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Original Research Article

Influence of autochthonous cultures on fatty acid composition, esterase activity and sensory profile of Argentinean goat cheeses



Natalia Taboada ^{a,1}, Carina Van Nieuwenhove ^{b,*}, Soledad López Alzogaray ^{a,1}, Roxana Medina ^b

- ^a Departamento de Ciencias de los Alimentos, Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero, Av. Belgrano 1912 (S), CP 4200 Santiago del Estero, Argentina
- b Laboratorio de Ecofisiología Tecnológica Centro de Referencia para Lactobacilos (Cerela-Conicet), Chacabuco 145, 4000 S.M. de Tucumán, Argentina

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ABSTRACT

In the present work conjugated fatty acid content, atherogenicity index, esterase activity and sensory analysis of semi-hard goat cheeses manufactured with autochthonous starter and adjunct or commercial cultures were analyzed. The comparison of cheeses obtained with or without addition of autochthonous cultures reported statistically significant differences in the fatty acid profile and estersase activity that could be due to a different degree of lipolysis in the cheeses, but did not significantly affect the gross composition of cheeses. Short chain and polyunsaturated fatty acid content was higher in products with autochthonous than commercial cultures. The inclusion of these cultures into the artisanal cheese manufacture improves the conjugated fatty acid content, flavour and the atherogenicity index of the final product. Though, conjugated linoleic acid (CLA) level, increased during ripening time in cheese containing autochthonous cultures from 0.6 to 1.0 g/100 g of fatty acids, offering for human consumers among 417–427 mg/100 g of cheese. Differences on esterase activity and atherogenicity index were also observed among cheeses, representing cheeses manufactured with autochthonous bacteria a healthier product than those elaborated with commercial cultures.

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1. Introduction

In South America there are 21 million of head of goats, with Argentina the second largest producer (FAO, 2010), and where the goat livestock is principally distributed in the northwest region using goat milk for the manufacture of cheese. Although some goat cheeses are produced under artisanal conditions, most are manufactured on an industrial scale (Taboada et al., 2014,2015). According to the Argentinean legislation (ANMAT, 2014), when the period of cheese ripening is minor to 60 days, the milk must be pasteurized. This thermal process inactivates both enzymes and native microbiota present in raw milk (Buffa et al., 2004), including lactic acid bacteria (LAB) and their beneficial contributions in dairy technology (Mäyrä-Mäkinen and Bigret, 2004). To reestablish the LAB population, bovine commercial starter cultures are used. This

fact is the responsible of products with great variation in chemical and sensorial characteristics.

Cheese lipolysis plays a crucial role in the development of flavour and other compounds (Bonanno et al., 2013; Pajor et al., 2012; Prandini et al., 2011; Van Nieuwenhove et al., 2009). The lipolysis due to the microbiota metabolism occurs via the esterase/lipase systems of lactic and propionic acid bacteria, non-starter lactic acid bacteria (NSLAB), surface microorganisms, yeasts and molds (McSweeney and Sousa, 2000). Esterases from starter and NSLAB are responsible for the release of short-chain fatty acids (SCFA) from milk fat at elevated $a_{\rm w}$ and the synthesis of short-chain ethyl esters as $a_{\rm w}$ decreases with ripening (Holland et al., 2005).

Moreover, during lipolysis some bioactive lipids such as conjugated linoleic (CLA) and linolenic (CLNA) acids could be produced by isomerase–desaturase complex enzymes. Conjugated linoleic acid (CLA), which is a generic name for a mixture of positional and geometrical isomers of linoleic acid (C18:2) with conjugated double bonds. It is present in ruminant meat and milk fat, being dairy products the main source for human consumption. Among its important biological function are anticarcinogenic activity (Lee et al., 2005), hipocolesterolemic (Lock et al., 2009) and

^{*} Corresponding author. Tel.: +54 3814311720; fax: +54 3814005600. E-mail address: carina@cerela.org.ar (C. Van Nieuwenhove).

¹ Tel.: +54 385 4509528.

antioxidant effects (Pariza et al., 2001), depending on the isomer (Van Nieuwenhove et al., 2012).

Modifications in ruminant diet can multiply concentrations of bioactive compounds in milk, such as some fatty acids (omega-3 fatty acids and CLA). The most effective strategies involved supplementing animal feed with different oils enriched in linoleic or omega-3 fatty acids (Ryhänen et al., 2005; Luna et al., 2008; Martínez Marín et al., 2011) acids. In contrast the effects of processing conditions, storage, and aging on the fatty acid profile of cheeses remain unclear. Luna et al. (2005) employed high temperature conditions in the manufacture of processed cheeses and they found that the production process exerted a negligible effect on the CLA content during manufacture. Although ripening period did not substantially modify the CLA content in different cheeses (Gnädig et al., 2004; Luna et al., 2007; Ryhänen et al., 2005) suggesting that CLA is a stable component, the role of initial starters in the production of that fatty acid is not well known.

In fermented dairy product manufacture, the selection of adequate cultures is crucial (Burns et al., 2012; Settanni and Moschetti, 2010). Medina et al. (2011) reported that in Argentinean goat and ewe cheeses, the autochthonous microbiota have enzymatic activities that produce aroma and flavour compounds from lactose, citrate, protein and lipid metabolism in cheese, as well as bioactive lipids (CLA). Thus, selected LAB with adequate metabolic activities can be used as starter or adjunct cultures for the manufacture of cheeses with typical organoleptic characteristics and potential functional properties. In Argentina, few data are available about the uses of autochthonous cultures for this proposes (Oliszewski et al., 2013; Taboada et al., 2014,2015).

The present work evaluates the contribution of selected autochthonous and commercial cultures on lipids metabolism of Argentinean goat cheeses. To achieve this aim, esterases activities, fatty acid profile and desaturase and atherogenic indexes of goat cheeses were determined.

2. Materials and methods

2.1. Microorganisms

The autochthonous strains used in this study were provided by the Universidad Nacional de Santiago de Estero (UNSE), Argentina. They were isolated from Argentinean goat milk and artisanal cheeses and carefully selected by their technological properties (Taboada et al., 2014,2015). On the basis of previous strain compatibility tests (Collins et al., 1991) different combinations between strains were used in cheese making, according to the schedule presented in Table 1.

