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Changes of free amino acid content of Teleme cheese made with different types of milk and culture

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ABSTRACT

The evolution of concentration of free amino acids in Teleme cheese made from sheep, goat or cow milk, using a thermophilic, mesophilic or a mixture of a thermophilic, a mesophilic culture throughout ripening was studied. The total free amino acid (TFAA) content increased at all stages of ripening, regardless of the milk and culture used. In general, the TFAA content was higher in cheeses made from cow's milk than that of the cheeses made from ewe's milk; cheese from goat's milk ranged over intermediate levels. Also, higher concentrations of TFAA were found in cheeses made with the thermophilic than with the mesophilic culture. Cheeses made with the mixture of thermophilic–mesophilic culture ranged over intermediate levels. The results of this study have shown that Leu, Glu, Phe, Val and Lys were the major FAA of Teleme cheese at all stages of ripening, regardless of the type of milk and culture used.

1. Introduction

Proteolysis is the most complex and, in many varieties, the most important biochemical event during ripening. The pattern of proteolysis can be summarized as follows: the caseins are hydrolyzed initially by residual coagulant activity retained in the curd and by plasmin and perhaps by other indigenous proteolytic enzymes to a range of intermediate-sized peptides, which are hydrolyzed by proteinases and peptidases from the starter lactic acid bacteria, non-starter lactic acid bacteria (NS-LAB) and secondary microflora to shorter peptides and amino acids (Upadhyay, McSweeney, Magboul, & Fox, 2004). Every type of cheese has its own characteristic free amino acid (FAA) pattern, resulting from the enzymatic degradation of peptides by various enzymes and also from amino acid interconversion and degradation (Polo, Ramos, & Sanchez, 1985). The concentrations of the different amino acids in a cheese are related to the manufacturing technology (type of curd, addition of starters, ripening conditions), duration of ripening and the extent and type of proteolysis (Christensen, Johnson, & Steele, 1995). Certain free amino acids (FAA) are extremely important in flavour and taste development, e.g. Arg is related to bitterness, while Pro, Ser and Asn are related to sweetness (Izco & Torre, 2000). Although the direct contribution of FAA to the typical cheese flavours are somewhat limited, they are associated with the volatile fraction of cheese (Salles et al., 2000) because they are precursors of many volatile aroma compounds (e.g. amines, carboxylic acids, thiols, esters, alcohols, aldehydes and thioesters).

White-brined cheese, like other types of ripened cheese, needs maturation to develop the required properties. White-brined cheeses are in great demand in warm climates and their preservation in brine is necessary. Teleme cheese is a soft white- brined cheese that can be produced using caprine, ovine or bovine milk alone or any mixture of them (Greek Codex Alimentarius, 1998). In Teleme cheese production, traditional yogurt is used as starter by the traditional cheese-makers, while mixtures of commercial starters, including mesophilic starters, are used in industrial production (Anifantakis, 1991). The kind of milk used in cheese production affects the activity of the starter culture and has a direct effect on the free amino acid profile of the cheese.

There are few publications available on amino acid profile of Teleme cheese. Mallatou, Pappa, and Boumba (2004) studied the profile of amino acids of Teleme cheese made from sheep's, goat's or mixture of sheep's-goat's (1:1) milks with mixed thermophilic–mesophilic cultures. Buriana and Farag (1983) determined the amino acid profile of Teleme cheese made from cow, sheep or mixtures (1:2) of these milks with *Lactococcus lactis* subsp. *lactis* and *Lb. casei*. Polychroniadou and Vlachos (1979) investigated the amino acid profile of Teleme cheese made from a mixture of goat's-sheep's (1:9) milk with thermophilic culture, whereas, Alichanidis, Polychroniadou, Tzanetakis, and Vafopoulou (1981) studied the amino acid profile of Teleme cheese from deep-frozen curd made

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with the same type of milk and culture. To our knowledge, there are no reports of the effect of different cultures on the amino acid profile of Teleme cheese.

The objective of this work was to study the effect of three commercial concentrated starters, namely a thermophilic, a mesophilic or a mixture of a thermophilic- mesophilic culture, on free amino acid release of Teleme cheese made from sheep's, goat's or cow's milk, during ripening. These results could serve as a model for future research in this field.

2. Materials and methods

2.1. Experimental design

The experimental design was that described elsewhere (Pappa, Kandarakis, Anifantakis, & Zerfiridis, 2006a). 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: (i) a freeze-dried yogurt thermophilic culture (T), FYS-11 (Rhone-Poulenc Laboratorium, 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 (Hansen's Laboratorium, Denmark), consisting of the microorganisms *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. (the ratio of thermophilic to mesophilic microorganismds was 1:1) and (iii) a freeze-dried concentrated mesophilic culture (M), R-703 (Hansen's Laboratorium, Denmark) consisting of the microorganisms *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. On the day of cheese-making, each kind of milk was used in making the three variables of cultures and nine lots of cheese were obtained. This was repeated five times, on a day after day basis.

2.2. Cheese-making and sampling

Teleme cheese was manufactured as described previously (Pappa et al., 2006a). Briefly, the milk was standardized to casein to fat ratios of 0.75, 0.64 and 0.73 for the sheep, goat and cow milk, respectively, to obtain a first quality cheese that is ⩽56% moisture and ⩾43% fat in dry matter (Greek Codex Alimentarius, 1998). The standardized milk was pasteurized at 63 °C for 30 min and then cooled at 37 °C. After cooling, one of the cultures already mentioned was added to it, at a quantity of 0.5% (v/v). To assist curdling of milk $CaCl_2$ solution (40%, w/v) was added. Rennet powder HA-LA (Hansen's Laboratorium, Horsholm, Denmark) was added at a quantity of 3.0 g per 100 kg milk, at 33 °C. Drainage was under pressure and salting took place in brine (14% NaCl, w/w) for 16 h at 19 °C.

After manufacture, cheeses were ripened in the warm room (19 °C), until both pH and moisture values reached the limits of ⩽4.6 and ⩽56%, respectively and afterwards cheeses were transferred to a cold room (5 °C) for up to 6 months. 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, 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 the cold room.

2.3. Analysis of free amino acids (FAA) and chemical analysis

The FAA were identified as phenylthiocarbamyl (PTC)-derivatives by RP-HPLC using the PICO-Tag amino acid analysis system (Waters, Milford, MA, USA). Samples were analyzed on a WATERS HPLC consisting of a controller (model 600), a solvent pump (model 600E), a helium degasser and a tunable absorbance detector (model 486). The chromatograms were processed using the Millennium 32 software package. Samples were applied using a rheodyne injector (model 7725i), equipped with a 50 μl sample loop. A volume of 20 μl of the amino acids was injected into the column. Separations were conducted at 45 °C and the absorbance was monitored at 254 nm. Run time was 67 min with flow rate 1 ml min^{-1}. HPLC analyses were done in duplicate.

The method of Bidlingmeyer, Cohen, and Tarvin (1984), as described in the PICO Tag amino acid analysis Operator's Manual (1984) was applied. Free amino acids were extracted from cheese as follows: 1 g of cheese was weighed and 10 ml of 0.1 M HCl, containing 0.4 mM methionine sulfone, was added as the internal standard. The mixture was homogenized using an Ultra-Turrax blender, sonicated in a water bath for 20 min and centrifuged at 3000*g* for 10 min; 500 μl of supernatant were diluted (1:1, v/v) with 40% trichloroacetic acid. The mixture was left for 10 min in an ice bath and then centrifuged at 20,000*g* for 10 min; 25 μl of the deproteinized supernatant were vacuum-dried in the PICO-Tag system. 20 μl of a redrying solution (triethylamine: methanol: 1 M sodium acetate 1:2:2, v/v/v) were added to the dried samples, which were vacuum-dried again. 20 μl of derivatizing solution (methanol: water: triethylamine: phenylisothiocyanate 7:1:1:1, v/v/v/v) were added to the dried samples. The samples were incubated for 10 min at room temperature and then they were vacuum-dried. Samples were suspended in 100 μl of Pico Tag sample diluent (Waters, Milford, MA, USA) and were filtered through a HV hydrophilic filter (Millipore) with 0.45 μm pore size before injection. A Waters reverse phase column (3.90 × 30 cm) was used. Identification of the cheese amino acids was achieved by comparing retention times with pure standards.

The working amino acid standard was prepared as described in detail in the PICO Tag amino acid analysis Operator's Manual (1984), except that glutamine (2.5 mM) was added, too (glutamine: acidic and neutral amino acids: basic amino acids 1:1:1, v/v/v). Methionine sulfone was added as an internal standard (standard solution: internal standard 1:1, v/v).

