FATTY ACID COMPOSITION AND MICROSTRUCTURE OF RIPENED GOAT CHEESE

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***Abstract*— The aim of this study was to evaluate the changes in the fatty acid composition and microstructure of goat cheeses during 80 days of ripening. Creole goat milk from the *Quebrada de Humahuaca* was used to produce cheeses. Samples were taken 5 hours and 10, 20, 30, 40, 60 and 80 days after preparation. The composition of fatty acids for each period was determined by gas chromatography. The changes in the matrix cheese were observed using electron microscopy. The medium-chain fatty acids and the unsaturated fatty acids decreased during the whole study but at two different velocities. They became established at the end of the ripening time, which is coincident with the decrease in fatty globule sizes: 2–4.6 μm at the beginning, 300 ηm at 30 days of ripening, and 140 ηm at the end of the maturation time studied.**

***Keywords*— Fatty acids, goat cheese, fat globules, ripening, microstructure.**

I. INTRODUCTION

In the northwestern province of Jujuy, Argentina, goat cheese is produced mainly by the farmers themselves or by small organizations to add value to the milk produced by their animals. Hand-made goat cheese production has increased in recent years in the region and is now an important source of income. Goat cheese is consumed fresh (unripened or uncured), and this consumption is associated with the customs of the rural population. However, there is an increasing tendency to consume ripe cheese made from goat milk. As the cheese ripens, different physical and chemical changes are expected to occur. In fact, cheese ripening is a complex procedure accompanied by pH variation, breakdown of proteins, slow accumulation of amino acids and lipid degradation (Delgado *et al.*, 2012; Hayaloglu *et al*., 2013).

Lipolysis releases fatty acids (FA), especially short- and medium-chain FA, which contributes directly to cheese flavor. In addition, FA also act as substrates for further reactions producing highly flavored catabolic end products (Collins *et al.*, 2003).

Lipid composition is one of the most important components of the technological and nutritional quality of goat milk. Lipids are involved in cheese yield (per kilogram of milk) and firmness, as well as in the color and flavor of goat dairy products (Delacroix-Buchet and Lamberet, 2000). Besides their quantitative contribution to the amount of dietary energy, the different FA (short- and medium-chain, saturated, branched, mono- and poly unsaturated, *cis* and *trans*, conjugated) are potentially involved as positive or negative predisposing factors for the health of human consumers (Williams, 2000). Furthermore, the peculiarities of goat milk lipolytic system and medium-chain FA could greatly change the content of free FA, playing a major role in the occurrence of the characteristic goat flavor (Chilliard *et al.*, 2003).

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). 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). In the northwest of Argentina, several studies have been carried out about the influence of autochthonic lactic acid bacterias on the CLA in goat cheeses (Medina *et al*.; 2011; Taboada *et al*., 2015). These studies showed that goat cheese is the important source of CLA for humans.

Furthermore, the physical state of cheese fat may differ, depending on the ripening temperature and the storage time. An investigation on the microscopic structure of cheeses is a useful approach to determine certain processing characteristics and flavor properties and also to provide information about cheese texture, and therefore a means of controlling cheese properties (Karami *et al.*, 2009).

Since the changes caused by the lipolysis in the composition of the fatty acids and in the microstructure of the goat cheeses of *Quebrada de Humahuaca* have not been described in the literature, the aims of this study were 1) to evaluate the changes in the fatty acid composition and the microstructure of goat cheeses during 80 days of ripening; and 2) to assess the nutritional indices associated with the composition of fatty acids in ripening goat cheeses.

II. METHODS

The goat milk from a flock comprised of native goats belonging to a goat dairy farm located in *Quebrada de Humahuaca*, Jujuy province, Argentina, was worked. Goat production on this farm is based on pasture feeding without supplementation. The milk was brought to the laboratory in chilled plastic drums immediately after milking.

For cheese making, raw milk was pasteurized (65 °C,

30 min). It was cooled to 38 ºC and lactic ferment CHR HANSEN 743 RST was added. Simultaneously, $CaCl_2$ was incorporated at a concentration of 0.02% (w/v). The CHYMAX commercial rennet (Chr. Hansen, Horsholm, Denmark), 100% chymosin, with a coagulating force of IMCU 77.93000 (50 ml/100 l) was added. The curd was cut into 1.5 cm cubes with a lyre and separated from the whey. The cheeses were salted during the molding stage by adding 2% of NaCl (common salt), in two layers, directly in the curd and during molding. The cheeses were placed in a hydraulic press, subjecting them to a pressure of 4 bars for 3 h. After that, the salty product was stored in the refrigeration chamber at 10 °C and 90% relative moisture. The goat cheeses obtained weighed 380 grams freshly made. Three replicates were made for each batch of cheese. At least three samples were taken from the ripening batch to observe the microstructure and study proteolysis and fatty acids, at the following times: t_1: 5 hours, t_2: 10 days, t_3: 20 days, t_4: 30 days, t_5: 40 days, t_6: 60 days and t_7: 80 days.

