BIOCHEMICAL AND MICROBIOLOGICAL CHANGES THROUGHOUT THE RIPENING OF ARGENTINEAN FRESH GOAT'S MILK CHEESES MADE WITH NATIVE CULTURES

Natalia Taboada[1], Roxana Medina[2], Martha Núñez[2] and Soledad López Alzogaray[1]

Address(es):
[1] Departamento de Ciencias de los Alimentos, Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero, Santiago del Estero, Argentina.
[2] Centro de Referencia para Lactobacilos (CERELA-CONICET), Tucumán, Argentina.

*Corresponding author: ntaboada2106@gmail.com

ABSTRACT

Two different cultures with native lactic acid bacteria (LAB) were designed, S (starter culture consisting of *Lactobacillus (L.) rhamnosus* UNSE308, *L. delbrueckii* subsp. *bulgaricus* UNSE309, *Streptococcus (S). thermophilus* UNSE314, *S. thermophilus* UNSE321) and A (starter culture S plus adjunct culture consisting of *L. plantarum* UNSE316, *L. plantarum* UNSE317, *Pediococcus (P). pentosaceus* UNSE22, *P. pentosaceus* UNSE253). Cultures S, A and C (lyophilized commercial culture of *Streptococcus thermophillus*) were used to manufacture fresh goat cheeses (CS, CA and CC) from pasteurized milk. The impact of these cultures on the microbiological, biochemical and physicochemical parameters were evaluated during ripening (30 days). Results evidenced that CC, CA and CS did not show significant differences in protein and fat content, NaCl, acidity, fat acidity and aw, at the same time of ripening. All experimental cheeses underwent moderate lipolysis since moderate levels of free fatty acids (FFA) at the end of ripening were detected (~1.5 g kg^{-1}) and made an important contribution to characteristic flavour and aroma. In all cases, a slight proteolysis was detected; CC showed the lowest levels during ripening. Values of NaCl (1.27%) were in accordance with those reported in most cheeses. During ripening, mesophilic LAB counts in MRS agar increased nearly 1 log cycle, total coliforms per gram at 30ºC showed counts below the maximum allowed by Argentinean legislation. Overall impression of CS and CA was qualified as good compared to CC that was scored as regular. The fresh goat cheeses made with native cultures presented suitable typical flavour and satisfying overall sensorial characteristics.

Keywords: Fresh goat cheese, native starter culture, native adjunct culture, physicochemical analysis, microbiological analysis

INTRODUCTION

The total of goats distributed around the world is 940 million animals, mainly distributed between Asia and Africa. In South America, there are 20.6 million goats, Brazil ranks first with 8.78 million goats and Argentina, second with 4.38 million goats (**FAO, 2013**). In 2012, milk production in Argentina has been evaluated in 2 million litres, mostly processed into cheeses in the northwest and central regions (**AACREA-Agroalimentos**). Goat's cheese production has increased greatly since the 1990s (**SAGPyA, 2007**).

Although some goat's milk cheeses are produced under artisanal conditions, most are manufactured at industrial scale (**AACREA-Agroalimentos**), especially semi-hard goat cheese, receiving little attention the fresh goat cheese. Although these cheeses have been produced in Argentina for more than a century ago, they have not been characterized as traditional cheeses. At present, little data on the biochemical changes produced during ripening which are responsible for the organoleptic and sensory characteristics of fresh goat cheese have been reported. According to Argentinean legislation, fresh cheeses must be made from pasteurized milk (**ANMAT, 2014**). But, pasteurization not only destroys pathogens but also the naturally occurring bacteria that contribute to flavor and might convey health benefits **Tunick, (2014)**. In this way, the drastic reduction in the number of propionibacteria and facultatively heterofermentative lactobacilli produced by milk pasteurization, modifies the catabolism of many organic acids, such as lactate and citrate (**Buffa et al. 2004**). Thus, the resulting cheese develops a less intense flavour and ripens more slowly than raw milk cheese (**Fox and Mc Sweeney, 2004**).

One way to intensify the flavour and improve the technological process is by the addition to pasteurized milk of adjunct and starter cultures, composed of selected lactic acid bacteria (LAB) (**Coppola et al., 2008**). Starter cultures cause rapid acidification of the milk through the production of organic acids, mainly lactic acid (**Leroy and De Vuyst, 2004**).

The composition of most mesophilic starters include species of *Lactococcus* and *Leuconostoc,* thermophilic organisms of starter belong to two genera,

Lactobacillus (*L.*) and *Streptococcus* (*S.*): *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus, L. delbrueckii* subsp. *lactis, L. helveticus* and *L. plantarum* (**Mäyrä-Mäkinen and Bigret, 2004; Reinheimer and Zalazar, 2006**). Adjunct cultures are mostly composed of facultative heterofermentative mesophilic lactobacilli including species such as *L. casei, L. paracasei, L. rhamnosus* and *L. plantarum*, as well as pediococci, *Leuconostoc* and micrococci (**Burns et al. 2012**) grown during the ripening process (**Settanni and Moschetti, 2010**).

In previous works, we have studied biochemical, technological and healthy properties of LAB isolated from goat milk and artisanal cheeses from northwest Argentina **Taboada et al. (2014a, b, 2015)** in order to select appropriate starter and adjunct cultures.

The purpose of the present work was to employ native and commercial cultures in the manufacture of fresh goat cheeses made from pasteurized milk and to evaluate the impact of these cultures on the microbiological, biochemical and physicochemical parameters of the end product.

MATERIALS AND METHODS

Microorganisms

The native 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 goat cheeses, phenotypically identified and carefully selected by their technological properties **Taboada et al. (2014 a, b)**. The following strains were used: *L. rhamnosus* UNSE308, *L. delbrueckii* subsp. *bulgaricus* UNSE309, *S. thermophilus* UNSE314, *S. thermophilus* UNSE321, *L. plantarum* UNSE316, *L. plantarum* UNSE317, *P. pentosaceus* UNSE22, *P. pentosaceus* UNSE253.

