Proteolysis, Texture and Microstructure of Goat Cheese

Burgos L., Pece N., Maldonado S.

Abstract— **Changes in the structure of cheese are mostly due to changes in the protein matrix, mainly because of the degradation of α- and β- and k-casein. Therefore, the objective of this work was to study the effect of proteolysis on the microstructure and texture of goat cheese during ripening. The cheeses were made using Creole goat milk from the Quebrada de Humahuaca in Jujuy and ripened at 10 °C and 90% RH. Samples were taken after 5 hours of preparation and after 10, 20, 30, 40, 60 and 80 days of ripening. Proteolysis was studied by the evolution of the major fractions of casein (α, β and para-κ) determined by HPLC and soluble nitrogen, allowing the calculation of the rate of maturation. The texture profile was determined using a texture analyzer QTS 25. The changes in the protein matrix of the cheese were observed by scanning electron microscopy using a JEOL JSM-6480 LV. We found that the α-casein was hydrolyzed at a low speed at the beginning and until 30 days, between 30 and 40 days of ripening, α-casein was hydrolyzed faster. After this time, this fraction content became stable until the end of the ripening. The rate of hydrolysis of para-κ-casein increased starting from 30 days up to 60 days of ripening, when it became stable. It was observed that the initial matrix of cheese protein was formed by free large cavities with a heterogeneous dispersion of casein particles. During ripening, the size of the cavities decreased and the cheese protein matrix became more compact. The size of the holes was reduced and the globular characteristic of the micelles was lost after 40 days of maturation, coinciding with accentuated hydrolysis α-caseins. The soluble nitrogen at pH 4.6, increased significantly until 30 days. After that, it remained statistically unchanged for 80 days. The velocity of maturation determined as soluble nitrogen in TCA, rose steadily until 60 days of ripening. Hardness, gumminess, adhesiveness and chewiness increased sharply at 40 days of maturation. After this time, these parameters increased slowly until the end of the sampling period, when the changes in the microstructure of the cheeses revealed the highest compaction of the matrix. This may be related to the formation of soluble nitrogen and degradation of α-caseins during ripening.**

Index Terms— **Casein, ripening, structure.**

I. INTRODUCTION

The ripening of cheese involves a complex series of biochemical events which lead to the flavor, aroma and texture characteristic of each variety. One of the main biochemical reactions is proteolysis [1]. It is characterized by

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the solubilization of the caseins, and results in an increase of soluble nitrogen. The first effect of proteolysis is the enzymatic breakdown of κ-casein and continues with the degradation of para-κ-casein. This process is mediated by the residual rennet and plasmin. Polypeptides resulting from enzymatic breakdown are degraded by proteases of lactic acid bacteria and secondary microflora [2]. Since the κ-casein and the colloidal calcium phosphate are functions of the dimensions of the micelles of casein, a larger size of the micelle produces a higher retention of solids particles, thus, improves the firmness of the clot and influences the greater conversion yield of milk into curds [3].

During proteolysis, amino acids and peptides are produced, these products form part of the soluble nitrogen fraction and which have an influence on the flavour and texture of the end product [1]. A part of the casein is converted into soluble nitrogenous compounds, such as peptides and amino acids. These peptides have different solubilities in water and other solvents. Therefore, extractions with different solvents and subsequent quantification of nitrogenous compounds in the cheese extract are used to study the extent of proteolysis in cheese [4]. The soluble nitrogen at pH 4.6 is the most common fraction. The extract contains whey proteins, medium and small-sized peptides, amino acids and other low molecular weight compounds. Different precipitants can be used to obtain sub-fractions of this extract, e.g., trichloroacetic acid (TCA) and phosphotungstic acid (PTA).

The protein network of cheese is formed by α and β-caseins, which form helical chains of cells. These cells retain fat globules, causing the ratio fat:protein in milk to become critical [5]. The electrostatic stability of the casein matrix is related to the mineral content of the curd. Changes in the structure of cheese are due mostly to modifications in the protein matrix, caused mainly by the degradation of α and β casein [6].