The characterization of goat cheese ripened was performed according to AOAC (1995): Total Protein: by the Kjeldahl method, AOAC 955.04 c. Ash: method AOAC 968.08. Moisture: AOAC 935.29. pH: with HANNA equipment, precision of 0.01 pH unit.

Lipids were extracted according to AOAC 933.05 method (AOAC, 1995). Fatty acid methyl esters (FAME) analyses were performed according to the method described by UNE 55-037-73 (Aenor, 1991). One microlitre of FAME, dissolved in hexane, was injected into an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph (HP 6890 Series GC System Plus, USA), equipped with a flame ionization detector and automatic injector into a DB-225 capillary column (30 m × 0.25 µm × 0.25µm, J&W Scientific). GC conditions were as follows: injector temperature, 240 ºC; the initial oven temperature of 100 ºC was increased to 180 ºC at 10 ºC/min, held for 5 min and then increased to 210 ºC at 5 ºC/min and held for 20 min. Detector temperature was 240 ºC. The total oven program was 40 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 were purchased from Merck (Darmstadt, Germany).

The estimation of nutritional indices was studied. The mammary gland of ruminants has substantial Δ^9-desaturase activity. Desaturase is an enzyme which can be measured indirectly by comparing the product:substrate ratio of certain fatty acids. Therefore, the $C_{14:1}/(C_{14:1}+C_{14:0})$ ratio is the best indicator of this activity because all C14:0 in milk fat comes from de novo synthesis in the mammary gland. Another parameter that has biological interest is the atherogenicity index (AI), which characterizes the atherogenicity of dietary fat, by using $AI = [C_{12:0}+(4*C_{14:0})+C_{16:0}]/(MUFA + PUFA)$ formula (MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids) and Desirable Fatty Acids (DFA) = $MUFA+PUFA+C_{18:0}$.

The microstructure was observed. A cylinder, 4 cm in length × 0.5 cm in diameter, was extracted from each cheese sample using a cork borer; three 1 mm thick slices were removed from approximately the center of each cylinder to obtain samples from the middle of the cheese. Cheese samples were prepared for scanning electron microscopy (SEM) analysis according to the procedure of Fallico *et al.* (2006) with some modifications. Samples were fixed in 2.5% (wt/vol) glutaraldehyde at 7 °C. Dehydration was carried out using graded ethanol (Fluka, Sigma-Aldrich) series (30, 50, 75 and 95%, vol/vol) for 30 min in each bath at room temperature and then absolute ethanol for 12 h at 7 °C. Samples were dried by the critical point method in CO_2 and then fractured into small pieces with a blade. The dried fractured samples were mounted on SEM aluminum stubs using a carbon adhesive and then coated (ca. 24 nm thick) with gold-palladium. Cheese samples were examined in a JEOL JSM-6480-LV scanning electron microscope (JEOL, Tokyo, Japan). Images were recorded at 5000X magnification.

All the formulations were prepared in triplicate. The experimental data were analyzed using Minitab 16.0 statistical software (Minitab Inc., State College, PA, USA). One-way analysis of variance was used to determine significant differences between means. The level of significance (p) was set at 0.05. Tukey's HSD test at 5% significance level was used as the multiple comparison tests on all main effect means.

III. RESULTS

The changes in chemical composition and pH of the samples are presented in Table 1.

Moisture content decreases significantly during ripening due to the conditions of the camera. According to the Argentine Food Code (Art. 605), goat cheeses turn since soft to hard during ripening time, due to a rapid loss of water demonstrated by the low moisture content. Protein and fat content increased during ripening due to changes in moisture and biochemical phenomena during it (Burgos *et al.*, 2016).

Table 2 shows the variation of total FA, at the different ripening times considered. A preponderance of palmitic acid was found, followed by oleic, stearic and myristic acids.

Table 1. Chemical composition of goat cheeses ripened (g/100g)

Days	Protein	Moisture	Fat	pH
5 hs.	18,6±0,7$^{(a)}$	50,1±0,7$^{(a)}$	24,1±0,5$^{(a)}$	6,51±0,05$^{(a)}$
10	21,8±0,4$^{(bc)}$	42,3±0,7$^{(b)}$	26,8±0,7$^{(b)}$	5,03±0,03$^{(b)}$
20	21,5±0,1$^{(b)}$	38,1±0,7$^{(c)}$	29,6±0,7$^{(c)}$	4,86±0,03$^{(c)}$
30	21,3±0,5$^{(b)}$	31,1±0,8$^{(d)}$	30,9±0,5$^{(c)}$	4,84±0,01$^{(c)}$
40	22,7±0,8$^{(c)}$	30,2±0,8$^{(d)}$	34,9±0,6$^{(e)}$	4,80±0,03$^{(c)}$
60	23,8±0,5$^{(d)}$	26,6±0,8$^{(e)}$	32,4±0,6$^{(d)}$	4,82±0,01$^{(c)}$
80	24,8±0,5$^{(e)}$	21,2±0,5$^{(f)}$	37,3±0,4$^{(f)}$	4,79±0,03$^{(c)}$

^{abc}Means within the same column and parameter followed by different superscripts are significantly different ($P < 0.05$).