In addition, cheese was manufactured using a lyophilized commercial culture of *Streptococcus thermophillus* (Diagramma SA, Santa Fe, Argentina). The autochthonous strains and the lyophilized commercial culture were previously activated in MRS broth (Merck, Darmstadt, Germany) and then multiplied in pasteurized

Table 1 Strain composition of autochthonous cultures.

Strains	Starter culture (%, <i>v</i> / <i>v</i>)	Starter culture plus adjunct culture $(\%, v/v)$
L. rhamnosus UNSE308	30	30
L. delbrueckii subsp. bulgaricus UNSE309	30	30
S. thermophilus UNSE314	20	18
S. thermophilus UNSE321	20	17
L. plantarum UNSE316	0	1.25
L. plantarum UNSE317	0	1.25
P. pentosaceus UNSE22	0	1.25
P. pentosaceus UNSE253	0	1.25

goat milk at 35 °C until coagulation. Cultures with 10^8 cfu mL⁻¹ were used for cheese manufacturing.

2.2. Milk for cheese manufacture

A single batch of raw goat's milk (Saanen breed) provided by a local farm was refrigerated and transported at 4 °C to the pilot plant of Instituto de Ciencia y Tecnología de Alimentos, UNSE (Santiago del Estero, Argentina) during a lactation period of 6 months. The chemical composition was expressed in percent (v/v) and determined by Lactostar analyser (Funke Gerber Lactostar, Funke-Dr. N. Gerber Labortechnik GmbH, Berlín, Alemania) were 4.30 ± 0.03 lactose; 3.42 ± 0.02 protein; 4.75 ± 0.03 fat; 8.41 ± 0.02 non-fat total solids. The pH value determined by a Metrohm 962 pHmeter (Herisau, Switzerland) was 6.6 ± 0.10 .

2.3. Cheeses manufacturing protocol

Semi hard experimental cheeses were prepared by the following the protocol: raw goat milk (35 L) was batch pasteurized at 65 °C for 30 min and after cooling at 38 °C, 0.2 g/L CaCl₂ (Merck, Darmstadt, Germany) was added, followed by an inoculums of 2% (v/v) of starter culture (CS) or starter plus adjunct culture (CA) or 0.1 g/L of commercial culture (CC; S. thermophillus, type DVS, Diagramma SA, Santa Fe, Argentina). Milk coagulation was achieved by adding 0.014 g/L chymosin (MAXIREN 150, Delft, The Netherlands). After 40 min of rest, the curd was cut to corn grain size and placed in cylindrical moulds (10 cm height, 12 cm diameter), pressed for 120 min. Cheeses were placed in cold storage at 5 °C for 12 h. Then, cheeses were salted by immersion in 16% (w/v) NaCl sterile solution at 5 °C for 2 h. Ripening was carried out at 12 °C and 85% relative humidity for 60 days, being vacuum packaged at 30 days of ripening. Cheeses of approximately 700 g were obtained. Three independent trials for each cheese were made and samples were taken at day 1 and after 60 days of ripening for it analysis.

2.4. Global composition of cheeses

The official AOAC (Association of Official Analytical Chemists) was used for fat content (Methods 933.05, AOAC 2006), NaCl content (Methods 975.20, AOAC 2006) and acidity (AOAC 920.124, AOAC 2006) determination. Dry matter and protein content were determined by FIL-IDF (1982) and Rossi et al. (2004), respectively. The pH values of the cheeses were obtained by directly inserting the tip of the probe (MV-TEMP pHmeter, Digital Instruments, Taiwan) into different portions of cheese samples. Water activity $(a_{\rm w})$ was determined using a ROTRONIC AwQuick carp instrument (New York, USA) according to manufacturer specifications.

2.5. Microbiological analysis

For microbiological analysis, 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 plated on specific media for viable counts. Microbiological counts were performed in triplicated after 1 and 60 days of ripening. Mesophilic and thermophilic lactic acid bacteria (LAB) were determined on MRS agar pH 6.5 after incubation at 35 °C and 42 °C, respectively, for 48 h.

2.6. Homogenate cheese preparation

Cheese samples (10 g) were dispersed in 90 mL of 100 mM sodium phosphate buffer, pH 7 solution, homogenized for 2 min in

a Stomacher (Laboratory Blender Stomacher model 400, Seward Medical, London, UK). Cheese debris was removed by centrifugation (10,000 \times g for 30 min) at 4 $^{\circ}$ C, and supernatants were used for esterase activity determination.

2.7. Cheese esterase activity

The esterase activity was determined in cheeses homogenate using on α -naphthyl derivatives of fatty acids of 2–8 carbon atoms as substrate (Sigma, St. Louis, MO) according to the method describe by Medina et al. (2004). The assay mixture contained 160 μ L of 100 mM sodium phosphate buffer, pH 7.0, 20 μ L of α naphthyl substrate (10 mM in ethanol), and 100 µL of cheese homogenate. After incubation for 1 h 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). Controls containing the reaction mixture plus glacial acetic acid were also incubated to test for the presence of background activity. A standard curve was prepared using α -naphthol. A unit of esterase activity was defined as the amount of enzyme that released 1 μ mol of α -naphthol per minute. Specific esterase activity was defined as units per milligram of protein.

2.8. Protein determination

Protein concentrations of cheese homogenate were determined using the method of Bradford (1976).

2.9. Fatty acid analysis

Lipids were extracted using chloroform/methanol solution (2:1, v/v) according to Folch et al. (1957). Fatty acid methyl esters (FAME) analyses were performed according to method described by Van Nieuwenhove et al. (2009, 2011). Briefly, lipids were hydrolyzed by addition of 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, then extracted with hexane and evaporated under a nitrogen stream. One microlitre of FAME, dissolved in hexane, was injected to an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph (Model 6890N, USA) equipped with a flame ionization detector and automatic injector (Model HP-88 column 7683, USA) into a capillary $(100 \text{ m} \times 0.25 \text{ mm} \times 0.20 \mu\text{m}, \text{ Agilent Technologies, USA}). GC$ conditions were: injector temperature, 255 °C; the initial oven temperature of 75 °C was increased to 165 °C at 8 °C/min and held there for 35 min, increased to 210 °C at 5.5 °C/min and held for 2 min and then increased to 240 °C at 15 °C/min and held for 3 min. Detector temperature was 280 °C. Oxygen-free nitrogen was used as carrier gas at flow rate of 18 mL/min, at 38 psi. The total time of the oven programme was 72 min. Fatty acids were identified by comparison of retention times with the methylated standards (99% pure; Sigma, St. Louis, MO, USA). Results were expressed as g/100 g of fatty acids. All reagents and solvents used were from Merck (Darmstadt, Germany).