Acidifying ability test

This test was performed to determine the acidifying capacity of strains in similar processing conditions to those in cheesemaking. **Hynes *et al.* (2000)** protocol was used with modifications. Bottles containing 100 mL of pasteurized goat milk (at 65ºC for 30 min) were kept in a water bath at 38ºC. Then, 0.2 g/L $CaCl_2$ (Merck, Darmstadt, Germany) was added, with a 2% (v/v) inoculum of an active culture with 10^8 cfu mL^{-1} of each of the strains mentioned above in 2.1. Then, 0.014 g/L chymosin (MAXIREN 150, Delft, The Netherlands) was added to coagulate milk. Coagulation time was controlled by rocking the bottles gently to test adhesion of casein to their sides. After coagulation, the bottles were maintained in a water bath at 38ºC to attain the appropriate curd strength. Cell growth and proliferation were followed by means of pH measurements. The pH determinations were made every 30 min, from inoculation until attaining the appropriate curd strength, at a pH of about 5.2, according to **Janhøj and Qvist (2010)**.

Strain composition of native cultures

Based on the acidifying activity of individual strains and compatibility tests (**Collins *et al.* 1991**) different combinations of strains were used in cheesemaking, according to the following schedule:

Strain composition of native cultures

Strains (%, v/v)	Starter culture (S) (%, v/v)	Starter culture plus adjunct culture (A)
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

In relation to the starter culture, for the selection of the species the criteria of **Kosikowski, (1978)** were followed.
Adjunct cultures contribute to desired cheese ripening events through their metabolic activities such as proteolysis and lipolysis which influence flavour and texture development of the product (**Barouei *et al*. 2011**). In previous works (**Taboada *et al*. 2014a**), both esterase and esterase-lipase activity were detected in the strains selected as adjunct cultures. Since it is desired to obtain a product that is accepted by the majority of consumers, intense lipolysis would not be desirable. According to our experience, native culture A was designed as follows, composed of 95% (v/v) starters plus 5% (v/v) adjunct strains, meaning 1.9 x 10^6 cfu mL^{-1} of starter strains and 10^5 cfu mL^{-1} of adjunct strains, in vat.

Optimal inoculation proportion test

In order to obtain the optimal proportion to inoculate the native cultures A and S, the acidifying ability test was performed by using an inoculum of 1, 1.5 and 2% (v/v).

Milk for cheese manufacture

Goat's milk (Creole breed) provided by a local farm was refrigerated and transported at 4ºC to the pilot plant of Instituto de Ciencia y Tecnología de Alimentos, UNSE (Santiago del Estero, Argentina). The chemical composition was expressed in percent (w/v) and determined by Lactostar analyser (Funke Gerber Lactostar, Funke-Dr. N. Gerber Labortechnik GmbH, Berlín, Germany). The official methods AOAC (Association of Official Analytical Chemists) were used for acidity (**Method 947.05, AOAC 2006**) and density (**Method 925.22, AOAC 2006**) determinations. The pH value was determined using a Metrohm 962 pHmeter (Herisau, Switzerland).

Cheese manufacturing protocol

Fresh cheeses were manufactured using native starter culture (S) or native starter plus adjunct culture (A) or a lyophilized commercial culture (C) of *S. thermophillus* (type DVS, Diagramma SA, Santa Fe, Argentina). The native strains and the lyophilized commercial culture were previously activated in MRS broth (Merck, Darmstadt, Germany) later they were multiplied in pasteurized goat milk with incubation at 35 ºC until coagulation. Cultures with 10^8 cfu mL^{-1} were used for cheese manufacturing.
Fresh experimental cheeses were prepared using the following standard protocol: raw goat milk (35 L) was batch pasteurized at 65ºC for 30 min and after cooling at 38ºC, 0.2 g/L $CaCl_2$ (Merck, Darmstadt, Germany) was added, followed by a 2% (v/v) inoculum of S or A cultures, or a 1% (v/v) of C culture. 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 5ºC for 12 h. Then, cheeses were salted by immersion in 16% (w/v) NaCl sterile solution at 5ºC for 2 h. Ripening was carried out at 12ºC and 85% relative humidity for 30 days, being vacuum packaged at the end of ripening. Cheeses of approximately 700 g were obtained. Three independent trials for each type of cheese (CS, cheese manufactured with native culture S; CA, cheese manufacture with native culture A; CC, cheese manufactured with commercial culture C) were made on different elaboration days, during a period of 3 months. Samples were taken at days 1 and 30 for physicochemical and microbiological analysis. Throughout the ripening period, the cheeses were turned over daily or every second day and, when necessary, the surface was brushed to eliminate the growth of mould.

Global composition of cheeses

Fat content (**Method 933.05, AOAC 2006**), NaCl content (**Method 975.20, AOAC 2006**) and acidity (**Method 920.124, AOAC 2006**) were measured. Dry matter and total nitrogen were determined by (**Standard IDF 4A, 1982)** and **Rossi *et al*. (2004)**, respectively. Non-protein nitrogen was determined by (**Standard IDF 25, 1964**) and fat acidity by (**Standard IDF 6A, 1969**). The pH values of the cheeses were obtained by directly inserting the tip of the probe (MV-TEMP pH meter, Digital Instruments, Taiwan) into different portions of cheese samples. Water activity (aw) was determined using a ROTRONIC a_w Quick carp instrument (New York, USA) according to manufacturer specifications.
Free fatty acids (FFA) in experimental cheeses were extracted from whole cheese fat by a column chromatographic separation step, using an alumina stationary phase and subsequent elution with solvents, according to **Deeth *et al.* (1983)**. FFA from C4:0 to C18:1 were determined by gas chromatography (GC) analysis (1:80 split injection) using a Shimadzu CG-17A gas chromatograph equipped with a flame ionisation detector (Shimadzu Corporation, Kyoto, Japan) and with a Nukol column (Supelco, Inc., Bellefonte, PA, USA, 30 m×0.25 mm ID×0.25 μm film thickness). The carrier gas was N_2 at 3 mL.min^{-1} flow rate, and the oven temperature programme was 100 °C (initial temperature), 16 °C.min^{-1}, 210 °C (30 min). Free fatty acids were detected at day 1 (FFA_1) and at the end of ripening (FFA_{30}).