Table 2. Fatty Acids Composition and Nutritional Indices in goat cheese during ripening.

Fatty acids	Goat cheeses						
	5 hs.	10 days	20 days	30 days	40 days	60 days	80 days
$C_{4:0}$	1.09±0.04$^{(f)}$	0.64±0.02$^{(b)}$	0.95±0.02$^{(d)}$	1.21±0.02$^{(e)}$	0.91±0.02$^{(c)}$	1.12±0.03$^{(d)}$	0.63±0.01$^{(a)}$
$C_{6:0}$	1.56±0.04$^{(g)}$	1.05±0.03$^{(b)}$	1.49±0.02$^{(e)}$	1.88±0.01$^{(f)}$	1.39±0.01$^{(c)}$	1.63±0.02$^{(d)}$	1.09±0.01$^{(a)}$
$C_{8:0}$	2.34±0.01$^{(f)}$	1.52±0.02$^{(b)}$	2.07±0.02$^{(d)}$	2.49±0.01$^{(e)}$	1.96±0.04$^{(c)}$	2.10±0.03$^{(c)}$	1.76±0.03$^{(a)}$
$C_{10:0}$	8.36±0.05$^{(g)}$	6.89±0.06$^{(d)}$	7.96±0.04$^{(e)}$	9.50±0.02$^{(f)}$	7.87±0.01$^{(c)}$	7.76±0.04$^{(b)}$	7.91±0.01$^{(a)}$
$C_{12:0}$	4.82±0.03$^{(f)}$	4.52±0.02$^{(e)}$	4.59±0.03$^{(d)}$	4.14±0.04$^{(b)}$	4.62±0.01$^{(c)}$	3.55±0.03$^{(a)}$	4.78±0.01$^{(b)}$
$C_{13:0}$	0.09±0.01$^{(a)}$	0.10±0.01$^{(a)}$	0.10±0.01$^{(a)}$	0.10±0.01$^{(a)}$	0.09±0.01$^{(a)}$	0.10±0.01$^{(a)}$	0.10±0.01$^{(a)}$
$C_{14:0}$	10.63±0.07$^{(f)}$	10.81±0.07$^{(e)}$	10.62±0.04$^{(d)}$	9.66±0.02$^{(b)}$	10.93±0.02$^{(c)}$	9.65±0.02$^{(a)}$	11.12±0.01$^{(b)}$
$C_{14:1\ 9\ cis}$	0.13±0.01$^{(e)}$	0.13±0.01$^{(de)}$	0.13±0.01$^{(cd)}$	0.09±0.02$^{(a)}$	0.14±0.01$^{(bcd)}$	0.12±0.01$^{(ab)}$	0.14±0.01$^{(bc)}$
$C_{15:0}$	0.94±0.02$^{(e)}$	0.98±0.07$^{(d)}$	0.95±0.04$^{(cd)}$	1.06±0.01$^{(cd)}$	0.93±0.01$^{(ab)}$	1.02±0.01$^{(bc)}$	0.94±0.01$^{(a)}$
$C_{16:0}$	28.55±0.01$^{(f)}$	29.51±0.04$^{(e)}$	28.73±0.03$^{(d)}$	28.53±0.02$^{(c)}$	28.94±0.03$^{(c)}$	26.45±0.03$^{(a)}$	29.28±0.03$^{(b)}$
$C_{16:1\ 9\ trans}$	0.46±0.01$^{(de)}$	0.56±0.01$^{(e)}$	0.57±0.01$^{(d)}$	0.45±0.01$^{(b)}$	0.49±0.01$^{(bc)}$	0.52±0.01$^{(c)}$	0.44±0.01$^{(a)}$
$C_{16:1\ 9\ cis}$	0.55±0.03$^{(d)}$	0.57±0.01$^{(c)}$	0.56±0.01$^{(b)}$	0.50±0.01$^{(a)}$	0.56±0.01$^{(a)}$	0.55±0.01$^{(a)}$	0.61±0.01$^{(a)}$
$C_{17:0}$	0.77±0.04$^{(e)}$	0.79±0.03$^{(d)}$	0.78±0.04$^{(cd)}$	0.77±0.02$^{(bc)}$	0.75±0.02$^{(ab)}$	0.84±0.03$^{(bc)}$	0.74±0.01$^{(a)}$
$C_{17:1\ 10\ cis}$	0.51±0.04$^{(e)}$	0.31±0.01$^{(c)}$	0.44±0.01$^{(d)}$	0.28±0.01$^{(ab)}$	0.35±0.03$^{(bc)}$	0.37±0.02$^{(bc)}$	0.30±0.01$^{(a)}$
$C_{18:0}$	11.21±0.05$^{(g)}$	12.23±0.06$^{(f)}$	11.67±0.01$^{(d)}$	13.55±0.03$^{(e)}$	11.67±0.04$^{(b)}$	13.23±0.01$^{(c)}$	11.81±0.01$^{(a)}$
$C_{18:1\ 9\ cis}$	21.78±0.08$^{(g)}$	23.22±0.06$^{(f)}$	23.04±0.06$^{(e)}$	20.71±0.01$^{(b)}$	22.22±0.01$^{(c)}$	24.26±0.02$^{(d)}$	22.32±0.03$^{(a)}$
$C_{18:1\ 11\ cis}$	1.06±0.04$^{(f)}$	1.11±0.02$^{(e)}$	1.03±0.01$^{(c)}$	1.23±0.02$^{(d)}$	1.02±0.01$^{(b)}$	1.14±0.01$^{(bc)}$	1.02±0.02$^{(a)}$
$C_{18:1\ 13\ cis}$	0.43±0.01$^{(f)}$	0.45±0.01$^{(e)}$	0.43±0.01$^{(cd)}$	0.50±0.01$^{(d)}$	0.44±0.01$^{(b)}$	0.48±0.02$^{(bc)}$	0.45±0.01$^{(a)}$