2.10. Estimation of atherogenicity and desaturase indexes

The mammary gland of ruminants has substantial Δ^9 -desaturase activity, enzyme which can be measured indirectly by comparing the product: substrate ratio of certain fatty acids. 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). CLA desaturase index was estimated by the following formula: CLA desaturase = cis-9,

trans-11/(*cis*-9, *trans*-11 + *trans*-11-C18:1). Other parameter that has biological interest is the atherogenicity index (AI), which characterizes the atherogenicity of dietary fat, by using AI = $[C12:0 + (4 \times C14:0) + C16:0]/(MUFA + PUFA)$ formula, described by Ulbricht and Southgate (1991).

2.11. Sensorial analysis

In order to characterize the sensorial profile of cheeses at the end of ripening (60 days) were subjected to sensorial evaluation by an internal panel consisting of 9 judges evaluating goat cheese. Panellists were chosen from a group prepared and trained in sensory analysis of cheeses, having among 30-45 years old, in gender proportion of 7 male and 2 female. The members were selected to define the descriptive terminology for the sensory attributes of goat cheese in three training sessions. Sensorial descriptive analysis was applied according to consensus technique (IRAM, 1997; Chamorro and Losada, 2002) and performed in three sessions with samples of each type of cheese served refrigerated (4 \pm 1 $^{\circ}$ C). The sensory attributes of odour and flavour were evaluated using a 5-point intensity scale ranging from less intense to more intense for most attributes. The average value of the three evaluations for each attribute of each panellist was statistically analyzed. Overall impression of the product was made 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).

2.12. Statistical analysis

All samples were analyzed at least by triplicate. Results were expressed as mean \pm standard deviation (SD). ANOVA analysis (InfoStat 2011, Grupo Infostat, FCA, Universidad Nacional de Córdoba, Argentina) was carried out to determine statistical differences (P < 0.05) between samples. Tukey test was used (P < 0.05).

3. Results and discussion

3.1. Goat milk fatty acids composition

The fatty acid profile of goat milk is shown in Table 2. Saturated fatty acids (SFA) were the main fatty acids (67.7 g/100 g of fatty acids), being palmitic (C16:0), stearic (C18:0) and myristic (C14:0) the most abundant among them. The monounsaturated fatty acids (MUFA) showed an average value of 28.0 g/100 g of fatty acids, oleic acid (cis-9 C18:1) was the predominant. Polyunsaturated fatty acids (PUFA) content was 4.27 g/100 g of fatty acids, linoleic and linolenic acid were the most abundant. Similar content of SFA, MUFA and PUFA in goat milk were reported by Lucas et al. (2006), Rodriguez-Alcalá et al. (2009) and Van Nieuwenhove et al. (2009). Conjugated linoleic acid (CLA) reached a value of 0.48 g/ 100 g of fatty acids, determined as *cis-*9, *trans-*11 isomer. The other isomer biologically important, trans-10, cis-12 CLA, was not detected in the milk. Our value of CLA are coincident to that reported by Medeiros et al. (2014) for goat milk and slightly lower than those informed by other authors (Bernard et al., 2005; Nudda et al., 2003; Pajor et al., 2012). The content of CLA is affected by many factors including feeding, stage of lactation, breed and age of animal (D'Urso et al., 2008).

3.2. Δ^9 -desaturase, CLA desaturase and atherogenicity indexes of goat milk

 Δ^9 -desaturase activity is important to maintenance of fluidity of milk fat. Δ^9 -desaturase index (C14) and CLA desaturase index (Table 2) showed a value of 0.054 and 0.23, respectively, similar to

Table 2 Fatty acid profile of goat's milk.

Fatty acid	Content
C4:0	$\textbf{0.18} \pm \textbf{0.02}$
C6:0	$\boldsymbol{0.85 \pm 0.02}$
C8:0	$\boldsymbol{1.91 \pm 0.10}$
C10:0	$\textbf{8.64} \pm \textbf{0.10}$
C12:0	$\textbf{4.62} \pm \textbf{0.20}$
C14:0	10.77 ± 0.30
C14:1	$\textbf{0.61} \pm \textbf{0.10}$
C15:0	$\boldsymbol{0.90 \pm 0.10}$
C16:0	26.05 ± 1.16
C16:1	$\boldsymbol{1.33 \pm 0.10}$
C17:0	$\boldsymbol{0.70 \pm 0.10}$
C17:1	$\boldsymbol{0.20\pm0.10}$
C18:0	12.59 ± 0.55
trans-10-C18:1	$\boldsymbol{0.19 \pm 0.03}$
trans-11-C18:1	$\boldsymbol{1.58 \pm 0.10}$
cis-9-C18:1	23.90 ± 1.05
C18:2n6t	$\boldsymbol{0.13 \pm 0.02}$
C18:2n6c	$\boldsymbol{1.83 \pm 0.21}$
C18:3n3	1.50 ± 0.03
cis-9,trans-11-CLA	$\boldsymbol{0.48 \pm 0.10}$
trans-10,cis-12-CLA	n.d.
C20:0	$\boldsymbol{0.52 \pm 0.04}$
C20:1	$\boldsymbol{0.18 \pm 0.04}$
C20:4	$\boldsymbol{0.15 \pm 0.02}$
C22:5	$\boldsymbol{0.18 \pm 0.03}$
Saturated (SFA)	67.73 ± 8.40
Monounsaturated (MUFA)	28.19 ± 10.50
Polyunsaturated (PUFA)	$\textbf{4.27} \pm \textbf{0.75}$
Δ^9 -desaturase index (C14)	$\boldsymbol{0.054 \pm 0.01}$
CLA desaturase index	$\boldsymbol{0.23 \pm 0.01}$
Atherogenicity index (AI)	2.28 ± 0.65

Fatty acids content was expressed as $g/100\,g$ of fatty acids. Values were expressed as mean \pm standard deviation. CLA, conjugated linoleic acid; n.d., not determined.

those reported by Van Nieuwenhove et al. (2009). The atherogenicity index reached a value of 2.28, within the range informed for goat milk (2.20–3.29) and coincident with Chilliard et al., 2003; Osmari et al., 2011; Van Nieuwenhove et al., 2009. Foods with high atherogenicity index are considered more detrimental for human health.

