

Improving the conjugated linoleic acid content and the sensorial characteristics of Argentinian semi-hard goat cheeses by adding cultures of native lactic acid bacteria

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Abstract

In this study the physicochemical, microbiological, and fatty acid compositions together with the specific esterase activities of semi-hard goat cheeses made from native strains as starter and adjunct cultures were evaluated and compared against those of manufactured using commercial culture cheeses. The physicochemical composition was similar among cheeses, while the lactic acid bacteria were the predominant microbiota in all samples. The highest specific esterase activities were detected in cheeses with native strains. The fatty acid profile was significantly affected by native strains during the ripening time (60 days) since the conjugated linoleic acid (CLA) level increased from 0.60 to 1.03 g 100 g⁻¹ of fatty acids, whereas cheeses with commercial starter showed a CLA content of about 0.60 g of fatty acids. In cheeses with native strains, it was detected the highest desirable fatty acids, $\Delta 9$ -desaturase and CLA desaturase indexes and the lowest atherogenicity index. The native strains inoculated as starter and adjunct cultures, grew conveniently in the cheese, developed their full potential as reflected by the profile of the metabolites released during ripening and in the global sensory perception of cheeses, and contributed thus to the development of a healthier food.

Key words: semi-hard goat cheese, starter cultures, adjunct cultures; fatty acid composition, conjugated linoleic acid, esterase activity

Introduction

Artisanal goat cheeses are traditional products highly appreciated by consumers due to their organoleptic and nutritional characteristics that are

often correlated to the enzymatic activities of the native microorganisms derived from the milk and the environment (Leroy and De Vuyst, 2004).

In South America there are 21 million goats, meaning, 2.5 % of the worldwide goat livestock. In Argentina, goat milk is mainly used for specialty

cheese production, either artisanal or industrial, especially in the north and central regions (Medina et al., 2011). According to the Argentinean legislation, cheeses must be manufactured from pasteurized milk when the ripening period is lower than 60 days (ANMAT, 2014). However, pasteurization eliminates not only pathogens but also the naturally occurring bacteria that could contribute to flavour and improve health benefits. Thus, pasteurization could produce flavour loss resulting in end products perceived as “boring” by consumers (Law, 2001).

Implementing selected lactic acid bacteria (LAB) as an adjunct culture for cheese manufacturing could improve *in situ* the expression of some desired properties of the product (Leroy and De Vuyst, 2004). LABs are either used as starter and adjunct cultures or could be found as secondary microbiota during cheese fermentation. Therefore, LABs used as starters produce food acidification that results in a tangy lactic acid taste, frequently exert proteolytic and lipolytic activities, and produce aromatic compounds from, for instance, amino acids upon further bioconversion (Van Kranenburg et al., 2002). The addition of non-starter LABs (NSLAB) as adjunct cultures could accelerate cheese ripening, and consequently increase the level of free aminoacids, peptides, and free fatty acids (FFA) that altogether leads to an increased flavour intensity (Crow et al., 2001).

Cheese flavour is one of the most important criteria determining consumer choice and acceptance (Hassan et al., 2013). Cheese flavour is the result of glycolysis, proteolysis, lipolysis and citrate metabolism carried out by cheese microbiota. The contribution of cheese microbiota to lipolysis occurs via the esterase/lipase systems. Esterases from starter and nonstarter lactic acid bacteria (NSLAB) are responsible for the release of short-chain fatty acids (SCFA) from milk fat at elevated water activity (a_w) and the synthesis of fruity flavour in dairy products (Taboada et al., 2014a).

During lipolysis, some bioactive compounds such as the conjugated linoleic acid (CLA) could be produced by the isomerase-desaturase complex enzymes (Taboada et al., 2015). The CLA term includes a mixture of linoleic acid (LA) isomers in which the double bonds are conjugated. Different CLA isomers can be found in the cis-trans, trans-cis and cis-cis or trans-trans geometrical configurations.

It has been reported that the CLA exhibits many health benefits such as anti-carcinogenic, anti-atherosclerosis and anti-obesity activities, and improves the immune system (Yadav et al., 2007) as well. The CLA is mainly produced by ruminal biohydrogenation of linoleic and linolenic fatty acids, and formed in the mammary gland through the desaturase activity on the vaccenic acid (C18:1 t11).

Therefore, dairy products are the most important source of CLA for humans. The CLA content in dairy products could be influenced by genetic and environmental factors affecting milk production, milk processing, the aging and storage of products and food manufacture (Bisig et al., 2007). Some strains of LABs such as *Bifidobacterium*, *Lactobacillus* (L), *Streptococcus* (S) are able to convert LA into CLA (Khosravi, 2015; Terán et al., 2015). While there are a lot of studies concerning the influence of LABs on the CLA in dairy products (Mohan et al., 2013; Carafa et al., 2019; Renes et al., 2019), there are only a few studies presenting data on the CLA in goat cheeses, influenced by LABs (Taboada et al., 2015).

The native LABs used for dairy products development are often associated with positive changes on the flavour and fatty acid profiles, higher acceptability by consumer and healthier functional attributes. Therefore, designing cultures using autochthonous strains to produce artisanal and functional cheeses is widely spread, especially for traditional goat and ewe dairy products (Mohan et al., 2013; Carafa et al., 2019; Picón et al., 2019). However, in Argentina, data about the use of selected autochthonous strains are scarce (Oliszewski et al., 2013; Taboada et al., 2015, 2017). Even though Taboada et al. (2015) designed a mix of autochthonous strains culture to be used for manufacturing cheese from Saanen goat milk the most widely breed used in Argentina is the Creole breed. As far as it is known, there are no studies providing data on the characterization of Creole semi-hard goat cheeses made out of native cultures and their potential contributions to consumers' health.

Therefore, the objective of this study was to evaluate the effect of especially designed autochthonous cultures, containing: *L. plantarum*, *L. bulgaricus*, *S. thermophilus* and *Pediococcus pentosaceus*, on the specific esterase activities together with the physicochemical, microbiological, and fatty acid compositions of Creole semi-hard goat cheeses.