$C_{18:2\ 9\ trans\ 12\ trans}$	0.36±0.02$^{(c)}$	0.44±0.02$^{(c)}$	0.41±0.02$^{(bc)}$	0.64±0.02$^{(d)}$	0.42±0.03$^{(ab)}$	0.45±0.03$^{(ab)}$	0.43±0.01$^{(a)}$
$C_{18:2\ 9\ cis\ 12\ cis}$	1.48±0.01$^{(f)}$	1.55±0.03$^{(e)}$	1.54±0.03$^{(d)}$	1.55±0.02$^{(bc)}$	1.51±0.03$^{(b)}$	1.71±0.03$^{(c)}$	1.40±0.01$^{(a)}$
$C_{18:2\ 9\ cis\ 11\ trans}$	1.23±0.03$^{(e)}$	1.25±0.02$^{(d)}$	1.24±0.03$^{(c)}$	1.12±0.01$^{(a)}$	1.22±0.02$^{(b)}$	1.36±0.01$^{(b)}$	1.20±0.02$^{(a)}$
$C_{18:3\ 9\ cis\ 12\ cis\ 15\ cis}$	1.24±0.04$^{(f)}$	1.27±0.01$^{(e)}$	1.24±0.02$^{(c)}$	1.46±0.01$^{(de)}$	1.23±0.01$^{(b)}$	1.53±0.01$^{(cd)}$	1.22±0.02$^{(a)}$
$C_{20:0}$	0.34±0.01$^{(d)}$	0.32±0.01$^{(c)}$	0.31±0.01$^{(b)}$	0.34±0.01$^{(b)}$	0.30±0.01$^{(a)}$	0.39±0.01$^{(bc)}$	0.33±0.01$^{(a)}$
SCFA ($C_{4:0}$-$C_{10:0}$) [a]	13.34±0.07$^{(e)}$	10.11±0.05$^{(a)}$	12.48±0.01$^{(c)}$	15.08±0.02$^{(f)}$	12.15±0.05$^{(c)}$	12.60±0.05$^{(d)}$	11.40±0.06$^{(b)}$
MCFA ($C_{12:0}$-$C_{16:0}$) [b]	45.04±0.04$^{(c)}$	45.94±0.08$^{(e)}$	45.00±0.01$^{(c)}$	43.49±0.02$^{(b)}$	45.52±0.04$^{(d)}$	40.78±0.02$^{(a)}$	46.22±0.03$^{(f)}$
LCFA ($C_{17:0}$-$C_{20:0}$) [c]	12.32±0.02$^{(a)}$	13.34±0.09$^{(c)}$	12.77±0.06$^{(b)}$	14.67±0.03$^{(e)}$	12.73±0.05$^{(b)}$	14.47±0.05$^{(d)}$	12.88±0.01$^{(b)}$
SFA[d]	70.7±0.1$^{(e)}$	69.4±0.1$^{(b)}$	70.2±0.1$^{(c)}$	73.25±0.03$^{(f)}$	70.4±0.06$^{(cd)}$	67.8±0.06$^{(a)}$	70.5±0.06$^{(d)}$
MUFA[e]	24.94±0.07$^{(b)}$	26.4±0.1$^{(e)}$	26.2±0.1$^{(d)}$	23.77±0.03$^{(a)}$	25.2±0.1$^{(c)}$	27.44±0.05$^{(f)}$	25.29±0.01$^{(c)}$
PUFA[f]	4.30±0.03$^{(ab)}$	4.52±0.05$^{(d)}$	4.4±0.1$^{(cd)}$	4.77±0.03$^{(e)}$	4.4±0.1$^{(bc)}$	5.1±0.1$^{(f)}$	4.26±0.05$^{(a)}$
Δ^9 desaturase Index (C_{14})	0.012±0.001$^{(b)}$	0.012±0.001$^{(b)}$	0.012±0.001$^{(b)}$	0.009±0.001$^{(a)}$	0.013±0.001$^{(b)}$	0.012±0.001$^{(b)}$	0.013±0.001$^{(b)}$
Atherogenicity index	2.60±0.01$^{(d)}$	2.50±0.01$^{(c)}$	2.47±0.02$^{(b)}$	2.50±0.01$^{(c)}$	2.61±0.02$^{(d)}$	2.11±0.01$^{(a)}$	2.66±0.01$^{(e)}$
DFA[g]	40.4±0.1$^{(a)}$	43.1±0.2$^{(d)}$	42.3±0.2$^{(c)}$	42.1±0.1$^{(c)}$	41.3±0.2$^{(b)}$	45.7±0.1$^{(e)}$	41.3±0.1$^{(b)}$
CLA[h] (mg/100 g cheese)	299±7$^{(a)}$	327±5$^{(b)}$	359±8$^{(d)}$	342±3$^{(c)}$	432±7$^{(e)}$	439±3$^{(e)}$	439±7$^{(e)}$
PUFA:SFA	0.061	0.065	0.063	0.065	0.062	0.074	0.060
ω3	1.24±0.04$^{(ab)}$	1.27±0.01$^{(b)}$	1.24±0.02$^{(ab)}$	1.46±0.01$^{(c)}$	1.23±0.01$^{(a)}$	1.53±0.01$^{(d)}$	1.22±0.01$^{(a)}$
ω6	1.83±0.01$^{(a)}$	2.00±0.05$^{(c)}$	1.95±0.05$^{(bc)}$	2.19±0.01$^{(d)}$	1.93±0.06$^{(b)}$	2.16±0.05$^{(d)}$	1.83±0.01$^{(a)}$
ω6 /ω3	1.48±0.04$^{(b)}$	1.57±0.03$^{(c)}$	1.57±0.04$^{(c)}$	1.50±0.01$^{(b)}$	1.57±0.03$^{(c)}$	1.41±0.03$^{(a)}$	1.50±0.01$^{(b)}$