The composition of the cheese, the structure of the matrix and the changes during ripening are distinctive to each type of cheese. The characterization of a cheese involves determining its composition and the variation thereof during ripening, but also the knowledge of the spatial organization of the constituents in the matrix and thus its microstructure [7]. The microstructure is a determining factor of the texture and sensory characteristics that define the final quality of the product. The interactions between the components of the finished product, such as the protein bonds and fat globules bonded to the casein micelles, determine the texture perceived by consumers [8].

The texture of cheese is one of the characteristics that determine its identity and quality. Different types of cheese have a unique structure, which together with flavor characteristics; determine its quality and consumer acceptability [9] [10].

We have determined the effects of proteolysis on the texture and microstructure variations during ripening of goat cheeses

made of milk from the Quebrada of Humahuaca (Jujuy) region, looking for a correlation for future prediction.

II. MATERIALS AND METHODS

Cheese manufacture

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We worked with milk from a flock comprised of native goats belonging to a goat dairy farm located in the Quebrada de Humahuaca, with pasture feeding without supplementation. The milk was brought to the laboratory in chilled plastic drums immediately after milking.

For making cheese, raw milk was pasteurized (65°C, 30 minutes). It was cooled to 38ºC and lactic ferment CHR HANSEN 743 RST was added. Simultaneously $CaCl_2$ was incorporated 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 and left to act for about 30 min. The curd was cut into 1.5 cm cubes with a lyre and was separated from the whey. The cheeses were salted during the molding stage by adding 2% of ClNa (common salt), i.e., 2 g of salt per 100 g of curd, in two layers, directly to the curd and during molding. The cheeses were pressed in a hydraulic press, subjecting them to a pressure of 4 bar for 3 hours. After that, the salty product was stored in the refrigeration chamber at 10 °C and 90% relative moisture. A minimum of three samples were taken of the ripening batch for studying proteolysis and observation of the microstructure, at the following times were extracted: 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.

Physicochemical analysis

The total protein content was determined by Kjeldahl method [11] (955.04 c) and the moisture content [11] (935.29) in a vacuum oven (Shel Lab, model 1410) at 60 ± 1 °C and 25 in Hg vacuum.

Proteolysis

The evolution of the nitrogenous fractions was studied. The soluble nitrogen fractions were determined, as described by Bernal et al. [12]: soluble nitrogen at pH 4.6 (SN pH4.6); trichloroacetic acid (12% w/v) soluble nitrogen (TCA-soluble nitrogen) and phosphotungstic acid (5% w/v) soluble nitrogen (PTA-soluble nitrogen). Total nitrogen of cheese and above fractions was determined by the Kjeldahl method [11] (955.04 c). The relationship between the fraction of soluble nitrogen and total nitrogen was expressed as the ripeness index (% of total nitrogen).

The variation of major fractions α, β and para-κ casein using HPLC ion exchange was studied, following the methodology described by Veloso et al. [13].

Texture profile analysis (TPA)

The texture analysis was performed in a texturometer Brookfield QTS-25. Samples were diced (2 cm x 2 cm x 2 cm) until texture profile analyses (TPA). The texture was measured at constant temperature (7 ℃). A cylinder probe was used (Acrylic cylinder probe, 26 mm diameter). The test was performed by two successive axial compression ramps to a value of 25% of the unloaded specimen height. The following texture parameters were measured from force–deformation curve: hardness, adhesiveness, cohesiveness, gumminess, springiness and chewiness.

Microstructure

Samples microstructure were observed by SEM. Small pieces taken from the inner parts of the cheeses were fixed, fractured using liquid N_2, gold coated and analyzed in high vacuum using a scanning electron microscope JEOL JSM-6480 LV (Japan).

Statistical analysis

Minitab statistic software version 16.1.0 was used for all statistical analysis. Analysis of variance (ANOVA) and the Tukey HSD mean comparison test were used to determine differences between samples. The significance level used was 0.05. Each treatment was evaluated using at least three determinations and each test by triplicate.

III. RESULTS AND DISCUSSION

Physicochemical characteristics

The changes in protein content and moisture of the samples are presented in Table 1.

Table 1. Protein content (g / 100 g dry matter) and moisture (g / 100g) of ripened cheese.