Materials and methods

Strain composition of cultures

The native strains used in this study were provided by the Universidad Nacional de Santiago de Estero (UNSE- Argentina), previously isolated from

Argentinean goat milk and cheese and carefully selected by their technological properties (Taboada et al., 2014 a, b). On the basis of strain compatibility tests (Taboada et al., 2015) different combinations of native strains in cheese making were used, according to the following schedule:

Strains	Starter culture plus adjunct culture C (% v/v)	Starter culture plus adjunct culture P (% v/v)
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> UNSE150 (starter)	30	30
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> UNSE313 (starter)	30	30
<i>Streptococcus thermophilus</i> UNSE228 (starter)	18	18
<i>Streptococcus thermophilus</i> UNSE282 (starter)	17	17
<i>Lactobacillus plantarum</i> UNSE287 (adjunct)	2.5	1.25
<i>Lactobacillus plantarum</i> UNSE218 (adjunct)	2.5	1.25
<i>Pediococcus pentosaceus</i> UNSE310 (adjunct)	0	1.25
<i>Pediococcus pentosaceus</i> UNSE315 (adjunct)	0	1.25

In addition, the lyophilized commercial culture *S. thermophilus* SLB95 (Diagramma SA, Santa Fe, Argentina) was used. Both, the native strains and the lyophilized commercial culture as well, were previously activated in de Man/Rogosa/Sharpe, MRS broth, (Merck, Darmstadt, Germany) and multiplied in goat milk pasteurized at 35 °C until coagulation. Native cultures with 10^8 cfu mL⁻¹ were used for cheese manufacturing.

Cheese manufacture

The raw goat milk samples (Creole Breed, n= 30) were obtained from a goat dairy farm (distant 40 km from the laboratory), refrigerated and transported at 4 °C to the pilot plant of the Instituto de Ciencia y Tecnología de Alimentos-UNSE (Santiago del Estero, Argentina). The goats were milked while they were between their second and fourth month of lactation. The chemical composition of the milk was determined using the Lactostar analyser (Funke Gerber Lactostar, Funke-Dr. N. Gerber Labortechnik GmbH, Berlín, Germany); the values of three determinations were averaged, showing the following results (expressed in percent, w/v): 3.90±0.03 protein; 4.93±0.03 fat; 8.46±0.03 non-fat total solids. The pH of the samples was determined through the Metrohm 962 pHmeter (Herisau, Switzerland): it was 6.63±0.13.

Semi-hard goat cheeses were made according to a standard process: thus 40 L of raw milk was pasteurized at 65 °C for 20 min, cooled at 38 °C and added with CaCl₂ (Merck, Darmstadt, Germany) to a final concentration of 0.2 g kg⁻¹.

The native strains were first suspended in sterile milk and then inoculated at 0.2 ml L⁻¹ (v/v) inoculum of either C or P culture or 0.1 g L⁻¹ of lyophilized commercial culture (*S. thermophilus* SLB95, Type DVS, Diagramma SA, Santa Fe, Argentina), according to the manufacturer's instructions.

The milk was coagulated by adding 0.014 g L⁻¹ of chymosin (Maxiren150, Delft, The Netherlands). When the curd reached the appropriate strength, it was cut to a corn grain size. The mixture of curd particles and whey was then gently stirred and heated to 47 °C at 1 °C min⁻¹. After the curd grains gained an adequate moisture level for semi-hard cheeses, the stirring was stopped and the whey was drained and discarded. The curd was placed in cylindrical molds (10 cm height, 12 cm diameter) and pressed (0.2-0.3 kg cm⁻²) for 24 h. The cheeses were salted by immersing them in brine 16 % (w/v) NaCl solution, pH 5.4 at 12 °C for 2 h.

Cheese ripening was carried out at 12 °C and 85 % of relative humidity for 60 days, being vacuum packaged after 30 days of ripening. Each cheese weighed approximately 700 g.

Three independent tests for each type of cheese (that is, CC, cheese manufactured using the autochthonous culture C; CP, cheese manufactured using the autochthonous culture P, and Commercial, cheese manufactured using the lyophilized commercial culture) were made and samples were taken on day 1, 45 and 60 of ripening for further analysis.

Physicochemical composition

The physicochemical composition of each cheese was performed on day 1, 45 and 60 of ripening. The AOAC (Association of Official Analytical Chemists) official methods were used for determining fat content (Methods 933.05 AOAC 2006), NaCl content (Methods 975.20 AOAC 2006) and acidity (AOAC 920.124 AOAC 2006). Dry matter and protein contents were determined according to Taboada et al. (2017). The pH values of the samples were obtained by inserting directly the tip of the probe (MV-TEMP pH meter, Digital Instruments, Taiwan) and their water activity (a_w) was determined using a RotronicAwQuick carp instrument (New York, USA).

Microbiological analysis

The cheese samples (10 g) were dispersed in 90 mL of 2 % (w/v) sodium citrate solution, homogenized for 2 min in a Stomacher (Laboratory Blender Stomacher model 400, Seward Medical, London, U.K.), diluted in peptone saline solution, and placed on specific media for viable counts.

The mesophilic and thermophilic LABs (MRS agar pH 6.5, at 35 °C and 42 °C, respectively, for 48 h), fungi and yeasts (Yeast Extract Glucose Chloramphenicol Agar, 5 days at 30 °C) and total aerobic mesophilic microorganisms (PCA, 35 °C, 48 h) were determined at different times of ripening.

According to the Argentinian legislation (ANMAT, 2014), coliforms at 30 °C and 45 °C (MPN method, three tubes series, Brilliant Green Lactose Bile Broth, 48 h at 30 °C and 45 °C, respectively) were also determined.

Fatty acid composition

Lipids in the raw milk and cheeses (on 1, 45 and 60 days of ripening) were extracted using a

chloroform/methanol solution (2:1, v/v) as the Folch's procedure (Folch and Lees, 1957). Fatty acid methyl esters (FAME) analyses were in turn performed according to the method described by Taboada et al. (2015). Briefly, the lipids were hydrolyzed by adding 3 mL of 0.9 % (w/v) NaOH in methanol at 50 °C for 35 min. Fatty acids were methylated using 4 % (v/v) HCl in methanol at 60 °C for 20 min, and then extracted with hexane and eventually evaporated under a nitrogen stream.

One microlitre of FAME, dissolved in hexane, was injected to an Agilent Technologies gas chromatograph (Model 6890N, Palo Alto, CA, USA) equipped with a flame ionization detector and automatic injector (Model 7683, USA) into a HP-88 capillary column (100 m x 0.25 mm x 0.20 µm, Agilent Technologies, USA). GC conditions were: injector temperature of 255 °C; the initial oven temperature of 75 °C was increased to 165 °C at 8 °C/min and held there for 35 min, then it was increased to 210 °C at 5.5 °C/min and held for 2 min and at last increased to 240 °C at 15 °C/min and held for 3 min. Detector temperature was 280 °C. Oxygen-free nitrogen was used as the carrier gas at a flow rate of 18 mL/min, at 38 psi. The total time of the oven programme was 72 min. The fatty acids were identified by comparing the retention times against the methylated standards (99 % pure; Sigma, St. Louis, MO, USA). The results were expressed as g 100 g⁻¹ of fatty acid methyl ester (FAME).

