ISSN 1330-9862 *original scientific paper*
doi: 10.17113/ftb.55.04.17.5338

Microbial Quality of and Biochemical Changes in Fresh Soft, Acid-Curd Xinotyri Cheese Made from Raw or Pasteurized Goat's Milk

Eleni C. Pappa[1], Thomas G. Bontinis[2], Maria Tasioula-Margari[2†] and John Samelis[1]*

[1]Dairy Research Department, Institute of Technology of Agricultural Products, Hellenic Agricultural Organization, DEMETER, Ethnikis Antistaseos 3, Katsikas, GR-45221 Ioannina, Greece

[2]Department of Chemistry, University of Ioannina, University Campus of Ioannina, GR-45110 Ioannina, Greece

Received: May 8, 2017
Accepted: October 10, 2017

Summary

The microbiological quality of and changes in the main physicochemical parameters, together with the evolution of proteolysis, lipolysis and volatile profiles of soft Xinotyri, a traditional Greek acid-curd cheese (pH≈4.4, moisture 65 %, salt 1 %) made from raw (RMC) or pasteurized (PMC) goat's milk without starters, were evaluated during aerobic storage at 4 °C for 60 days. No statistically significant differences between the total nitrogen (TN) and nitrogen fraction (% of TN) contents, the degradation of intact α_s- or β-caseins, total free amino acid (FAA) contents, and the ratio of hydrophilic and hydrophobic peptides in the water-soluble fraction of RMC and PMC were found. Threonine, alanine and lysine were the principal FAAs. Oleic, palmitic, capric and caprylic acids, and ethyl hexonate, ethyl octanoate, ethyl decanoate, ethanol, 3-methyl butanol, phenyl ethyl alcohol and acetone were the most abundant free fatty acids and volatile compounds, respectively. Cheese lipolysis evolved slowly at 4 °C, and milk pasteurization had no significant effect on it. Mesophilic lactic acid bacteria (LAB) were predominant in fresh cheese samples. PMC samples had significantly lower levels of enterococci and enterobacteria than RMC samples, while yeasts grew at similar levels during storage at 4 °C. All cheese samples (25 g) were free of *Salmonella* and *Listeria monocytogenes*. Coagulase--positive staphylococci exceeded the 5-log safety threshold in fresh RMC samples, whereas they were suppressed (<100 CFU/g) in all PMC samples. Consequently, pasteurization of raw goat milk's and utilization of commercially defined or natural mesophilic LAB starters are recommended for standardizing the biochemical, microbial and safety qualities of fresh soft Xinotyri cheese.

Key words: microbiological and physicochemical characteristics, goat's milk, soft Xinotyri cheese, lipolysis, volatiles, proteolysis

Introduction

Traditional raw milk cheese is an authentic dairy product characterized by a rich and diverse microbiota and generally superior sensorial qualities compared to similar types or varieties of pasteurized milk cheese produced industrially with commercial starter cultures (*1*). The desirable sensorial characteristics of raw milk cheese are attributed to its microecological complexity and biodiversity, which in turn desirably affect cheese biochemistry (*2-4*). Cheese ripening involves the evolution of complex biochemical processes, including glycolysis, proteolysis and lipolysis (*2,5*). Flavour compounds are produced as catabolic products and play a critical role in the quality of the final cheese (*6*). Cheese flavour is the result of a

*Corresponding author: Phone: +30 26 510 94 785; Fax: +30 26 510 92 523; E-mail: pappa.eleni@yahoo.gr
ORCID IDs: 0000-0002-6471-2088 (Pappa), 0000-0002-1738-3584 (Bontinis), 0000-0002-4921-5649 (Samelis)
† In memory of Professor Maria Tasioula-Margari

complex balance among volatile and non-volatile chemical compounds from milk fat, milk proteins, and carbohydrates during ripening (*7-9*).