Days	Protein	Moisture
Initial	$37.3 \pm 0.5^{(a)}$	$50.1 \pm 0.7^{(a)}$
10	$36.9 \pm 0.7^{(a)}$	$42.3 \pm 0.7^{(b)}$
20	$34.7 \pm 0.1^{(b)}$	$38.1 \pm 0.7^{(c)}$
30	$31.6 \pm 0.5^{(c)}$	$31.1 \pm 0.8^{(d)}$
40	$32.4 \pm 0.4^{(c)}$	$30.2 \pm 0.8^{(d)}$
60	$32.5 \pm 0.6^{(c)}$	$26.6 \pm 0.8^{(e)}$
80	$31.4 \pm 0.6^{(c)}$	$21.2 \pm 0.5^{(f)}$

Means within the same column without a common superscript are significantly different (P<0.05).

Moisture content decreases significantly during ripening due to the conditions of the camera. Cheeses turn since soft to hard during maturation time, according to the Argentine Food Code (Art. 605) There was a rapid loss of water demonstrated by the low moisture content, coinciding with reports of Delgado et al. [14], Ferrandini et al. [15] and Pino et al. [16].

The total protein decreased significantly to 30 days of ripening, after that it become stable, coinciding with the evolution of cheese samples to hard type, similar to that reported by Ferrandini et al. [15]. This could be related to microbial catabolism of amino acids which may be degraded by four groups of enzymes: decarboxylase, transaminase, deaminase and lyases. This catabolic process continues degrading molecules to volatile compounds such as ammonia, ketones, aldehydes, acids or sulfur-containing compounds that are part of aroma most cheeses [17].

Changes on proteolysis of goat milk cheese

Figure 1 shows the evolution of soluble nitrogenous fraction (SN) at pH 4.6; trichloroacetic acid fraction (TCA) and phosphotungstic acid fraction (PTA).
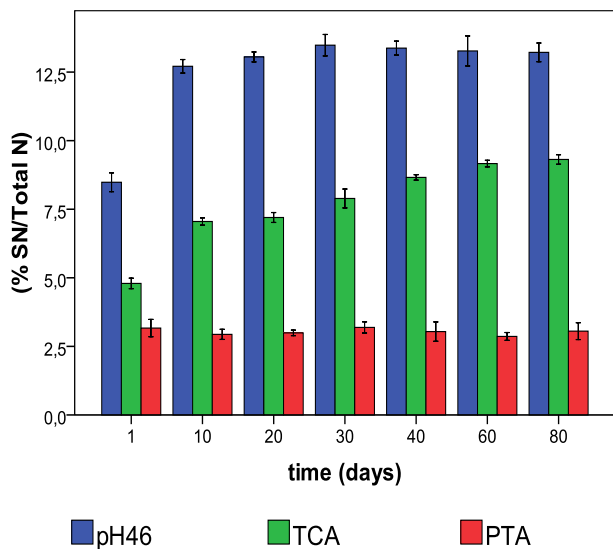
Figure 1. Nitrogenous fractions of goat cheese.

Figure 2. Evolution of fractions α, β and para-κ caseins during ripening of goat cheese.

The extent of proteolysis, determined by the soluble nitrogen fraction at pH 4.6 increased significantly until 30 days. After this time the SN fraction keeps statistically constant to the end of the study. After 30 days, the values found were lower than those reported by Tejada et al. [18], Pino et al. [16], Delgado et al. [14] and Oliszewski et al. [19]. This showed that a low proteolysis occurs in 30 days of ripening.

The TCA-soluble nitrogen fraction increased continuously from the beginning up to 60 days of ripening; between 60 and 80 days ripening there was no significant differences in TCA values. The PTA-soluble nitrogen showed no significant differences.

According Bustamante et al. [20] soluble nitrogen (pH 4.6) fraction increases during the early stages of maturation, caused by endogenous protease activity, by the residual coagulant and to a lesser extent by the residual protease activity of the yeast.

The Soluble Nitrogen (pH 4.6) showed no changes from 30 days until the end of the study, which could be because of the small peptides produced by the degradation of caseins, which coincides with the continued increase of TCA-soluble nitrogen fraction to 60 days of ripening. This fraction is considered an index of the depth of ripening, as it is composed mainly of ammonium nitrogen, small peptides of 2 to 20 residues and free amino acids produced by microbial enzymes acting on large peptides released by chymosin and on α-casein [21]. Increasing the fraction of TCA-soluble nitrogen with the ripening time was also found by Tejada et al. [18], who reported proteolysis of intermediate depth up to 60 days in ripened goat cheeses.

