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Original article

## Influence of starter cultures on the proteolysis of Teleme cheese made from different types of milk

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**Abstract** – Teleme cheese was made from ovine, caprine and bovine milk with thermophilic (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*), mesophilic (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*) and mixed thermophilic-mesophilic (1:1) cultures. Nine lots of cheese each time (five repetitions) were made in order to study the proteolysis of cheeses during ripening and storage up to 6 months. All cheeses showed similar profiles of proteolysis. However, the rate of proteolysis was different. The thermophilic and the mesophilic cultures resulted in cheeses with the highest and the lowest degree of proteolysis, respectively. Proteolysis was of a descending order in cheeses made from sheep's, goat's and cow's milk when estimated on the basis of nitrogenous fractions (total nitrogen, water soluble nitrogen, nitrogen soluble in 5% phosphotungstic acid and nitrogen soluble in 12% trichloroacetic acid), whereas it was highest in cow's milk cheeses and lowest in goat's milk cheeses when estimated on the basis of electrophoresis and RP-HPLC. Multivariate analysis of the data revealed that cheeses could be more correctly clustered by the type of milk or culture than by the stage of ripening.

**Teleme cheese / white-brined cheese / proteolysis / starter culture**

**摘要** - 不同发酵剂对不同类型原料奶生产的泰拉米 (Teleme) 干酪蛋白质水解的影响。分别以绵羊奶、山羊奶和牛奶为原料加工泰拉米干酪 (Teleme cheese), 每种原料奶分别接种嗜热菌 (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*)、嗜温菌 (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*) 及嗜热和嗜温混合菌 (1:1) 3 种不同的发酵剂, 共进行 9 个批次的试验, 每一批样品进行 5 个重复实验。系统地研究了泰拉米干酪在 6 个月成熟和贮藏期内蛋白质的水解情况。试验结果表明, 所有干酪蛋白质水解特性相似, 然而, 每一批干酪的蛋白质水解率不同。嗜热菌和嗜温菌发酵剂生产的干酪分别具有最高和最低的蛋白质水解率。根据成熟干酪中含氮部分 (总氮、可溶性氮、5% 磷钨酸中可溶性氮、12% 三氯乙酸中可溶性氮) 来评价蛋白质水解率时, 绵羊奶、山羊奶和牛奶加工的干酪中蛋白质水解率呈依次降低的顺序; 然而根据电泳和反相高效液相色谱测定的结果来评价蛋白质水解率时, 则是以牛奶加工的干酪中蛋白质水解率最高, 而以山羊奶加工的干酪中蛋白质水解率最低。根据测定数据的多因素方差分析结果表明, 干酪中蛋白质水解与发酵剂的种类和原料奶的类型具有相关性, 但与干酪成熟过程关系不显著。

**泰拉米干酪 / 白卤干酪 / 蛋白质水解 / 发酵剂**

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**Résumé – Influence du type de lait et de levain sur la protéolyse de fromage Télémé au cours de l’affinage.** Du fromage Télémé a été fabriqué avec du lait de brebis, du lait de chèvre et du lait de vache en utilisant des levains lactiques thermophiles (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*), mésophiles (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*) ou mixtes thermophiles-mésophiles (1 : 1). Neuf fabrications (cinq répétitions) ont été réalisées pour étudier la protéolyse des fromages au cours de l’affinage pendant 6 mois. Tous les fromages avaient un profil protéolytique similaire, mais le degré de protéolyse était différent. Les fromages fabriqués avec des levains thermophiles étaient les plus protéolysés et ceux fabriqués avec des levains mésophiles les moins protéolysés. La protéolyse observée, estimée par les fractions azotées (azote total, azote soluble en phase aqueuse, azote soluble dans le PTA 5 % et azote soluble dans le TCA 12 %), diminuait dans l’ordre lait de brebis, lait de chèvre et lait de vache mais elle était la plus forte pour le fromage préparé avec du lait de vache et la plus faible pour le fromage préparé avec du lait de chèvre quand elle était estimée par électrophorèse et HPLC phase inverse. L’application de l’analyse multivariée a révélé que la classification des fromages était plus correcte avec le type de levains lactiques ou de lait utilisés qu’avec le degré de maturation.

**Télémé / fromage en saumure / protéolyse / levain / maturation**

## 1. INTRODUCTION

Proteolysis is an important biochemical process in the ripening of most cheese varieties. Proteolysis, which contributes to the development of cheese texture and flavor, is due to the action of enzymes from (a) coagulant, (b) milk (general plasmin), (c) starter bacteria, (d) the adventitious non-starter microflora and (e) secondary starter (in some cheese varieties). The proteolytic system of lactic acid bacteria (LAB) consists of proteinases, which hydrolyze caseins, and large peptides and peptidases, which are responsible for the liberation of small peptides and free amino acids (FAA), an essential step in cheese ripening [43].

White-brined cheeses, in which Teleme cheese is included, form a special category of cheeses. Their main characteristic is that they are ripened and stored in brine. Traditionally, Teleme cheese was manufactured from non-pasteurized milk, but nowadays it is manufactured on an industrial scale. Rigorous control of the production and maturation process is necessary. The selection of the starter culture is one of the most important steps in the manufacture of high-quality cheeses. In traditional Teleme cheese manufacture, yogurt is used as a starter. A mixture of commercial starters, including mesophilic starters, are used in industrial production [2]. Studies concerning the effect of the starter cultures used on the ripening of Teleme cheeses have not been previously reported in a systematic way [20,

45]. In this study we investigated the effect of three commercial concentrated starters, i.e. a thermophilic, a mesophilic and a mixture of a thermophilic-mesophilic culture, on the proteolysis of Teleme cheese made from sheep’s, goat’s or cow’s milk during ripening (at 19 °C) and storage (5 °C) of up to 180 d.

## 2. MATERIALS AND METHODS

### 2.1. Experimental design

The experimental design was that described in a previous paper [33]. That is, Teleme cheese was manufactured using (a) sheep’s milk of the “Boutsiko” breed from the Agricultural Station of Ioannina, (b) goat’s milk from a local native goat population and (c) cow’s milk from a herd of Friesian cows, near the Dairy Research Institute where the cheese-makings took place. Each kind of milk was used in making Teleme cheese with three starter culture variables, that is: (i) a freeze-dried yogurt thermophilic culture (T), FYS-11 (Rhodia, France), consisting of the microorganisms *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, (ii) a freeze-dried concentrated mixture of thermophilic-mesophilic culture (MX), FRC-60 (Chr. Hansen, Denmark), consisting of the microorganisms *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp.

*bulgaricus* and *Streptococcus thermophilus* - the ratio of thermophilic to mesophilic microorganisms was 1:1, and (iii) a freeze-dried concentrated mesophilic culture (M), R-703 (Hansen's Laboratory, Denmark) consisting of the microorganisms *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. For one week, on a day after day basis, one batch of cheese-making of sheep's, goat's or cow's milk took place. On the day of the cheese-making, the milk (sheep's, goat's or cow's) was divided into three equal parts and in each part one of the three cultures was used. Therefore nine lots of cheese were obtained in one week. This procedure was repeated during five subsequent weeks. The reported results are the averages of five trials.

## 2.2. Cheese-making and sampling

Teleme cheese was manufactured as described previously [33]. Briefly, the milk was standardized to casein to fat ratios of 0.75, 0.64 and 0.73 for the sheep's, goat's and cow's milk, respectively, to obtain a first quality cheese that was  $\leq 56\%$  moisture and  $\geq 43\%$  fat in dry matter [15]. The standardized milk was pasteurized at  $63\text{ }^{\circ}\text{C}$  for 30 min and then cooled at  $37\text{ }^{\circ}\text{C}$ . After cooling, one of the cultures mentioned already was added to it, at a quantity of  $0.5\%$  (v/v). To assist curdling of the milk  $\text{CaCl}_2$  solution ( $40\%$ , w/v) was added. Rennet powder HA-LA (Hansen's Laboratory, Horsholm, Denmark) was added at a quantity of 3.0 g per 100 kg milk, at  $33\text{ }^{\circ}\text{C}$ . Drainage was under pressure and salting took place in brine ( $14\%$  NaCl, w/w) for 16h at  $19\text{ }^{\circ}\text{C}$ .