Traditional cheese, however, may also harbour undesirable or harmful bacteria, such as *Staphylococcus aureus* or *Listeria monocytogenes*, which are common raw milk contaminants or originate from other diverse environmental contamination sources (*10*). Depending on the cheese type and the manufacturing and hygienic practices, pathogens may survive or grow in the cheese matrix at population levels likely to cause disease (*11,12*). Therefore, milk pasteurization prior to cheese processing is recommended or demanded by food regulators in many countries, including Greece, because it protects public health and results in the manufacture of a uniform and safe product of constant quality. Pasteurization, however, alters the biochemistry of cheese ripening by suppressing the indigenous microbiota of the milk, by partial or complete inactivation of certain indigenous enzymes which contribute to ripening, and/or by slight denaturation of whey proteins (*13-16*).

Soft, spreadable, acid-curd cheese types constitute a major group of traditional cheese varieties with specific technological, physical, chemical, microbiological and sensorial characteristics (*17*). Several acid-curd cheese varieties also are traditionally manufactured in Greece from raw, thermized or pasteurized milk and consumed fresh or after varying ripening times, depending on local consumer habits and needs (*18*). Usually the milk is curdled and acidified with the aid of its native microbiota at ambient temperatures for 1-2 days and then, depending on the cheese variety, the fresh curd is drained at various extents (60-75 % moisture), salted with 1-4 % dry sea salt, placed in leather sacks, wooden barrels, tins or cans, and transferred for ripening and cold storage in cellars, other dry cool places or, to date, refrigerated rooms for a few days to several weeks before selling to consumers. Sometimes the milk is boiled and salted before the curdling. Addition of rennin is a common empirical practice to improve the cheese curd firmness and ripening quality. Natural or commercial starter cultures may also be added to enhance acidification, particularly when the milk is pasteurized before cheese making (*17,18*). The most popular Greek acid-curd cheese varieties for which published data exist are the protected designation of origin (PDO) (*18*) cheese varieties Galotyri (*19,20*), Anevato (*21*), Katiki (*22*), Kopanisti (*23*) and Pichtogalo Chanion (*24*). While these cheese varieties are still artisan-made in rural areas from raw milk, their manufacturing technology has been standardized and/or industrialized. Industrial products are made from pasteurized milk with commercially defined starter cultures and distributed by central food retailers and supermarkets (*19,24-26*). However, additional acid-curd cheese varieties are still produced to date by traditional practices in small dairies and homesteads in Greek mountainous areas or islands and they are marketed and consumed locally. These cheese varieties are considered to have clear positive impact on the national economy and agro-tourism, despite still being 'unknown', unstudied and unreachable by central markets. Because traditional Greek cheese processing technologies were developed with the primary aim to preserve the milk of small ruminants, the resultant artisan products may be eaten fresh or ripened; thus, quite often different cheese varieties of similar origin, but technologically distinct, are meant under a given local name.

Xinotyri is an artisanal, fully ripened, hard raw goat's milk cheese of increasing popularity that has been recently studied (*27,28*), whereas soft Xinotyri is the acid-curd goat's milk cheese variety that is not shaped and consumed fresh or ripened and cold-stored as mentioned above. Both cheese types are still produced traditionally on the island of Naxos in the Cyclades complex, Aegean Sea (*27*). Although the popularity of soft Xinotyri is also increasing, no published data exist on this cheese. There is only one previous relevant study on the biochemical characterization of indigenous *Lactobacillus plantarum* strains from a traditional Greek raw milk cheese named Xynotyri, but its production area was not reported (*29*). At Naxos, soft Xinotyri cheese is typically made from raw milk. Usage of pasteurized milk is however increasing in response to current safety concerns and regulatory or consumer demands. Therefore, the purpose of this study is to follow the evolution of the ripening and cold storage processes and evaluate the influence of using raw milk in comparison with using pasteurized milk on the main microbiological, physicochemical and biochemical characteristics of soft Xinotyri cheese during refrigerated (4 °C) storage, with the aim of contributing to their quality and safety.