Standard mixtures of basic, acidic and neutral amino acids and methionine sulfone and glutamine were purchased from Sigma (St. Louis, MO, USA). Phenylisothiocyanate (PITC) (sequential grade) and thiethylamine were from Pierce (Rockford, IL, USA). The solvents were of HPLC grade. All other reagents were analytical grade. The solvent system consisted of two eluents made according to the Operator's Manual (1984). Eluent A comprised 70 mM sodium acetate adjusted to pH 6.55 with acetic acid containing 2.5% acetonitrile. Eluent B was 45% acetonitrile, 40% water and 15% methanol. Before each injection, the column was equilibrated with solvent A for 20 min. Gradient elution was as follows: 0–3% eluent B over 13.5 min using curve 11, then 3–6% eluent B over 10.5 min using curve 8, then 6–9% eluent B over 6 min using curve 5, then 9–34% eluent B over 20 min using curve 6, holding at 34% eluent B for 12 min, then followed by a purge at 100% eluent B for 4.5 min and re-equlibration to 100% eluent A. Curves 5, 6, 8 and 11 are gradients programmed by the Waters HPLC control station. Curves 5, 6 and 8 are convex, linear and concave gradients, respectively, and curve 11 maintains starting conditions until the next step.

The dry matter (DM) of the cheese samples was determined according to the IDF standard (IDF, 1986), and the results were expressed on a dry matter basis.

2.4. Statistical analysis

A three-way split–splot analysis of variance (ANOVA) was applied to the data, considering the type of culture (thermophilic, mixed thermophilic–mesophilic or mesophilic) and the kind of milk (sheep's, goat's or cow's) as factors and ripening time as split–splot factor. Moreover, a comparison of the values of each parameter of Teleme cheese manufactured with one type of milk (sheep's, goat's or cow's) with the three types of culture (thermophilic, mixed thermophilic–mesophilic or mesophilic), at each age, was made. One-way analysis of variance was used for the comparison of the data of each parameter of Teleme cheese made with one type of milk and one type of culture, at different ages. The least significant differences were obtained using the LSD test ($P < 0.05$). Principal component analysis (PCA) was used to reduce the dimensionality of the data and stepwise discriminant analysis was also applied to establish those amino acids capable of discriminating and classifying cheeses by the type of starter, the type of milk and the stage of ripening. Wilk's lambda (λ) criterion was used for selecting discriminant variables. Linear regression analyses were applied to fit the evolution of free amino acids with ripening time.

Statistical analysis was performed using statistical packages (SPSS for Windows version 14.0, SPSS Inc., Chicago, IL, USA, STATGRAPHICS Plus for Windows version 5.2, Manugistics Inc., Rockville, MD, USA and GraphPad Prism version 4, San Diego, CA, USA).

3. Results and discussion

3.1. General

Amino acids were identified according to their retention times by comparison with a standard mixture solution chromatogram. During the extraction and derivatization process, a number of unidentified peaks were present, together with the intact FAA. However, generally, these peaks did not interfere with the identified peaks. When necessary, standard solutions of pure amino acids were co-injected with the standard mixture solution to identify peaks in the standard solution.

Principal component analysis (PCA) was applied to pooled data in order to reduce the dimensionality of the data and to detect the most important causes of variability, since a great correlation between the free amino acid contents was noticed. PCA of the 27 free amino acids resulted in eight principal components with eigenvalues greater than 1.0, a common statistical cut-off point. The eight selected components accounted for 79.14% of the total variability. In Fig. 1, free amino acids were represented as a function of both first and second principal components. The first principal component explained 30.75% of the total variability, and was mainly defined by TFAA, Asn, Gly and Lys. These amino acids were placed closed together on the negative side of the horizontal axis, indicating that they were positively correlated with each other. They were away from the axis origin, suggesting that they were well represented from the first PC, which could be considered as representative of the ripening time, because all cheeses, from 1 to 60 days old, were clustered on the positive side of PC1, while cheeses 120 to 180 days old were placed on the negative side of PC1 (Fig. 1). All the cheeses that were located on the left of Fig. 1 are ripened 180 d old cheeses, having the highest contents of TFAA, Asn, Gly and Lys. The second principal component explained

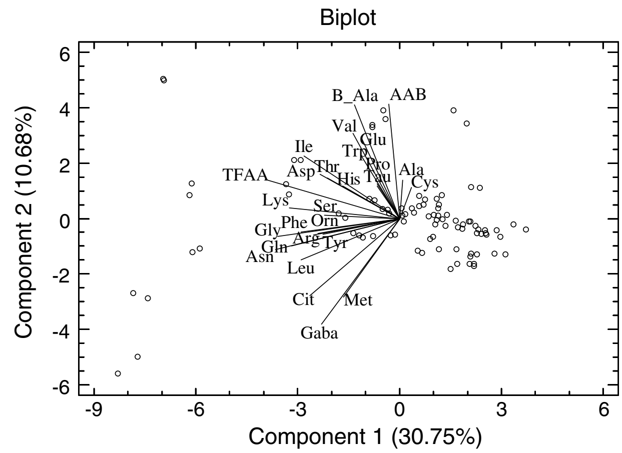

Fig. 1. Principal component analysis. Plot of the free amino acids as a function of the two first principal components.

10.68% of the total variability and was defined by AAB, β-Ala (positive values) and Gaba (negative value). In Fig. 2 AAB and β-Ala were placed close together on the positive side of PC2, indicating a high positive correlation. On the other hand, these amino acids were lying opposite to Gaba; therefore they were negatively correlated to this. Cheeses made with thermophilic culture were clustered near AAB and β-Ala and therefore had higher contents of these amino acids, than cheeses made with the other cultures, which represented the opposite characteristics. Consequently the second principal component could be a representative of the type of culture. The third principal component explained another 10.42% of the total variability and was defined by Pro, Ala (negative values) and Ser (positive value). Cheeses made from goat's milk were clustered on the negative side of PC3, near Pro and Ala and therefore had the highest contents of Pro and Ala (Fig. 2). On the other hand, these cheeses had the lowest content of Ser. Cheeses made from sheep's or cow's milk had the opposite characteristics and were placed on the positive side of PC3. Consequently, the third principal component could be a representative of the kind of milk. The first three principal components were the most important; together they accounted for 51.85% of the total variability and had eigenvalues greater than 2.8.

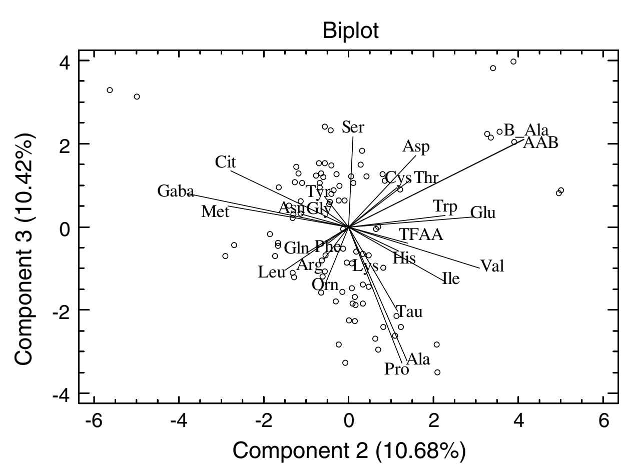

Fig. 2. Principal component analysis. Plot of the free amino acids as a function of the second and third principal components.

3.2. Effect of ripening time

The results of the three-way split–splot ANOVA (Table 1) showed that the main effects, kind of milk, type of culture and ripening time, were significant ($P < 0.001$) for the total free amino acids (TFAA) of Teleme cheese and for all of the individual free amino acids, except Ala, which did not change significantly during ripening time ($P > 0.05$) and Tau, which was not significantly different with respect to starter addition ($P > 0.05$). All two-way and three-way interactions between the factors were significant ($P < 0.05$) for the TFAA and for all individual free amino acids. Table 1 also shows the effect size (>0.8 in most cases), which describes the proportion of total variability attributable to a factor. Fig. 3 presents the evolution of mean total free amino acids (TFAA) of Teleme cheese during ripening. Table 2 shows the concentrations of individual FAA of Teleme cheese made with different starter cultures and milks during ripening. In Table 2 it can be seen that the concentrations of almost all FAA showed a clear tendency to increase with ripening time, as was expected since, during proteolysis, these compounds are released by proteolytic agents. Pappa, Kandarakis, Anifantakis, Zerfiridis, and Sotirakoglou (2006b) reported an increase of proteolysis during cheese maturation, as estimated by the increase of nitrogen faction, decrease of the intact casein content, increase of hydrophilic peptides and decrease of hydrophobic peptides. The increase of TFFA at all stages of ripening, in this study, is in accordance with the results found by Mallatou et al. (2004). Buriana and Farag (1983) found that the TFFA decreased after 180 days of ripening and re-increased rapidly until day 360.

Generally, TFFA mean content of Teleme cheese increased significantly from 295 mg 100 g^{-1} DM in 1 day old cheese to 316 mg 100 g^{-1} DM on the day cheeses were transferred to the cold room, to 424 mg 100 g^{-1} DM in 60 day old cheese, to 492 mg 100 g^{-1} DM in 120 day old cheese and finally to 675 mg 100 g^{-1} DM in180 day old cheese ($P < 0.001$). The levels of TFFA found in this study, are within the range reported from other studies. Barcina, Ibanez, and Ordonez (1995) reported an increase from 200 mg 100 g^{-1} DM in 1 day old to 1300 mg 100 g^{-1} DM in Idiazabal cheese. The FAA in Manchego cheese typically contribute 1728 mg 100 g^{-1} DM, in Cheddar cheese 1592 mg 100 g^{-1} DM, in Edam cheese 1056 mg 100 g^{-1} DM and in Romano cheese 650 mg 100 g^{-1} DM (Tavaria, Franco, Javier Carballo, & Xavier Malcata, 2003).