3.3. Global composition of cheeses

Evolution of global composition of semi hard goat cheeses during ripening is shown in Table 3. Results evidenced that cheeses CC, CA and CS did not show significant differences (P < 0.05) in: acidity, salt, $a_{\rm w}$ and dry matter at the same time of ripening. However, pH values of CS and CA (5.04 and 5.09, respectively) were slightly lower than CC pH values (5.29) after 60 days of ripening.

Dry matter and fat values are according to the range established by Argentinean legislation for semi hard cheeses (ANMAT, 2014). Water activity ($a_{\rm w}$) slightly decreased with ripening time. Fat, protein and salt content increased after 60 days of ripening, due to the drying of cheeses. The chemical composition of cheeses was similar to those from Italian, Spanish and Argentinean semi hard goat cheeses (Caridi et al., 2003; Oliszewski et al., 2013; Peláez Puerto et al., 2004).

In three cheeses of 1 day of ripening contained a cell count of $\sim\!\!7.17$ to 7.45 log cfu/g (Table 4). At 60 days of ripening, in three cheeses was observed an increase of cell counts from $\sim\!\!8.04$ to 8.40 log cfu/g (1 log cycle), which is statistically significant. Similar results were reported by Oliszewski et al. (2013) for goat cheeses. These results would indicate that mesophilic and termophilic LAB remain viable in cheeses at 60 days of ripening, thus contributing to cheese lipolysis.

3.4. Fatty acid composition of cheeses

The fatty acid composition of semi hard goat cheeses is shown in Table 5. As in milk, SFA were predominant with a mean value for CC, CS and CA of 68.25, 65.52 and 68.29 g/100 g of fatty acids, respectively, at 1 day, decreasing after 60 days of ripening to 65.31, 63.0 and 63.7 g/100 g of fatty acids, respectively. These results were similar to those cited on the literature for fresh goat cheese (Medeiros et al., 2014; Van Nieuwenhove et al., 2009). Among SFA, palmitic acid was found at the highest level, followed by stearic (C18:0) and myristic (C14:0) acids.

The most important variation attributed to strain addition was determined on short chain fatty acids (SCFA) content. Though, initially, all cheeses presented values from $2.84-3.35 \, g/100 \, g$ of fatty acids, increasing after 60 days of ripening to $3.37-5.01 \, g/100 \, g$ of fatty acids, the highest value in CS and CA cheeses. Butyric acid (C4:0) showed the most important increase, $\sim 4 \, times$, varying from $0.12-0.14 \, g/100 \, g$ of fatty acids at 1 day to $0.39-0.68 \, g/100 \, g$ of fatty acids at 60 days. Large variations during of ripening were also observed for C8:0 content. This fatty acid increased in CS cheese from $1.88 \, to \, 2.77 \, g/100 \, g$ and in CA cheese from $1.93 \, to \, 2.60 \, g/100 \, g$ of fatty acids. It is remarkable that goat and ewe cheese result a richer source of short chain fatty acids (C4 to C8) than cow milk (Chilliard et al., 2006; Luna et al., 2008) and this fact was also observed in the present study.

No changes on monounsaturated fatty acids (MUFA) content were observed over time. Though, MUFA ranged from 27 to 30 g/100 g of fatty acids at 1 day and near 31.0 g/100 g of fatty acids at 60 days. Oleic acid (*cis*-9 C18:1) was the most abundant fatty acid in this group, representing among 85–90% of total MUFAs and showing a significant increment over time. High vaccenic (*trans*-11 C18:1) acid content, around 2 g/100 g of fatty acids was determined in all cheeses, while *trans*-10 C18:1 content was

Table 3 Global composition of experimental semi-hard goat cheeses during ripening.^a

Day of ripening	Cheese ^b	Protein (%, w/w)	Protein (%TS)	Fat (%, w/w)	Dry matter (%, w/w)	NaCl (%, w/w)	рН	Acidity (%, w/w)	a_{W}
1	CC CS CA	$\begin{array}{c} 21.09 \pm 0.30^C \\ 21.82 \pm 0.23^B \\ 21.94 \pm 0.25^B \end{array}$	$38.10 \pm 0.13^D \\ 38.31 \pm 0.13^D \\ 39.46 \pm 0.16^C$	$\begin{array}{c} 33.22 \pm 0.22^D \\ 33.78 \pm 0.34^C \\ 33.80 \pm 0.25^C \end{array}$	$\begin{array}{c} 55.50 \pm 0.30^B \\ 55.65 \pm 0.30^B \\ 55.60 \pm 0.35^B \end{array}$	$\begin{aligned} 0.98 \pm 0.02^B \\ 1.05 \pm 0.02^B \\ 1.04 \pm 0.01^B \end{aligned}$	$\begin{aligned} 5.21 &\pm 0.02^A \\ 5.20 &\pm 0.02^A \\ 5.24 &\pm 0.03^A \end{aligned}$	$\begin{array}{c} 0.70 \pm 0.03^B \\ 0.71 \pm 0.07^B \\ 0.72 \pm 0.03^B \end{array}$	$\begin{aligned} 0.988 &\pm 0.003^A \\ 0.987 &\pm 0.002^A \\ 0.986 &\pm 0.004^A \end{aligned}$
60	CC CS CA	$\begin{aligned} 27.15 \pm 0.45^A \\ 27.19 \pm 0.40^A \\ 27.35 \pm 0.30^A \end{aligned}$	$\begin{array}{l} 43.30 \pm 0.17^B \\ 43.90 \pm 0.18^B \\ 44.93 \pm 0.11^A \end{array}$	$\begin{array}{c} 38.28 \pm 0.21^B \\ 39.95 \pm 0.24^A \\ 40.00 \pm 0.39^A \end{array}$	$\begin{aligned} 61.78 &\pm 0.20^A \\ 61.66 &\pm 0.40^A \\ 61.60 &\pm 0.24^A \end{aligned}$	$\begin{aligned} &1.40 \pm 0.02^A \\ &1.46 \pm 0.02^A \\ &1.45 \pm 0.02^A \end{aligned}$	$\begin{aligned} 5.29 \pm 0.03^A \\ 5.04 \pm 0.04^B \\ 5.09 \pm 0.03^B \end{aligned}$	$\begin{aligned} &1.00 \pm 0.07^A \\ &1.03 \pm 0.03^A \\ &1.04 \pm 0.03^A \end{aligned}$	$\begin{aligned} &0.980 \pm 0.003^B \\ &0.978 \pm 0.003^B \\ &0.977 \pm 0.004^B \end{aligned}$

^a Values were expressed as mean \pm SD. Different letters in the same column correspond to statistically significant differences (P<0.05).

b Experimental cheeses, CC (cheese manufactured with commercial culture), CS (cheese manufactured with starter culture), CA (cheese manufacture with starter and adjunct autochthonous cultures). Acidity values were expressed as g of lactic acid/100 g of cheese. Protein, fat, dry matter and NaCl values were expressed as g/100 g of cheese.