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.), serial dilutions in peptone saline solution were performed and plated on specific media for viable counts. Microbiological counts were performed in triplicate after 1 and 30 days of ripening. Mesophilic LAB (MRS agar pH 6.5, at 35ºC for 48 h), fungi and yeasts (Yeast Extract Glucose Chloramphenicol Agar, 5 days at 30ºC) and total aerobic mesophilic microorganisms (PCA, 35ºC, 48 h) were determined. According to Argentinean legislation (**ANMAT, 2014**), coliforms at 30ºC and 45ºC (MPN method, three tubes series, Brilliant Green Lactose Bile Broth, 48h at 30ºC and 45ºC, respectively) were determined.

Sensorial analysis

In order to characterize the sensorial profile of cheeses at the end of ripening (30 days) they were subjected to sensorial evaluation by an internal panel consisting of 9 judges. Panellists were chosen from a group prepared and trained in sensory analysis of cheeses, having among 30–45 years old, in gender proportion of 6 male and 3 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 ± 1ºC). 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).

Statistical analysis

Results were mean of three independent trials for each type of cheese, during a period of 3 months. Results were expressed as mean ± 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).

RESULTS AND DISCUSSION

Acidifying ability test

In cheesemaking, it has been observed that lowering the pH of milk (e.g. by the activity of starter) leads to a shorter coagulation time and a faster initial increase of gel firmness (**Brule and Lenoir, 1990**). For individual strains, the results are shown in **Table 1**. Of all studied strains, *L. plantarum* UNSE316 and *S. thermophilus* UNSE314 presented the highest acidifying activity. As *L.*

plantarum strains were used as adjunct cultures they were placed in a smaller proportion in culture A. *S. thermophilus* strains, employed as starters, reached the desired pH 5.2 after 5 h of incubation, while *L. bulgaricus* strains did after 6 h of incubation.

It is generally agreed that κ-casein is predominantly located at the casein micelle surface with the hydrophobic para-κ-casein part (residues 1–105) linked to the micelle, and the hydrophilic and negatively charged caseinomacropeptide (CMP) part (residues 106–169), rich in carbohydrates, protruding into the solution. Upon hydrolysis of κ-casein by the coagulant (chymosin in this case), CMP is released, leaving para-κ-casein attached to the micelle. The removal of CMP from the micelle surface leads to a decrease in electrostatic repulsion between micelles and the steric stabilisation is also decreased. The loss of electrostatic repulsion and steric stabilisation allows attractive forces to come into play, and the micelles start to aggregate, forming a gel that eventually can expel liquid by syneresis. Aggregation is also very dependent on the concentration of calcium (**Harboe et al, 2010**) and the pH (**Janhøj and Qvist, 2010**). A decreasing pH leads to a decrease in the negative charge of the caseins that may favour aggregation. On the other hand, it also leads to dissolution of calcium phosphate from the casein micelles working in the opposite direction to increase the negative charge. The balance between these effects can probably explain the effect of pH on gel firming. Decreasing the pH, at least down to pH 5.3, leads to a large increase in aggregation and greatly enhances syneresis facilitating the flow of whey out of the grains (**Janhøj and Qvist, 2010**).

Table 1 Acidifying ability of the selected native lactic acid bacteria

	pH Time (hours)						
Strains	1	2	3	4	5	6	7
L plantarum							
UNSE316	6.25 ± 0.03[D]	6.17 ± 0.02[D]	5.97 ± 0.03[E]	5.41 ± 0.02[F]	5.29 ± 0.03[E]	4.99 ± 0.02[G]	4.97 ± 0.02[D]
UNSE317	6.23 ± 0.03[D]	6.19 ± 0.03[D]	6.00 ± 0.01[D]	5.46 ± 0.03[E]	5.34 ± 0.02[D]	5.04 ± 0.02[F]	4.95 ± 0.02[D]
L. rhamnosus							
UNSE308	6.42 ± 0.02[AB]	6.33 ± 0.03[B]	6.21 ± 0.03[A]	5.85 ± 0.03[C]	5.49 ± 0.02[C]	5.36 ± 0.02[C]	5.17 ± 0.03[C]
L. bulgaricus							
UNSE309	6.44 ± 0.03[AB]	6.29 ± 0.02[C]	6.18 ± 0.02[B]	5.78 ± 0.02[D]	5.46 ± 0.02[C]	5.28 ± 0.02[D]	5.15 ± 0.02[C]
P. pentosaceus							
UNSE22	6.46 ± 0.03[A]	6.38 ± 0.03[A]	6.21 ± 0.01[A]	6.10 ± 0.02[A]	5.96 ± 0.02[A]	5.57 ± 0.02[B]	5.40 ± 0.02[B]
UNSE253	6.41 ± 0.02[B]	6.36 ± 0.01[A]	6.11 ± 0.02[C]	6.02 ± 0.03[B]	5.92 ± 0.03[B]	5.65 ± 0.03[A]	5.50 ± 0.02[A]
S.thermophilus							
UNSE314	6.39 ± 0.01[C]	6.27 ± 0.03[C]	5.82 ± 0.02[F]	5.45 ± 0.03[E]	5.28 ± 0.01[E]	5.05 ± 0.03[F]	4.97±0.03[D]
UNSE321	6.41 ± 0.02[B]	6.32 ± 0.02[B]	6.00 ± 0.03[D]	5.75 ± 0.02[D]	5.34 ± 0.03[D]	5.22 ± 0.02[E]	5.19 ± 0.02[C]

Legend: Values are expressed as mean ± SD.
[A-G] Different letters in the same column indicate statistically significant differences (P < 0.05). The initial pH of milk was 6.50±0.10.

Optimal inoculation proportion test

The results obtained are shown in **Figures 1A and 1B**. Cultures S and A at 2% (v/v) reached the desired pH 5.2 in curd (see above **Acidifying ability test)** at 4 h of incubation. A four hour period from milk inoculation until para-κ-casein gel formation (optimum at pH 5.2) is considered to lead to a more efficient process of cheesemaking (**Hynes et al., 2000).** Therefore, the 2% (v/v) inoculum was selected for cheese manufacture in this study.