Estimation of desaturase and atherogenicity indexes

The mammary gland of ruminants has substantial Δ^9 -desaturase activity, enzyme which can be measured indirectly by comparing the product: substrate ratio. Therefore, C14:1/C14:1 + C14:0 ratio is the best indicator of this activity because all C14:0 in milk fat come from *de novo* synthesis in the mammary gland (Lock et al., 2005). The CLA desaturase index was estimated using the following equation: CLA_{desaturase} = cis-9, trans-11 / (cis-9, trans-11 + trans-11-C18:1). Other food parameter of biological interest is the atherogenicity index (AI), which characterizes the atherogenicity of dietary fat and represents the relationship between hypercholesterolemic and protective fatty acids.

The AI was calculated by the following formula: $AI = [C12:0 + (4 \times C14:0) + C16:0] / (MUFA + PUFA)$ described by Ulbricht and Southgate, (1991).

Specific esterase activity

The cheese samples (10 g) were dispersed in 90 mL of 100 mM sodium phosphate buffer solution of pH 7, homogenized for 2 min in a Stomacher (Laboratory Blender Stomacher model 400, Seward Medical, London, UK). The debris was removed by centrifugation (10,000 x g for 30 min) at 4 °C, and supernatants were then used for determining the esterase activity.

The specific esterase activity (EA) was determined in the cheese samples homogenate using α -naphthyl derivatives of 2-8 carbon atom fatty acids as substrate (Sigma, St. Louis, MO) according to the method described by Taboada et al. (2014b). The sample mixture contained 160 μ L of a 100 mM sodium phosphate buffer, pH 7.0, 20 μ L of α -naphthyl substrate (10 mM in ethanol), and 100 μ L of cheese homogenate. After 1 h incubation at 37 °C, color was developed by adding 0.6 mL of Fast Garnet GBC (Sigma) solution (5 mg/mL in 10 % w/v SDS) and further incubation at room temperature for 15 min. The absorbance was measured at 560 nm in a spectrophotometer (CECIL 2021, Cambridge, UK).

Control samples containing the reaction mixture and glacial acetic acid were also incubated for testing background activity. A standard curve was prepared using α -naphthol. A unit of esterase activity was defined as the amount of enzyme releasing 1 μ mol of α -naphthol per minute. The specific esterase activity was defined as units per milligram of protein. Protein concentrations in homogenate cheese samples were determined according to Taboada et al., (2014b).

Sensorial analysis

The sensorial analyses of the cheese samples were performed at the end of the ripening time (60 days) by an internal panel consisting of nine trained judges, among 30-60 years old, in a 4 male and 5 female gender proportion. All the panelists were previously trained in four 1 h sessions using regional artisanal and commercial goat cheese. In these sessions, the attributes (flavour and odor) were

defined and qualified. The samples of each goat cheese were refrigerated at 4 ± 1 °C for 60 days of ripening time and randomly identified with 3-digit code and served to the judges on individual dishes at room temperature (22 ± 1 °C) along with mineral water to clean their palates between samples.

The odour and flavour of the cheese samples were evaluated using a 5-point intensity scale ranging from less to more intense attributes. The average value of the three evaluations for each attribute of each panelist was statistically analyzed. Overall impression of the product was determined considering the intensity of flavour notes present as well as the mixtures thereof. It was rated as good (high intensity), regular (medium intensity) and poor (low intensity).

Statistical analysis

The results of the three independent trials for each cheese sample were expressed as mean \pm standard deviation (SD). The ripening time, global composition, microbial count, fatty acid composition and specific sterase activity data were all subjected to one-way analysis of variance (ANOVA) using the InfoStat 2016 statistical software (Grupo Infostat, FCA, Universidad Nacional de Córdoba, Argentina) to detect differences between commercial and experimental cheeses. The Tukey's multiple comparison test was applied when significant differences ($P < 0.05$) in mean values were detected.

Results and discussion

Physicochemical composition

According to Argentinean legislation (ANMAT, 2014), cheeses with humidity values between 36 and 45.9 %, are classified as medium humidity cheeses (generally known as semi-hard cheeses).

The evolution of physicochemical composition of semi-hard goat cheeses during ripening is shown in Table 1. For all cheese samples, values of fat and dry matter are according to the range established by Argentinean legislation for semi-hard cheeses. The physicochemical composition of cheeses was similar to that previously informed for other semi-hard goat cheeses of the region (Oliżewski et al., 2013; Taboada et al., 2015).

TABLE 1. Physicochemical composition during ripening of semi-hard goat cheeses (cheese manufactured with culture C-CC, cheese manufactured with culture P-CP and cheese manufactured with lyophilized commercial culture-Commercial)

Days of ripening	Cheese	Protein	Fat	Dry matter	NaCl	pH	a _w
1	CC	21.08±0.34 ^A	33.68±0.22 ^A	55.53±0.27 ^A	1.01±0.11 ^A	5.23±0.03 ^A	0.988±0.002 ^A
	CP	21.18±0.26 ^A	33.84±0.25 ^A	55.62±0.35 ^A	1.02±0.12 ^A	5.20±0.05 ^A	0.987±0.003 ^A
	Commercial	21.02±0.23 ^A	33.12±0.27 ^A	55.32±0.30 ^A	0.99±0.12 ^A	5.18±0.02 ^A	0.986±0.002 ^A
45	CC	25.23±0.22 ^A	38.28±0.26 ^B	59.22±0.24 ^B	1.26±0.19 ^A	4.98±0.02 ^A	0.984±0.003 ^A
	CP	25.33±0.35 ^A	36.96±0.20 ^A	58.20±0.30 ^A	1.22±0.22 ^A	4.94±0.45 ^A	0.985±0.004 ^A
	Commercial	25.04±0.27 ^A	38.02±0.26 ^B	58.02±0.25 ^A	1.20±0.30 ^A	5.12±0.30 ^A	0.983±0.003 ^A
60	CC	27.21±0.22 ^A	39.54±0.18 ^A	60.56±0.22 ^B	1.40±0.22 ^A	5.02±0.02 ^A	0.978±0.003 ^A
	CP	26.97±0.26 ^A	40.00±0.35 ^A	61.60±0.24 ^A	1.43±0.24 ^A	5.08±0.30 ^A	0.979±0.003 ^A
	Commercial	27.10±0.33 ^A	38.32±0.26 ^B	61.13±0.20 ^A	1.38±0.27 ^A	5.00±0.25 ^A	0.978±0.004 ^A

Values are mean of three trials for each type of cheese ± standard deviation (SD). Different superscript letters in each column at the same time of ripening indicate statistically significant differences ($P < 0.05$). Protein, Fat, Dry matter and NaCl values are expressed as g 100 g⁻¹ of cheese.