The PTA-soluble nitrogen fraction showed no significant differences during the study, in similarity to those reported by Delgado et al. [15] and Pino et al. [16]. This fraction provides an index of the production of free amino acids during cheese ripening and is related to the ammoniacal nitrogen, reflecting the ability of deamination exerted on the free amino acids by microbial biota [21]. At present no changes during the 80 days of ripening could assume a low capacity deamination lactic culture used.

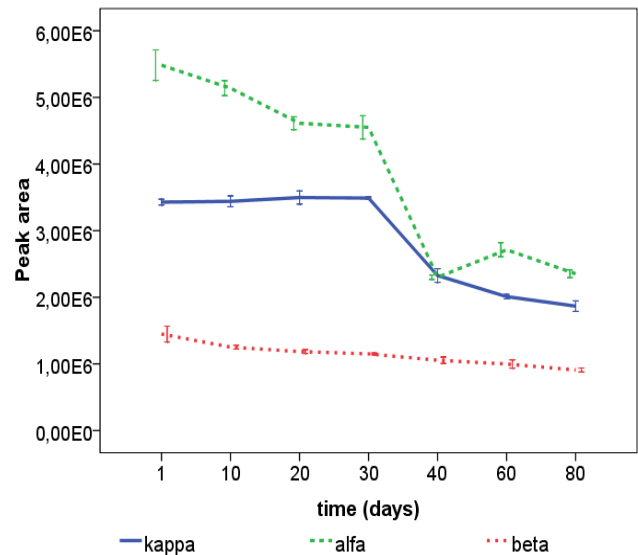Figure 2 presents the evolution of the peak areas of the caseins fractions, determined by HPLC.

The α-caseins were hydrolyzed at a low speed at the beginning of the study until 30 days, then it was accentuated until 40 days when it became stable to the end of the study. The para-κ-caseins did not hydrolyze until 30 days; between 30 and 40 days were hydrolyzed to the highest speed and after 40 days the rate of hydrolysis continued decreasing till 60 days of ripening. No significant differences were found between the values of 60 and 80 days so we assume that the hydrolysis of the para-κ-casein becomes stable at 60 days of ripening, under the study conditions. The β-caseins were hydrolyzed slowly in the whole study period.

These results allow us to affirm that he initial hydrolysis of caseins was catalyzed first by the residual rennet and to a lesser extent by plasmin. The residual rennet is one of the main proteolytic agents in soft or semihard cheese paste. This is due to the relatively high proportion of residual coagulant in the protein matrix in these varieties of cheeses (to 30% of the original amount added to milk) [22], which produces a corresponding increase in the gel strength on proteolysis of para-κ- and α-caseins, since the rearrangement of the particles form a more compact structure of degradation to smaller peptides [23]. In this case the proteolysis of α-casein increased between 30 and 40 days, coinciding with the fraction of soluble nitrogen at pH 4.6 (Figure 2). So, the major changes in the extent of proteolysis occur at 40 days of maturation.

Degradation of β-caseins were moderate coinciding with Holt and Roginski [24] and Prieto et al. [21], who reported that the β-casein have a lower degree of hydrolysis that α-casein during ripening, due to low activity of plasmin and its resistance to chymosin. This behavior of β- and para-κ-caseins matches the fraction TPA-soluble nitrogen, which can be stated that proteolysis in the analyzed cheeses occurs more deeply at 60 days of ripening.