After manufacture, cheeses were ripened in a warm room ( $19^{\circ}\text{C}$ ), until pH and moisture values reached the limits of  $\leq 4.6$  and  $\leq 56\%$ , respectively, and afterwards cheeses were transferred to a cold room ( $5\text{ }^{\circ}\text{C}$ ) for up to 6 months (period of sampling). The relative humidity of the rooms was medium to avoid rusting of the tins, since Teleme cheese is ripened and stored in sealed tins. Teleme cheeses made from sheep's milk using thermophilic, thermophilic-mesophilic or mesophilic culture were transferred to the cold room at 24, 16

and 12 days, respectively, whereas cheeses made from goat's milk using thermophilic, mixed or mesophilic culture were transferred to the cold room at 22, 13 and 11 days, respectively, and cheeses made from cow's milk using thermophilic, mixed or mesophilic culture were transferred to the cold room at 23, 17 and 15 days, respectively.

Cheeses were sampled for analysis on days 1, 60, 120 and 180, as well as on the day they were transferred to  $5\text{ }^{\circ}\text{C}$  (cold room). Due to Teleme cheese being ripened and stored in brine, the excess of brine was drained off by leaving the sample on an absorbing paper for 1–2 min during cheese sampling.

## 2.3. Nitrogen fractions

Cheeses were analyzed for total nitrogen (TN), water-soluble nitrogen (WSN), nitrogen soluble in  $5\%$  phosphotungstic acid (PTA-N) and nitrogen soluble in  $12\%$  trichloroacetic acid (TCA-N) as described by Pappa and Anifantakis [32].

## 2.4. Urea-polyacrylamide gel electrophoresis (Urea-PAGE)

Pure reference caseins (CN) from bovine, ovine or caprine milk as well as cheese samples and their water-soluble extracts were analyzed in duplicate by urea-PAGE as described by Mallatou et al. [25]. From the densitograms the levels of residual  $\alpha_s$ - and  $\beta$ -casein in the aged cheeses were calculated in comparison with the level present in reference samples of 1-day-old cheese. The zones of pure whole casein samples of the corresponding milk were used in electrophoresis for the identification of different bands. The levels of peptides in the WSN extract of cheeses were calculated in comparison with the level present in the reference sample of 1-day-old WSN extract of cheese.

## 2.5. Reversed-phase high performance liquid chromatography (RP-HPLC)

Peptide profiles of the water-soluble fraction of the cheeses made from the different kinds of milk and culture used were determined by RP-HPLC using a

Waters HPLC system (WATERS Associates, Milford, MA, USA), as described by Mallatou et al. [25]. After each run, the integration area of peptides was determined and divided into two regions with the criterion being the elution time of the peaks. The first group consists of the hydrophilic peptides (HL) with retention times from 0 to 67.5 min (0–55% eluent B). The second group consists of hydrophobic peptides (HB) with retention times from 67.6 to 110 min (55.1–100% eluent B).

### 2.6. Statistical analysis

The statistical analysis of results was done by a multifactor analysis of variance using the software STATGRAPHICS Plus for Windows v.5.2 (1995, Manugistics, Inc, Rockville, MD, USA). The least significant difference of the data is reported ( $P < 0.05$ ). Principal component analysis (PCA) and linear discriminant analysis (LDA) were also applied to the data using the same statistical program.

## 3. RESULTS

The composition of cheeses made with different kinds of milk and cultures has been reported in a previous paper [33]. The pH of all mature cheeses ranged between 4.26 and 4.38.

The changes in nitrogenous fractions during ripening of Teleme cheese made from different kinds of milk and cultures are shown in Table I. The values of TN ( $\text{g} \cdot 100 \text{ g}^{-1}$  cheese on a wet basis) of mature cheeses (over 60 days old) made from sheep's, goat's or cow's milk ranged from 2.22–2.48, 2.37–2.55 and 2.16–2.36, respectively, regardless of the kind of culture used (Tab. I). Mature cheeses made with the thermophilic culture had lower TN values (2.66–2.16) than cheeses made from the other two cultures, regardless of the kind of milk used ( $P < 0.05$ ). Cheeses made with the mixed (2.55–2.30) or mesophilic (2.53–2.29) cultures did not show any significant difference ( $P > 0.05$ ) in their TN values. From Table I, it can also be seen that the level of the %WSN, %PTA-TN and %TCA-TN increased during ripening ( $P < 0.05$ ). The % WSN/TN content of

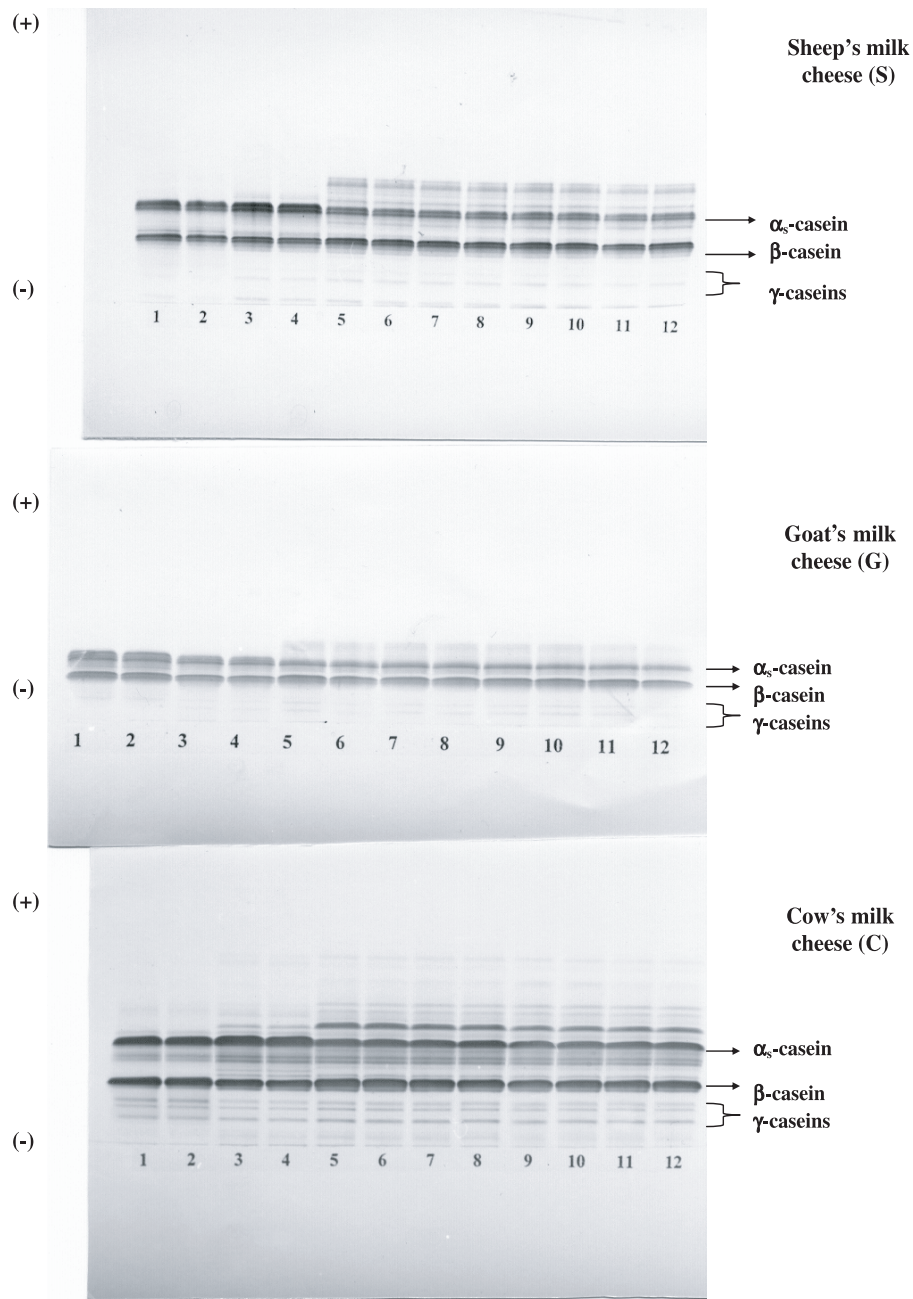
cheeses made from different types of milk ranged from 3.10–19.02%, 3.69–13.00% and 4.99–17.10% for sheep's, goat's and cow's milk, respectively, regardless of age or culture used. The % WSN/TN content of cheeses made with the thermophilic, mixed and mesophilic culture varied from 8.75–19.02%, 4.60–12.54% and 3.10–10.15%, respectively. The values of PTA-N of cheeses made from sheep's, goat's or cow's milk ranged from 1.12–2.69%, 1.10–2.86% and 1.10–2.94%, respectively, regardless of the kind of culture used. The values of PTA-N of Teleme cheeses made with the thermophilic, the mixed or the mesophilic culture in this study ranged from 1.36–2.94%, 1.10–2.42% and 1.10–2.28%, respectively, regardless of the kind of milk used. The TCA-N content of cheeses made from sheep's, goat's and cow's milk ranged from 2.14–11.92%, 1.80–10.32% and 1.80–10.31%, respectively, regardless of the culture used. The TCA-N content of cheeses made with the thermophilic, mixed and mesophilic culture varied from 2.80–11.92%, 1.99–9.11% and 1.80–8.51%, respectively.