Materials and Methods

Cheese production and sampling

Three independent soft Xinotyri cheese production trials were carried out at a small creamery located at a mountain village of Naxos, where the hard Xinotyri cheese previously analyzed by Bontinis *et al.* (*27,28*) was produced. According to the local cheese maker, raw goat's milk derived from a local native goat population was used to produce the soft raw milk cheese (RMC) samples, whereas the same milk after an open-batch pasteurization at 63 °C for 30 min was used to produce the pasteurized milk cheese (PMC) counterparts. No defined starter cultures were added to PCM. However, to enhance curdling of both milk types, commercial calf rennet (1:10000 strength, 3-4 mL/100 L of milk) was used in all trials. The milk with rennet was left to curdle for 20 h at room temperature (18-20 °C). The curd was cut in cubes (1-2 cm), knitted and drained through a cheesecloth placed in pierced plastic containers for 10 h in a cool room (16 °C). After 30 h at ambient temperature, the above RMC and PMC curds represented the fresh (day 1) unsalted Xinotyri cheese from which separate samples were kept for analyses. On the next day, edible sea salt (approx. 1.5 g per 100 kg of fresh cheese) was distributed uniformly in the curd, which was then packed in tins (containing approx. 1.5 kg cheese mass). The tins were shipped from Naxos to our laboratory at Ioannina in insulated iceboxes and placed in a refrigerator at 4 °C for ripening and cold storage. Samples were taken from each RMC or PMC batch on day 1 (the fresh unsalted cheese curd) and after aerobic storage of the cheese samples in the tins at 4 °C for one (day 8), two (day 15), four (day 30) and eight (day 60) weeks.

Microbiological analyses

The microbiological quality of the soft Xinotyri samples was determined by analyzing the microbial groups listed in Table 1. The microbiological methods were selected according to the PDO acid-curd Galotyri cheese studied previously (*19*). Briefly, on each sampling day, 25 g of cheese were homogenized with 225 mL of 0.1 % g per 100 mL of buffered peptone water (Merck, Darmstadt, Germany) in stomacher bags (Lab Blender, Seward, London, UK) for 60 s at room temperature. The homogenates were serially diluted in the same diluent and then spread (0.1-mL samples) or poured (1-mL samples) in duplicate on the different agar medium plates, as appropriate. In addition to the microbial quantification analyses shown in Table 1, the presence of *Salmonella* sp. and *Listeria* sp./*L. monocytogenes* in 25-gram cheese samples was determined by culture enrichment, as reported previously (*19,27*).

Physicochemical analyses

Moisture, fat, fat-in-dry matter (FDM), protein, NaCl and ash content of cheese samples were analyzed as described by Bontinis *et al.* (*28*). The pH was measured by the micro-pH 2001 meter (Crison, Barcelona, Spain) and water activity (α_w) by Novasina unit Thermoconstanter, Hamidat-TH-2/RTD-33/BS (Novasina AG, Zurich, Switzerland). Total nitrogen (TN) fractions, namely water soluble nitrogen (WSN), nitrogen soluble in 5 % phosphotungstic acid (PTA-SN) and nitrogen soluble in 12 % trichloroacetic acid (TCA-SN), were determined as described by Mallatou *et al.* (*30*). All analyses were carried out in duplicate.

Electrophoretic analysis

Pure reference caseins (CN) from caprine milk as well as cheese samples were analyzed in duplicate by urea-PAGE as described by Mallatou *et al.* (*30*). Electrophoresis was performed using a vertical slab unit (gel electrophoresis apparatus GE-2/4; Pharmacia, Upsalla, Sweden) with 180 mm×140 mm×1.5 mm slabs, equipped with a Hetofrig cooling bath type CB 60 and an electrophoresis power supply (EPS 500/400;, Pharmacia Upsalla). From the densitograms the levels of residual α_s- and β-casein in the aged cheese were calculated in comparison with the level present in the reference sample of the fresh (day 1) cheese. The zones of pure whole casein samples of the corresponding milk were used in electrophoresis for the identification of different bands. All analyses were carried out in duplicate.