Fatty acid composition

MICROBIOLOGICAL ANALYSIS

The evolution of microorganisms in semi-hard goat cheeses during the ripening time is shown in Table 2. The total coliforms count at 30 °C was lower than what the Argentinean legislation ($m=1000$; $M=5000$) allows for medium humidity cheeses (36.0-45.9 % moisture) (ANMAT, 2014); whereas coliforms at 45 °C were not even detected.

Along ripening, fungi, yeast and aerobic mesophilic bacteria counts showed non-statistically significant differences ($P > 0.05$).

These findings indicate that mesophilic and thermophilic LABs remain viable even on day 60. The microbiological characteristics of the cheese samples are coincident to those previously reported for Argentinean semi-hard goat cheeses (Oliszewski et al., 2013).

TABLE 2. Microbiological counts during ripening of semi-hard goat cheeses (cheese manufactured with culture C-CC, cheese manufactured with culture P-CP and cheese manufactured with lyophilized commercial culture-Commercial)

Days of ripening	Cheese ^a	Lactic acid bacteria (LAB)		Fungi and yeast	Aerobic mesophilic bacteria
		Mesophyle	Termophyle		
1	CC	7.27±0.26 ^B	7.02 ±0.32 ^B	1.94±0.27 ^A	3.63±0.40 ^A
	CP	7.39±0.20 ^B	7.17 ±0.30 ^B	1.83±0.25 ^A	3.70±0.30 ^A
	Commercial	6.40±0.40 ^A	6.20 ±0.30 ^A	2.20±0.33 ^A	3.90±0.27 ^A
45	CC	8.40±0.26 ^A	8.18±0.32 ^B	2.46±0.26 ^A	3.96±0.28 ^A
	CP	7.78±0.40 ^{AC}	7.85±0.40 ^{BC}	2.58±0.40 ^A	4.02±0.30 ^A
	Commercial	7.05±0.40 ^{BC}	7.00±0.30 ^{AC}	2.88±0.30 ^A	4.20±0.40 ^A
60	CC	7.95±0.30 ^B	7.82 ± 0.32 ^B	2.72±0.22 ^A	4.22±0.25 ^A
	CP	8.32±0.33 ^B	8.20 ± 0.40 ^B	2.88±0.20 ^A	4.31±0.23 ^A
	Commercial	6.90±0.40 ^A	6.70 ±0.40 ^A	2.50±0.40 ^A	4.60±0.30 ^A

Values are mean ± SD of three trials for each type of cheese. Counts of microbial groups are expressed as log CFUg⁻¹. Different superscript letters in each microbial group and at the same ripening time indicate statistically significant differences ($P < 0.05$)

The fatty acid (FA) composition of the goat raw milk is shown in Table 3. The saturated fatty acids (SFA) were predominant (~ 68 g 100 g⁻¹ of FAME), being the palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids the most abundant among them. The oleic acid (cis-9, C18:1) was the main FA detected among the monounsaturated fatty acids (MUFA, ~28 g 100 g⁻¹ of FAME),

The linoleic (C18:2) and linolenic (C18:3) acids shown the highest levels among the polyunsaturated fatty acids (PUFA, near 5 g 100 g⁻¹ of FAME) while the conjugated linoleic acid (CLA) reached a value of 0.73 g/100 FAME, being cis-9, trans-11 the unique isomer determined, in agreement with previous studies on goat milk (Rodríguez-Alcalá et al., 2009; Savoini et al., 2010).

TABLE 3. Fatty acid composition of Creole breed goat milk (g 100 g⁻¹ of FAME)

Fatty acid	Value*	Fatty acid	Value*
C4:0	0.22±0.03	C18:2 n6t	0.17±0.03
C6:0	0.89±0.02	C18:2 n6c	1.99±0.24
C8:0	1.97±0.12	C18:3 n3	1.64±0.04
C10:0	8.72±0.14	<i>cis</i> 9, <i>trans</i> -11-CLA	0.63±0.12
C12:0	4.68±0.26	<i>trans</i> 10, <i>cis</i> -12- CLA	n.d
C14:0	10.82±0.28	C20:0	0.45±0.03
C14:1	0.68±0.12	C20:1	0.18±0.02
C15:0	0.97±0.08	C20:4	0.17±0.02
C16:0	26.18±1.10	C22:5	0.20±0.03
C16:1	1.38±0.14	SFA ^a	67.87±7.99
C17:0	0.67±0.10	MUFA ^b	28.29±10.40
C17:1	0.30±0.10	PUFA ^c	4.64±0.82
C18:0	12.30±0.67	Δ9-desaturase index (C14) ^d	0.057±0.01
<i>trans</i> 10-C18:1	0.20±0.02	CLA desaturase index ^e	0.28±0.01
<i>trans</i> 11-C18:1	1.63±0.12	Atherogenicity index (AI) ^f	2.25±0.65
<i>cis</i> 9-C18:1	24.30±1.17		

Values are expressed as mean ± standard deviation. CLA, conjugated linoleic acid.

^aSFA, saturated fatty acids.

^bMUFA, monounsaturated fatty acids.

^cPUFA, polyunsaturated fatty acids.

^dΔ9-desaturase index, C14:1/C14:1+C14:0.

^eCLA desaturase index: CLA / (CLA + *trans*-11C18:1).

^fAtherogenicity index = [(C12:0 + (4 × C14:0) + C16:0)] / (MUFA + PUFA). n.d, not determined

Desaturase index is important to maintain milk fat fluidity. It reached a value of 0.057 in goat raw milk while the CLA desaturase index was 0.26 which agrees with values previously reported for both indexes in the goat milk from the region (Van Nieuwenhove et al., 2009). The atherogenicity index (AI), which in-

dicates the negative effect of foods on health, reached a value of 2.25 that falls within the range informed in the literature for goat milk (Van Nieuwenhove et al., 2009; Osmari et al., 2011).