Table 4 Lactic acid bacteria (LAB) in goat cheeses.

Cheeses ^b	Day of ripening	Lactic acid bacter of cheese) ^a	Lactic acid bacteria (Log cfu/g of cheese) ^a	
		Mesophilics	Thermophilics	
СС	1 60	$7.45 \pm 0.30^A \\ 8.30 \pm 0.30^B$	$7.27 \pm 0.25^A \\ 8.10 \pm 0.25^B$	
CS	1 60	$\begin{aligned} 7.44 \pm 0.25^A \\ 8.04 \pm 0.30^B \end{aligned}$	$\begin{aligned} 7.28 &\pm 0.30^A \\ 8.16 &\pm 0.30^B \end{aligned}$	
CA	1 60	$\begin{aligned} 7.39 \pm 0.20^A \\ 8.19 \pm 0.33^B \end{aligned}$	$\begin{aligned} 7.17 \pm 0.32^A \\ 8.12 \pm 0.33^B \end{aligned}$	

^a Means in the same column for each cheese with different letters are significantly different (P<0.05).

near 10 times lower (range from 0.14 to 0.24 g/100 g of fatty acids).

After 60 days, cheeses manufactured with autochthonous strains (CS and CA) showed the highest content of PUFAs (5.77 and 5.10 g/100 g of fatty acids, respectively) compared with CC (4.0 g/100 g of fatty acids). Conjugated linoleic acid (CLA) values varied among 0.52-0.60 g/100 g of fatty acids in CC, at 1 day and 60 days of ripening, respectively. After the inclusion of autochthonous cultures, it content increased from 0.66 to 1.07 g/100 g of fatty acids in CS and from 0.64 to 1.04 g/100 g of fatty acids in CA cheese. cis-9, trans-11 was the major CLA isomer determined in goat cheeses, coincident with the literature for goat and ewe dairy products (Bernard et al., 2005; Zlatanos et al., 2002). The initial value of CLA is according to other authors who reported values from 0.56 to 1.07 g/100 g of fatty acids for cis-9, trans-11 isomer (Medeiros et al., 2014; Osmari et al., 2011; Prandini et al., 2011). Goats seem to be unable to produce CLA by ruminal biohydrogenation intermediate product as trans-10, cis-12 form. However, both cis-9, trans-11 and trans-10, cis-12 CLA, as well as mixtures of