The results obtained by urea-PAGE electrophoresis for Teleme cheeses and their WSN, made with the three cultures, were qualitatively similar. The quantitative results were different and are presented in Table II. Qualitative results from cheeses and their WSN made from the three types of milk with the mixed culture are shown in representative electrophoregrams (Figs. 1 and 2, respectively). Considering that caseins are intact on day 1, then the highest  $\alpha_s$ -CNs hydrolysis (estimated as the difference of the lowest percentage of residual  $\alpha_s$ -CNs from 100) ranged from 100 to 54.4%, 100 to 73.4% and 100 to 34.4% while hydrolysis of  $\beta$ -CN ranged from 100 to 75.9%, 100 to 84.2% and 100 to 70.2% for cheeses made from sheep's, goat's or cow's milk, respectively, regardless of the kind of culture used. Therefore, Teleme cheeses made from cow's, goat's or sheep's milk had the highest, lowest and intermediate degree of hydrolysis, respectively ( $P < 0.05$ ). The hydrolysis of  $\alpha_s$ -CNs ranged from 100 to 34.4%, 100 to 50.3% and 100 to 61.7% and the hydrolysis of  $\beta$ -CN ranged from 100 to

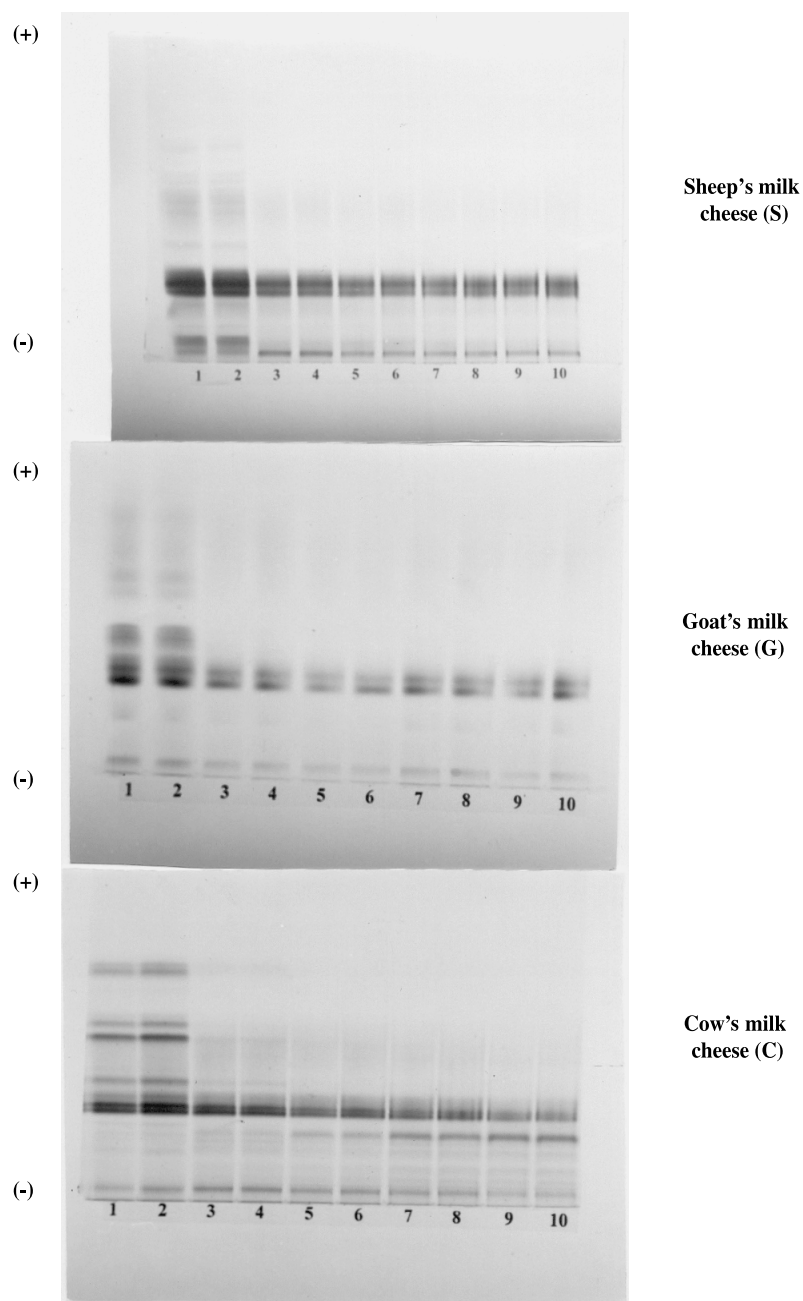
**Table I.** Changes of nitrogenous fractions of Teleme cheese made from sheep's (S), goat's (G) or cow's (C) milk using a thermophilic (T), a mesophilic (M) or a mixture of thermophilic and mesophilic (MX) starter during ripening and storage<sup>(i)</sup>.