The FA composition of semi-hard goat cheeses is shown in Table 4.

TABLE 4. Fatty acid composition during ripening of semi-hard goat cheeses (cheese manufactured with culture C-CC, cheese manufactured with culture P-CP and cheese manufactured with lyophilized commercial culture - Comercial)

Fatty acids	Ripening time (days)								
	1			45			60		
	CC	CP	Commercial	CC	CP	Commercial	CC	CP	Commercial
C4:0	0.14 ±0.02 ^A	0.15±0.03 ^A	0.12±0.02 ^A	0.47±0.03 ^B	0.17±0.03 ^A	0.15±0.02 ^A	0.66 ± 0.02 ^B	0.62±0.03 ^B	0.56 ± 0.02 ^A
C6:0	1.28±0.16 ^A	1.30±0.14 ^A	1.02±0.20 ^A	1.39±0.20 ^A	1.34±0.14 ^A	1.20±0.14 ^A	1.60±0.16 ^A	1.57±0.05 ^A	1.43±0.10 ^B
C8:0	1.61±0.19 ^A	1.88±0.22 ^A	1.50±0.20 ^A	1.82 ± 0.23 ^A	1.93±0.05 ^A	1.78± 0.13 ^A	2.26 ±0.04 ^B	2.67±0.12 ^B	1.98±0.10 ^A
C10:0	9.98±0.90 ^B	7.68±0.25 ^A	9.40±0.22 ^B	9.68 ± 0.26 ^B	8.52±0.20 ^A	9.12±0.22 ^{AB}	9.08 ±0.08 ^B	8.02±0.20 ^A	8.86±0.22 ^B
C12:0	4.95±0.22 ^A	5.40±0.18 ^{AB}	5.60±0.20 ^B	4.67±0.28 ^B	3.80±0.14 ^A	4.86±0.20 ^B	4.02±0.15 ^B	3.42±0.25 ^A	4.30±0.20 ^B
C14:0	10.67±0.23 ^A	12.55±0.49 ^B	12.70±0.20 ^B	9.62±0.49 ^A	11.30±0.40 ^B	11.46±0.50 ^B	8.80±0.07 ^A	8.68±0.20 ^A	9.50±0.28 ^B
C14:1	0.58±0.04 ^A	0.59±0.06 ^A	0.55±0.07 ^A	0.55±0.08 ^A	0.62±0.04 ^A	0.52±0.03 ^A	0.52±0.02 ^A	0.53±0.03 ^A	0.50±0.04 ^A
C15:0	0.84±0.03 ^A	0.86±0.03 ^A	0.90±0.05 ^A	0.82±0.06 ^A	0.90±0.04 ^A	0.92±0.05 ^A	0.78±0.06 ^A	0.82±0.05 ^A	0.86±0.04 ^A
C16:0	23.60±0.40 ^A	24.63±0.45 ^A	24.40±0.50 ^A	24.82±0.45 ^A	24.77±0.40 ^A	24.50±0.35 ^A	25.36±0.45 ^A	25.27±0.33 ^A	25.95±0.22 ^A
C16:1	1.19±0.10 ^A	1.43±0.07 ^A	1.02±0.20 ^A	1.48±0.49 ^A	1.82±0.04 ^A	1.08±0.20 ^A	1.70±0.21 ^B	0.54±0.02 ^A	0.50±0.02 ^A
C17:0	0.95 ±0.04 ^B	0.69±0.09 ^A	0.98 ±0.04 ^B	0.77±0.03 ^A	0.64±0.07 ^A	0.70±0.03 ^A	0.58±0.05 ^A	0.52±0.02 ^A	0.54±0.02 ^A
C17:1	0.28±0.03 ^A	0.35±0.03 ^A	0.26±0.02 ^A	0.33±0.02 ^A	0.35±0.01 ^A	0.30±0.02 ^A	0.38±0.03 ^A	0.39±0.02 ^A	0.33±0.03 ^A
C18:0	12.65±0.45 ^A	12.18±0.33 ^A	12.78±0.30 ^A	11.52±0.33 ^A	11.74±0.35 ^A	11.70±0.30 ^A	10.55±0.21 ^A	11.23±0.03 ^A	11.43±0.04 ^A
<i>trans</i> 10-C18:1	0.21±0.05 ^A	0.17 ± 0.04 ^A	0.15 ± 0.03 ^A	0.18± 0.02 ^A	0.20 ±0.05 ^A	0.17 ± 0.03 ^A	0.15± 0.02 ^A	0.21± 0.02 ^A	0.15± 0.02 ^A
<i>trans</i> 11-C18:1	1.84±0.14 ^A	2.00±0.10 ^A	1.80±0.10 ^A	1.87±0.20 ^A	2.01±0.12 ^A	1.83±0.10 ^A	1.98±0.035 ^A	2.06±0.11 ^A	1.88±0.20 ^A
<i>cis</i> 9- C18:1	23.22±0.45 ^A	24.98±0.42 ^B	23.03±0.30 ^A	24.17±0.94 ^{AB}	25.75±0.61 ^A	24.02±0.60 ^B	25.86±0.64 ^A	27.90±0.30 ^B	26.02±0.40 ^A
C18:2	1.95±0.09 ^A	2.05±0.14 ^A	1.88±0.14 ^A	2.06±0.15 ^A	2.06±0.15 ^A	1.90±0.13 ^A	2.27±0.19 ^A	2.42±0.12 ^A	2.03±0.13 ^A
C18:3	1.17±0.12 ^A	1.35±0.07 ^A	1.02±0.10 ^A	1.28±0.09 ^{AB}	1.41±0.06 ^B	1.18±0.06 ^A	1.39±0.12 ^A	1.94±0.09 ^B	1.21±0.04 ^A
<i>cis</i> 9, <i>trans</i> 11-C18:2 (CLA)	0.63±0.06 ^A	0.60±0.03 ^A	0.50±0.04 ^A	0.68±0.04 ^{AB}	0.78±0.05 ^B	0.57±0.05 ^A	0.92±0.05 ^B	1.03±0.02 ^B	0.62±0.03 ^A
<i>cis</i> 12, <i>trans</i> 10-C18:2 (CLA)	0.06±0.01	n.d	n.d	0.06±0.014	n.d	n.d	0.05±0.01	n.d	n.d
S CLA	0.69±0.03 ^B	0.60±0.03 ^{AB}	0.50±0.04 ^A	0.74±0.04 ^B	0.78±0.05 ^B	0.57±0.04 ^A	0.97±0.05 ^B	1.03±0.02 ^B	0.62±0.03 ^A
C20:0	0.52±0.02 ^A	0.50±0.05 ^A	0.53±0.02 ^A	0.51±0.014 ^A	0.47±0.02 ^A	0.52±0.02 ^A	0.44±0.03 ^A	0.43±0.04 ^A	0.50±0.02 ^A
C20:1 <i>cis</i> 11	0.19±0.07 ^A	0.18±0.02 ^A	0.17±0.03 ^A	0.21±0.02 ^A	0.20±0.02 ^A	0.19±0.03 ^A	0.21±0.01 ^A	0.20±0.04 ^A	0.19±0.01 ^A
C20:4	0.12 ±0.02 ^A	0.13±0.02 ^A	0.10±0.02 ^A	0.15±0.02 ^A	0.16±0.02 ^A	0.12±0.02 ^A	0.19 ±0.02 ^A	0.20±0.02 ^A	0.18±0.02 ^A
C22:5	0.24±0.07 ^A	0.19±0.02 ^A	0.18±0.02 ^A	0.22±0.014 ^A	0.20±0.02 ^A	0.18±0.02 ^A	0.23±0.02 ^A	0.20±0.02 ^A	0.19±0.02 ^A
SCFA ^d	3.03±0.17 ^{AB}	3.33±0.18 ^B	2.64±0.18 ^A	3.68±0.19 ^A	3.44±0.15 ^A	3.13±0.12 ^A	4.48±0.15 ^B	4.86±0.12 ^B	3.97±0.13 ^A
MCFA ^e	27.02±0.42 ^A	27.08±0.40 ^A	29.15±0.30 ^B	25.34±0.49 ^A	25.14±0.63 ^A	26.88±0.67 ^A	23.20±0.46 ^B	21.57±0.68 ^A	24.02±0.47 ^B
LCFA ^f	68.22±0.25 ^A	71.47±0.39 ^B	68.80±0.28 ^A	70.33±0.31 ^B	72.55±0.36 ^C	68.96±0.27 ^A	69.99±0.31 ^A	74.58±0.48 ^B	71.53±0.49 ^A
SFA ^g	67.19±0.42 ^A	67.80±0.67 ^A	69.93±0.75 ^B	66.09±0.52 ^A	65.58±0.62 ^A	66.91±0.58 ^A	64.09±0.52 ^A	63.35±0.50 ^A	65.91±0.55 ^A
MUFA ^h	27.51±0.52 ^A	29.52±0.35 ^A	26.81±0.31 ^A	28.63± 0.72 ^A	30.75±0.43 ^A	27.92±0.50 ^A	30.8±0.41 ^A	31.63±0.30 ^A	29.57±0.42 ^A
PUFA ⁱ	4.11±0.11 ^A	4.06±0.10 ^A	3.68±0.08 ^A	4.45±0.10 ^A	4.24±0.11 ^A	3.95±0.10 ^A	5.05±0.11 ^A	5.39±0.13 ^A	4.23±0.09 ^A
D9-desaturase index	0.052±0.01 ^B	0.045±0.01 ^A	0.041±0.01 ^A	0.054±0.01 ^B	0.052±0.01 ^B	0.043±0.01 ^A	0.056±0.01 ^B	0.057±0.01 ^B	0.050±0.01 ^A
CLA desaturase index	0.25±0.01 ^A	0.25±0.01 ^A	0.22±0.01 ^A	0.28±0.01 ^A	0.28±0.01 ^A	0.24±0.01 ^A	0.33±0.01 ^B	0.33±0.01 ^B	0.25±0.01 ^A
AI	2.25±0.40 ^A	2.39±0.54 ^A	2.65±0.33 ^A	2.05±0.28 ^A	2.11±0.33 ^A	2.36±0.26 ^A	1.80±0.32 ^B	1.71±0.34 ^B	2.02±0.32 ^A
DFA ^t	44.27	45.74	43.27	44.60	46.73	43.57	46.40	48.25	45.23
SFA:UFA ⁿ	2.12	2.02	2.29	1.99	1.87	2.10	1.79	1.71	1.95