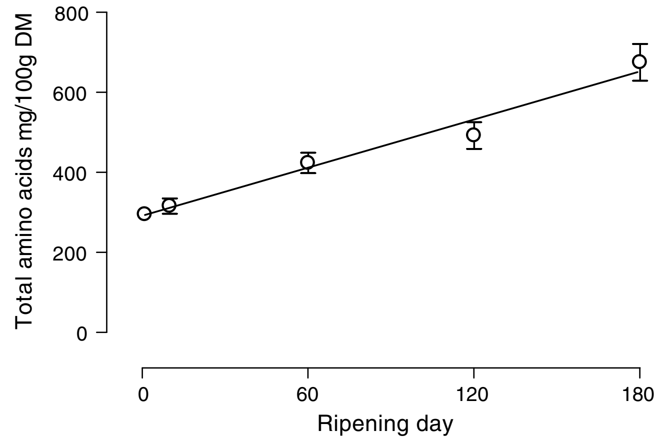

Fig. 3. Evolution of total free amino acids during the ripening time.

From Table 2 and Fig. 4 it can be seen that Leu, Glu, Phe, Val and Lys were the major FAA of Teleme cheese at all stages of ripening, regardless of the type of milk and culture used. At the age of 180 days, they accounted for 41.5% of the TFFA, regardless of the type of milk and culture used. The same FAA were found in abundance in Teleme cheese by Mallatou et al. (2004) and Polychroniadou and Vlachos (1979), as well as in other cheeses (Hayaloglou, Guven, Fox, & McSweeney, 2005; Hayaloglou, Guven, Fox, Hannon, & McSweeney, 2004; Vicente, Ibanez, Barcina, & Barron, 2001; Barcina et al., 1995). The proteolysis of α_{s1}-casein, which has a high content

Table 1
Significance levels and size effect of the three-way split–splot ANOVA for the effects, kind of milk (M), type of culture (C) and ripening time (T)

Amino acids	M		C		T		MC		MT		CT		MCT	
	Sig.	Effect size	Sig.	Effect size	Sig.	Effect size	Sig.	Effect size	Sig.	Effect size	Sig.	Effect size	Sig.	Effect size
AAB	***	0.990	***	0.993	***	0.634	***	0.993	***	0.909	***	0.577	***	0.938
Ala	***	0.988	***	0.965	NS	0.078	***	0.925	***	0.590	***	0.732	***	0.740
Arg	***	0.915	***	0.942	***	0.883	***	0.896	***	0.875	***	0.620	***	0.833
Asn	***	0.979	***	0.974	***	0.996	***	0.979	***	0.971	***	0.948	***	0.954
Asp	***	0.966	***	0.897	***	0.940	***	0.980	***	0.911	***	0.905	***	0.952
Bala	***	0.988	***	0.986	***	0.736	***	0.996	***	0.683	***	0.493	***	0.824
Cit	***	0.994	***	0.965	***	0.991	***	0.989	***	0.990	***	0.983	***	0.991
Cys	***	0.861	*	0.509	***	0.932	***	0.938	***	0.867	***	0.598	***	0.921
Gaba	***	0.966	***	0.852	***	0.934	**	0.823	***	0.971	***	0.901	***	0.931
Gln	***	0.938	***	0.943	***	0.970	***	0.940	***	0.863	***	0.882	***	0.913
Glu	***	0.984	***	0.993	***	0.990	***	0.939	***	0.980	***	0.979	***	0.953
Gly	***	0.946	***	0.923	***	0.971	***	0.849	***	0.912	***	0.902	***	0.839
His	***	0.939	***	0.965	***	0.958	***	0.973	***	0.872	***	0.880	***	0.980
Ile	***	0.806	***	0.982	***	0.974	**	0.824	***	0.868	***	0.964	***	0.934
Leu	***	0.985	***	0.925	***	0.986	***	0.985	***	0.958	***	0.866	***	0.967
Lys	***	0.964	***	0.991	***	0.994	***	0.977	***	0.960	***	0.936	***	0.949
Met	***	0.944	***	0.860	***	0.970	***	0.960	***	0.957	***	0.829	***	0.930
Orn	***	0.986	***	0.982	***	0.983	***	0.952	***	0.962	***	0.903	***	0.984
Phe	***	0.980	***	0.993	***	0.964	***	0.940	***	0.926	***	0.961	***	0.900
Pro	***	0.886	***	0.994	***	0.913	***	0.894	***	0.912	***	0.903	***	0.930
Ser	***	0.990	*	0.550	***	0.908	***	0.984	***	0.869	***	0.811	***	0.962
Tau	***	0.910	NS	0.434	***	0.742	*	0.668	**	0.423	***	0.796	***	0.900
Thr	***	0.975	***	0.990	***	0.934	***	0.989	***	0.928	***	0.907	***	0.916
Trp	***	0.995	***	0.990	***	0.908	***	0.993	***	0.949	***	0.966	***	0.984
Tyr	***	0.985	***	0.937	***	0.966	***	0.988	***	0.959	***	0.951	***	0.970
Val	***	0.985	***	0.981		0.984	***	0.989	***	0.978	***	0.953	***	0.973
TFFA	***	0.988	***	0.997	***	0.996	***	0.994	***	0.926	***	0.939	***	0.914

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, NS: not significant.

Table 2a

Changes of amino acid contents (mg 100 g^{-1} DM[A]) 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