Table 2. Texture parameters ripened cheeses

Time	Initial	10	20	30	40	60	80
(days)	value						
Hardness (N)	$4.5\pm0.4^{(a)}$	$5.3\pm0.6^{(a)}$	$10.1\pm0.4^{(b)}$	$34\pm5^{(c)}$	$114\pm3^{(d)}$	$115\pm2^{(d)}$	$126\pm4^{(e)}$
Gumminess (N)	$3.7\pm0.2^{(a)}$	$6.9\pm0.9^{(ab)}$	$5.1\pm0.9^{(a)}$	$10\pm5^{(b)}$	$83\pm3^{(c)}$	$93\pm4^{(d)}$	$94\pm2^{(d)}$
Adhesiveness (N s)	$-0.24\pm0.01^{(a)}$	$-0.25\pm0.01^{(a)}$	$-0.12\pm0.01^{(b)}$	$-0.03\pm0.01^{(d)}$	$-0.04\pm0.01^{(c)}$	$-0.02\pm0.01^{(d)}$	$-0.02\pm0.01^{(d)}$
Cohesiveness	$-0.83\pm0.04^{(b)}$	$-1.53\pm0.02^{(a)}$	$-0.57\pm0.02^{(d)}$	$-0.77\pm0.03^{(c)}$	$-0.75\pm0.02^{(c)}$	$-0.76\pm0.02^{(c)}$	$-0.75\pm0.02^{(c)}$
Chewiness (N cm)	$0.64\pm0.05^{(a)}$	$1.2\pm0.3^{(a)}$	$1.6\pm0.1^{(a)}$	$3.2\pm0.9^{(b)}$	$32\pm5^{(d)}$	$15\pm1^{(c)}$	$14.85\pm0.05^{(c)}$
Springiness (cm)	$0.16\pm0.01^{(b)}$	$0.16\pm0.03^{(b)}$	$0.24\pm0.01^{(c)}$	$0.16\pm0.01^{(ab)}$	$0.36\pm0.01^{(d)}$	$0.15\pm0.01^{(a)}$	$0.155\pm0.005^{(ab)}$

Means within the same line without a common superscript are significantly different ($P<0.05$).
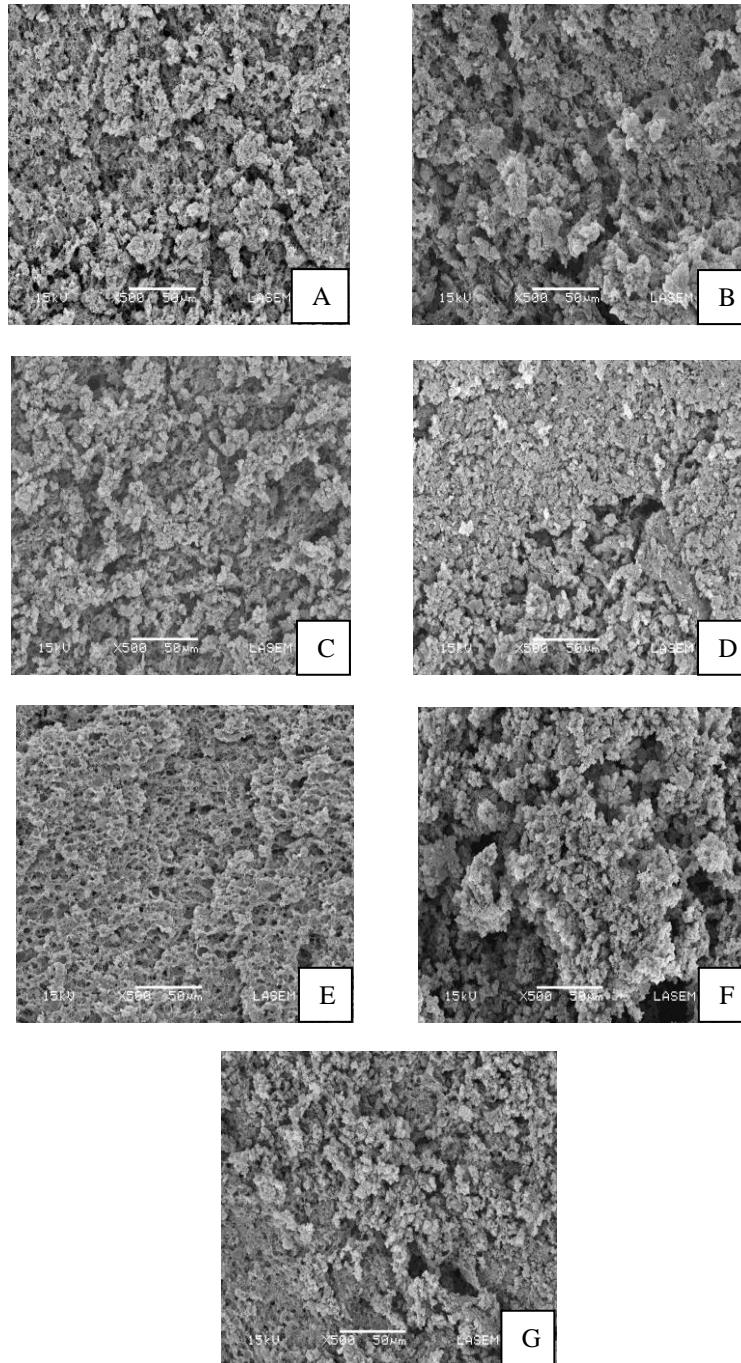

Figure 3. SEM images obtained from goat cheeses of different ripening times A) 5 hours B) 10 days C) 20 days D) 30 days E) 40 days F) 60 days G) 80 days.