Age (days)	Kind of milk	TN, (g·100 g <sup>-1</sup> cheese in wet basis)			WSN, %TN			PTA-N, %TN			TCA-N, %TN		
		T	MX	M	T	MX	M	T	MX	M	T	MX	M
1	S	2.47 <sup>1a</sup>	2.40 <sup>2a</sup>	2.42 <sup>12a</sup>	8.75 <sup>1a</sup>	4.60 <sup>2a</sup>	3.10 <sup>3a</sup>	1.72 <sup>1a</sup>	1.30 <sup>2a</sup>	1.12 <sup>3</sup>	3.10 <sup>1a</sup>	2.81 <sup>2a</sup>	2.14 <sup>3a</sup>
	G	2.51 <sup>1a</sup>	2.49 <sup>b</sup>	2.50 <sup>b</sup>	6.99 <sup>1b</sup>	5.17 <sup>2b</sup>	3.69 <sup>3b</sup>	1.60 <sup>1b</sup>	1.10 <sup>2b</sup>	1.10 <sup>2</sup>	2.80 <sup>1b</sup>	1.99 <sup>2b</sup>	1.80 <sup>3b</sup>
	C	2.28 <sup>1b</sup>	2.35 <sup>2a</sup>	2.30 <sup>12c</sup>	7.15 <sup>1c</sup>	5.20 <sup>2b</sup>	4.99 <sup>3c</sup>	1.36 <sup>1c</sup>	1.18 <sup>2c</sup>	1.10 <sup>3</sup>	2.90 <sup>1c</sup>	2.00 <sup>2b</sup>	1.90 <sup>3c</sup>
R <sup>(ii)</sup>	S	2.53 <sup>1a</sup>	2.43 <sup>2ab</sup>	2.45 <sup>2a</sup>	13.83 <sup>1a</sup>	6.53 <sup>2a</sup>	5.87 <sup>3a</sup>	2.09 <sup>1</sup>	1.62 <sup>2</sup>	1.92 <sup>1a</sup>	7.32 <sup>1</sup>	7.11 <sup>1a</sup>	5.04 <sup>2a</sup>
	G	2.66 <sup>a</sup>	2.47 <sup>a</sup>	2.53 <sup>b</sup>	9.92 <sup>1b</sup>	8.27 <sup>2b</sup>	7.62 <sup>2b</sup>	2.04 <sup>1</sup>	1.53 <sup>2</sup>	1.39 <sup>2b</sup>	6.95 <sup>1</sup>	5.61 <sup>2b</sup>	5.49 <sup>2ab</sup>
	C	2.30 <sup>1b</sup>	2.39 <sup>2b</sup>	2.29 <sup>1c</sup>	10.41 <sup>1b</sup>	8.17 <sup>2b</sup>	8.57 <sup>2c</sup>	1.99 <sup>1</sup>	1.66 <sup>2</sup>	1.68 <sup>2c</sup>	6.90 <sup>1</sup>	5.68 <sup>2b</sup>	5.70 <sup>2b</sup>
60	S	2.26 <sup>1a</sup>	2.39 <sup>2a</sup>	2.44 <sup>2a</sup>	16.08 <sup>1a</sup>	10.19 <sup>2a</sup>	11.05 <sup>2a</sup>	2.14 <sup>1</sup>	1.93 <sup>2a</sup>	1.76 <sup>3</sup>	8.18 <sup>1a</sup>	7.56 <sup>2a</sup>	6.88 <sup>3a</sup>
	G	2.46 <sup>1b</sup>	2.55 <sup>2b</sup>	2.50 <sup>12a</sup>	10.19 <sup>1b</sup>	8.24 <sup>2b</sup>	7.41 <sup>2b</sup>	2.11 <sup>1</sup>	1.68 <sup>2b</sup>	1.62 <sup>2</sup>	8.43 <sup>1a</sup>	6.29 <sup>2b</sup>	5.81 <sup>2b</sup>
	C	2.32 <sup>c</sup>	2.36 <sup>a</sup>	2.29 <sup>b</sup>	10.11 <sup>1b</sup>	9.66 <sup>c</sup>	9.06 <sup>c</sup>	2.00 <sup>1</sup>	1.99 <sup>1a</sup>	1.81 <sup>2</sup>	7.04 <sup>b</sup>	6.87 <sup>ab</sup>	6.73 <sup>a</sup>
120	S	2.35 <sup>1</sup>	2.38 <sup>12</sup>	2.48 <sup>2a</sup>	17.19 <sup>1a</sup>	12.49 <sup>2a</sup>	11.28 <sup>2a</sup>	2.04 <sup>1</sup>	1.99 <sup>1a</sup>	1.82 <sup>2a</sup>	8.74 <sup>ab</sup>	8.68 <sup>a</sup>	8.07 <sup>a</sup>
	G	2.38 <sup>12</sup>	2.38 <sup>1</sup>	2.46 <sup>2ab</sup>	11.71 <sup>1b</sup>	9.37 <sup>2b</sup>	7.76 <sup>3b</sup>	1.99 <sup>1</sup>	1.76 <sup>2b</sup>	1.57 <sup>2b</sup>	10.05 <sup>1a</sup>	6.44 <sup>2b</sup>	5.90 <sup>2b</sup>
	C	2.29 <sup>1</sup>	2.36 <sup>12</sup>	2.38 <sup>2b</sup>	11.75 <sup>1b</sup>	9.68 <sup>2b</sup>	9.04 <sup>2c</sup>	2.20 <sup>1</sup>	1.90 <sup>2ab</sup>	1.87 <sup>2a</sup>	7.89 <sup>1b</sup>	6.40 <sup>2b</sup>	6.05 <sup>2b</sup>
180	S	2.22 <sup>1ab</sup>	2.36 <sup>2a</sup>	2.42 <sup>2a</sup>	19.02 <sup>1a</sup>	12.54 <sup>2a</sup>	11.74 <sup>2a</sup>	2.69 <sup>1</sup>	2.32 <sup>12ab</sup>	2.14 <sup>2ab</sup>	11.92 <sup>1a</sup>	9.11 <sup>2a</sup>	8.51 <sup>2a</sup>
	G	2.37 <sup>1a</sup>	2.45 <sup>2b</sup>	2.42 <sup>12a</sup>	13.00 <sup>1b</sup>	9.19 <sup>2b</sup>	9.00 <sup>2b</sup>	2.86 <sup>1</sup>	2.06 <sup>2a</sup>	1.95 <sup>2a</sup>	10.32 <sup>1b</sup>	6.71 <sup>2b</sup>	6.35 <sup>2b</sup>
	C	2.16 <sup>1b</sup>	2.30 <sup>2a</sup>	2.30 <sup>2b</sup>	17.10 <sup>1c</sup>	10.28 <sup>2c</sup>	10.15 <sup>2c</sup>	2.94 <sup>1</sup>	2.42 <sup>2b</sup>	2.28 <sup>2b</sup>	10.31 <sup>1b</sup>	7.53 <sup>2b</sup>	7.31 <sup>2c</sup>

<sup>(i)</sup> Means of five cheese-making trials. <sup>(ii)</sup> Day cheeses were transferred to cold room (see text for details).a,b,c Means of each parameter in the same column of the same day with different letters are significantly different ( $P < 0.05$ ).1,2,3 Means of each parameter in the same row of the same day with different numbers are significantly different ( $P < 0.05$ ).



**Figure 1.** Electrophoregrams of Teleme cheese made with sheep's, goat's or cow's milk with the mixed (thermophilic-mesophilic) culture during ripening and storage. Lanes 1–2: total sheep's casein; 3–4: 1-d cheese; 5–6: when cheese was transferred to cold room (see text for details); 7–8: 60-d cheese; 9–10: 120-d cheese; 11–12: 180-d cheese.



**Figure 2.** Electrophoregrams of the WSE of Teleme cheese made with sheep's, goat's or cow's milk with the mixed (thermophilic-mesophilic) culture during ripening and storage. Lanes 1-2: 1-d cheese; 3-4: when cheese was transferred to cold room (see text for details); 5-6: 60-d cheese; 7-8: 120-d cheese; 9-10: 180-d cheese.



**Table II.** Changes in residual  $\beta$ -casein (%),  $\alpha_s$ -casein (%) and in water-soluble nitrogen (WSN, %), in polyacrylamide gel, of Telene cheese made from sheep's (S), goat's (G) or cow's (C) milk using a thermophilic (T), a mesophilic (M) or a mixture of thermophilic and mesophilic (MX) starter during ripening and storage<sup>(i)</sup>.

Age (days)	Kind of milk	$\beta$ -casein (%)			$\alpha_s$ -casein (%)			WSN (%) in polyacrylamide gel		
		T	MX	M	T	MX	M	T	MX	M
1	S	100	100	100	100	100	100	100	100	100
	G	100	100	100	100	100	100	100	100	100
	C	100	100	100	100	100	100	100	100	100
R <sup>(ii)</sup>	S	90.0 <sup>1ab</sup>	94.7 <sup>2a</sup>	96.7 <sup>2</sup>	72.1 <sup>1a</sup>	83.7 <sup>12a</sup>	87.5 <sup>2ab</sup>	61.3 <sup>1a</sup>	66.8 <sup>12a</sup>	70.7 <sup>2a</sup>
	G	95.0 <sup>a</sup>	95.0 <sup>a</sup>	98.6	91.0 <sup>b</sup>	91.5 <sup>b</sup>	93.9 <sup>a</sup>	83.9 <sup>b</sup>	85.7 <sup>b</sup>	88.9 <sup>b</sup>
	C	86.1 <sup>1b</sup>	91.4 <sup>2b</sup>	94.0 <sup>2</sup>	55.7 <sup>1c</sup>	73.4 <sup>2c</sup>	77.8 <sup>2b</sup>	40.8 <sup>1c</sup>	57.2 <sup>2c</sup>	58.8 <sup>2c</sup>
60	S	87.9 <sup>1a</sup>	90.6 <sup>2a</sup>	92.5 <sup>3a</sup>	58.1 <sup>1a</sup>	75.0 <sup>2a</sup>	78.9 <sup>3a</sup>	54.2 <sup>1a</sup>	60.6 <sup>2a</sup>	67.5 <sup>3a</sup>
	G	93.2 <sup>b</sup>	94.5 <sup>b</sup>	94.5 <sup>b</sup>	85.7 <sup>b</sup>	86.2 <sup>b</sup>	89.0 <sup>b</sup>	73.7 <sup>b</sup>	77.3 <sup>b</sup>	81.0 <sup>b</sup>
	C	83.7 <sup>c</sup>	84.7 <sup>c</sup>	85.3 <sup>c</sup>	49.2 <sup>1c</sup>	54.4 <sup>1c</sup>	72.9 <sup>2c</sup>	42.2 <sup>1c</sup>	47.2 <sup>12c</sup>	47.9 <sup>2c</sup>
120	S	86.4 <sup>1a</sup>	86.5 <sup>2a</sup>	91.3 <sup>3a</sup>	57.3 <sup>1a</sup>	64.9 <sup>2a</sup>	71.4 <sup>3a</sup>	43.7 <sup>1a</sup>	58.5 <sup>2a</sup>	62.3 <sup>2a</sup>
	G	86.2 <sup>a</sup>	89.7 <sup>a</sup>	89.7 <sup>a</sup>	83.5 <sup>b</sup>	84.9 <sup>b</sup>	85.0 <sup>b</sup>	67.5 <sup>1b</sup>	71.3 <sup>12b</sup>	75.4 <sup>2b</sup>
	C	75.1 <sup>1b</sup>	76.8 <sup>1b</sup>	82.7 <sup>2b</sup>	47.7 <sup>1c</sup>	52.7 <sup>1c</sup>	64.4 <sup>2a</sup>	37.9 <sup>1a</sup>	45.0 <sup>2c</sup>	45.2 <sup>2c</sup>
180	S	75.9 <sup>1a</sup>	82.7 <sup>2a</sup>	84.1 <sup>3ab</sup>	54.4 <sup>1a</sup>	59.9 <sup>2a</sup>	63.8 <sup>2a</sup>	37.4 <sup>1a</sup>	55.0 <sup>2a</sup>	59.3 <sup>3a</sup>
	G	84.2 <sup>b</sup>	84.7 <sup>a</sup>	85.4 <sup>a</sup>	73.4 <sup>b</sup>	79.0 <sup>b</sup>	79.1 <sup>b</sup>	65.2 <sup>1b</sup>	69.6 <sup>12b</sup>	73.5 <sup>2b</sup>
	C	70.2 <sup>1c</sup>	74.2 <sup>12b</sup>	77.6 <sup>2b</sup>	34.4 <sup>1c</sup>	50.3 <sup>2a</sup>	61.7 <sup>3a</sup>	32.7 <sup>1c</sup>	43.0 <sup>2c</sup>	44.1 <sup>3c</sup>