[abc]Means within the same row and parameter followed by different superscripts are significantly different ($P < 0.05$).

Fatty acid values were expressed as g/100 g of fatty acids. [a] SCFA: short chain fatty acids. [b] MCFA: medium chain fatty acids. [c]LCFA: long chain fatty acids. [d] SFA: saturated fatty acids. [e] MUFA: monounsaturated fatty acids. [f] PUFA: polyunsaturated fatty acids. [g] DFA: desirable fatty acids. [h] CLA: conjugated linoleic acid.

[abc]Means within the same line and parameter followed by different superscripts are significantly different ($P < 0.05$).

The evolution of short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), and long-chain fatty acids (LCFA) throughout the maturation process of goat cheese, presents in Table 2.

Moisture content of samples varied significantly during ripening (Table 1). Therefore, the values of the fatty acid (FA) content were expressed in dry basis, so that their behavior could be analyzed. The initial proportion of SCFAs (26.72±0.05 g/100g FA d.b.) decreased until day 10 (17.51±0.04 g/100g FA d.b.) of ripening and then it increased until day 30 (21.89±0.08 g/100g FA d.b.). After that, the content of these fatty acids diminished again until day 80 (14.46±0.05 g/100g FA d.b.) of ripening. Collins *et al.* (2003) related the fatty acids decrease with the increase on their rate of volatilization in relation to their degradation velocity at the beginning of the study. This situation reversed between days 10 and 30 of ripening when the increase in the content of short-chain fatty acids was observed, only $C_{4:0}$ and $C_{6:0}$ presented a significant increase at 60 day. This was coincident with the decrease in the medium- lization rate seemed to be higher again, producing a great proportion of volatile fatty acids, indicating degradation. This behavior could explain the decrease observed in the concentration of short-chain fatty acids until day 80 of ripening.