The test was also performed with the commercial culture, but C at 2% (v/v) reached the desired pH 5.2 in curd at 4.5 h of incubation (data not shown).
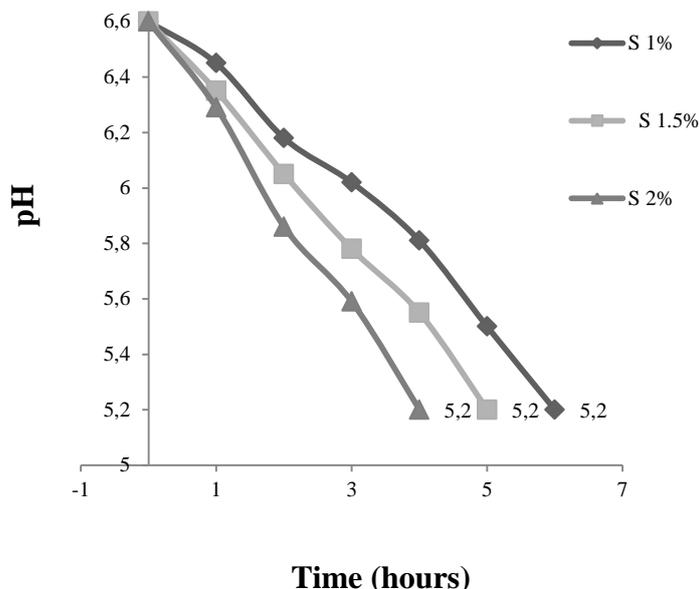
Figure 1A Acidification curve for cheesemaking with native starter culture (S) in different inoculum (1, 1.5, 2%).
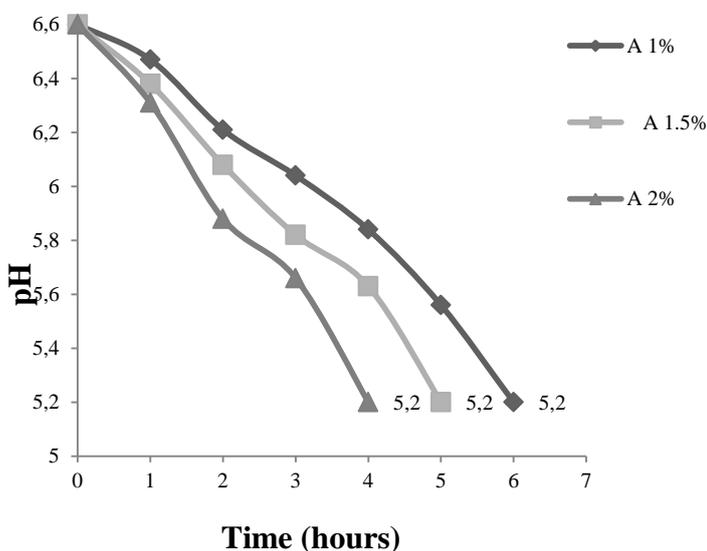

Figure 1B Acidification curve for cheesemaking with native starter plus adjunct culture (A) in different inoculum
(1, 1.5, 2%).

Chemical composition of milk

The values (means ± standard deviations) of the physicochemical parameters of the milk used in the manufacture of the experimental cheeses were % (w/v): lactose, 4.80±0.01; protein, 3.82±0.01; fat, 5.85±0.04; non-fat total solids, 9.40±0.01. Freezing point (ºC), -0.523 ± 0,000, acidity, 0.17% (w/w) lactic acid; density (at 15ºC) (g/ml) 1.0320 ± 0.0023 and pH was 6.5±0.10.

The composition of milk and the yield of cheese derived from a given quantity of milk is determined by a multiplicity of factors, which include the animal species (e.g. cow, goat or sheep), the breed of animal, the stage of lactation, nutrition, the lactation number and animal health (**Banks, 2007**). This study was performed in winter, when goats received rations of lower nutritional value than the rest of the year seasons and this had impact in decreased protein content. The two most important constituents with respect to cheese yield are milk fat and casein (**Banks, 2007**). According to this, the cheese yield obtained in this study was 10-11%, lower than that obtained by **Oliszewski et al. (2002).**

Global composition of cheese

Evolution of global composition of fresh goat cheeses during ripening is shown in **Table 2.**

Values of fat in dry matter and moisture are in accordance with the range established by Argentinean legislation (**ANMAT, 2014**) for semi fat cheeses (25.0-44.9%) and fresh cheeses (46.0-55.0%), respectively.

Results evidenced that CC, CA and CS did not show significant differences (P< 0.05) in: protein, fat, NaCl, acidity, fat acidity and a_w, at the same time of ripening. Fat and protein contents were close to those reported in similar type of cheese (**Franco et al. 2003; Janštová et al. 2010**).

In all experimental cheeses, titratable acidity increased progressively during ripening. Final acidity values (1.05 %) doubled those found by **López Alzogaray et al. (2007)** in goat milk cheeses made without selected starter addition but were slightly lower than those reported by **González and Zárate, (2012)** (1.26%) in goat cheeses made with native starter culture.

Final pH values of CS (4.92) and CA (4.88) were slightly higher than those reported by **Areti Asteri et al. (2010)** (4.53) in fresh cheese. Of all experimental cheeses, CC showed the highest final value of pH (5.05). pH values lower than 4.0 make the cheese very acid and maybe brittle, values higher than 5.0 are not proper and safe for good keeping quality of this type of cheese (**Alichanidis and Polychroniadou, 2008**).

In relation to fat acidity, final average values of 1.79-1.76 mg of KOH 0.1 N /g of fat (equivalent to ~3.2 mEq of acid/100 g of fat) were detected. Slightly higher value (1.92 mg of KOH 0.1 N /g of fat) was found in artisanal cheeses made with raw goat milk (**López Alzogaray et al. 2007**).

Table 3. shows the average concentrations of FFA determined at the end of experimental cheese ripening. The total FFA content was obtained by summing individual FFA concentrations. The values ranged from approximately 1472 to 1527 mg kg^{-1} cheese. The results are in agreement with those described for different types of goat's milk cheese. **Nouira et al. (2011)** found total FFA values between 1448.5 and 1495.7 mg kg^{-1} in semi-soft Cheddar-type caprine cheeses after 30 days of ripening. **Franco et al. (2003)** obtained a value of 1561.4 mg kg^{-1} for Babia-Laciana cheese at 15 days of ripening. **Juan et al. (2015)** reported similar FFA values (1444 mg.kg^{-1} cheese) in a similar type of goat's milk cheese. Total FFA contents showed significant differences between CC and cheeses made with native cultures, CS and CA.Variability in the FFA contents among replicate trials of cheesemaking was observed.