<sup>(i)</sup> Means of five cheese-making trials. <sup>(ii)</sup> Day cheeses were transferred to cold room (see text for details).

a,b,c Means of each parameter in the same column of the same age with different letters are significantly different ( $P < 0.05$ ).

1,2,3 Means of each parameter in the same row of the same age with different numbers are significantly different ( $P < 0.05$ ).

72.2%, 100 to 74.2% or 100 to 77.6% for cheeses made with thermophilic, mixed or mesophilic culture, respectively, regardless of the kind of milk used. The decrease in the total area of the WSN in PAGE varied from 100 to 37.4%, 100 to 65.2% and 100 to 32.7% for cheeses made from sheep's, goat's and cow's milk, respectively, regardless of the kind of culture used and from 100 to 32.7%, 100 to 43.0% and 100 to 44.1% for cheeses made with the thermophilic, the mixed or the mesophilic culture, regardless of the kind of milk used.

The changes in the peptide profile of Teleme cheese made from different kinds of milk and cultures during ripening and storage are shown in Table III. The use of thermophilic, mixed or mesophilic cultures did not cause any major qualitative differences in the RP-HPLC profiles of cheeses made from different types of milk. A representative peptide profile of Teleme cheeses made with sheep's, goat's or cow's milk with the mixed culture is shown in Figure 3. However, differences were observed in the profile of cheeses made from the three types of milk, regardless of the culture used. It is known that each peak represents at least one peptide, and the area is, in principle, proportional to the concentration of the peptide(s). The peak with retention time ~44 min in sheep's milk cheese was the highest in quantity and the one eluted in the same retention time in goat's milk cheese was the lowest, regardless of the type of culture used. On the contrary, the peak eluted in ~74 min in cow's milk cheese was higher in quantity than the one eluted in the same retention time in sheep's or goat's milk cheeses. Table III shows that the area of hydrophilic (HL) peptides expressed as % of the total area (%TA) increased during ripening and storage ( $P < 0.05$ ), especially until cheeses were transferred to the cold room. On the contrary, the area of hydrophobic (HB) peptides (%TA) and the ratio HB/HL decreased during ripening ( $P < 0.05$ ), regardless of the type of milk and culture used. The area of the HL (%TA) peptides of cheeses made with the thermophilic, mixed and mesophilic culture varied from 48.51–79.16%, 39.57–72.53% and 33.24–68.81%, respectively, regard-

less of the type of milk used. The area of the HL peptides (%TA) of cheeses made with sheep's, goat's and cow's milk varied from 33.24–76.11%, 40.74–75.25% and 51.25–79.16%, respectively, regardless of the type of culture used.

## 4. DISCUSSION

### 4.1. Effect of ripening time

It can be seen (Tab. I) that, regardless of the type of milk and culture used, TN values showed a slight decrease during ripening. This can be attributed to hydrolysis of proteins to water-soluble nitrogen compounds that are lost due to their diffusion into brine [1]. Also, the high degree of hydrolysis contributes to the protein decrease as the migration of such compounds of cheese into brine is determined by factors such as size and hydrophobicity of the water-soluble nitrogenous compounds [29]. Higher TN values than those found in this study were reported for white-brined cheese of similar age by other researchers [3, 18, 24]. These differences can be attributed to the different composition of milks and to the different cheese-making procedures. As expected, the greater part of nitrogen fraction changes occurred during the warm room (19 °C) ripening. The rate of proteolysis of Teleme cheese in this study, as estimated by the % WSN/TN content (Tab. I), was similar to those reported by other researchers [16, 24, 32, 49] though slower than the rate found by Antoniou et al. [3] and Kehagias et al. [20] for white-brined cheeses made with similar types of milk and cultures. The PTA-N values of mature Teleme cheese in this study (Tab. I) were, generally, lower than the values found for other mature white-brined cheese by other researchers [28, 32, 42]. The ratios of % TCA-N/TN and % PTA-N/TN have been widely used as indicators of maturation of cheese. The % TCA-N/TN values of mature Teleme cheese in this study were similar to or lower than those found by others [3, 18, 24, 32, 42] for mature white-brined cheeses made with similar types of milk and cultures.

From Table II and Figures 1-2, it can be seen that no major qualitative differences

**Table III.** Changes in the area of hydrophilic (HL) and hydrophobic (HB) peptides (%TA) and the ratio of HB/HL of the water-soluble fraction of Teleme cheese made from sheep's (S), goat's (G) or cow's (C) milk using a thermophilic (T), a mesophilic (M) or a mixture of thermophilic and mesophilic (MX) starter during ripening and storage<sup>(i)</sup>.