Fatty acids values were expressed as g 100 g⁻¹ of fatty acids (mean ± standard deviation). Different superscript letters in the same row correspond to statistically significant differences (P<0.05) among fatty acids content of each time.

SCFA (C4:0-C8:0): short chain fatty acids. MCFA (C10:0-C15:0): medium chain fatty acids. LCFA (>C16:0): long chain fatty acids. SFA, saturated fatty acids. MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids. UFA, unsaturated fatty acids.

^tDFA (desirable fatty acids) = (MUFA+PUFA + C18:0).

n.d: not determined.

The most important variation linked to the inoculation of native strains was noted on the short chain fatty acids (SCFA) content. Initially, all the cheese samples presented showed values vary-

ing from 2.64 to 3.33 g 100 g⁻¹ of FAME, which increased during maturation up to 3.97-4.86 g 100 g⁻¹ FAME, the highest value determined in CC and CP after 60 days. In turn, the butyric acid (C4:0)

content increased significantly in the cheeses manufactured using native strains (~4.5 fold), varying from 0.14-0.15 to 0.62-0.66 g 100 g⁻¹ of FAME on day 1 and 60, respectively. The variations of the caprylic acid (C8:0) content over the ripening time were also determined. They increased from 1.88 to 2.67 g 100 g⁻¹ of FAME in CP and from 1.61 to 2.26 g 100 g⁻¹ of FAME in CC, while in the commercial cheeses, the increment was lower (1.50 to 1.98 g 100 g⁻¹ FAME).

The MUFA content was not significantly affected by train addition and varied between 26.81-31.63 g 100 g⁻¹ of FAME in different samples (Table 4). The predominant MUFA was the oleic acid (cis-9, C18:1) whose content ranged from 23.03 to 27.90 g 100 g⁻¹ of FAME. Among the other MUFAs present in the cheese fat, the vaccenic (trans-11, C18:1) acid content was of around 2 g 100 g⁻¹ of FAME in all the cheese samples. Beneficial properties of this FA, such as inhibition of cancer cell growth, anti-inflammatory effect and influence on the functions of the immune system have been documented (Field et al., 2009).