Age, (days)	1									R[B]								
Milk	S			G			C			S			G			C		
Culture	T	MX	M	T	MX	M	T	MX	M	T	MX	M	T	MX	M	T	MX	M
Asp	1.5^{1a}	9.9^{2a}	1.5^{1a}	0.8^{1ab}	2.7^{2b}	1.7^{12a}	0.2^{1b}	3.0^{2b}	2.1^{2a}	1.9^{1a}	2.0^{1a}	1.0^{2a}	1.8^{1a}	3.4^{1a}	2.4^{1a}	1.1^{1b}	2.9^{1a}	2.7^{1a}
Glu	30.9^{1a}	16.9^{2a}	15.4^{2a}	20.8^{12ab}	12.8^{1a}	25.3^{12b}	15.0^{1b}	16.7^{1a}	33.7^{2c}	36.2^{1a}	29.1^{2a}	26.5^{2a}	46.8^{1b}	26.4^{2a}	21.5^{3b}	55.0^{1c}	37.9^{2b}	33.4^{3c}
Ser	3.8^{1a}	6.7^{12a}	8.1^{2a}	1.1^{1b}	0.0^{1b}	0.8^{1b}	4.8^{1a}	8.8^{2a}	5.2^{1c}	6.6^{1a}	1.2^{2a}	0.3^{3a}	6.3^{1a}	0.0^{2b}	0.5^{2a}	3.9^{1b}	7.0^{2c}	4.9^{1b}
Asn	12.0^{1a}	4.2^{2a}	5.5^{2a}	0.6^{1b}	5.9^{2a}	2.9^{3b}	1.2^{1b}	3.4^{2a}	4.4^{2ab}	7.0^{1a}	2.1^{2a}	4.0^{2a}	4.2^{1b}	5.1^{1b}	1.5^{2b}	3.3^{1b}	3.3^{1ab}	1.1^{2b}
Gly	6.1^{1a}	2.4^{2a}	1.4^{2a}	0.8^{1b}	1.7^{12a}	2.4^{2a}	5.5^{1ab}	6.1^{1b}	4.2^{1b}	1.9^{1a}	0.7^{2a}	0.5^{2a}	8.1^{1b}	2.7^{2b}	2.0^{2b}	7.2^{1b}	7.8^{1c}	2.2^{2b}
Gln	7.1^{1a}	3.7^{1ab}	13.0^{2a}	9.0^{1a}	1.7^{2a}	4.7^{3b}	17.8^{1b}	15.0^{1b}	5.9^{1b}	2.1^{1a}	11.1^{2ab}	5.3^{3a}	23.2^{1b}	9.9^{2a}	7.9^{2a}	26.5^{1b}	19.8^{1b}	7.9^{2a}
β-Ala	16.2^{1a}	3.1^{2ab}	0.1^{2a}	7.8^{1b}	1.0^{2a}	4.9^{3b}	0.8^{1c}	5.8^{2b}	8.6^{3c}	18.6^{1a}	1.7^{2a}	6.8^{3a}	3.8^{1b}	3.2^{1a}	1.0^{2b}	2.0^{1b}	6.9^{2b}	5.1^{2c}
Tau	2.3^{1a}	3.9^{1a}	6.0^{2a}	11.8^{1b}	5.0^{2a}	0.3^{3b}	0.7^{1a}	3.3^{1a}	1.3^{1b}	5.0^{1a}	2.6^{2a}	2.4^{2a}	3.9^{1a}	5.8^{1a}	6.7^{1b}	0.4^{1b}	3.2^{1a}	3.0^{1a}
His	4.7^{1a}	11.2^{2a}	11.8^{2a}	0.1^{1b}	16.8^{2a}	17.4^{2b}	11.1^{12c}	19.7^{1a}	5.9^{2c}	1.0^{1a}	8.2^{2a}	10.9^{3a}	21.2^{12b}	14.4^{1a}	25.1^{2b}	11.4^{1c}	22.0^{2b}	1.4^{3c}
Gaba	3.6^{1a}	3.7^{1a}	0.6^{2a}	0.1^{1b}	6.7^{2b}	13.4^{3b}	2.4^{1a}	4.4^{2a}	8.3^{3c}	0.7^{1a}	3.6^{2a}	2.2^{12a}	3.2^{1b}	5.5^{2a}	7.7^{2b}	3.7^{1b}	5.7^{1a}	10.3^{2b}
Cit	0.9^{1a}	0.9^{1a}	0.0^{2a}	0.1^{1a}	1.0^{12a}	1.2^{2a}	0.6^{1a}	1.3^{1a}	6.0^{2b}	2.8^{1a}	0.5^{2a}	1.0^{2a}	2.7^{1a}	0.7^{2a}	0.2^{2a}	1.3^{1a}	3.0^{1b}	14.8^{2b}
Thr	5.2^{1a}	8.4^{12a}	10.2^{2a}	8.7^{1a}	6.0^{1a}	6.1^{1b}	17.1^{1b}	24.4^{2b}	7.0^{3b}	84.2^{1a}	13.5^{2a}	9.1^{2a}	23.2^{1b}	13.4^{2a}	10.9^{2a}	13.8^{1b}	12.4^{1a}	1.3^{2b}
Ala	3.2^{1a}	1.0^{2a}	0.0^{3a}	20.9^{1b}	1.6^{2a}	7.9^{3b}	8.0^{1a}	0.2^{2a}	0.2^{2a}	1.7^{1a}	1.8^{1a}	0.0^{2a}	15.6^{1b}	3.2^{2a}	11.4^{12b}	4.0^{1a}	0.4^{2a}	0.3^{2a}
Arg	27.6^{1a}	6.0^{2a}	11.5^{2a}	32.8^{1a}	9.6^{2ab}	2.6^{3b}	15.9^{1b}	14.8^{1b}	6.8^{2c}	2.5^{1a}	15.0^{2a}	10.5^{3a}	34.3^{1b}	16.9^{2a}	16.5^{2a}	35.7^{1b}	28.0^{1b}	28.2^{1b}
Pro	7.8^{1a}	9.2^{1a}	13.3^{2a}	16.1^{1b}	19.1^{1b}	38.5^{2b}	7.0^{1a}	6.8^{1a}	0.7^{2c}	4.5^{1a}	8.1^{2a}	11.8^{3a}	22.1^{1b}	22.7^{1b}	23.6^{1ab}	9.8^{1c}	8.1^{1a}	29.8^{2b}
AAB	26.2^{1a}	10.4^{2a}	3.9^{3a}	28.0^{1a}	0.0^{2b}	0.1^{2a}	3.1^{1b}	10.7^{2a}	34.3^{3b}	56.2^{1a}	14.0^{2a}	20.2^{3a}	7.2^{1b}	7.2^{1b}	4.4^{1b}	12.8^{1b}	12.6^{1a}	13.5^{1c}
Tyr	11.4^{1a}	1.8^{2a}	5.0^{2a}	36.8^{1b}	7.8^{2b}	3.8^{2a}	9.4^{1a}	16.1^{2c}	5.6^{1a}	4.2^{1a}	6.3^{12a}	7.2^{2a}	2.7^{1a}	5.1^{2a}	5.0^{2ab}	13.8^{1b}	19.5^{2b}	3.9^{3b}
Val	16.3^{1a}	49.2^{2a}	3.2^{3a}	51.3^{1b}	5.3^{2b}	8.9^{2b}	18.4^{1a}	52.3^{2a}	34.1^{3c}	67.6^{1a}	11.9^{2a}	3.7^{3a}	43.5^{1b}	16.4^{2b}	4.9^{3a}	9.0^{1c}	4.1^{2c}	8.7^{1b}
Met	18.9^{12a}	23.9^{1a}	13.7^{2a}	35.4^{1b}	23.3^{2a}	16.3^{3a}	19.5^{1a}	13.9^{1b}	14.2^{2a}	84.8^{1a}	17.5^{2a}	3.1^{3a}	29.8^{1b}	68.0^{2b}	58.3^{2b}	23.9^{1b}	16.1^{2a}	32.4^{3c}
Cys	6.0^{1a}	3.5^{2a}	0.9^{3a}	19.6^{1b}	7.3^{2b}	17.0^{1b}	5.2^{1a}	2.7^{1a}	13.1^{2b}	4.1^{1}	2.5^{2a}	2.0^{2a}	6.2^{1a}	3.9^{1a}	3.1^{1a}	6.7^{1a}	10.3^{1b}	10.0^{1b}
Ile	40.3^{1a}	8.8^{2a}	12.0^{2a}	1.9^{1b}	11.5^{2a}	15.0^{3b}	0.9^{1b}	4.6^{2b}	0.7^{1c}	8.4^{1a}	21.1^{2a}	10.8^{1a}	9.1^{1a}	18.3^{2a}	11.1^{1a}	2.9^{1b}	1.6^{1b}	2.7^{1b}
Leu	23.1^{1a}	11.2^{2a}	9.7^{2a}	38.0^{1a}	17.6^{2a}	29.4^{12b}	25.2^{12a}	32.8^{1b}	17.5^{2a}	3.6^{1a}	14.2^{2a}	15.9^{2a}	44.5^{1b}	24.4^{2a}	31.3^{2b}	22.3^{1c}	37.0^{2b}	0.5^{3c}
Phe	22.0^{1a}	8.5^{2a}	11.1^{2a}	12.5^{1a}	12.8^{1ab}	19.5^{2b}	33.8^{1b}	24.4^{1b}	7.9^{2a}	16.1^{1a}	35.0^{2a}	8.2^{1a}	37.1^{1b}	31.5^{12a}	26.5^{2b}	21.8^{1a}	43.6^{2a}	35.5^{2b}
Trp	2.8^{1a}	4.5^{1a}	2.8^{1a}	0.9^{1b}	3.6^{1a}	1.8^{1a}	4.2^{1c}	3.7^{1a}	1.5^{1a}	2.1^{12a}	3.3^{1a}	1.0^{2a}	9.1^{1b}	1.2^{2a}	3.1^{2a}	1.8^{1a}	1.2^{1a}	0.8^{1a}
Orn	14.3^{1a}	6.1^{2a}	6.7^{2a}	0.2^{1b}	11.1^{2b}	12.3^{2b}	7.3^{12c}	11.5^{1b}	3.8^{2c}	0.8^{1a}	8.6^{2a}	6.0^{2ab}	16.4^{1b}	13.5^{1b}	8.8^{2a}	6.0^{1c}	3.3^{1c}	2.7^{1b}
Lys	66.7^{1a}	14.9^{2a}	7.5^{2a}	64.9^{1a}	73.0^{1b}	38.2^{2a}	63.6^{1a}	14.6^{2a}	40.3^{3a}	2.7^{1a}	6.9^{2a}	8.1^{3a}	19.6^{1b}	6.8^{2a}	7.6^{3a}	17.3^{1b}	23.4^{1b}	2.1^{2b}

[1–3]: For each age and each kind of milk, different numbers indicate significant differences ($P < 0.05$) between kinds of cultures.
[a–c]: For each age and each kind of culture, different letters indicate significant differences ($P < 0.05$) between kinds of milk.
[A] DM: Dry Matter, %.
[B] R: Day cheeses were transferred to cold room.

Table 2b

Changes of amino acids content (mg 100 g^{-1} DM[A]) 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