Table 5Fatty acids composition of semi hard goat milk cheeses.^a

Fatty acids (%)	Cheeses samples ^b					
	1 day			60 days		
	СС	CS	CA	СС	CS	CA
C4:0	0.14 ± 0.03^{D}	0.15 ± 0.03^{D}	$0.12\pm0.04^{\text{D}}$	0.39 ± 0.02^{C}	$0.52 \pm 0.03^{\text{B}}$	$0.68 \pm 0.02^{\text{A}}$
C6:0	1.05 ± 0.21^B	1.30 ± 0.14^B	1.30 ± 0.18^B	1.05 ± 0.11^B	$1.17\pm0.05^{\text{B}}$	$1.73\pm0.18^{\text{A}}$
C8:0	1.65 ± 0.06^{C}	$1.88 \pm 0.22^{\text{B}}$	1.93 ± 0.10^B	1.93 ± 0.13^{B}	$2.77\pm0.12^{\text{A}}$	2.60 ± 0.12^{A}
C10:0	$9.76\pm0.10^{\text{A}}$	$6.68 \pm 0.25^{\text{C}}$	$9.63\pm0.35^{\text{A}}$	8.85 ± 0.64^B	8.09 ± 0.20^B	8.93 ± 0.20^{B}
C12:0	$5.00\pm0.12^{\text{A}}$	5.40 ± 0.08^A	4.70 ± 0.11^B	4.24 ± 0.25^B	$3.42\pm0.27^{\text{C}}$	$3.71 \pm 0.14^{\circ}$
C14:0	12.05 ± 0.77^{A}	12.55 ± 0.49^{A}	11.60 ± 0.42^{A}	9.60 ± 0.53^{B}	$8.68 \pm 0.20^{\text{C}}$	7.69 ± 0.13^{D}
C14:1	$0.59 \pm 0.04^{\text{A}}$	$0.61 \pm 0.07^{\text{A}}$	$0.57 \pm 0.02^{\text{A}}$	$0.63\pm0.21^{\text{A}}$	0.36 ± 0.02^B	0.37 ± 0.02^{B}
C15:0	$0.82\pm0.03^{\text{A}}$	$0.88 \pm 0.03^{\text{A}}$	$0.81 \pm 0.12^{\text{A}}$	$0.96\pm0.49^{\text{A}}$	$0.92 \pm 0.49^{\text{A}}$	$0.85\pm0.44^{\text{A}}$
C16:0	23.35 ± 0.63^{B}	23.33 ± 0.72^{B}	$24.80 \pm 0.59^{\text{A}}$	$25.46\pm0.69^{\text{A}}$	25.27 ± 0.13^{A}	25.78 ± 0.53
C16:1	$1.38\pm0.05^{\text{A}}$	$1.43\pm0.07^{\text{A}}$	$1.48\pm0.12^{\text{A}}$	$1.39 \pm 0.14^{\text{A}}$	0.40 ± 0.02^{C}	$0.52\pm0.02^{\text{B}}$
C17:0	$0.95 \pm 0.04^{\text{A}}$	0.69 ± 0.09^B	$0.93 \pm 0.05^{\text{A}}$	0.62 ± 0.02^{B}	$0.51\pm0.02^{\text{C}}$	0.50 ± 0.02^{C}
C17:1	$0.31\pm0.03^{\text{A}}$	$0.35\pm0.03^{\text{A}}$	$0.31 \pm 0.04^{\text{A}}$	$0.39\pm0.02^{\text{A}}$	$0.38\pm0.02^{\text{A}}$	0.36 ± 0.02^{A}
C18:0	12.95 ± 0.21^{A}	12.16 ± 0.33^{A}	12.0 ± 0.42^{A}	11.74 ± 1.77^{A}	11.16 ± 0.53^{A}	10.79 ± 0.23
trans-10-C18:1	0.21 ± 0.05^{A}	$0.22\pm0.03^{\text{A}}$	$0.19 \pm 0.04^{\text{A}}$	0.16 ± 0.03^A	0.16 ± 0.02^{A}	0.14 ± 0.04^{A}
trans-11-C18: 1	$1.98\pm0.04^{\text{A}}$	$2.00\pm0.10^{\text{A}}$	1.40 ± 0.12^{AB}	$2.0\pm0.64^{\text{A}}$	$2.0\pm0.35^{\text{A}}$	$2.0\pm0.50^{\text{A}}$
cis9-C18:1	$23.31 \pm 0.35^{\circ}$	25.61 ± 0.42^{B}	24.30 ± 0.70^{B}	26.08 ± 0.49^{B}	27.90 ± 0.30^{A}	27.84 ± 0.36
C18:2 n6t	0.10 ± 0.02^{C}	0.10 ± 0.02^{C}	$0.08\pm0.01^{\text{C}}$	0.22 ± 0.03^{B}	$0.26\pm0.05^{\text{A}}$	0.33 ± 0.03^{A}
C18:2	2.01 ± 0.10^{A}	$1.71 \pm 0.04^{\text{B}}$	1.61 ± 0.09^{B}	1.65 ± 0.15^{B}	2.15 ± 0.02^{A}	1.93 ± 0.12^{A}
C18:3	$1.02\pm0.14^{\text{C}}$	$1.51 \pm 0.07^{\text{B}}$	$0.83\pm0.06^{\text{C}}$	1.17 ± 0.35^{B}	$1.89\pm0.09^{\text{A}}$	1.46 ± 0.14^{B}
cis-9,trans-11-CLA ^c	0.52 ± 0.02^{C}	0.60 ± 0.03^B	0.64 ± 0.03^B	0.60 ± 0.02^{B}	$1.07\pm0.02^{\text{A}}$	1.04 ± 0.05^{A}
trans10,cis12-CLA	n.d	0.06 ± 0.01	n.d	n.d	n.d	n.d
TOTAL CLA	$0.52 \pm 0.02^{\text{C}}$	$0.66\pm0.03^{\text{B}}$	0.64 ± 0.03^B	0.60 ± 0.02^B	$1.07 \pm 0.02^{\text{A}}$	$1.04\pm0.05^{\text{A}}$
C20:0	$0.53\pm0.02^{\text{A}}$	$0.50\pm0.05^{\text{A}}$	$0.47 \pm 0.03^{\text{A}}$	$0.47\pm0.21^{\text{A}}$	$0.48 \pm 0.04^{\text{A}}$	$0.43\pm0.05^{\text{A}}$
C20:1	$0.19\pm0.02^{\text{A}}$	$0.19\pm0.02^{\text{A}}$	$0.17\pm0.02^{\text{A}}$	0.20 ± 0.05^A	0.20 ± 0.14^A	0.12 ± 0.09^{A}
C20:4	0.18 ± 0.02^{A}	$0.12\pm0.02^{\text{A}}$	0.14 ± 0.02^{A}	0.17 ± 0.02^{A}	0.20 ± 0.02^{A}	0.19 ± 0.03^{A}
C22:5	$0.19\pm0.02^{\text{A}}$	0.20 ± 0.02^{A}	$0.18\pm0.02^{\text{A}}$	0.19 ± 0.14^{A}	0.20 ± 0.02^A	0.15 ± 0.03^{A}
SCFA ^d (C4:0-C8:0)	2.84 ± 0.45^{B}	3.33 ± 0.87^{B}	3.35 ± 0.32^{B}	3.37 ± 0.21^{B}	4.46 ± 0.50^{A}	5.01 ± 0.71^{A}
MCFA ^e (C10:0-C15:0)	$28.22 \pm 4.02^{\text{A}}$	26.12 ± 4.35^{A}	27.31 ± 2.15^{A}	$24.28\pm4.02^{\text{A}}$	21.47 ± 2.86^{B}	21.55 ± 2.93
LCFA ^f (C16:0-C22:0)	69.00 ± 6.99^{A}	70.78 ± 8.41^{A}	$69.53 \pm 7.09^{\text{A}}$	$72.51 \pm 7.35^{\text{A}}$	$74.23\pm8.85^{\text{A}}$	73.58 ± 9.33
Saturated fatty acids SFA	$68.25 \pm 7.48^{\text{A}}$	65.52 ± 7.80^{A}	$68.29 \pm 7.69^{\text{A}}$	$65.31 \pm 6.27^{\text{A}}$	$63.00 \pm 7.59^{\text{A}}$	63.70 ± 7.55
Monounsaturated fatty acids MUFA	27.97 ± 9.33^{A}	30.41 ± 10.77^{A}	$28.42\pm9.65^{\text{A}}$	30.85 ± 9.93^{A}	31.40 ± 12.24^{A}	31.34 ± 13.7
Polyunsaturated fatty acids PUFA	3.99 ± 0.73^{B}	4.30 ± 0.70^A	3.48 ± 0.58^B	4.00 ± 0.31^{B}	$5.77\pm0.88^{\text{A}}$	5.10 ± 0.70^{A}
Δ 9-desaturase index (C14)	0.047 ± 0.01^B	0.046 ± 0.01^{B}	0.047 ± 0.01^B	0.061 ± 0.01^A	0.047 ± 0.01^B	0.046 ± 0.01
CLA desaturase index	0.21 ± 0.01^B	0.23 ± 0.01^B	$0.31 \pm 0.01^{\text{A}}$	0.24 ± 0.01^B	$0.32\pm0.01^{\text{A}}$	0.31 ± 0.01^{A}
Atherogenicity index	2.41 ± 0.61^A	$2.29\pm0.64^{\text{A}}$	$2.39\pm0.62^{\text{A}}$	1.96 ± 0.32^{A}	$1.67 \pm 0.34^{\text{B}}$	$1.66\pm0.32^{\mathrm{B}}$
DFA ^g	44.91	46.87	43.90	46.59	48.33	47.23
SFA:UFA	2.14	1.89	2.14	1.88	1.69	1.75
PUFA:SFA	0.058	0.065	0.051	0.06	0.09	0.08
MUFA:SFA	0.41	0.46	0.42	0.47	0.50	0.49

 $^{^{}a}$ Values were expressed as mean \pm SD. Different letters in the same row correspond to statistically significant differences (P<0.05).

n.d., not determined.