Reversed-phase high performance liquid chromatography analysis

Peptide profiles of the water-soluble fraction of the cheese samples were determined by RP-HPLC using a Waters HPLC system (Waters Corporation, Milford, MA, USA), as described by Mallatou *et al.* (*30*). After each run, the integration area of peptides was determined and divided into two regions with the criterion being the elution time of peaks. The first group consisted of the hydrophilic peptides (HL) with retention times from 0 to 67.5 min (0-55 % eluent B). The second group consisted of hydrophobic peptides (HB) with retention times from 67.6 to 110 min (55.1-100 % eluent B). Eluent A was 0.1 % (by volume) trifluoroacetic acid (TFA) in deionized water, and eluent B was 0.085 % (by volume) TFA in 60:40 (by volume) acetonitrile/

Table 1. Microbiological analyses performed for evaluation of the microbiological quality of fresh soft Xinotyri cheese samples made from raw or pasteurized goat's milk and for monitoring microbial changes during aerobic storage of the cheese samples at 4 °C

Microbial group	Microbiological enumeration medium[a]	Incubation conditions		
		Temperature/°C	*t*/h	O_2
Total viable mesophilic bacteria	Casein-peptone soymeal-peptone (CASO) agar with 0.6 % yeast extract	30	72	aerobically
Total lactic acid bacteria (LAB)	M17 agar	37	48	aerobically
Total mesophilic LAB	de Man, Rogosa, Sharpe (MRS) agar	30	72	anaerobically[d]
Enterococci	Kanamycin aesculin azide (KAA) agar	37	48	aerobically
Aerobic pseudomonad-like bacteria	Cephalothin-fucidin-cetrimide (CFC) agar[b]	25	48	aerobically
Total Enterobacteriaceae	Violet red bile glucose (VRBG) agar	37	18-24	microaerophilic[e]
Catalase-positive bacteria	Mannitol salt agar	30	72	aerobically
Total staphylococci	Baird-Parker (BP) agar base with egg yolk tellurite[c]	37	24-48	aerobically

[a]All microbiological media and supplements were purchased from Lab M (Bury, Lancashire, UK) except CASO agar, which was purchased from Merck (Darmstadt, Germany). For enterobacteria, the detection limit was 10 CFU/g. For all other subdominant non-LAB microbial groups, the detection limit was 100 CFU/g

[b]*Pseudomonas* agar base supplemented with cephalothin-fucidin-cetrimide (supplement X108, Lab M)

[c]Staphylococcal colonies surrounded by characteristic zones of lecithinase activity were confirmed for agglutination using the rapid Staph Microscreen test (Microgen Bioproducts, Camberley, UK)

[d]Plates were incubated in anaerobic jars using envelopes of the Gas-Pack System (BBL, Becton, Dickinson, Sparks, MD, USA)

[e]Poured plates were overlayed with 5 mL of sterile VRBG medium for the creation of microaerophilic incubation conditions

deionized water. The ratio of hydrophobic to hydrophilic peaks (HB/HL) of water-soluble fraction was calculated as the ratio of the area of peaks eluted within 67.6–110 min to that of peaks eluted within 0–67.6 min. All analyses were carried out in duplicate.

Analysis of free amino acids

The free amino acids (FAAs) were identified as phenylthiocarbamyl (PTC) derivatives by RP-HPLC using the Pico-Tag amino acid analysis system (Waters Corporation). 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). 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 a flow rate of 1 mL/min. Free amino acids were extracted from cheese as described by Pappa and Sotirakoglou (*31*). The results were expressed on a dry matter basis. HPLC analyses were done in duplicate. Amino acids were identified according to their retention times by comparison with a standard mixture solution chromatogram. During extraction and derivatization, 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. All analyses were carried out in duplicate.