Age, (days)	60									120								
Milk	S			G			C			S			G			C		
Culture	T	MX	M	T	MX	M	T	MX	M	T	MX	M	T	MX	M	T	MX	M
Asp	5.1^{1a}	1.4^{2ab}	0.5^{2a}	0.1^{1b}	0.3^{1a}	0.5^{1a}	1.6^{1c}	2.1^{1b}	4.3^{2b}	8.0^{1a}	2.1^{2a}	1.1^{2a}	2.4^{1b}	2.3^{1a}	0.4^{2a}	1.4^{1b}	9.1^{2b}	4.4^{3b}
Glu	122.9^{1a}	91.1^{2a}	13.6^{3a}	28.4^{1b}	37.9^{1b}	30.9^{1b}	157.0^{1c}	147.0^{1c}	84.0^{2c}	114.0^{1a}	78.5^{2a}	28.9^{3a}	140.0^{1a}	38.3^{2b}	16.1^{2a}	134.0^{1b}	101.0^{12a}	64.0^{2b}
Ser	5.8^{1a}	2.9^{2a}	6.7^{1a}	0.5^{1b}	0.0^{1b}	5.8^{2a}	6.2^{1a}	8.7^{2c}	3.9^{1a}	4.3^{1a}	3.8^{1a}	7.5^{1a}	5.0^{1a}	0.0^{2b}	0.1^{2b}	3.1^{1a}	12.8^{2c}	6.4^{1a}
Asn	5.8^{1a}	1.2^{2a}	6.4^{1a}	0.5^{1b}	0.9^{1a}	0.8^{1b}	6.7^{1a}	4.8^{2b}	2.1^{3b}	3.5^{1a}	2.6^{1a}	7.2^{2a}	6.0^{1b}	5.5^{1b}	1.4^{2b}	3.5^{1a}	11.6^{2c}	2.8^{1b}
Gly	5.8^{1a}	3.3^{1a}	3.5^{1a}	1.1^{1b}	1.8^{1a}	3.1^{2a}	0.9^{1b}	10.7^{2b}	1.8^{1a}	5.6^{1a}	2.2^{2a}	4.5^{12a}	7.9^{1b}	3.8^{2a}	1.7^{3b}	4.9^{1a}	4.4^{1a}	4.5^{1a}
Gln	8.9^{1a}	24.3^{2a}	7.9^{1a}	3.9^{1a}	12.3^{12a}	16.7^{2b}	30.5^{1b}	15.2^{2a}	5.1^{2a}	12.2^{1a}	15.7^{1a}	28.9^{2a}	32.3^{1b}	14.3^{2a}	2.9^{3b}	17.0^{1a}	30.1^{2b}	8.6^{1c}
β-Ala	23.0^{1a}	3.7^{2a}	7.5^{2a}	2.9^{1b}	4.1^{1a}	3.0^{1a}	2.4^{1b}	6.6^{2a}	6.0^{2a}	23.9^{1a}	4.5^{2a}	6.8^{2a}	7.9^{1b}	6.4^{12a}	2.6^{2b}	1.7^{1c}	7.4^{2a}	8.8^{2a}
Tau	3.0^{1a}	3.3^{1a}	2.2^{1a}	3.0^{1a}	11.7^{2b}	6.5^{3b}	3.8^{1a}	2.9^{12a}	1.0^{2a}	3.1^{1a}	5.3^{1a}	4.2^{1a}	5.8^{12b}	3.3^{1a}	8.1^{2b}	2.3^{1a}	6.8^{1a}	1.5^{1a}
His	13.3^{1a}	13.3^{1a}	17.4^{1a}	8.9^{1b}	17.2^{12a}	26.6^{2b}	0.8^{1c}	40.2^{2b}	23.6^{3ab}	9.1^{1a}	11.4^{1a}	19.5^{2a}	27.7^{1b}	18.7^{2ab}	23.3^{12a}	14.0^{1a}	27.7^{2b}	48.2^{3b}
Gaba	3.0^{1ab}	3.1^{1a}	4.7^{1a}	5.3^{1a}	4.2^{1ab}	13.0^{2b}	1.1^{1b}	6.2^{2b}	5.7^{2a}	5.0^{1a}	3.8^{1a}	4.4^{1a}	3.6^{1a}	8.5^{2b}	4.7^{1a}	3.9^{1a}	7.9^{1b}	14.4^{2b}
Cit	3.0^{1a}	1.6^{2a}	0.9^{2ab}	0.5^{1b}	1.6^{2a}	0.1^{1a}	1.0^{1b}	2.7^{2a}	1.2^{1b}	2.0^{1ab}	3.7^{1a}	3.2^{1a}	3.1^{1a}	2.6^{1a}	0.0^{2b}	1.6^{1b}	3.9^{1a}	3.1^{1a}
Thr	15.5^{1a}	13.4^{1a}	13.7^{1a}	8.0^{1b}	9.5^{1a}	9.7^{1a}	19.4^{1c}	41.8^{2b}	26.6^{3b}	22.4^{1a}	20.3^{1a}	13.4^{1a}	31.5^{1b}	15.4^{2a}	12.4^{2a}	18.2^{1a}	18.0^{1a}	23.7^{1b}
Ala	4.0^{1a}	1.0^{2a}	0.0^{3a}	12.0^{1b}	11.1^{1b}	7.4^{1b}	3.9^{1a}	0.3^{2a}	0.3^{2a}	3.4^{1a}	4.4^{1a}	0.0^{2a}	13.2^{3b}	4.5^{1a}	11.8^{1b}	5.8^{1a}	0.1^{2b}	0.5^{a}
Arg	29.4^{1a}	16.5^{2a}	11.9^{2a}	20.4^{12b}	25.2^{1b}	14.6^{2a}	13.9^{1c}	15.6^{1b}	15.3^{1a}	20.2^{1a}	20.6^{1a}	16.4^{1a}	40.0^{1b}	25.0^{2ab}	10.9^{3a}	37.4^{1b}	34.2^{1b}	34.8^{1b}
Pro	8.1^{1a}	11.0^{1a}	8.4^{1a}	16.1^{1b}	33.7^{2b}	21.6^{1b}	19.2^{1b}	9.0^{2a}	10.2^{2a}	15.6^{1a}	30.5^{2a}	8.7^{3a}	31.9^{1b}	20.6^{2b}	23.1^{2b}	11.4^{1a}	5.4^{2c}	10.4^{1a}
AAB	46.2^{1a}	10.7^{2a}	12.9^{2a}	21.6^{1b}	3.8^{2b}	20.8^{1b}	17.1^{1c}	12.0^{2a}	5.2^{3c}	46.1^{1a}	20.8^{2a}	18.1^{2a}	12.6^{1b}	0.5^{2b}	12.8^{1ab}	8.9^{1b}	11.6^{2c}	9.9^{12a}
Tyr	6.1^{1a}	21.4^{2a}	6.1^{1a}	4.9^{1a}	11.2^{2b}	12.1^{2b}	12.6^{1b}	26.5^{2a}	30.7^{2c}	11.0^{1a}	6.4^{2a}	13.2^{1a}	4.0^{1b}	7.6^{2a}	3.4^{1b}	13.5^{1a}	44.1^{2b}	34.8^{2c}
Val	28.1^{1a}	6.4^{2a}	9.5^{2a}	38.4^{1b}	11.8^{2a}	37.9^{1b}	17.5^{1c}	28.3^{2b}	67.2^{3c}	70.2^{1a}	40.6^{2a}	13.6^{3a}	103.0^{1b}	77.1^{1b}	135.0^{2b}	52.4^{1a}	28.5^{2a}	36.6^{2c}
Met	20.0^{12a}	22.6^{1a}	15.4^{2a}	36.0^{1b}	22.4^{2a}	19.3^{2ab}	21.6^{1a}	37.0^{2b}	22.9^{1b}	16.6^{1a}	6.1^{2a}	12.2^{1a}	29.4^{1b}	10.0^{2b}	9.9^{2a}	12.9^{1a}	48.2^{2c}	61.5^{3b}
Cys	6.7^{1a}	4.5^{1a}	5.7^{1a}	38.5^{1b}	10.9^{2b}	3.2^{2a}	4.9^{1a}	14.0^{2c}	10.3^{3b}	5.1^{1a}	2.2^{2a}	1.8^{2a}	7.7^{1a}	3.9^{2a}	3.2^{2a}	4.3^{1a}	11.9^{2b}	16.5^{3b}
Ile	35.6^{1a}	18.5^{2a}	10.5^{3a}	35.3^{1a}	4.4^{2b}	13.0^{3a}	0.9^{1b}	5.5^{2b}	4.2^{2b}	22.9^{1a}	2.6^{2a}	11.0^{3a}	9.3^{1b}	13.9^{2b}	5.7^{3b}	3.2^{1c}	24.7^{2c}	3.1^{1c}
Leu	16.4^{1a}	26.2^{2a}	20.4^{12a}	132.2^{1b}	33.4^{2a}	38.0^{2b}	17.1^{1a}	51.5^{2b}	32.4^{3b}	18.7^{1a}	21.4^{1a}	23.0^{1a}	52.8^{1b}	36.1^{2b}	19.8^{3a}	22.7^{1a}	51.6^{2c}	59.2^{2b}
Phe	67.0^{1a}	4.7^{2a}	2.7^{2a}	72.7^{1a}	25.1^{2b}	3.0^{3a}	43.1^{1b}	3.2^{2a}	3.5^{2a}	26.3^{1a}	38.7^{2a}	14.7^{3a}	43.8^{1b}	19.1^{2b}	8.0^{3a}	41.7^{1b}	46.7^{1c}	38.8^{1b}
Trp	7.2^{1a}	3.4^{1a}	5.3^{1a}	8.5^{12a}	10.9^{1b}	5.9^{2a}	9.1^{1a}	1.3^{2a}	1.1^{2b}	6.3^{1a}	9.5^{2a}	8.1^{12a}	15.6^{1b}	8.2^{2a}	3.0^{3b}	9.1^{1a}	30.2^{2b}	3.3^{3b}
Orn	7.6^{1a}	6.3^{1a}	4.7^{1a}	6.5^{1a}	9.2^{1ab}	7.9^{1b}	1.1^{1b}	11.8^{2b}	6.7^{3ab}	4.1^{1a}	9.9^{2a}	3.3^{1a}	49.3^{1b}	27.6^{2b}	9.8^{3b}	8.0^{1a}	28.4^{2b}	16.0^{3c}
Lys	59.2^{1a}	24.0^{2a}	5.9^{3a}	31.5^{1b}	77.4^{2b}	58.6^{2b}	51.9^{1a}	18.4^{2a}	42.8^{3c}	66.8^{1a}	11.0^{2a}	5.0^{2a}	71.1^{1a}	81.9^{2b}	40.7^{3b}	48.5^{1b}	34.1^{2c}	7.9^{3a}

[1–3]: For each age and each kind of milk, different numbers indicate significant differences ($P < 0.05$) between kinds of cultures.
[a–c]: For each age and each kind of culture, different letters indicate significant differences ($P < 0.05$) between kinds of milk.
[A] DM: Dry Matter, %.