Regarding the PUFA content, the cheeses manufactured with the addition of native strains (CC and CP) showed the highest content (5.05 and 5.39, respectively) compared with the commercial cheese (4.23) after 60 days of maturation. The values for the conjugated linoleic acid (CLA) varied between 0.50 and 0.62 in commercial cheeses, on days 1 and 60 of ripening, respectively. After the inoculation of native strains, the CLA content increased from 0.69 to 0.97 in CC and from 0.60 to 1.03 in CP cheese.

The *cis*-9, *trans*-11 was the major CLA isomer determined in the goat cheese samples, which is coincident with the literature for goat dairy products (Van Nieuwenhove et al., 2009). Goats seem to be unable to produce CLA by ruminal biohydrogenation intermediate product as *trans*-10, *cis*-12 form. Although *cis*-9, *trans*-11 is the most representative CLA isomer in food in quantitative terms, the *trans*-10, *cis*-12 isomer is present in negligible amounts in foods (Yamasaki and Yanagita, 2013).

The results concerning the increased CLA levels during maturation are comparable with the data reported by Rodrigues et al. (2012), where higher

CLA levels in probiotic and symbiotic cheeses manufactured with bacteria able to form CLA *in vitro* was evidenced. Moreover, other authors informed higher CLA levels in ripened than in soft cheeses (Lobos-Ortega et al., 2012). It has also been informed that several probiotic strains are able to enhance the CLA content in a yogurt manufactured with fruit-fiber (do Espírito Santo et al., 2012). The CLA increment in cheeses by using autochthonous strains was informed for goat and ewe cheeses (Mohan et al., 2013; Renes et al., 2019) as well.

DESATURASES AND ATHEROGENICITY INDEXES IN GOAT CHEESES

The $\Delta 9$ -desaturase (C14) and CLA desaturase indexes increased during cheese maturation, reaching the maximum values at 60 days in all cheeses added with native cultures (Table 4).

In contrast, lower AI values were determined in cheeses inoculated with autochthonous strains as to commercial cheeses after 60 days (1.80 and 1.71 vs. 2.02). Foods with low AI could help to prevent the occurrence of micro- and macro-coronary diseases (Ulbricht and Southgate, 1991).

The percentage of desirable fatty acids (DFA), expressed as the sum of unsaturated fatty acids plus stearic acid (Osmari et al., 2011), allows to infer the content of those beneficial for health. The results obtained showed that the DFA contents were higher in ripened cheeses added with native cultures that reached values of up to of 46-48 g 100 g⁻¹ of FAME, respectively which are like those values reported for goat cheeses by Medeiros et al. (2014) (42 g 100 g⁻¹ FAME).

CONTRIBUTION OF CLA IN GOAT DAIRY PRODUCTS

To evaluate the CLA contribution of goat dairy products to human consumption, the values were expressed as mg 100 g⁻¹ of product. The samples of milk used in cheese manufacturing presented an average value of 36 mg 100 g⁻¹ of milk (data not shown).

Cheeses from Creole breed goat milk represent a good source of CLA for human consumers, reaching their highest CLA contents on day 60 (Fig. 1) supplying about (384 mg and 412 mg 100 g⁻¹ for CC and CP respectively) which is higher than those detected in commercial cheeses (238 mg 100 g⁻¹).

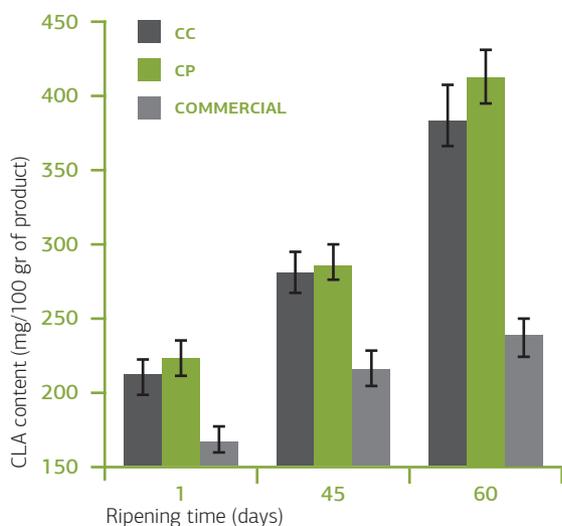


FIGURE 1. The conjugated linoleic acid (CLA) content in semi-hard goat cheeses during ripening expressed as mg 100 g⁻¹ of product

SPECIFIC ESTERASE ACTIVITY IN CHEESES

Important specific esterase activity (EA) was determined in cell free extracts from each LAB strains that constitute the native cultures used in this work previously (Taboada et al., 2014b). To evaluate if esterases remain active in the cheese matrices, the EA was determined in cheeses homogenates of on days 1, 45 and 60 of ripening. The esterase activities were detected in all the cheeses during the ripening stage. CP showed a near to 2 or 2.5-fold increase over time in all EA (C2, C3, C4 and C8 as shown Table 5). For CC cheese, the EA levels increased 1.30-1.73 times during ripening and the highest EA levels was observed using α -NA acetate as substrate. Therefore, the highest EA₆₀/EA₁ ratio for all the substrates analyzed was determined in CP cheeses.

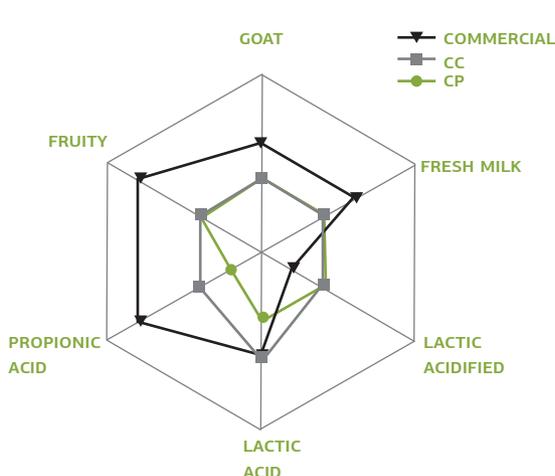


FIGURE 2A. The quantitative sensory profile of the three types of semi hard goat cheeses (Comercial, CC, CP) at the end of the ripening process (60 days) (*p<0.05): (A) odour attribute