According to **Collins et al. (2003)**, in cheeses that underwent moderate lipolysis, FFA in the order of 2000–7000 mg kg^{-1} are liberated during ripening and make an important contribution to characteristic flavour and aroma in both raw and pasteurized milk cheese. The lipolysis degree was determined as ratio total FFA_{30}/FFA_1. The highest ratio was detected in CA.The FFA profile of experimental cheeses indicated that the most abundant acids were palmitic (C16:0), oleic (C18:1), stearic (C18:0), capric (C10:0) and lauric (C12:0) (in decreasing order), representing together about 77.6% of the total FFA content. A similar pattern has been reported by **Juan et al. (2015)**.

Proteolysis is the most complex event that occurs during ripening and gives a significant contribution to flavour, via the formation of amino acids and small peptides (**Fox and Cogan, 2004**). Experimental cheeses underwent a slight proteolysis since NPN values were lower (4.54- 4.49%) than those reported in cheeses subject to moderate proteolysis (18.20%) (**Fontecha et al. 1990**) but higher than those found by **Olarte el al. (2000)** (4.12%) and **Albenzio et al. (2006)** (0.2-0.4%). Of all experimental cheeses, CC showed the lowest levels of proteolysis during ripening.

The salt level markedly influences cheese flavour and aroma and, hence, overall quality (**Guinee, 2004**). Average values of salt content (1.27%) were in accordande with those reported in most of cheeses (0.5 to 2%) (**Hardy, 1990**). As a consequence, the cheesemaking protocol described in this work allowed the successful control of the factors that affect salt uptake and distribution in cheese. Among these factors, the most important are the pH and aw of the cheese, temperature and concentration of the brine and standing time in brine (**Hardy, 1990).** Thus moderate values of salt content were measured without affecting the cheese overall quality.

Table 2 Global composition of experimental fresh goat cheeses at the beginning and end of ripening.

Global composition of experimental cheeses

Days of ripening	Cheeses[a]	Protein (% TS)	Fat (% w/w)	Total solids (% w/w)	NaCl (% w/w)	pH	Titratable acidity g of lactic acid/100g of cheese	NPN (%TN)	Fat acidity mg KOH 0,1N/ g of fat	Moisture (%, w/w)	aw
1	CS	32.73 ± 0.30^B	30.27 ± 0.32^B	48.52 ± 0.30^B	0.98 ± 0.02^B	5.22 ± 0.20^A	0.75 ± 0.07^B	1.48 ± 0.34^C	0.180 ± 0.007^A	51.80 ± 0.62^A	0.992 ± 0.004^B
	CA	32.80 ± 0.32^B	30.52 ± 0.28^B	48.73 ± 0.33^B	0.96 ± 0.02^B	5.20 ± 0.28^A	0.73 ± 0.04^B	1.43 ± 0.22^C	0.182 ± 0.005^A	51.83 ± 0.56^A	0.991 ± 0.003^B
	CC	32.48 ± 0.20^B	30.12 ± 0.36^B	48.40 ± 0.23^B	0.97 ± 0.01^B	5.24 ± 0.26^A	0.70 ± 0.07^B	1.41 ± 0.20^C	0.183 ± 0.005^A	51.78 ± 0.56^A	0.990 ± 0.002^B
30	CS	34.90 ± 0.30^A	33.70 ± 0.39^A	52.50 ± 0.30^A	1.29 ± 0.02^A	4.92 ± 0.30^C	1.05 ± 0.07^A	4.54 ± 0.22^A	$1.790\pm0\cdot006^B$	49.71 ± 0.53^B	0.979 ± 0.004^A
	CA	34.98 ± 0.25^A	33.83 ± 0.35^A	52.55 ± 0.28^A	1.27 ± 0.03^A	4.88 ± 0.32^C	1.04 ± 0.07^A	4.49 ± 0.22^A	$1.782\pm0\cdot005^B$	49.76 ± 0.42^B	0.980 ± 0.003^A
	CC	34.68 ± 0.22^A	33.23 ± 0.33^A	52.68 ± 0.28^A	1.25 ± 0.02^A	5.05 ± 0.24^B	1.02 ± 0.06^A	4.18 ± 0.22^B	$1.757\pm0\cdot008^B$	49.68 ± 0.35^B	0.978 ± 0.004^A

Legend: Values are mean of three trials for each type of cheese. Different letters in each column at the same time of ripening indicate statistically significant differences (P<0.05) between experimental cheeses.

[a]Experimental cheeses, CC (cheese manufactured with commercial culture C), CS (cheese manufactured with autochthonous starter culture S), CA (cheese manufactured with autochthonous starter and adjunct culture A).

Titratable acidity values are expressed as g of lactic acid/100 g of cheese. Fat, Total solids, NaCl and Moisture values are expressed as g/100 g of cheese. Protein values are expressed as percentage of Total solids. Non protein nitrogen (NPN) values are expressed as percentage of total nitrogen.

Table 3 Free fatty acid concentrations of experimental fresh goat cheeses at end of ripening

FFA group[b]	Type of cheese[a]		
	CS	CA	CC
SCFA	138 ± 20^A	144 ± 18^A	126 ± 31^B
MCFA	407 ± 50^A	411 ± 42^A	390 ± 38^B
LCFA	963 ± 60^A	972 ± 54^A	956 ± 45^A
Total FFA	1.508 ± 21^A	1.527 ± 28^A	1.472 ± 27^B
Ratio total FFA_{30}/FFA_1	2.90	2.94	2.88

Legend: Values are mean of three trials for each type of cheese. Different letters in each row indicate statistically significant differences (P<0.05) between experimental cheeses.

FFA, Free fatty acids concentrations are expressed in $mg.kg^{-1}$ cheese.

$FFA_{30,}$ free fatty acid concentration at the end of ripening; $FFA_{1,}$ free fatty acid concentration at day 1.

[a] CS, cheese manufactured with native culture S; CA, cheese manufacture with native culture A; CC, cheese manufactured with commercial culture C.

[b] SCFA, short chain fatty acids (C4:0–C8:0); MCFA, medium-chain fatty acids (C10:0–C14:0), LCFA, long-chain fatty acids (C16:0–C18:2).

