong	16 ^A	21 ^A	20 ^A

^a = In a column, trials sharing a same superscript capital letter in a same sampling period did not differ significantly ($P > 0.05$).

For judgements with $n = 30$, it would be necessary a number of correct responses equal or greater than 15 to reject the assumption of "no significant difference" considering a $P < 0.05$ according to Mailgaard et al. (1999) and IAL (2008)

textural results obtained in the present study for ST and ST-LC beverages were slightly reduced in comparison to those found by Buriti et al. (2014) for fermented whey-based beverages added of guava or soursop pulp, for which consistency values were between $311.25-331.18 \text{ N} \times 10^2 \times s (= 3.11-3.31 \text{ N} \times s)$, with firmness and consistency of about $15 \text{ N} \times 10^2$ and $8.5 \text{ N} \times 10^2$, respectively ($= 150 \text{ mN}$ and 85 mN , respectively). The slightly firmer products obtained in that study probably resulted from the addition of whole goat milk to the fermented dairy base (not added in the present study), collaborating with a gelling net with casein, beyond the addition of the fruit pulps, sources of pectin that can act as texturizer. Similarly, González-Martínez et al. (2002) verified lesser consistent yogurts in the back extrusion test and with a more open structure observed by cryo-scanning electron microscopy as the proportion of whey powder in products increased and the skimmed milk powder decreased. According to those authors, the aspects observed in the formulations with whey powder added made them suitable for production of drinkable yogurts.

The results of sensory evaluation of ST and ST-LC dairy beverages are shown in Table 3. The ST and ST-LC dairy beverages did not differ significantly after 7, 14 and 21 days of storage ($P > 0.05$). The consumers reported in the answer sheets information such as “very similar flavour” and “difficult to distinguish” (data not shown). These results showed that the addition of *L. casei* did not result in any change concerning the visual and flavour aspects of dairy beverage when compared with the ST, which were in agreement with the pH and textural parameters data that also did not differ significantly between the trials studied ($P > 0.05$).

Conclusion

The use of the potential probiotic strain *L. casei* BGP93 as adjunct culture in the production of dairy beverages processed with goat Coalho cheese whey powder and *S. thermophilus* TA-40 as starter culture did not modified the physicochemical, texture and sensory characteristics when compared with the control product, without *L. casei*. The acidification pattern during fermentation of the goat whey base with the adjunct culture was similar to the control

product and post-acidification throughout the storage was not verified. The goat dairy beverage with *L. casei* BGP93 was, therefore, stable over 21 days and it could be a strategy of product with aggregated value based on goat cheese whey.

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Instrumentalno određivanje teksture i senzorskih svojstava fermentiranih mliječnih napitaka pripremljenih od rekonstituirane kozje sirutke i starter-kultura Streptococcus thermophilus i Lactobacillus casei

Sažetak

U ovom radu ispitivan je utjecaj soja *Lactobacillus casei* BGP93 kao pomoćne kulture (ST-LC napitak) i *Streptococcus thermophilus* TA-40 kao starter kulture na fizikalno-kemijska, teksturalna i senzorska svojstva mliječnih napitaka pripremljenih od rekonstituirane kozje sirutke zaostale u proizvodnji sira Coalho. Nisu zabilježene značajne razlike između ispitivanih uzoraka ST i ST-LC u odnosu na kinetiku zakiseljavanja tijekom procesa fermentacije ($P > 0,05$). Ni u jednom od ispitivanih uzoraka nije zapaženo naknadno zakiseljavanje s obzirom da su njihove pH vrijednosti bile stabilne i bez značajnih razlika tijekom 21 dana čuvanja pri 4 ± 1 °C. Tijekom cijelog razdoblja skladištenja oba su uzorka (ST

i ST-LC) bila stabilna s obzirom na pH, čvrstoću, kohezivnost, viskoznost i senzorsku procjenu ($P > 0,05$).

Ključne riječi: zakiseljavanje, poboljšanje nusproizvoda, kozji mliječni napitci, *Lactobacillus* dodatak kulture, stabilnost tijekom skladištenja

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