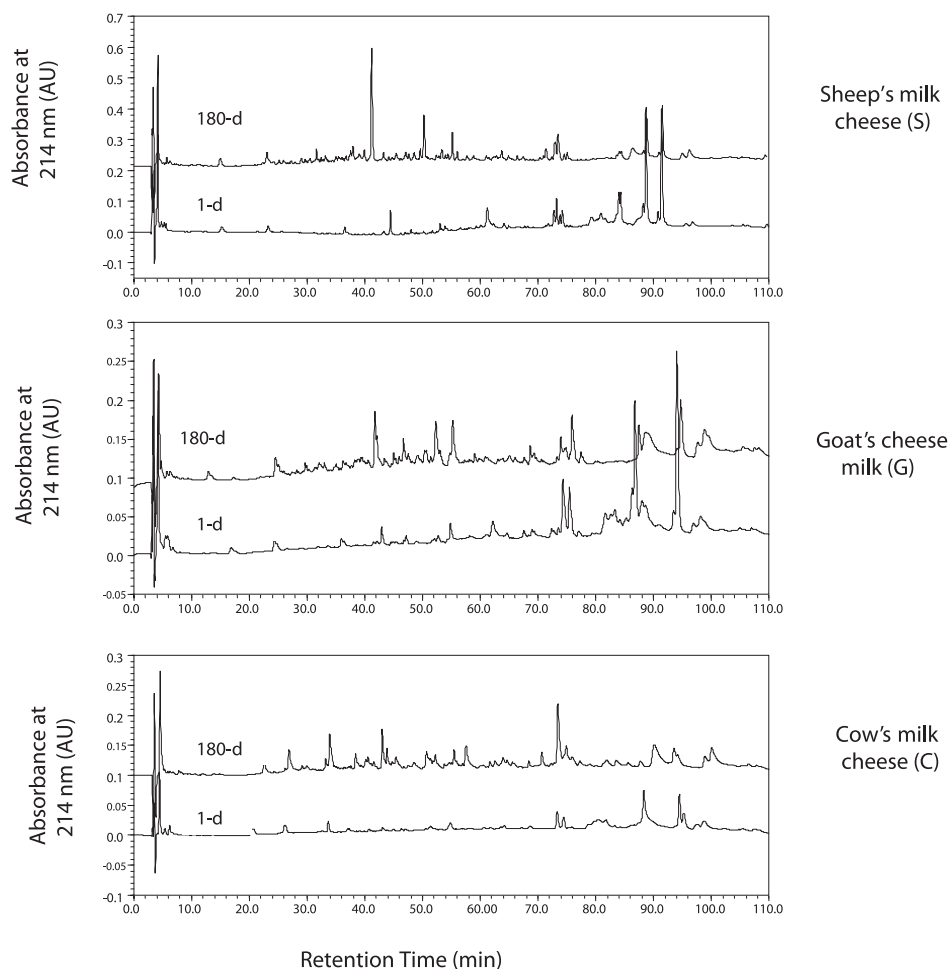
Age (days)	Kind of milk	HL (%TA) <sup>(iii)</sup>			HB (%TA) <sup>(iii)</sup>			HB/HL		
		T	MX	M	T	MX	M	T	MX	M
1	S	48.51 <sup>1a</sup>	39.57 <sup>2a</sup>	33.24 <sup>3a</sup>	51.49 <sup>1a</sup>	60.43 <sup>2a</sup>	66.76 <sup>3a</sup>	1.04 <sup>1a</sup>	1.53 <sup>2a</sup>	2.00 <sup>3a</sup>
	G	49.26 <sup>1a</sup>	44.08 <sup>2b</sup>	40.74 <sup>3b</sup>	50.74 <sup>1a</sup>	55.92 <sup>2b</sup>	59.26 <sup>3b</sup>	1.03 <sup>1a</sup>	1.27 <sup>2b</sup>	1.45 <sup>3b</sup>
	C	53.54 <sup>b</sup>	52.2 <sup>c</sup>	51.25 <sup>c</sup>	46.46 <sup>b</sup>	47.8 <sup>c</sup>	48.75 <sup>c</sup>	0.87 <sup>b</sup>	0.92 <sup>c</sup>	0.95 <sup>c</sup>
R <sup>(ii)</sup>	S	68.61 <sup>1a</sup>	61.17 <sup>2a</sup>	58.73 <sup>2a</sup>	31.39 <sup>1a</sup>	38.83 <sup>2a</sup>	41.27 <sup>2a</sup>	0.46 <sup>1a</sup>	0.63 <sup>2a</sup>	0.70 <sup>2a</sup>
	G	65.54 <sup>1a</sup>	55.44 <sup>2b</sup>	50.48 <sup>3b</sup>	34.96 <sup>1a</sup>	44.56 <sup>2b</sup>	49.52 <sup>3b</sup>	0.53 <sup>1a</sup>	0.80 <sup>2b</sup>	0.98 <sup>3b</sup>
	C	73.24 <sup>1b</sup>	66.86 <sup>2c</sup>	58.05 <sup>3a</sup>	26.76 <sup>1b</sup>	32.48 <sup>2c</sup>	41.95 <sup>3a</sup>	0.37 <sup>1b</sup>	0.49 <sup>2c</sup>	0.72 <sup>3a</sup>
60	S	73.12 <sup>1ab</sup>	66.43 <sup>2a</sup>	61.62 <sup>3a</sup>	26.88 <sup>1ab</sup>	33.57 <sup>2a</sup>	38.38 <sup>3a</sup>	0.37 <sup>1ab</sup>	0.51 <sup>2a</sup>	0.62 <sup>3a</sup>
	G	70.89 <sup>1a</sup>	63.88 <sup>2a</sup>	56.71 <sup>3b</sup>	29.11 <sup>1a</sup>	36.12 <sup>2b</sup>	43.29 <sup>3b</sup>	0.41 <sup>1a</sup>	0.57 <sup>2a</sup>	0.76 <sup>3b</sup>
	C	75.35 <sup>1b</sup>	69.80 <sup>2b</sup>	64.54 <sup>3c</sup>	24.65 <sup>1b</sup>	30.20 <sup>2c</sup>	35.46 <sup>3c</sup>	0.33 <sup>1b</sup>	0.43 <sup>2b</sup>	0.55 <sup>3c</sup>
120	S	75.67 <sup>1a</sup>	68.33 <sup>2a</sup>	63.34 <sup>3a</sup>	24.33 <sup>1a</sup>	31.67 <sup>2a</sup>	36.66 <sup>3a</sup>	0.32 <sup>1a</sup>	0.46 <sup>2a</sup>	0.58 <sup>3a</sup>
	G	73.44 <sup>1a</sup>	66.44 <sup>2b</sup>	60.66 <sup>3b</sup>	26.55 <sup>1a</sup>	33.56 <sup>2b</sup>	39.34 <sup>3b</sup>	0.36 <sup>1a</sup>	0.51 <sup>2b</sup>	0.65 <sup>3b</sup>
	C	78.69 <sup>1b</sup>	70.69 <sup>2c</sup>	66.18 <sup>3c</sup>	21.35 <sup>1b</sup>	29.31 <sup>2c</sup>	33.82 <sup>3c</sup>	0.27 <sup>1b</sup>	0.41 <sup>2c</sup>	0.51 <sup>3c</sup>
180	S	76.11 <sup>1a</sup>	70.33 <sup>2a</sup>	65.59 <sup>3a</sup>	23.89 <sup>1a</sup>	29.67 <sup>2a</sup>	34.41 <sup>3a</sup>	0.31 <sup>1a</sup>	0.42 <sup>2a</sup>	0.53 <sup>3a</sup>
	G	75.25 <sup>1a</sup>	68.08 <sup>2b</sup>	63.67 <sup>3b</sup>	24.75 <sup>1a</sup>	31.89 <sup>2b</sup>	36.33 <sup>3b</sup>	0.33 <sup>1a</sup>	0.47 <sup>2b</sup>	0.57 <sup>3b</sup>
	C	79.16 <sup>1b</sup>	72.53 <sup>2c</sup>	68.81 <sup>3c</sup>	20.84 <sup>1b</sup>	27.47 <sup>2c</sup>	31.19 <sup>3c</sup>	0.26 <sup>1b</sup>	0.38 <sup>2c</sup>	0.45 <sup>3c</sup>

<sup>(i)</sup> Means of three cheese-making trials. <sup>(ii)</sup> Day cheeses were transferred to cold room (see text for details).

<sup>(iii)</sup> Results are expressed as % of the total area (TA) of the chromatogram.

a,b,c Means of each parameter in the same column of the same age with different letters are significantly different ( $P < 0.05$ ).

1,2,3 Means of each parameter in the same row of the same age with different numbers are significantly different ( $P < 0.05$ ).



**Figure 3.** RP-HPLC chromatograms of the WSE of Teleme cheese with sheep's, goat's or cow's milk with the mixed (thermophilic-mesophilic) culture at 1 and 180 days.

were observed on any sampling day, suggesting that the mode of casein breakdown was similar in all cheeses. It can be seen that there were peptides produced with faster electrophoretic mobility ( $R_f$ ) than  $\alpha_s$ -caseins, with mobility slower than  $\beta$ -casein and with mobility between  $\alpha_s$ - and  $\beta$ -caseins. Abd El-Salam and Alichanidis [1] found that the hydrolysis products in white-brined cheeses with  $R_f$  faster than  $\alpha_s$ -CN are produced from the action of chymosin on  $\alpha_{s1}$ -CN, whereas the products with

mobility slower than  $\beta$ -CN are produced from the action of indigenous milk enzymes on  $\beta$ -CN ( $\gamma$ -CNs). In this study, it was also observed that there was a continuous decrease in the intensity of bands of  $\alpha_s$ - and  $\beta$ -CNs in all cheeses, regardless of the kind of milk and culture starter used. Also, the rate of hydrolysis of  $\beta$ -CN is slower than that of  $\alpha_s$ -CNs in all cheeses, regardless of the kind of milk and culture used. This is in agreement with the results found for other cheeses from sheep's, goat's or cow's milk