The initial proportion of MCFAs (90.24±0.05 g/100g FA d.b.) and the MUFA (49.97±0.07 g/100g FA d.b.) diminished during the whole ripening time studied, showing a decrease in their rate of degradation from day 30 (63.11±0.05 and 34.5±0.05 g/100g FA d.b., respectively), until day 80 (58.63±0.02 and 32.08±0.04 g/100g

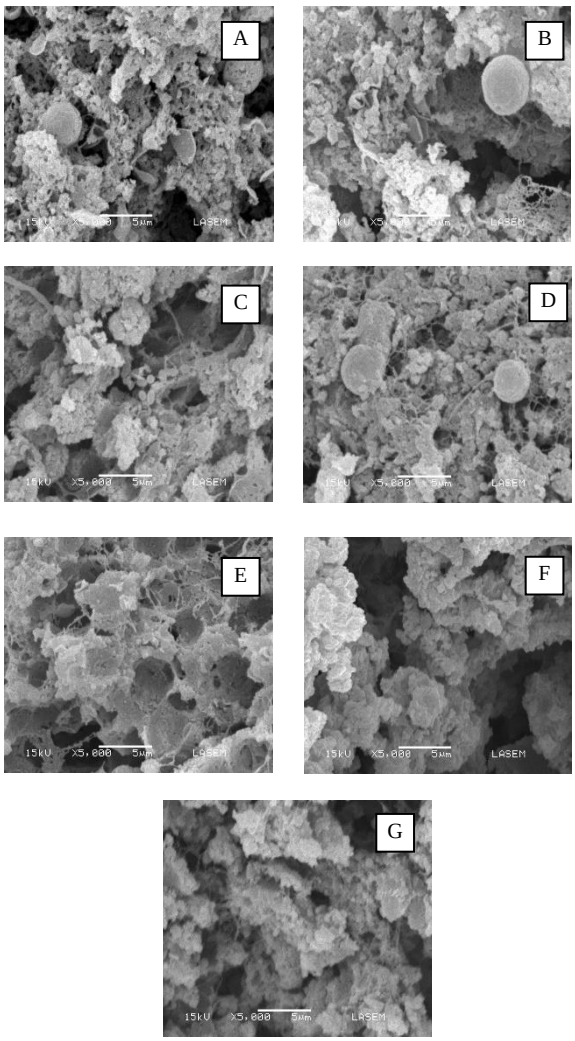

Fig. 1. SEM images (5000X) obtained from goat cheeses of different ripening times A) 5 hours B) 10 days C) 20 days D) 30 days E) 40 days F) 60 days G) 80 days.

d.b., respectively) of ripening. The LCFAs (24.69±0.01 g/100g FA d.b.) and the polyunsaturated fatty acids (8.63±0.01 g/100g FA d.b.) decreased slowly during the study (16.33±0.05 and 5.41±0.02 g/100g FA d.b., respectively).

Table 2 also shows the different nutritional indices obtained for goat cheeses during ripening. Saturated fatty acids (SFA) were predominant (70±3 g/100g FA), followed by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), which presented values of 26±2 and 4.7±0.6 g/100g FA, respectively.

Fig. 1 presents the micrographs obtained for each ripening time, with the SEM shown at 5000×magnification. The heterogeneous initial distribution of the large-sized fat globules: 4.6 to 2 μm (Fig. 1A) was observed. As the maturation progressed, the size of the fat globules decreased. The coexistence of globules of different sizes at day 10 of ripening, ranging between 3.7 and 1.31 μm (Fig. 1B) was noted.

After 20 days of ripening, uniform groupings of fat globules (1±0.1 μm) in the cheese matrix (Fig. 1C), were observed. From day 30 of ripening, globule size was 300 ηm (Fig. 1D) and it slowly diminished to 140 ηm at the end of the maturation (Fig.1G). This is coincident with the decrease in the rate of hydrolysis of the medium-chain Fatty acids and the monounsaturated Fatty acids, both observed from day 30 of ripening (Table 2).

IV. DISCUSSION

The protein and fat content increased by the effect of the concentration associated with the loss of water throughout the ripening time (Table 1). Protein and fat did not show significant differences between 20 and 30 days, which could suggest that despite the loss of moisture, biochemical events occurred slowly between these days and the samples were similar. Taboada *et al.* (2015), Ferrandini *et al.* (2011) and Delgado *et al.* (2012) reported similar behavior in goat cheeses ripened for 60 days and Pino *et al.* (2009) in goat cheeses ripened for 90 days.