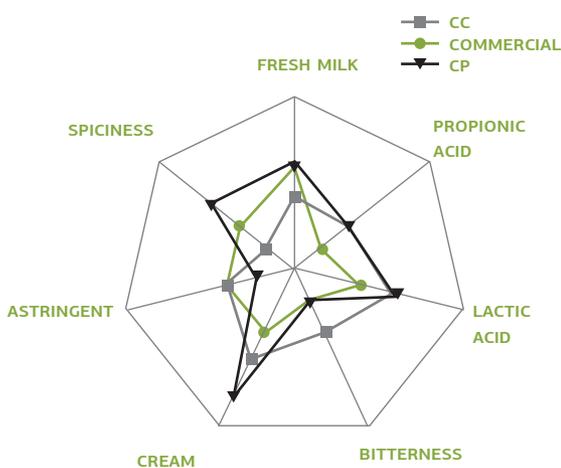


FIGURE 2B. The quantitative sensory profile of the three types of semi hard goat cheeses (Comercial, CC, CP) at the end of the ripening process (60 days) (*p<0.05): (B) flavour attribute

TABLE 5. Specific esterase activity during ripening of semi-hard goat cheeses (cheese manufactured with culture C-CC, cheese manufactured with culture P-CP and cheese manufactured with lyophilized commercial culture-Commercial)

Cheese samples	Day of ripening	Substrate a-naphthyl derivative			
		Acetate (C2)	Propionate (C3)	Butyrate (C4)	Caprylate (C8)
CC	1	10.94±1.10 ^B	11.54±0.80 ^B	10.06±0.90 ^B	11.94±1.25 ^A
	45	13.88±0.70 ^B	12.32±1.20 ^B	13.32±1.40 ^A	14.24±0.90 ^A
	60	18.93±1.45 ^A	15.72±1.30 ^A	14.83±1.30 ^A	15.47±0.30 ^A
	Ratio EA ₆₀ /EA ₁	1.73	1.36	1.47	1.30
CP	1	8.42±1.20 ^C	7.11±1.20 ^C	8.14±1.30 ^B	7.85±1.10 ^C
	45	12.55±1.10 ^B	11.20±0.80 ^B	13.83±0.70 ^A	14.40±0.90 ^B
	60	18.16±0.85 ^A	16.65±1.20 ^A	15.38±0.40 ^A	20.32±1.30 ^A
	Ratio EA ₆₀ /EA ₁	2.16	2.34	1.89	2.59
Commercial	1	7.60±0.88 ^C	6.33±1.10 ^C	10.50±1.20 ^B	11.02±1.30 ^B
	45	7.92±1.25 ^C	12.27±1.30 ^B	11.20±1.10 ^B	12.02±1.20 ^B
	60	8.32±0.60 ^C	14.02±0.70 ^A	12.70±1.20 ^B	13.50±0.80 ^B
	Ratio EA ₆₀ /EA ₁	1.09	2.21	1.21	1.22

Means in the same column for each type of cheese with different letters are significant different ($P < 0.05$). *EA = specific esterase activity (U/mg protein).

SENSORIAL ANALYSIS

The sensory evaluations of goat cheeses are presented in Fig. 2A and 2B. The three types of cheeses showed significant differences for odour attribute such as fresh milk, fruity and propionic acids. The CP cheese samples received the highest score for fruity and propionic acid attributes (Fig. 2A).

As to flavour, the three types of cheeses presented significant differences for flavour attributes such as propionic acid, bitterness, cream and spiciness. However, the CP received the highest score for cream and spiciness but the lowest for bitterness (Fig. 2B). As overall impression, the cheeses manufactured using autochthonous cultures (CC and CP) were qualified as good when compared to the commercial cheese which was scored as regular.

Conclusion

The isolation and characterization of native strains in order to design cultures with specific technological properties allows producing goat cheeses with distinctive sensorial profile and potential functional properties. Our results indicate that autochthonous strain carefully selected by its technological property (acidi-

fying and esterase activity on food matrix) may have a positive effect to produce new goat cheeses with distinctive sensorial attributes and fatty acid profile improvement without altering the global composition. The selection of a mix of native strains as starter and adjunct cultures evidenced a positive effect on the fatty acid profile, enhancing the beneficial conjugated fatty acids content and the percentage of desirable fatty acids (oleic and linoleic acids), contributing altogether to the development of new healthier foods compared with commercial cheeses. Moreover, the inclusion of autochthonous selected strains impacted directly on the sensory analysis having a better global perception in comparison with cheeses manufactured with commercial strains. The new flavour and tasty goat cheeses may represent a viable alternative to offer new products to the market to satisfy the high consumer demand of distinctive regional goat products.

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Povećanje udjela konjugirane linolne kiseline i poboljšanje senzorskih svojstava argentinskih polutvrdih kozjih sireva primjenom nativnih bakterija mliječne kiseline

Sažetak

U ovom radu uspoređivani su fizikalno-kemijski i mikrobiološki parametri, te sastav masnih kiselina i aktivnost specifičnih esteraza u polutvrdim kozjim sirevima proizvedenim pomoću nativnih sojeva bakterija mliječne kiseline (BMK) u odnosu na sireve proizvedene pomoću komercijalnih starter kultura. Fizikalno-kemijski parametri bili su slični, a mikrofloru su uglavnom sačinjavali BMK kao dominantni sojevi u svim sirevima. Najviše vrijednosti specifičnih esteraza utvrđene su kod sireva s nativnim sojevima BMK. Sastav masnih kiselina značajno je ovisio o prisutnosti nativnih sojeva BMK tijekom razdoblja zrenja (60 dana), s obzirom da je koncentracija konjugirane linolne kiseline (CLA) porasla s 0,60 na 1,03 g 100 g⁻¹, dok su sirevi s komercijalnim starter kulturama sadržavali oko 0,60 g CLA na 100 g ukupnih masnih kiselina. Nadalje, u sirevima s nativnim sojevima BMK utvrđena je najviša koncentracija poželjnih masnih kiselina, kao i $\Delta 9$ -desaturaza te CLA desaturaza indeksi; dok im je indeks aterogenosti bio najniži. Nativni sojevi korišteni kao starter ili kao pomoćne kulture pokazali su dobru sposobnost rasta u siru, a njihovi pozitivni učinci odrazili su se putem metabolita nastalih tijekom procesa zrenja na poželjna senzorska svojstva sireva, što u konačnici doprinosi procesu razvoja zdrave hrane.

Ključne riječi: polutvrđi kozji sir, starter kulture, pomoćne kulture, sastav masnih kiselina, konjugirana linolna kiselina, aktivnost esteraza

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