Microbiological analysis

Evolution of microorganisms in experimental fresh goat cheeses during ripening is shown in **Table 4**.

Results evidenced that CC, CA and CS did not show significant differences (P < 0.05) in total counts of aerobic mesophilic microorganisms and coliforms at 30ºC, at the same time of ripening. Total coliforms per gram at 30ºC showed counts below the maximum allowed by Argentinean legislation (m=5000; M=10000), for cheeses of high humidity (46.0-55.0% moisture) (**ANMAT, 2014**), coliforms at 45ºC were not detected; these results confirm that the manufacturing protocol used allowed making microbiologically safe cheeses. At the end of ripening, there were no significant differences in mesophilic LAB counts and fungi and yeast counts between experimental cheeses. Microbial counts showed a growth of LAB in all experimental cheeses between 8.02 and 8.34 log CFU g^{-1}. These results indicate that LAB remained viable during ripening thus contributing to the moderate lipolysis and slight proteolysis that were detected in all cheese samples.

Table 4 Counts of the main microbial groups during ripening of experimental fresh goat cheeses.

Day of ripening	Cheeses[a]	Lactic acid bacteria (log CFUg^{-1})	Total mesophilic (log CFU g^{-1})	Coliforms at 30ºC (MPN/g)	Fungi and yeasts (log CFU g^{-1})
		Microbial counts in experimental cheeses			
1	CS	7.54±0.35[B]	4.04±0.25[B]	3.09±0.14[A]	5.27±0.20[B]
	CA	7.86±0.38[B]	4.10±0.30[B]	3.13±0.26[A]	5.38±0.30[B]
	CC	6.98±0.33[C]	4.12±0.20[B]	3.17±0.22[A]	5.30±0.22[B]
30	CS	8.26±0.25[A]	4.18±0.30[A]	2.18±0.16[B]	5.40±0.20[B]
	CA	8.34±0.42[A]	4.26±0.23[A]	2.22±0.12[B]	5.48±0.30[B]
	CC	8.02±0.33[A]	4.30±0.25[A]	2.29 ±0.18[B]	5.67±0.32[B]

Legend: Values are mean of three trials for each type of cheese. Counts of microbial groups are expressed as log CFUg^{-1}. Different letters in each column at the same time of ripening indicate statistically significant differences (P<0.05) between experimental cheeses. [a]Experimental cheeses, CS (cheese manufactured with autochthonous starter culture), CA (cheese manufactured with autochthonous starter and adjunct cultures) and CC (cheese manufactured with commercial culture).

Sensorial analysis

The sensory profiles of the three types of cheeses are shown in **Figures 2A** and **2B**. The results of the sensory evaluation showed that CA received the highest score, followed by CS, in comparison with CC at 30 days of ripening. The CA and CS showed significant differences for odour attributes as fresh milk, fruity and propionic acid. CA presented the highest score for fruity and propionic acid attributes, as well as stronger goaty note than commercial cheese (**Fig. 2A**). Respect to flavour (**Fig. 2B**), all types of cheeses showed significant differences for flavour attributes as cream, spiciness propionic acid and bitterness. CA was highlighted by its highest score for cream and spiciness and lowest score for bitterness attributes. Overall impression of cheeses elaborated with native cultures (CS and CA) was qualified as good compared to CC that was scored as regular.
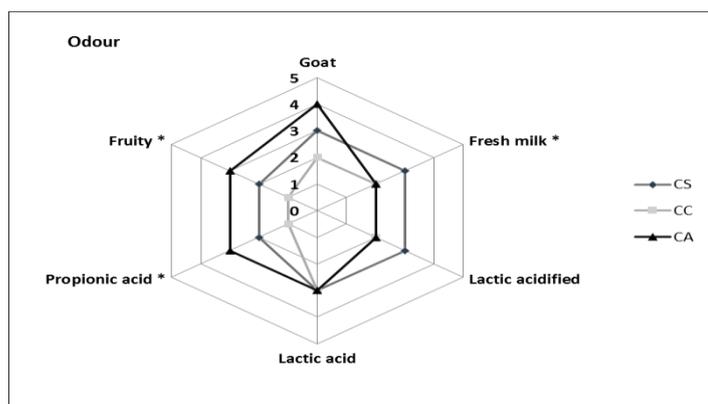

Figure 2A Quantitative sensory profile of three types of fresh goat cheeses (CS, CA,CC) at the end of ripening (30 days)(*p < 0.05): (A) odour attribute.
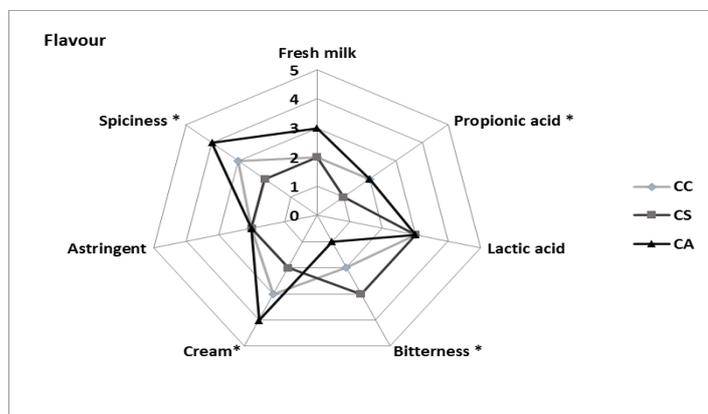

Figure 2B Quantitative sensory profile of three types of fresh goat cheeses (CS, CA, CC) at the end of ripening (30 days)(*p < 0.05): (B) flavour attribute.

CONCLUSION

Fresh goat cheeses made with both native and commercial cultures presented moderate lipolysis and slight proteolysis during ripening (30 day-old-cheese), obtaining a product of similar global composition. Native cultures S and A showed a better perfomance in cheesemaking.

The fresh goat cheeses made with native cultures S and A were greater scored for their suitable typical flavour and satisfying overall sensorial characteristics. These results should be of great interest for the eventual manufacture of this type of cheese on an industrial scale. However, more studies are needed to conveniently perform the scaling up.