The fatty acids content of samples decreased from day 1 to day 60, indicating the significant effect of ripening time on cheese lipolysis, in agreement with Juan *et al.* (2015), at days 30 and 60 of ripening, the lower amounts of total levels of Fatty acids (TFA) were observed in pasteurized cheeses. No statistical differences in medium-chain fatty acids (MCFA) were found in pasteurized cheese at days 30 and 60 of ripening. Taboada *et al.* (2015) found no changes in monounsaturated Fatty acids (MUFA) content in semi-hard goat cheeses over time, though MUFA ranged from 27 to 30 g/100 g of fatty acids at day 1 and near 31.0 g/100 g of fatty acids at day 60.

Lipolysis in cheese can be produced by natural lipases, such as lipoprotein lipase (LPL) or by microbial lipases from the starter and non-starter lactic acid bacteria. The indigenous LPL is nearly completely inactivated by pasteurization (Collins *et al.*, 2003). The endogenous LPL, lipases, and esterases of bacteria appear could be the some lipolytic agents in the lipolysis found in cheeses in the present study.

On the other hand, the differences found in the levels of lipolysis (FA) between ripening times could be caused by the release of intracellular lipase, the availability of fat, and the activity of the enzymes, produced by ripening. In this sense, lipolysis is initiated by physical damage to the milk fat globule membrane (MFGM), which allows the lipase access to the fat substrate. Both the incorporation of proteins such as caseins, which brings LPL into intimate contact with fat, and the increase in fat surface area render fat globules more susceptible to lipolysis (Deeth, 2006).

The goat cheeses showed a lower rate of lipolysis during the last 30 days of ripening, coincident with Delgado *et al.* (2011), who informed that a low rate may be caused by a diminished enzymatic activity due to micro-environmental changes in cheese (e.g., moisture or pH). Similar results have been reported in Babia–Laciana cheese (raw goat milk) (Franco *et al.*, 2003) and other pasteurized goat milk cheeses (Poveda and Cabezas, 2006).

During the ripening of the goat cheese, values higher than 0.06 were obtained for the PUFA/SFA ratio, in agreement with the result obtained by Medeiro *et al.* (2014). This low value indicates a high atherogenicity.

To assess the atherogenic potential, the atherogenicity index (AI) was obtained. The values of AI can be associated with the risk of suffering from atherosclerosis. The AI values (Table 2) were in agreement with those reported by Taboada *et al.* (2015) and Van Nieuwenhove *et al.* (2009) in goat cheeses.

The CLA contribution represented by the consumption of 100g of cheese would be approximately 300 at the beginning and 430 (mg/100 g cheese) at day 40, without significant differences until day 80. Considering that after 40 days, the cheeses presented globules of smaller size (Fig. 1), the increase in the content of CLA coincides with that found by Michalski *et al.* (2005), who attribute the highest content of CLA in hard cheeses to the smaller size of their fat globules. After day 40, there were no significant differences, as reported by Abd El Salam and El Shibiny (2014) during the ripening of goat cheese. CLA results were similar to those reported by Taboada *et al.* (2015) in goat cheese with 60 days of ripening. These values allow us to infer that goat cheese is a good source of CLA.

The cis-9, trans-11 is produced either as an intermediate product of the ruminal biohydrogenation of linoleic acid or in tissues, such as the mammary gland, by Δ^9-desaturase activity from vaccenic acid (Griinari *et al.*, 2000). The Δ^9-desaturase indices were lower than those found by Taboada *et al.* (2015) and Van Nieuwenhove *et al.* (2009) in semi-hard goat cheese. Abd El Salam and El Shibiny (2014) reported that the proportion and type of pasture grass in the diet of dairy goats are directly related to the CLA content of milk, so the results found in this study are probably influenced by the diversity in the flora of the *Quebrada de Humahuaca*.

The Desirable Fatty Acids (DFA) makes it possible to infer the content of beneficial fatty acids for health. The values obtained in the goat cheeses studied (Table 2) were similar to those reported by Medeiros *et al.* (2014). These authors managed to increase the DFA by supplementing the diet of the goats with vegetable oils.

Regarding the ω_6/ω_3 ratios in the cheeses (Table 2), the values found were lower than those recommended by Simopoulos (2008), between 4/1 - 2/1 and by FAO (2010) of 2/1. The values were in agreement with those found by Santurino *et al.* (2017) in goat cheese enriched with CLA and ω_3. Besides, lower ω_6 and ω_3 contents were found than those reported by Cossignani *et al.* (2014) and Medeiros *et al.* (2014). Regarding the values of ω_3 found (0.3-0.5 g/100 g cheese); the goat cheeses studied are within the acceptable range of distribution of this macronutrient, since FAO (2010) recommended 0.25-2 g/day. The ω_3 play an important role in the prevention and control of arterial diseases, hypertension, diabetes, arthritis, autoimmune diseases and cancer (Simopoulos, 2008). The ω_6 values obtained (0.4 - 0.7 g / 100 g cheese) were lower than the range recommended by FAO (2010) of 4.4-6.7 g / day. The functions of ω_6 include the reduction of LDL cholesterol, which is another risk factor for heart disease. It can also reduce blood triglyceride levels after a meal or as a means to stop adverse cardiac arrhythmias (Simopoulos, 2008).