Table 2c
Changes of amino acids content (mg 100 g^{-1} DM[A]) 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

Age, (days)	180								
Milk	S			G			C		
Culture	T	MX	M	T	MX	M	T	MX	M
Asp	15.3^{1a}	4.7^{2a}	3.3^{2a}	1.6^{1b}	1.9^{1b}	2.5^{1a}	4.1^{1c}	8.1^{2c}	7.0^{2b}
Glu	86.1^{1a}	38.2^{2a}	59.9^{3a}	63.9^{1b}	19.4^{2b}	66.1^{1b}	61.0^{1b}	31.6^{2c}	23.1^{3c}
Ser	16.2^{1a}	5.9^{2a}	6.2^{2a}	5.5^{1b}	0.0^{2b}	0.1^{2b}	1.1^{1c}	14.8^{2c}	26.2^{3c}
Asn	49.2^{1a}	23.0^{2a}	24.5^{2a}	62.1^{1b}	23.3^{2a}	14.0^{3a}	56.0^{1b}	74.4^{2b}	52.6^{1b}
Gly	14.5^{1a}	4.1^{2a}	5.0^{2a}	14.0^{1a}	5.5^{2b}	5.8^{2a}	21.3^{1b}	14.2^{1c}	16.6^{12b}
Gln	32.3^{1a}	23.3^{12}	16.2^{2a}	42.3^{1b}	24.0^{2}	23.8^{2a}	79.8^{1c}	34.6^{2}	34.7^{2b}
β-Ala	25.8^{1a}	0.9^{2a}	9.2^{3a}	7.0^{1b}	2.4^{2a}	5.2^{3b}	5.0^{1b}	9.4^{2b}	5.9^{12b}
Tau	6.4^{1a}	5.8^{1a}	8.5^{2a}	7.4^{1a}	3.2^{1a}	13.6^{2b}	1.0^{1b}	6.0^{2a}	7.1^{2a}
His	52.1^{1a}	30.9^{2a}	6.2^{3a}	29.6^{1b}	36.0^{2a}	34.5^{12b}	1.0^{1c}	3.2^{1b}	67.6^{2c}
Gaba	1.8^{1a}	7.1^{2a}	1.7^{1a}	3.3^{1b}	5.6^{2a}	6.0^{2b}	29.9^{1c}	36.4^{1b}	12.4^{2c}
Cit	33.9^{1a}	14.6^{2a}	3.5^{3a}	18.4^{1b}	28.1^{2b}	4.0^{3a}	15.5^{1b}	7.7^{2c}	9.4^{2b}
Thr	31.6^{1a}	20.0^{2a}	20.4^{2a}	38.3^{1a}	25.5^{2a}	34.2^{12b}	45.6^{1b}	41.5^{1b}	30.9^{2ab}
Ala	4.7^{1a}	1.2^{2ab}	8.9^{3a}	9.5^{1b}	3.6^{2a}	9.8^{1a}	0.2^{1c}	0.1^{1b}	0.1^{1b}
Arg	39.4^{1a}	16.2^{2a}	1.5^{3a}	105.0^{1b}	9.7^{2b}	4.4^{2b}	28.6^{1a}	53.7^{2c}	32.7^{1c}
Pro	34.4^{1a}	18.2^{2a}	34.4^{1a}	36.4^{1a}	22.8^{2b}	37.0^{1a}	17.4^{1b}	6.9^{2c}	11.2^{12b}
AAB	34.5^{1a}	7.2^{2a}	5.5^{3a}	27.1^{1a}	2.7^{2b}	7.7^{2a}	4.8^{1b}	7.5^{1a}	17.9^{2b}
Tyr	31.2^{1a}	22.0^{2a}	16.4^{3a}	31.5^{1a}	6.1^{2b}	23.6^{3b}	15.6^{1b}	48.7^{2c}	7.3^{3c}
Val	94.5^{1a}	33.2^{2a}	15.3^{3a}	63.0^{1b}	97.4^{2b}	30.8^{3b}	30.5^{1c}	29.4^{1a}	39.8^{2c}
Met	10.9^{1a}	5.9^{2a}	5.3^{2a}	17.8^{1b}	5.0^{2a}	7.8^{2b}	19.4^{1b}	44.1^{2b}	25.1^{1c}
Cys	5.4^{1a}	3.2^{12a}	2.6^{2a}	5.9^{1a}	3.4^{12a}	0.6^{2a}	14.9^{1b}	7.7^{1b}	24.2^{1a}
Ile	34.0^{1a}	8.8^{2a}	7.3^{2a}	27.4^{1ab}	11.5^{2b}	15.6^{3b}	25.1^{1b}	1.6^{2c}	6.3^{2a}
Leu	36.1^{12a}	46.7^{1a}	23.0^{2a}	108.0^{1b}	53.4^{2a}	46.9^{2b}	72.4^{1c}	92.5^{2b}	81.7^{12c}
Phe	38.7^{1a}	43.6^{1a}	16.9^{2a}	60.0^{1b}	34.9^{2a}	26.1^{2b}	81.6^{1c}	57.9^{2b}	60.6^{2c}
Trp	7.7^{1a}	2.7^{2a}	0.0^{3a}	7.6^{1a}	5.6^{1b}	3.3^{2b}	12.3^{1b}	46.1^{2c}	10.7^{1c}
Orn	14.2^{1a}	21.6^{2a}	9.2^{3a}	9.4^{1a}	50.3^{2b}	8.5^{1a}	42.5^{1b}	19.5^{2a}	27.8^{3b}
Lys	150.0^{1a}	89.5^{2a}	63.0^{3a}	112.0^{1a}	70.0^{2b}	56.2^{3b}	132.0^{1c}	112.0^{1c}	105.0^{3c}

[1–3]: For each age and each kind of milk, different numbers indicate significant differences ($P < 0.05$) between kinds of cultures.
[a–c]: For each age and each kind of culture, different letters indicate significant differences ($P < 0.05$) between kinds of milk.
[A] DM: Dry Matter, %.

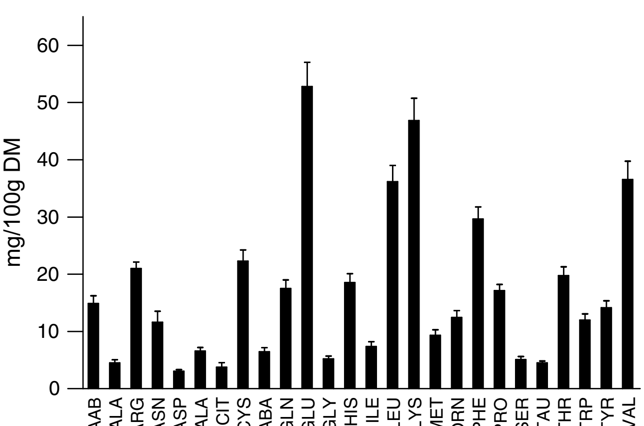

Fig. 4. Free amino acid mean content of Teleme cheese.

of the amino acids Leu, Phe and Val, occurs during the first days of ripening, which predominates over the proteolysis of β-casein.

Teleme cheese samples had high contents of Glu (Fig. 4). Amino acid transamination is catalyzed by aminotranferases and results in the formation of α-ketoacids while the α-ketoacidacceptor, often α-ketoglutarate, is transformed to the corresponding amino acid Glu (Yvon & Rijnen, 2001). In contrast to TFAA and most of the other free amino acids, Glu decreased with cheese ripening and presented a maximum around day 60 or 120. A similar decrease with cheese ripening was found for AAB, Cys and Ala.

Pro mean concentration was 13.2 mg 100 g^{-1} DM in the 1 day old cheese and increased to 24.3 mg 100 g^{-1} DM in 180 day old cheese. The low concentration of Pro in Teleme cheeses could be because this amino acid is normally released as a dipeptide (Pritchard & Coolbear, 1993).

The mean concentration of Arg was 14.2 mg 100 g^{-1} DM in the 1 day old cheese and reached 26.0 mg 100 g^{-1} DM in 180 day old cheese. Arg is responsible for unpleasant or bitter taste (Polo et al., 1985), therefore it is important that this amino acid concentration does not increase with ripening time.

Gaba (which is produced from Glu by a decarboxylase and is present in low-quality cheeses) was found at low concentrations in Teleme cheese. Despite the fact that the mean concentration of this amino acid increased continuously throughout the ripening period (from 4.8 mg 100 g^{-1} DM in 1day old cheese to 11.6 mg 100 g^{-1} DM in 180 day old cheese), its content represented less than 2% of the TFAA content, at 180 days of ripening.