REFERENCES

AACREA-Caprinos, Agroalimentos II. *Disponible en http://www.produccion-animal.com.ar/produccion_caprina/produccion_caprina/22-produccion_caprinos_aacrea.pdf.*

Albenzio, M., Caroprese, M., Marino, R., Muscio, A., Santillo, A., & Sevi, A. (2006). Characteristics of Garganica goat milk and Cacioricotta cheese. *Small Rumin Res*, 64, 35–44. http://dx.doi.org/10.1016/j.smallrumres.2005.03.010

Alichanidis, E., & Polychroniadou, A. (2008). Characteristics of major traditional regional cheese varieties of East-Mediterranean countries: a review. *Dairy Sci Technol*, 88,495–510. http://dx.doi.org/10.1051/dst:2008023

ANMAT. (2014). Alimentos lácteos In: Chapter 8 in Código Alimentario Argentino. *http://www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp (retrieved 02.04.14).pp93-94.*

AOAC. (2006). Titrimetric acidity of milk, official method 947.05. Dairy Products. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemistry, Washington, DC, USA.

AOAC. (2006). Density, official method 925.22. Dairy Products. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemistry, Washington, DC, USA.

AOAC. (2006). Titrimetric Acidity of cheese, official method 920.124. Dairy Products. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemistry, Washington, DC, USA.

AOAC. (2006). Titrimetric salt in cheese, official method 975.20. Dairy Products. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemistry, Washington, DC, USA.

AOAC. (2006). Titrimetric fat in cheese, official method 933.05. Dairy Products. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemistry, Washington, DC, USA.

Areti Asteri, I., Kittaki, N., &Tsakalidou, E. (2010). The effect of wild lactic acid bacteria on the production of goat's milk soft cheese. *Int J Dairy Technol*, 63,234-242. http://dx.doi.org/10.1111/j.1471-0307.2010.00564.x

Banks, J.M. (2007). *Cheese yield:Cheese Problems Solved*. McSweeney, P.L.H. (ed.). Woodhead Publishing series. Cambridge.pp 100-110.

Barouei, J., Karbassi, A., Ghoddusi, H.B., Mortazavi, A., Ramezani, R., & Moussavi, M. (2011). Impact of native *Lactobacillus paracasei* subsp. *Paracasei* and *Pediococcus* spp. as adjunct cultures on sensory quality of Iranian white brined cheese. *Int J Dairy Technol*, 64, 526-535. http://dx.doi.org/10.1111/j.1471-0307.2011.00720.x

Brule, G., & Lenoir, J. (1990). *The general mechanisms of transformation of milk into cheese. Coagulation of milk*, Omega editions. Barcelona, Spain. pp. 3-21.

Buffa, M., Guamis, B., Saldo, J., & Trujillo, A.J. (2004). Changes in organic acids during ripening of cheeses made from raw, pasteurized or high pressure-treated goats' milk. *LWT – Food Sci Technol*, 37, 247–253. http://dx.doi.org/10.1016/j.lwt.2003.08.006

Burns, P., Cuffia, F., Milesi, M., Vinderola, G., Meinardi, C., Sabbag, N., & Hynes, E. (2012). Technological and probiotic role of adjunct cultures of non-starter lactobacilli in soft cheeses. *Food Microbiol*, 30, 45–50. http://dx.doi.org/10.1016/j.fm.2011.09.015

Chamorro, C., & Losada, M.M. (2002). *El análisis sensorial de los quesos*, AMV Ediciones. Mundi-Prensa, Madrid, España.

Collins, C.H., Lyne, P.M., & Grang, J.M. (1991). *Antimicrobial susceptibility and assay tests:Microbiological Methods* 6th ed. Butterworth-Heinemann, Oxford, UK, pp. 155–168.

Collins, Y.F., McSweeney, P.L.H., & Wilkinson, M.G. (2003). Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. *Int Dairy J*, 13,841–866. http://dx.doi.org/10.1016/S0958-6946(03)00109-2

Coppola, S., Blaiotta, G., & Ercolini, D. (2008). *Dairy Products: Molecular Techniques in the Microbial Ecology of Fermented Foods*. Cocolin, L., Ercolini, D., eds. New York, USA, pp.35. http://dx.doi.org/10.1007/978-0-387-74520-6

Deeth, H.C., Fitzgerald, C.H., & Snow, A.J. (1983). A gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products. *New Ireland J Dairy Sci Technol*,18, 13-20.

FAO. (2013). *FAOSTAT Database. Available*: *http://faostat3.fao.org. accessed: June 9, 2016.*

FIL-IDF (1964). Determination of the Non-protein nitrogen content of the processed cheese products. IDF Standard 25. International Dairy Federation, Brussels, Belgium.

FIL-IDF (1969). Milk fat products and butter. Determination of fat acidity. IDF Standard 6A. International Dairy Federation. Brussels, Belgium.

FIL-IDF (1982). Cheese and Processed Cheese – Determination of total solids content. IDF Standard 4A. International Dairy Federation, Brussels, Belgium.

Franco I, Prieto B, Bernardo A, González Prieto J., & Carballo J. (2003). Biochemical changes throughout the ripening of a traditional Spanish goat cheese variety (Babia-Laciana). *Int Dairy J*,13,221–230. http://dx.doi.org/10.1016/S0958-6946(02)00154-1

Fox, P.F., & McSweeney, P.L.H (2004). *Cheese: An Overview in Cheese: chemistry, physics and microbiology*. Fox, P.F., McSweeney, P.L., Cogan, T.M., Guinee, T.P., eds.London: Elsevier Academic Press, pp. 8.

Fox, P.F., & Cogan, T.M. (2004). *Factors that affect the quality of cheese. Cheese: chemistry, physics and microbiology*. Fox, P.F., McSweeney, P.L., Cogan, T.M., Guinee, T.P., eds. Elsevier Academic Press, London, pp. 583-608.