The fat in cheese appears as globules in varying degrees of coalescence, as confirmed by the presence of fissures, or irregularly-shaped openings in the para-casein matrix, which remain as degradation products during ripening (Guinee, 2016).

Fat can be entrapped in the cheese matrix as an inert filler or as a copolymer within the casein matrix. Fat globules can exist as singular globular shapes, coalesced fat globules, or non-globular fat (free fat) (Everett and Auty, 2008). Coalesced fat globules occur when two or more fat globules combine to make a large, irregularly shaped fat molecule. This molecule still possesses its protective globular membrane (Ong and Shah, 2009), and non-globular fat, also known as free fat, are pools of fat which possess no milk fat globule membrane (MFGM) (Lopez *et al.*, 2007). Fat retention influences texture, cheese yield, and mouthfeel as more fat retention in a cheese results in a less dense protein matrix, an increase in available water, and a softer texture with improved meltability. The retention of fat within the cheese matrix is heavily influenced by the initial fat content of milk used for cheese making, in relation to the protein content, and by the cutting of the curd, as curd particle size influences moisture retention, which in turn determines the fat content (Everett and Auty, 2008).

The cheese samples presented uniform aggregates of milk fat globules (1±0.1 µm) at day 20 of ripening in this study. Clumping and coalescence are the results of shear stress imposed on the fat globule membrane by various manufacturing steps (e.g., cutting, stirring, pressing, and plasticization) and the shrinkage of the surrounding para-casein network, which forces the occluded globules into close contact. Shrinkage of the para-casein network coincides with its dehydration during the various stages of cheese-making, e.g., acidification, heating, and pressing. In the temperature range used for cheese making (~30–55 °C) most, or all, of the milk fat is liquid and therefore flows on the application of stress (Guinee, 2016).

Lopez *et al.* (2007) identified four forms of fat present in Emmental cheese: (1) intact milk fat globules, which are small, spherical globules dispersed throughout the cheese; (2) aggregates of milk fat globules, which appear as clumps of circular globules; (3) coalesced milk fat globules, which are spherical but larger than the typical milk fat globules; and (4) non-globular fat or free fat. The first two of these forms were identified in the micrographs in the current study.

In this study, the aggregates of milk fat globules changed their spatial conformation probably due lipolysis during ripening. The coexistence of globules of large size (residuals of intermediate maturation and a great number of small-sized fat globules (the result of the hydrolysis of the FA) was observed. Michalski *et al.* (2005) investigated the effect of milk fat globule (MFG) size prior to cheese manufacture on the quality of Emmental and Cheddar cheese. They found cheese made from milk with small MFG had higher moisture content, while

cheese made from milk with large MFG showed a significantly lower protein content. As expected, small MFG cheese had a lower fat content than the other cheeses due to the loss of small fat globules in the whey. Large MFG cheese was found to have a significantly higher rate of fatty acid liberation than the small MFG cheese, which could affect the rate of cheese ripening.

The size of globules will impact upon texture. Larger globules with a smaller pressure of deformability will decrease the firmness of cheese. Conversely, small globules are much harder to deform and rupture, and are also less likely to disrupt the casein matrix, thus contributing to a firmer cheese (Everett and Auty, 2008).

V. CONCLUSIONS

The fatty acid profile of goat cheeses manufactured in the province of Jujuy, Argentina, shows higher proportion of medium chain fatty acids and of palmitic, oleic, stearic, and myristic acids. The medium-chain Fatty acids and the monounsaturated Fatty acids decreased during the study. The rate of hydrolysis of these Fatty acids decreased at day 30 of ripening, which agrees with the changes in microstructure, evidenced by the decrease in the size of the fatty globules. For this reason, it can be state that the effect of lipolysis on the composition of FA and the microstructure of fatty globules decreases after 30 days of ripening in goat cheeses, under the conditions used in this study.

Goat cheeses can be considered good sources of CLA and ω_3, according to the nutritional indices found in this work. Consequently, they could produce beneficial effects on human nutrition.

Furthermore, in view of the changes observed in the fatty acid profile and in the microstructure of goat cheeses during the studied ripening time, it is recommended to mature the goat cheese for at least 30 days, before consumption or sale. Particularly because of the fatty acids are responsible of the flavor of ripened cheese, which means a high value added to the local goat product.

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Received: June 5, 2019
Sent to Subject Editor: August 23, 2019
Accepted: October 25, 2020
Recommended by Subject Editor María Luján Ferreira