**Table IV.** Spearman correlation coefficients between the different variables investigated<sup>(i)</sup>.

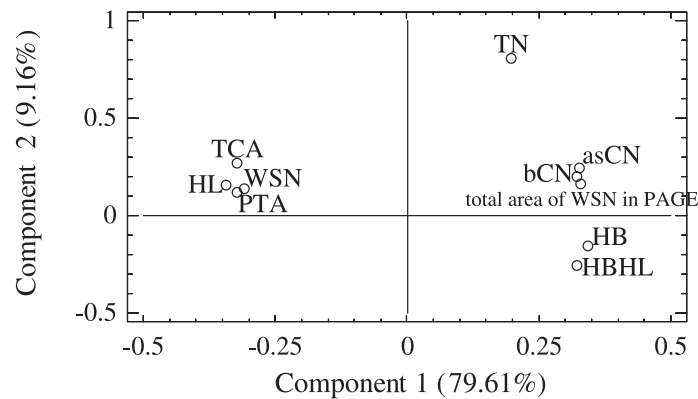
	WSN	PTA	TCA	TN	HL	HB	HB/HL	$\alpha_s$ -CN	$\beta$ -CN
WSN									
PTA	0.88**								
TCA	0.93**	0.88**							
TN	-0.38*	-0.40**	-0.35*						
HL	0.85**	0.88**	0.85**	-0.53**					
HB	-0.85**	-0.88**	-0.85**	0.53**	-0.99**				
HB-HL	-0.85**	-0.88**	-0.85**	0.53**	-0.99**	0.99**			
$\alpha_s$ -CN	-0.79**	-0.80**	-0.75**	0.58**	-0.87**	0.87**	0.87**		
$\beta$ -CN	-0.75**	-0.83**	-0.75**	0.53**	-0.86**	0.86**	0.86**	0.91**	
Total area									
of the WSN	-0.74**	-0.76**	-0.72**	0.63**	-0.85**	0.85**	0.85**	0.98**	0.88**
in PAGE									

<sup>(i)</sup>Abbreviations of the variables are explained into the text.\* $P < 0.05$ ; \*\* $P < 0.01$ .

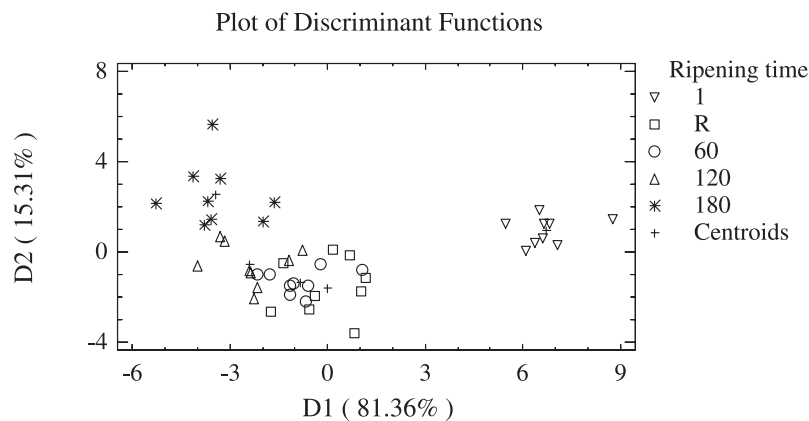
[8, 27] and it might be explained by the low pH (4.26–4.38) during ripening and storage of Teleme cheeses that does not favor the hydrolysis of  $\beta$ -CN by plasmin. Moreover, the high NaCl content of white-brined cheeses results in retarding  $\beta$ -CN hydrolysis [7]. The decrease ( $P < 0.05$ ) in the total area of bands of the WSN in PAGE of all cheeses during the ripening period is due to the continuous hydrolysis of the large peptides to small peptides and amino acids, and to diffusion of whey proteins into brine. Moreover, peptides with  $MW < 6 \text{ kg} \cdot \text{mol}^{-1}$  were not colored with Coomassie Blue [22] and therefore, their total area was shown to decrease during ripening.

From Table III and Figure 3 it can be seen that, regardless of the kind of milk and culture used, new peaks appeared while others decreased or disappeared during ripening and storage. As said before, the area of HL (%TA) peptides increased, whereas the area of HB (%TA) and the ratio HB/HL decreased during ripening ( $P < 0.05$ ), regardless of the type of milk and culture used (Tab. III). A similar trend was found by others [14, 22]. The reasons for the decrease in the HB/HL peptides are the degradation of water-soluble HB peptides [22] and the diffusion of the whey proteins into the brine [28].

Table IV shows Spearman correlation coefficients between the different variables investigated. All variables were strongly correlated. WSN, PTA and TCA were positively correlated with each other. HB had a high positive correlation with HB/HL and both HB and HB/HL were negatively correlated with HL.  $\alpha_s$ -CN,  $\beta$ -CN and total area of the WSN in PAGE were also positively correlated with each other. TN was positively correlated with  $\alpha_s$ -CN,  $\beta$ -CN, total area of the WSN in PAGE, HB and HB/HL and was negatively correlated with WSN, PTA, TCA and HL. Since there were many highly correlated variables a principal component analysis (PCA) was performed to reduce the variables to a smaller number of principal components. Prior to multivariate procedures, variables were scaled to zero mean and unit variance. The first principal component explained 79.61% of the total variability and was considered to retain most information existing in the original data, since it was the only component which had an eigenvalue greater than unit. Even though one PC was enough to explain most of the variation, the first two components were retained in order to obtain a two-dimensional mapping for the variables, more readable than a one-dimensional plot



**Figure 4.** The principal component analysis for the variables investigated.



**Figure 5.** Linear discriminant functions (D1 and D2) for cheese samples at 1, 60, 120, 180 days of ripening time as well as the day cheeses entered the cold room (R). Both D1 and D2 were significant ( $P < 0.001$ ).

or table (Fig. 4). The first two principal components explained 88.77% of the total variability of the data set (PC1 79.61% and PC2 9.16%). The total N (TN) determined the second principal component and all the other variables contributed to the first PC. WSN, PTA, TCA and HL were close together on the component plot (Fig. 4), indicating a high positive correlation, as was also found by the correlation coefficients of Table IV. On the other hand, these variables were lying opposite to HB and HB/HL, and therefore they were negatively

correlated to these.  $\alpha_s$ -CN,  $\beta$ -CN and the total area of the WSN in PAGE were clustered on the component plot because of their high positive correlation. All variables were away from the axis origin, suggesting that they were represented by the two PCs.

Taking into account the results of PCA, linear discriminant analysis (LDA) was performed to look into the possibility of correctly grouping cheeses by the stage of ripening. The percentage of the samples that were classified into the correct group by the type of ripening was 82.22% (Fig. 5).

Some overlapping between R, 60- and 120-day-old samples was observed, which caused the misclassification. As ripening is a continuous process, the groups may not be completely separated. The most important predictor variables in classifying samples into the correct group were TN, TCA, HL and the total area of the WSN in PAGE.

#### 4.2. Effect of type of culture

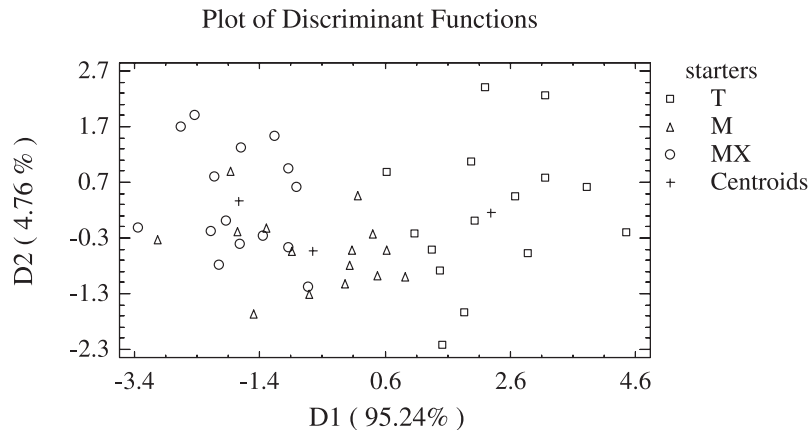
Analysis of the TN and soluble N, as estimated with the Kjeldahl method (Tab. I), showed that the lowest TN values of mature cheeses made with the thermophilic culture were due to the fact that this culture was the most proteolytic, and therefore the loss of water-soluble nitrogenous compounds in the brine was higher than the other two cultures. Cheeses made with the thermophilic and the mesophilic cultures showed the highest and the lowest % WSN/TN values, respectively. This is in agreement with the results of other studies for white-brined cheese made with cultures similar to those of this study [20, 32]. Thermophilic culture resulted in cheeses with higher PTA-N content than the cheeses made with the other two cultures. Generally, the latter did not differ significantly in the PTA-N content ( $P > 0.05$ ). Similar results were also found by Gomez and Malcata [12]. The % TCA/TN values of cheeses made with the thermophilic culture were significantly higher ( $P < 0.05$ ) than those of cheeses made with the other two cultures. This is in agreement with the results found by Pappa and Anifantakis [32] and Gomez and Malcata [12]. From this study, it is observed that the thermophilic and the mesophilic cultures resulted in cheeses with the highest and the lowest ( $P < 0.05$ ) production of HL peptides, respectively (Tab. III). This is in agreement with the results of Steffen et al. [40] and Garde et al. [9].