Fontecha, J., Peláez,C., Juárez, M., Requena, T., & Gómez, C.(1990). Biochemical and Microbiological Characteristics of Artisanal Hard Goat's Cheese. *J Dairy Sci*,73,1150-1157. http://dx.doi.org/10.3168/jds.S0022-0302(90)78776-0

González, L., & Zárate, V. (2012). Influence of an autochthonous starter culture and a commercial starter on the characteristics of Tenerife pasteurised goats' milk cheese. *Int J dairy technol*, 65, 542-547. http://dx.doi.org/10.1111/j.1471-0307.2012.00862.x

Guinee, T.P. (2004). Salting and the role of salt in cheese. *Int J Dairy Technol*,57,99-109. http://dx.doi.org/10.1111/j.1471-0307.2004.00145.x

Harboe, M., Broe, M.L., & Qvist, K.B. (2010). *The Production, Action and Application of Rennet and Coagulants:Technology of Cheesemaking Second Edition.*. Law, B.A., Tamime, A.Y. Eds., Wiley & Sons, Ltd., Publication. Singapore, pp98-102. http://dx.doi.org/10.1002/9781444323740.ch3

Hardy, J. (1990). *The cheese. The general mechanisms of transformation of milk into cheese. Water activity and salty cheeses*. Omega editions. Barcelona, Spain, pp. 35-55.

Hynes, E., Ogier, J.C., & Delacroix-Buchet, A. (2000) Protocol for the manufacture of miniature washed curd cheeses under controlled microbiological conditions. *Int. Dairy. J*, 70,733-37. http://dx.doi.org/10.1016/S0958-6946(00)00102-3

IRAM 20012:1997. Instituto Argentino de Normalización y Certificación. Métodos para determinar el perfil de flavour. Buenos Aires, Argentina.

Janštová, B., Dračková, M., Cupáková, Š., Přidalová, H., Pospíšilová, M., Karpíšková, R., & Vorlová, L. (2010). Safety and Quality of Farm Fresh Goat's Cheese in the Czech Republic. *Czech J. Food Sci*, 28,(1), 1–8.

JanhØj, T., & Qvist, K.B. (2010).*The formation of cheese curd Technology of Cheesemaking*. Second Edition. Law B.A., Tamime, A.Y. (Eds). U.SA.pp.130-156. http://dx.doi.org/ 10.1002/9781444323740.ch4

Juan, B., Quevedo, J. M., Zamora, A., Guamis, B., & Trujillo., A.J. (2015). Lipolysis of cheeses made from goat milk treated by ultra-high pressure homogenization**.** *Food Sci Technol*, 60, 1034-1038. http://dx.doi.org/10.1016/j.lwt.2014.10.003

Kosikowski, F. V. (1978). *Cultures and starters*: *Cheese and fermented milk foods.* Kosikowski and associates eds. Brooktondale, New York, pp.16-36.

Leroy, F., & De Vuyst, L. (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol*, 15,67-78. http://dx.doi.org/10.1016/j.tifs.2003.09.004

López Alzogaray, S., Taboada, N., Neme, H., & Núñez, M. (2007). Contributions to the Characterization of Artisanal Goat Cheese in the Province of Santiago del Estero. *Latin American Dairy Technol*,47, 46-9.

Mäyrä-Mäkinen, A., & Bigret, M. (2004). *Industrial use and production of lactic acid bacteria*. Salminen, S., Von Wright, A., Ouwehand, A.C., eds. *Lactic Acid Bacteria: Microbiological and Functional Aspects*. New York: Marcel Dekker, pp. 175–198.

Nouira,W., Park, Young, W., Guler, Z., & Terrill, T. (2011). Comparison of free fatty acid composition between low-fat and full-fat goat milk cheeses stored for 3 months under refrigeration. *Open J Anim Sci*,1, 17-23. http://dx.doi.org/10.4236/ojas.2011.12003

Olarte, C., Sanz, S., Gonzalez-Fandos, E., & Torre, P. (2000).The effect of a commercial starter culture addition on the ripening of an artisanal goat's cheese (Cameros cheese). *J Applied Microbiol*, 88,421–429. http://dx.doi.org/10.1046/j.1365-2672.2000.00980.x

Oliszewski, R., Rabasa, A.E., Fernández, J.L., Poli, M.A., &Núñez de Kairúz, M.S. (2002). Composición química y rendimiento quesero de la leche de cabra Criolla Serrana del noroeste argentino. *Zootecnia Tropical*, 20, 179-189

Reinheimer, J., & Zalazar, C (2006). *Cheese Ripening and its Control: Advances in Microbiology, Biochemistry and Technology of Cheese*. Reinheimer, J., and Zalazar, C. A. (eds). Santa Fé, Argentine: National University of Litoral, pp.177-244.

Rossi, A.M., Villarreal, M., Juárez, M.D., & Samman, N. (2004). Nitrogen content in food: comparison between the Kjeldhal and Hach method. *J Argentine Chem Society*, 92, 99–108.

SAGPyA. (2007). *Cadena Caprina en la Región Noroeste. VI Foro federal de la industria Noroeste Argentino. Unión industrial Argentina. Disponible en http://www.agroindustria.gob.ar/site/ganaderia/caprinos/05-informacion_caprina/_archivos/000001 Comercializaci%C3%B3n/000010_Cadena%20caprina%20en%20la%20regi%C3%B3n%20noroeste.pdf*

Settanni, L., & Moschetti, G. (2010). Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. *Food Microbiol*. 27:691–697. http://dx.doi.org/ 10.1016/j.fm.2010.05.023

Taboada, N., Núñez, M., Medina, R., & López Alzogaray, M.S. (2014a). Characterization and technological properties of lactic acid bacteria isolated from traditional Argentinean goat's milk products. *Food Biotechnol*, 28, 123–141. http://dx.doi.org/10.1080/08905436.2014.895944

Taboada, N., López Alzogaray, S., Abeijon Mukdsi, M., & Medina, R. (2014b). Esterase activities and biochemical properties of lactic acid bacteria isolated from goat's milk cheese in Argentina. *J Agr. Sci Tech B*, 4, 752–760. http://dx.doi.org/10.17265/2161-6264/2014.09.008

Taboada, N.,Van Nieuwenhove, C., López-Alzogaray, S., & Medina, R. (2015). Influence of autochthonous cultures on fatty acid composition, esterase activity and sensory profile of Argentinean goat cheeses. *J Food Comp Anal*, 40, 86–94. http://dx.doi.org/10.1016/j.jfca.2014.12.013

Tunick, M. (2014). *The Science of Cheese*. Oxford University Press. New York, USA, p.26.