Analysis of free fatty acids

Free fatty acids (FFAs) of cheese samples were extracted following the method described by De Jong and Badings (*32*). A Shimadzu model GC-17A gas chromatograph (Shimadzu Scientific Instruments Inc, Columbia, MD, USA), equipped with an on-column injector and a flame ionization detector (FID) was used. The column used was SGE, BP21-FFAP (15 m×0.53 mm×0.5 μm i.d). The chromatographic conditions were as described previously (*33*). The quantification of the FFA in cheese samples was performed using the internal standardization technique, *i.e.* with C9:0 as an internal standard and processing the chromatograms with the CLASS-VP™ software (*34*). All analyses were carried out in duplicate.

Analysis of volatile compounds by CG/MS

Volatile compounds were analysed by gas chromatography–mass spectrometry (GC–MS), using solid phase microextraction (SPME); a 15-gram sample was homogenized in an analytical blender with an internal standard aqueous solution containing 0.5 mg/mL of cyclohexanone (Sigma-Aldrich, Alcobendas, Spain) as described before (*28*). Headspace volatile compounds were analyzed using a GC-2010 Shimadzu series gas chromatograph coupled to a GCMS-QP2010 mass spectrometer detector (Shimadzu, Kyoto, Japan). Data were recorded and analyzed with the GC-MS solution (*35*). Peak identification was performed by comparing the mass spectra with the NIST library (*36*) and comparison of their retention times with authentic standards when available. Peak areas (arbitrary units) were calculated from the total ion current. The relative abundance of a particular compound was calculated as the sum of the peak areas of its characteristic ions divided by the sum of the peak areas of the characteristic ions of cyclohexanone employed as internal standard. Samples were analyzed in triplicate.

Statistical analysis

Three replicate trials for each cheese type (RMC or PMC) were processed on independent milk collection and production days in the creamery. The microbiological data were converted to log CFU/g and along with the physicochemical and biochemical parameters were analyzed statistically by a multifactor analysis of variance using the software Statgraphics Plus for Windows (*37*). The least significant difference of the data is reported ($p<0.05$).

Results and Discussion

Main physicochemical attributes of soft Xinotyri cheese

The pH and water activity (a_w) values and the moisture, fat, protein, salt and ash contents of the fresh unsalted Xinotyri raw (RMC) and pasteurized milk cheese (PMC) curds (day 1) and their subsequent changes in the salted cheese products during storage at 4 °C for 60 days are shown in Table 2. No significant differences were observed between the initial pH of the RMC and PMC samples which were 4.44±0.04 and 4.38±0.03, respectively. These acid pH values of the fresh curds provided evidence that both milk types had undergone sufficient lactic fermentation during the initial 30-hour holding period at 16-20 °C. Specifically for the PMC samples, probably the post-thermal contamination of the milk with adventitious LAB under the artisanal cheese making conditions was high enough to proliferate and reduce the fresh curd pH comparatively to that of the RMC samples after 30 h of fermentation at ambient temperature. The pH of both cheese types also slightly decreased during 30 days of storage to average values from 4.23 to 4.40. Thus, having a pH≤4.4, and despite their high moisture and a_w values (Table 2), both soft Xinotyri cheese products were in compliance with the current EU microbiological specifications for ready-to-eat foods, particularly with regard to being non-supportive for the growth of *L. monocytogenes* during retail storage (*38*). Indeed, thanks to their low pH of 3.7 to 4.4, neither Galotyri nor Katiki or other traditional Greek PDO soft acid-curd cheeses (*e.g.* Pictogalo Chanion) supported growth of *L. monocytogenes*, according to recent validation studies (*19,22,24*). The pH values of both Xinotyri cheeses slightly increased above the threshold pH=4.4 after 60 days; this increase in pH, which was higher ($p<0.05$) in the PCM samples (Table 2), was previously observed also in Galotyri cheese (*19,20*). Trends of the increase of pH in aged acid-curd cheese are most likely associated with lactate assimilation by acid-tolerant spoilage yeasts and may constitute a 'delayed' safety concern upon a 'tailing' survival potential of dormant cells of *L. monocytogenes* or other acid-resistant pathogenic bacteria, such as *Escherichia coli* O157:H7, in the high-moisture cheese matrix (*19,22,39*).