Thermophilic culture showed the highest hydrolysis of  $\alpha_s$ - and  $\beta$ -CN, and mesophilic the lowest when cheeses were made from sheep's or cow's milk, as estimated with urea-PAGE (Tab. II). This is in agreement with the results reported by others [4, 9, 13, 44]. However, the three cultures showed the same rate of proteolysis ( $P > 0.05$ ), in rip-

ened Teleme cheeses made with goat's milk. This may be attributed to a balanced presence of starter and NS-LAB in the cheeses. Further investigation on this is necessary. Moreover, the results of this study showed that the highest and the lowest decrease in the total area of the WSN in PAGE was observed in cheeses made with the thermophilic and the mesophilic cultures, respectively, regardless of the kind of milk used ( $P < 0.05$ ).

Linear discriminant analysis (LDA) was performed to look into the possibility of correctly grouping cheeses by the type of starter. The percentage of the samples that were classified into the correct group by the type of starter was 86.67%. Cheeses were successfully clustered by the type of starter, although some overlapping between mixed and mesophilic cultures was observed (Fig. 6).

The highest levels of proteolysis observed in Teleme cheeses made with the thermophilic culture, regardless of the type of milk used, could be due to an earlier lysis of thermophilic starter bacteria that could have enhanced the early release of intracellular enzymes into the cheese matrix and could have increased the rate of proteolysis [6]. The overall proteolytic activity of lactobacilli has been found to be higher than that of *Lc. lactis* because lactobacilli possess additional peptidases and because their peptidases have higher expression levels [21, 38]. Also, *Str. thermophilus* possesses two additional peptidases (an oligopeptidase and aminopeptidase PepS) with respect to *Lc. lactis* and shows higher specific activities of PepX, PepN and PepC [36]. Oumer et al. [30] concluded that thermophilic adjunct culture, when inoculated into milk, showed considerably higher aminopeptidase activity than mesophilic adjunct culture. Garde et al. [10] found similar results when they manufactured Hispanico cheese with thermophilic or mesophilic adjunct culture. Another reason might be the different proteolytic enzyme system of the starters used. Genetic studies suggested that the proteinases found in thermophilic lactobacilli may represent a novel type of proteinase and that the genes encoding them may differ from those described in lactococci [39].



**Figure 6.** Linear discriminant functions (D1 and D2) for cheese samples made using a thermophilic (T), a mesophilic (M) and a mixture of thermophilic-mesophilic (MX) culture. D1 was significant ( $P < 0.001$ ) but D2 was not significant ( $P > 0.05$ ).

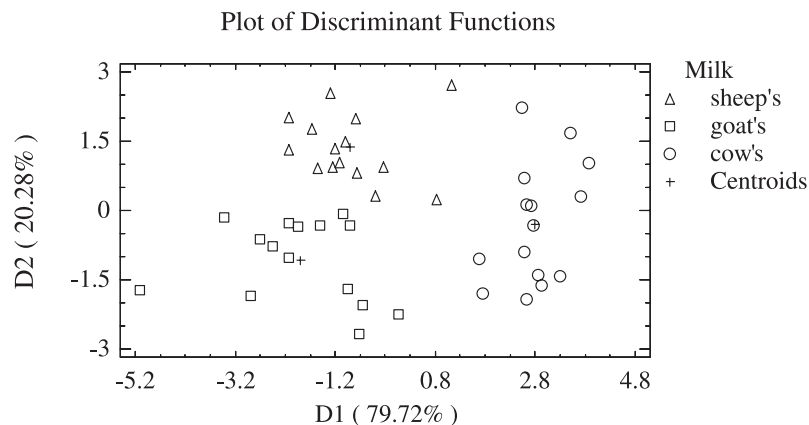
NSLAB play an important role in cheese ripening. They contribute to an increase in peptidase activity in cheese and enhance the production of peptides and amino acids [34]. The low pH and the high salt content of white-brined cheeses seem to favor the growth of lactobacilli. In Teleme cheese made from ewe's milk *Lb. plantarum* was the main species isolated. *Lb. paracasei*, *Lb. casei* and *Lb. brevis* were also identified. Other NSLAB belong to enterococci and pediococci [41]. In Feta cheese, *Lb. plantarum*, *Lb. paraplantarum* and *Lb. pentosaceus* were the most common mesophilic lactobacilli found during cheese ripening [26]. In addition, the presence of *Lb. paracasei* as well as *Lb. plantarum* has been isolated from white-brined cheeses made from goat's [48] and ewe's milk [47]. Poveda et al. [35] used *Lb. plantarum* as an adjunct culture and studied the proteolysis of Manchego cheese made from ewe's milk. They concluded that, perhaps, the effects of lactobacilli are not the same in cow's milk cheese as in ewe's milk cheese. Sasaki et al. [38] found that strains of *Lb. paracasei* ssp. *paracasei* possessed higher levels of proteinase, PepN and proline iminopeptidase activities than strains of *Lb. plantarum*. NSLAB influence is probably dependent on the strain and the final bacte-

rial count. The starter strain used also plays a role in the growth of NSLAB and their influence on cheese ripening [17].

#### 4.3. Effect of type of milk

It can be seen that the TN values of cheeses made from sheep's or goat's milk were higher ( $P < 0.05$ ) than those of cheeses made from cow's milk. Ozer et al. [31] also found similar results. The formation of the %WSN and TCA-TN was more pronounced in cheeses made from ovine milk than those made from bovine or caprine milk. This is in agreement with the results found by others [19, 24, 31]. From Table II it can be seen that Teleme cheeses made from cow's, goat's or sheep's milk had the highest, lowest and intermediate degree of hydrolysis, respectively ( $P < 0.05$ ). This is in agreement with the results reported by others [5, 20, 46]. Other reasons such as the inaccessibility of enzymes from rennin, LAB and NSLAB and possibly plasmin to hydrolyze specific bonds of caseins, or the possibility that the genetic variants of local caprine milk could be responsible for the low hydrolysis of goat's Teleme cheese are discussed by Mallatou et al. [25].  $\gamma$ -Caseins were fainter in goat's milk cheeses than in cow's milk cheeses





**Figure 7.** Linear discriminant functions (D1 and D2) for cheese samples made from sheep's, goat's and cow's milk. Both D1 and D2 were significant ( $P < 0.001$ ).

during ripening. This is in agreement with Saric et al. [37] for Travnicki, a white-brined cheese.

The highest and the lowest decrease in the total area of bands of the WSN in PAGE (Tab. II) and of production of HB (%TA) peptides (Tab. III) were observed in cheeses made from cow's and goat's milk, respectively, regardless of the kind of culture used ( $P < 0.05$ ). Lopez-Fandino et al. [23] using urea-PAGE studied the WSN of six cheeses and Gobetti et al. [11] the WSN of Gorgonzola cheese and found that bands that exist in young cheeses do not appear in old cheeses, but no quantitative results were mentioned.

Linear discriminant analysis (LDA) was performed to look into the possibility of correctly grouping cheeses by the type of milk. 97.78% of the samples were classified correctly by the type of milk (Fig. 7).

## 5. CONCLUSIONS

The results in this study show that the use of the different starter cultures affected the rate of proteolysis of Teleme cheese made from different types of milk. It is concluded that the mixed (thermophilic-mesophilic) or the thermophilic cultures might be more appropriate in the making of Teleme cheese

from sheep's, cow's or goat's milk, as they resulted in cheeses of higher levels of proteolysis.

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