^b Experimental cheeses, CC (cheese manufactured with commercial culture), CS (cheese manufactured with starter culture), CA (cheese manufacture with starter and adjunct autochthonous cultures).

b Cheeses, CC (manufactured with commercial culture), CS (manufactured with starter culture), CA (cheese manufacture with starter and adjunct autochthonous cultures). Fatty acids values were expressed as g/100 g of fatty acids (mean ± standard deviation).

^c CLA, conjugated linoleic acid.

^d SCFA, short chain fatty acids.

e MCFA, medium chain fatty acids.

f LCFA, long chain fatty acids.

g DFA (desirable fatty acids)=(UFA+C18:0).

these isomers, showed anticarcinogenic activity (Churruca et al., 2009). Several authors that informed CLA variation on fermented products due to bacterial metabolism (Collomb et al., 2006; Domagala et al., 2010).

On the other hand, some studies reported no changes on CLA content during ripening of Emmental (Gnädig et al., 2004), Edam (Ryhänen et al., 2005) and goat cheeses (Luna et al., 2007), but the microbiota of these products were not monitored. Our results about the increment of CLA level during ripening are comparable with Rodrigues et al. (2012), whose informed higher CLA levels in probiotic and synbiotic cheeses manufactured with bacteria able to form CLA *in vitro* (Rodriguez-Alcalá et al., 2011). Zlatanos et al. (2002) also informed higher CLA concentration after ripening of hard cheeses. Furthermore, Luna et al. (2007) determined a slight increase on CLA content in one Mahón cheese between two days and 60 days of ripening, showing the great variability of CLA content among dairy products.

In the present work, all conditions of manufacture were the same except bacteria included as cultures. In fact, cheeses showed an increase of one log of viable cell count for thermophilic and mesophilic LAB (Table 4), but only cheeses with autochthonous cultures showed a significant increment of CLA after 60 days. So, the increment observed throughout ripening time could be associated to the inclusion of the autochthonous strains.

3.5. Desaturases and atherogenicity indexes in goat cheeses

 Δ^9 -desaturase index (C14) was lower when autochthonous starter and adjunct cultures were used compared with commercial starter (0.047, 0.046 vs. 0.061, respectively), at 60 days. However, higher values of CLA desaturase index were determined in CS, CA than CC cheese (0.32, 0.31 vs. 0.24) respectively at 60 days, explaining the higher CLA level in both CS and CA cheeses (Table 5).

Regarding to AI, lower values were detected in cheeses added with autochthonous strains than CC at 60 days (1.67 and 1.66 vs. 1.96, respectively). Foods with high atherogenicity index are considered more detrimental to human health. Our AI values (2.57 and 2.55, respectively) were lower than those reported for goat cheeses by Van Nieuwenhove et al. (2009) and Medeiros et al. (2014).

Desirable fatty acid (DFA) allows inferring the content of those benefic fatty acids for health, and can be determined by summing [(UFA) + C18:0] (Osmari et al., 2011). Our results were also higher for ripened cheeses, reaching values of 46–48 g/100 g of fatty acids, higher than value (42.43 g/100 g of fatty acids) informed by Medeiros et al. (2014).

3.6. Contribution of CLA in dairy goat products

To evaluate the contribution of CLA by each individual cheese for human consumption, values were expressed as mg/100 g of product (Table 6). Goat cheeses result a good source of CLA for human consumers, offering among 172.7–229.9 mg/100 g of sample at 1 day, similar to values determined by Van Nieuwenhove et al. (2009) for goat cheeses. However, more CLA was supported by semi hard goat cheese after 60 days of ripening, varying among 229.7–427.5 mg/100 g of product, cheeses manufactured with autochthonous starter (CS) and adjunct (CA) cultures represented the highest CLA source.

3.7. Cheese esterase activity

The main role of the esterases in fermented dairy products is to hydrolyze triglycerides from milk fat releasing short chain fatty acid (SCFA). These acids (SCFA) such as acetic, propionic, butyric

Table 6Conjugated linoleic acid (CLA) content in goat dairy products.

CLA content ^a (mg/100 g sample)	Day of ripening	Samples ^b	
		1	60
cis-9,trans-11-CLA	Cheese CC Cheese CS Cheese CA	$\begin{aligned} 172.7 \pm 6.6^A \\ 222.9 \pm 11.14^A \\ 216.3 \pm 10.13^A \end{aligned}$	$\begin{array}{c} 229.7 \pm 7.65^B \\ 427.5 \pm 18.99^B \\ 417.0 \pm 20.04^B \end{array}$
cis-9,trans-11-CLA	Goat milk	23.0	± 2.54

- ^a CLA content was calculated by using mean value of each time.
- ^b Cheeses, CC (cheese manufactured with commercial culture), CS (cheese manufactured with starter culture), CA (cheese manufactured with starter and adjunct autochthonous cultures).

and caprylic acids are responsible for the typical spicy flavours of goat and sheep cheese (Abeijón Mukdsi et al., 2009a,b; Haenlein, 1996). Esterases can also catalyze ester synthesis under certain environmental conditions. In some foods, BL esterases are involved in the synthesis of esters of SCFA, particularly ethyl acetate, ethyl butanoate and ethyl caprylate. These compounds, at appropriate concentrations, are responsible for flavour and fruity flavours (Abeijón Mukdsi et al., 2009a; Liu et al., 2004).