Stepwise discriminant analysis selected only nine amino acids for discriminating among the stages of ripening (Fig. 5). Four discriminant functions were statistically significant ($P < 0.05$). Asn and Tau contributed the most to discrininant function *D*1(54%), Leu and Met contributed mainly to discriminant function *D*2 (35%), Glu and Leu to discriminant function D3 (7.66%) and Orn and Leu to discrininant function D4 (2.08%). The percentage of the samples that were classified into the correct group by the stage of ripening was 80.0%. (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 distances between the group centroids corresponding to R, 60 and 120 day old samples were lower than the distances corresponding to 1 and 180 day old samples.

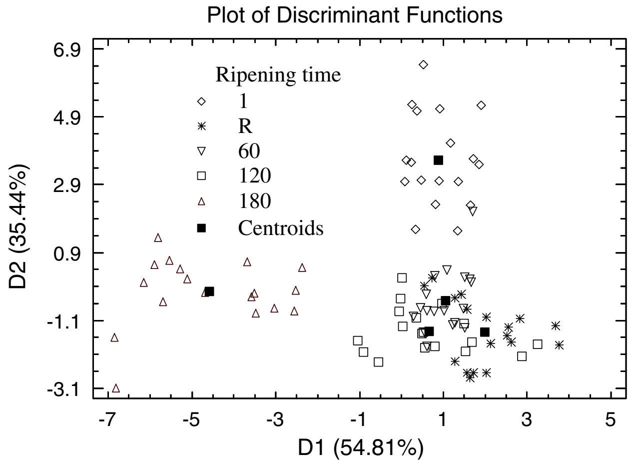

Fig. 5. Linear discriminant functions (*D*1 and *D*2) for cheese samples at 1, 60, 120, 180 days of ripening as well as the day cheeses entered the cold room (R). Both *D*1 and *D*2 were significant ($P < 0.05$).

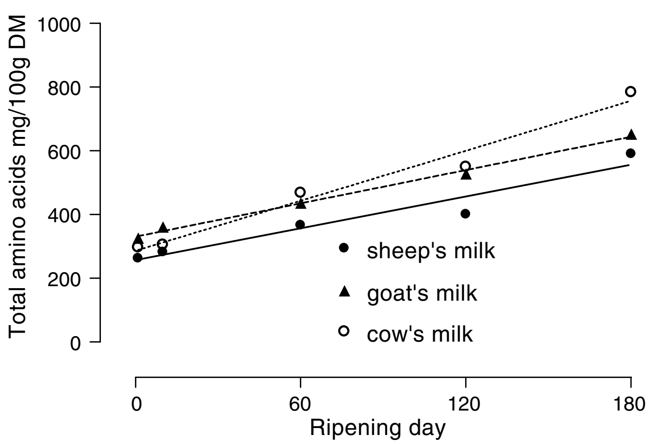

Fig. 6. Total free amino acid mean content in cheeses made with sheep's, goat's and cow's milk.

3.3. Effect of type of milk

Fig. 6 shows the evolution of TFAA for the three types of milk during ripening time. In ovine cheese, there was an increase in the mean TFAA concentration from 263 mg 100 g^{-1} DM in 1 day old cheese to 590 mg 100 g^{-1} DM in 180 day old cheese, in caprine cheese from 326 mg 100 g^{-1} DM to 652 mg 100 g^{-1} DM and in bovine cheese from 297 mg 100 g^{-1} DM to 784 mg 100 g^{-1} DM. The overall statistical analysis showed that, the TFAA in cow's milk cheese had a mean content 481 mg 100 g^{-1} DM and was significant higher ($P < 0.01$) than those of goat's (460 mg 100 g^{-1} DM) or sheep's (380 mg 100 g^{-1} DM) milk cheese. Cheese made with goat's milk was also significant higher than that of sheep's milk cheese ($P < 0.001$). Freitas et al. (1998) found that the TFFA content in Picante cheese made from sheep's milk was lower than the cheese made from goat's milk too. In contrast, Buriana and Farag (1983) found that the TFFA in ovine Teleme cheese was higher than that of bovine cheese. Mallatou et al. (2004) found that the TFAA contents of Teleme cheeses made from ewe's or goat's milk did not differ ($P > 0.05$).

Orn has been found in Teleme cheeses from the first day (Table 2). Orn (as well as Cit, AAB and GABA) did not originate from casein, but accumulated as a metabolic product of microorganisms. The mean concentration of Orn in cheeses made from ewe's milk was 8.2 mg 100 g^{-1} DM and was significant lower ($P < 0.001$) than that of cow's (13.1 mg 100 g^{-1} DM) milk cheese. Orn in goat's milk cheese had a mean content of 16.0 mg 100 g^{-1} DM and was significantly higher ($P < 0.001$) than that of the cheeses made from the other two types of milk.

According to Mehaia and Al-Kanhal (1992), Tau (which originates from Cys) is the most abundant FAA in goat's milk. In this study, Tau was found in low concentrations in goat's milk cheeses, as well as in ewe's and cow's milk cheeses (<1% of TFAA, in 180 day old cheese, regardless of the kind of milk and culture used). Low Tau contents have also been reported for Picante cheese (Freitas et al., 1998) and Idiazabal cheese (Barcina et al., 1995).

Stepwise discriminant analysis selected 18 amino acids for discriminating among the three kinds of milk (Fig. 7). Two discriminating functions were statistically significant ($P < 0.05$). TFAA and Phe contributed the most to discrininant function *D*1 (80.86%), while TFAA and Asn contributed mainly to discriminant function *D*2 (19.14%). As it can be observed in Fig. 7, the cheeses were correctly classified according to the kind of milk (100% of the total cases).

3.4. Effect of type of culture

The mean concentration of TFAA in Teleme cheese made with the thermophilic culture increased from 366 mg 100 g^{-1} DM in 1day old cheese to 877 mg 100 g^{-1} DM in 180 day old cheese, in Teleme cheese made with the mixed (thermophilic- mesophilic) culture, from 273 mg 100 g^{-1} DM to 619 mg 100 g^{-1} DM and in cheese made with the mesophilic culture from 246 mg 100 g^{-1} DM to 530 mg 100 g^{-1} DM, on the corresponding days (Fig. 8). Generally, the overall statistical analysis showed that the mean content of TFAA in Teleme cheese made with the thermophilic culture was 551 mg 100 g^{-1} DM and was significantly higher than that in mixed (421 mg 100 g^{-1} DM) or mesophilic (350 mg 100 g^{-1} DM) culture ($P < 0.001$). Cheeses made with the mesophilic culture had significantly lower ($P < 0.001$) mean concentration of TFAA than had cheeses made with the other two types of culture.

The highest TFAA contents of 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 in cheese matrix and could have increased the rate of proteolysis (Chapot-Chartier, 1996). Moreover, it is known that *S. thermophilus* possesses two additional peptidases (an oligopeptidase and aminopeptidase PepS) with respect to *Lc. lactis* and shows higher specific

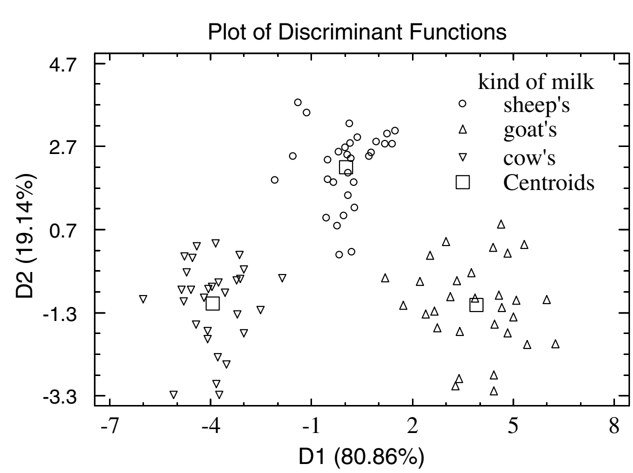

Fig. 7. Linear discriminant functions (*D*1 and *D*2) for cheese samples made from sheep's, goat's and cow's milk. Both *D*1 and *D*2 were significant ($P < 0.05$).

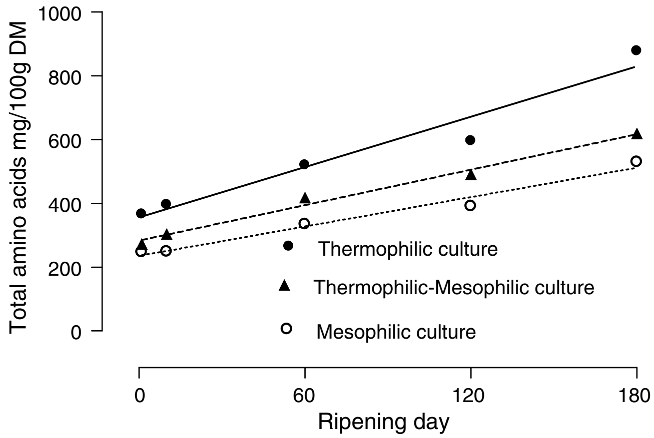

Fig. 8. Total free amino acid mean content in cheeses made with the thermophilic culture, the mesophilic culture or the mixture of thermophilic–mesophilic culture.