Texture evaluation of cheese

The changes in texture parameters during maturation were shown in Table 2.

The hardness and gumminess increased during the study period, both increased significantly in 40 days of ripening. This may relate to the changes occurring in the matrix of caseins, since the α-caseins presented significant changes in the same period of maturation. These results show a positive correlation between hardness and α-caseins.

Chewiness increased significantly, reaching a maximum at the 40 days of ripening. After 60 days, chewiness decrease without presenting changes to the end of the study. The decrease observed is influenced by changes in elasticity, since more matured the cheese more energy is required to chew because both, the hardness and the cohesion, increased in the same proportion.

As shown in Table 2 adhesiveness and cohesiveness showed a net increase at the end of ripening, which can be related to the formation of soluble nitrogen and casein degradation, coinciding as described by Álvarez et al. [25] and Osorio et al. [26]. Adhesiveness showed no significant changes from 30 days of ripening, coinciding with results obtained for β and para-κ- caseins and the fraction of TCA-soluble nitrogen effect.

Cohesiveness presented a maximum (1.53) at 10 days of ripening and the springiness at 40 days (0.358 cm), due to the increase in elasticity and consequently in the resistance of the matrix to deformation because of the flexibility of internal links, so also increased the cohesiveness. Van Hekken et al. [27] and Delgado et al. [28] reported similar behavior in goat cheese soft paste.

Microstructure

In Figure 3 are presented the micrographs obtained for each ripening time.

It was noted that the initial protein matrix of cheese had free large cavities with a heterogeneous dispersion of casein particles. Cavities size decreased during the progress of ripening, the protein matrix was compacted, the size of the holes appeared visibly reduced and native globular nature of the micelles was lost after 40 days of ripening, coinciding with the accentuated hydrolysis α- caseins (Figure 1). Similar differences were reported by Fallico et al. [7] and Buffa et al. [29] for the microstructure of a goat's milk cheese produced from raw and pasteurized milk. The more open structure observed in nontraditional cheeses might have been determined by increased hydration of the protein matrix resulting from milk pasteurization. This effect is known to be a consequence of the decreased fusion of casein micelles caused by the formation of a complex between denatured β-lactoglobuline and para-κ-caseins and to the lower level of soluble Ca in cheese produced by heat treatment of milk.

Free spaces appear in the matrix of caseins after 60 days of ripening, which occur as result of the solubilization of calcium phosphate and spatial rearrangement of the casein micelles, according Holt and Roginski [24]. Calcium phosphate in fresh cheeses occupies small spaces in dense regions of the casein micelles. As elapses maturation, calcium phosphate solubilization increases the spacing between dense regions. Micelles are assembled without a defined orientation, which imparts an amorphous cheese texture [7].

According Buffa et al. [29] the open structure of the matrix

to a higher degree was associated of primary proteolysis. This is because the lower degree of curd fusion and the greater surface area of the protein matrix, at the protein-void interface, increased the accessibility of the paracasein to proteases and thereby contributed to a higher level of primary proteolysis in cheese.

IV. CONCLUSION

The proteolysis of casein occurred in the α-caseins from the beginning to 40 days of ripening, coincident with the increase and stabilization of soluble nitrogen to pH 4.6 fraction, indicating that the proteolysis have a short extension.

Hardness, gumminess and chewiness increased sharply after 40 days of ripening, coinciding with decrease in moisture content and in the content of α-caseins. This situation was reflected in the changes of microstructure of the cheeses with different ripening times. The matrix presented a reordering of casein micelles with a greater compactness of the matrix after 40 days of ripening. The main changes can be associated to the hydrolysis of α-casein, demonstrating the influence of proteolysis on the microstructure and texture of goat cheese.

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