We previously determined esterase activities of strains that constitute the starter and adjunct cultures used in this work for cheeses manufacture (Taboada et al., 2014,2015). To assess if esterases remain active in the cheese matrices, specific esterase activities were determined in cheeses homogenates of 1 and 60 days of ripening. Esterases activities were detected in all evaluated cheeses. However, esterases activities levels of CS cheese were higher than in other cheeses tested and increased 2.15-2.99 times during ripening, highest activities were obtained with propionate (Table 7). These results would indicate that an increased release of fatty acids or short chain ester synthesis with fruit flavors could be conducted during cheese ripening (Liu et al., 2004). In CA cheese, esterase activity levels increased 1.68-1.85 times during ripening and the high activity levels were observed with acetate and caprylate as substrate. On the other hand, no statistically significant differences on esterases activities was observed during ripening cheeses without autochthonous strains (CC), and only an increase in activity (2.32 times) was observed when α -NA-propionates was used as substrate.

These results are in agreement with the increase in short chain fatty acids (butyric and caprylic) that was observed during ripening for CA and CS cheeses (Table 5). Under the environmental conditions present in the cheese, low pH (\sim 5) and $a_{\rm w}$ (\sim 0.979), esterase enzymes maintained their activities. Thus, strains used in it manufacture may contribute to the release of short chain fatty acid or ester synthesis.

LAB esterase can catalyze both the hydrolysis and the synthesis of esters according to the environmental conditions. Thus, in the early stages of cheese ripening hydrolysis process prevails, when a_w is still high. Over the course of the ripening the a_w decrease, becoming important ester synthesis (Liu et al., 2004).

Cheeses manufactured with autochthonous starter cultures showed higher activities than CC cheeses. Both in cheese elaborated with commercial and autochthonous starter cultures log of LAB detected at day 60 were similar (~8.04 to 8.40 log cfu/g). These results would indicate that higher esterase activities detected in CS and CA that CC is possibly due to a better adaptation to the goat milk of autochthonous starter cultures. Several studies concerning to detection of esterase activity in lactic bacteria strains of dairy origin were reported (Gobbetti et al., 1996; Vafopoulou-Mastrojiannaki et al., 1996; Oliszewski et al., 2007; Taboada et al., 2014,2015). At present, there are few

Table 7 Specific esterase activity (EA)^a in goat cheeses.

Cheeses ^b	Day of ripening	Substrate α-naphthy	Substrate α -naphthyl derivative				
		Acetate (C2)	Propionate (C3)	Butyrate (C4)	Caprylate (C8)		
СС	1 60 Ratio EA ₆₀ /EA ₀	7.77 ± 1.20^{A} 8.04 ± 0.30^{A} $n.a.^{c}$	$\begin{aligned} 6.45 &\pm 1.20^B \\ 14.97 &\pm 0.80^A \\ 2.32 \end{aligned}$	$10.75 \pm 1.10^{A} \\ 12.79 \pm 1.40^{A} \\ n.a.$	11.04 ± 1.45^{A} 13.88 ± 1.25^{A} n.a.		
CS	1 60 Ratio EA ₆₀ /EA ₀	$\begin{array}{c} 8.64 \pm 1.20^B \\ 18.55 \pm 1.40^A \\ 2.15 \end{array}$	$7.93 \pm 0.80^B \\ 23.74 \pm 1.40^A \\ 2.99$	$7.53 \pm 1.25^B \\ 19.89 \pm 1.40^A \\ 2.64$	$\begin{aligned} &6.23 \pm 0.20^B \\ &16.37 \pm 1.25^A \\ &2.63\end{aligned}$		
CA	1 60 Ratio EA ₆₀ /EA ₀	$10.03 \pm 1.10^B \\ 18.60 \pm 1.30^A \\ 1.85$	$\begin{array}{c} 9.52 \pm 1.30^B \\ 16.29 \pm 1.10^A \\ 1.71 \end{array}$	$\begin{aligned} 11.99 \pm 0.90^B \\ 20.11 \pm 1.30^A \\ 1.68 \end{aligned}$	$12.83 \pm 1.40^B \\ 23.58 \pm 1.30^A \\ 1.84$		

^a EA = specific esterase activity was expressed as units per milligram of protein. Means in the same column for each cheese with different letters are significantly different (P < 0.05).

c n.a., not applicable.

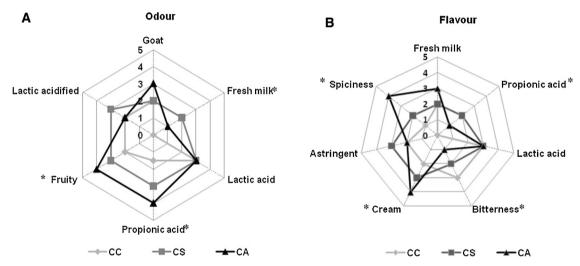


Fig. 1. Quantitative sensory profile of goat cheeses (*p < 0.05): (A) odour attribute and (B) flavour attribute.

studies about esterase activity in cheese. Morales et al. (2005, 2010) detected tributyrin esterase in cheese inoculated with *Micrococcus* sp. INIA 528 and in cheeses added with high enzymatic activity curds.

3.8. Sensorial analysis

The sensory profiles of goat cheeses are shown in Fig. 1. The three cheeses showed significant differences for odour attribute as fresh milk, fruity and propionic acid. CA cheese presented the highest score for fruity and propionic acid attributes (Fig. 1 A).

Regarding to flavour (Fig. 1B), three cheeses showed significant differences for flavour attributes as propionic acid, bitterness, cream and spiciness. CA cheese presented the highest score for cream and spiciness and lowest score for bitterness attributes (Fig. 1B).

Overall impression of cheeses elaborated with autochthonous cultures (CS and CA) were scored as good compared to CC cheese, manufactured with commercial culture, that was scored as regular.

Our results to indicate that autochthonous cultures present enzymatic activities (esterase and lipases) that contribute to development flavour during ripening of cheeses, thus improving the sensory characteristics thereof.

4. Conclusions

To the best of our knowledge, this is the first work that reports about the influence of autochthonous cultures on fatty acid profile, esterase activity, sensorial properties and chemical composition of Argentinean goat cheeses. The inclusion of these cultures into the artisanal cheese manufacture improves the conjugated fatty acid content, flavour and the atherogenicity index of the final product. The uses of autochthonous cultures could impact on the development of dairy products with beneficial effect on health, having a strong influence on the economy of the region, where goat cheeses are the main dairy product produced for human consumption.

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^b Cheeses, CC (cheese manufactured with commercial culture), CS (cheese manufactured with starter culture), CA (cheese manufacture with starter and adjunct autochthonous cultures).

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