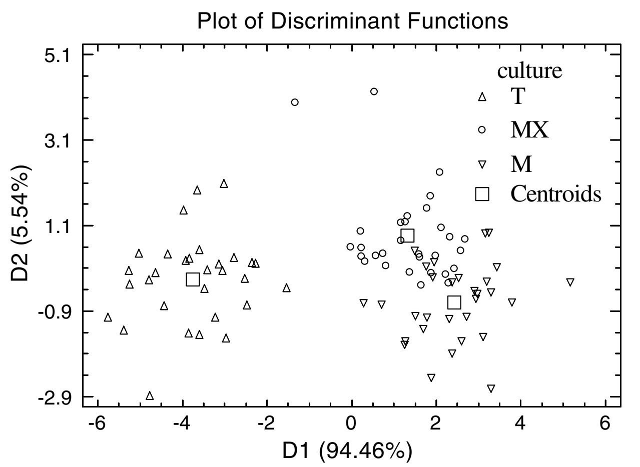

Fig. 9. Linear discriminant functions (*D*1 and *D*2) for cheese samples made using a thermophilic (T), a mesophilic (M) or a mixture of thermophilic–mesophilic (MX) culture. Both *D*1 and *D*2 were significant ($P < 0.05$).

activities of PepX, PepN and PepC (Rul & Monnet, 1997). Also, 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 (Khalid & Marth, 1990; Sasaki, Bosman, & Tan, 1995). Oumer, Garde, Medina, and Nunez (2001) concluded that thermophilic adjunct culture, when inoculated into milk, showed considerably higher aminopeptidase activity than did mesophilic adjunct culture. Garde, Tomillo, Gaya, Medina, and Nunez (2002) found similar results when they manufactured Hispanico cheese with thermophilic or mesophilic adjunct culture.

Stepwise discriminant analysis selected 13 amino acids for discriminating among the three types of culture (Fig. 9). Two discriminating functions were statistically significant ($P < 0.05$). TFAA and Leu contributed the most to discrininant function *D*1 (94.46%), while TFAA and Asn contributed mainly to discriminant function *D*2 (5.54%). The percentage of the samples classified into the correct group by the type of culture was 88.89%. Cheeses were successfully clustered by the type of starter, although some overlapping between mixed and mesophilic cultures was observed (Fig. 9). The distance between the group centroids corresponding to these cultures was lower than the distances corresponding to thermophilic culture from mixed or mesophilic culture.

4. Conclusions

The free amino acid content of Teleme cheese was considerably affected by the kind of milk used, the kind of culture added and the ripening time. The total free amino acid (TFAA) content increased with ripening time. The TFAA contents were highest in cheeses made with the thermophilic culture and lowest in cheeses made with the mesophilic culture. Cheeses made with the mixed thermophilic–mesophilic culture ranged over intermediate levels. Moreover, cheeses made with cow's milk had higher TFAA contents than had cheeses made with ewe's milk. Goat cheeses ranged over intermediate levels.

References

Alichanidis, E., Polychroniadou, A., Tzanetakis, N., & Vafopoulou, A. (1981). Teleme cheese from deep-frozen curd. *Journal of Dairy Science, 31*, 732–739.

Anifantakis, E. M. (1991). Greek cheeses – A tradition of centuries. In *National Dairy Committee of Greece* (pp. 27–42). Athens.

Barcina, Y., Ibanez, F. C., & Ordonez, A. I. (1995). Evolution of free amino acids during Idiazabal cheese ripening. *Food Control, 6*, 161–164.

Bidlingmeyer, B. A., Cohen, S. A., & Tarvin, L. A. (1984). Rapid analysis of amino acids using precolumn derivatisation. *Journal of Chromatography, 336*, 93–104.

Buriana, L. M., & Farag, S. I. (1983). The variation of amino acids in Telemea cheese during pickling. *Egyptian Journal of Dairy Science, 11*, 53–59.

Chapot-Chartier, M. P. (1996). Les autolysins des bactéries lactiques. *Lait, 76*, 91–109.

Christensen, J. E., Johnson, M. E., & Steele, J. L. (1995). Production of Cheddar cheese using a *Lactococcus lactis* ssp. *cremoris* SK11 derivative with enhanced aminopeptidase activity. *International Dairy Journal, 5*, 367–379.

Freitas, A. C., Fresno, J. M., Prietro, B., Franco, I., Xavier Malcata, F., & Carballo, J. (1998). Influence of milk source and ripening time on free amino acid profile of Picante cheese. *Food Control, 9*, 187–194.

Garde, S., Tomillo, J., Gaya, P., Medina, M., & Nunez, M. (2002). Proteolysis in Hispanico cheese manufactured using a mesophilic starter, a thermophilic starter and bacteriocin-producing *Lactococcus lactis* subsp. *lactis* INIA 415 adjunct culture. *Journal of Agricultural and Food Chemistry, 50*, 3479–3484.

Greek Codex Alimentarius (1998). *Official journal of the hellenic republic Vol. B no. 899 article 83 paragraph D3C*. Athens, GR: National Printing Office.

Hayaloglou, A. A., Guven, M., Fox, P. F., Hannon, J. A., & McSweeney, P. L. H. (2004). Proteolysis in Turkish white-brined cheese made with defined strains of *Lactococcus*. *International Dairy Journal, 14*, 599–610.

Hayaloglou, A. A., Guven, M., Fox, P. F., & McSweeney, P. L. H. (2005). Influence of starters on chemical, biochemical and sensory changes in Turkish white-brined cheese during ripening. *Journal of Dairy Science, 88*, 3460–3474.

IDF (1986). *Cheese and processed cheese products. Determination of dry matter. (FIL-IDF standard no. 4)*. Brussels: International Dairy Federation.

Izco, J. M., & Torre, P. (2000). Characterization of volatile flavour compounds in Roncal cheese extracted by the purge and trap method and analyzed by GC–MS. *Food Chemistry, 70*, 409–417.

Khalid, N. M., & Marth, E. H. (1990). Lactobacilli-their enzymes and role in ripening and spoilage of cheese: A review. *Journal of Dairy Science, 73*, 2669–2684.

Mallatou, H., Pappa, E. C., & Boumba, V. A. (2004). Proteolysis in Teleme cheese made from sheep's, goat's or a mixture of sheep's and goat's milk. *International Dairy Journal, 14*, 977–987.

Upadhyay, V. K., McSweeney, P. L. H., Magboul, A. A. A., & Fox, P. F. (2004). Proteolysis in cheese during ripening. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.), *Cheese: chemistry, physics and microbiology* (3rd ed., pp. 391–433). Amsterdam: Elsevier Academic Press.

Mehaia, M. A., & Al-Kanhal, M. A. (1992). Taurine and other free amino acids in milk of camel, goat, cow and man. *Milchwissenschaft, 47*, 351–353.

Operator's Manual No. 88140. (1984). *Pico Tag Amino Acids Analysis System*. Midford, MA, USA: Waters Association.

Oumer, A., Garde, P., Medina, M., & Nunez, M. (2001). The effects of cultivating lactic acid cultures with bacteriocin-producing lactic acid bacteria. *Journal of Food Protection, 64*, 81–86.

Pappa, E. C., Kandarakis, I., Anifantakis, E. M., & Zerfiridis, G. K. (2006a). Influence of types of milk and culture on the manufacturing practices, composition and sensory characteristics of Teleme cheese during ripening. *Food Control, 17*, 570–581.

Pappa, E. C., Kandarakis, I., Anifantakis, E. M., Zerfiridis, G. K., & Sotirakoglou, K. (2006b). Influence of starter cultures on the proteolysis of Teleme cheese made from different types of milk. *Lait, 86*, 273–290.

Polo, C., Ramos, M., & Sanchez, R. (1985). Free amino acids by high performance liquid chromatography and peptides by gel electrophoresis in Mahon cheese during ripening. *Food Chemistry, 16*, 85–96.

Polychroniadou, A., & Vlachos, J. (1979). Les acides aminés du fromage Télémé. *Le Lait*, 234–243.

Pritchard, G. G., & Coolbear, T. (1993). The physiology and biochemistry of the proteolytic system in lactic acid bacteria. *FEMS Microbiology Reviews, 12*, 179–206.

Rul, F., & Monnet, V. (1997). Presence of additional peptidases in *Streptococcus thermophilus* CNRZ 302 compared to *Lactococcus lactis*. *Journal of Applied Microbiology, 82*, 695–704.

Salles, C., Herve, C., Septier, C., Demaizieres, D., Lesschaeve, I., Issanchou, S., et al. (2000). Evaluation of taste compounds in water-soluble extract of goat cheeses. *Food Chemistry, 6*, 429–435.

Sasaki, M., Bosman, B. W., & Tan, P. S. T. (1995). Comparison of proteolytic activities in various lactobacilli. *Journal of Dairy Research, 62*, 601–610.

Tavaria, F. K., Franco, I., Javier Carballo, F., & Xavier Malcata, F. (2003). Amino acid and soluble nitrogen evolution throughout ripening of Serra da Estrela cheese. *International Dairy Journal, 13*, 537–545.

Vicente, M. S., Ibanez, F. C., Barcina, Y., & Barron, L. J. R. (2001). Changes in the free amino acid content during ripening of Idiazabal cheese: Influence of starter and rennet type. *Food Chemistry, 72*, 309–317.

Yvon, M., & Rijnen, L. (2001). Cheese flavour formation by amino acid catabolism. *International Dairy Journal, 11*, 185–201.