Table 2. Changes in physicochemical parameters of soft Xinotyri cheese made from raw (RMC) or pasteurized (PMC) milk during aerobic storage at 4 °C

t/day	Type of milk	pH	*w*(moisture)%	*w*(fat)/%	*w*(fat in dm)/%	a_w/%	*w*(ash)/%	*w*(NaCl)/%	*w*(protein)/%
1	RMC	$(4.44\pm0.04)^{aA}$	$(73.4\pm0.3)^{bA}$	$(15.7\pm0.5)^{aA}$	$(59.0\pm1.2)^{aA}$	–	$(0.84\pm0.06)^{aA}$	$(0.28\pm0.01)^{aA}$	$(9.34\pm0.04)^{bA}$
	PMC	$(4.38\pm0.03)^{aA}$	$(71.0\pm0.2)^{cA}$	$(16.0\pm0.0)^{aA}$	$(55.1\pm0.3)^{aA}$	–	$(0.75\pm0.01)^{aA}$	$(0.22\pm0.03)^{aA}$	$(9.79\pm0.06)^{cA}$
15	RMC	$(4.24\pm0.03)^{aB}$	$(65.0\pm1.0)^{aB}$	$(18.2\pm0.9)^{aB}$	$(51.6\pm1.2)^{aB}$	$(98.4\pm0.7)^{aA}$	$(1.5\pm0.2)^{aB}$	$(0.9\pm0.2)^{aB}$	$(15.0\pm0.6)^{bB}$
	PMC	$(4.23\pm0.04)^{aB}$	$(67.2\pm1.4)^{aB}$	$(17.3\pm0.7)^{aB}$	$(52.4\pm0.8)^{aB}$	$(97.9\pm0.2)^{aA}$	$(1.6\pm0.1)^{aB}$	$(1.2\pm0.1)^{aB}$	$(10.9\pm0.5)^{cA}$
30	RMC	$(4.33\pm0.02)^{aB}$	$(65.7\pm0.8)^{aAB}$	$(17.0\pm0.6)^{aAB}$	$(49.5\pm0.6)^{bB}$	$(98.0\pm0.32)^{aA}$	$(1.5\pm0.3)^{aB}$	$(1.0\pm0.2)^{aB}$	$(13.8\pm0.2)^{bB}$
	PMC	$(4.40\pm0.03)^{aA}$	$(67.2\pm1.3)^{aB}$	$(17.7\pm0.7)^{aB}$	$(53.9\pm0.3)^{cAB}$	$(98.0\pm0.1)^{aA}$	$(1.6\pm0.3)^{aB}$	$(1.0\pm0.2)^{aB}$	$(11.00\pm0.2)^{cA}$
60	RMC	$(4.41\pm0.03)^{bB}$	$(63.2\pm0.8)^{aB}$	$(18.7\pm0.8)^{aB}$	$(50.7\pm1.1)^{aB}$	$(96.7\pm0.3)^{aA}$	$(1.4\pm0.3)^{aB}$	$(1.0\pm0.2)^{aB}$	$(15.0\pm0.7)^{bB}$
	PMC	$(4.55\pm0.02)^{cC}$	$(66.8\pm1.2)^{aB}$	$(17.8\pm0.7)^{aB}$	$(53.6\pm0.7)^{aAB}$	$(97.5\pm0.3)^{aA}$	$(1.5\pm0.2)^{aB}$	$(1.0\pm0.2)^{aB}$	$(11.2\pm0.2)^{cA}$

Results present mean values of six measurements (three cheese-makings and duplicate analyses)±standard error. Mean values of each parameter in the same column of the same day with different lower case letters (a-c) are significantly different ($p<0.05$). Mean values of each parameter in the same column of the same type of milk with different capital letters (A-C) are significantly different ($p<0.05$). dm=dry mass, a_w=water activity

No constant significant differences in the moisture, fat, protein, salt and ash contents were observed during storage at 4 °C between the cheese samples after draining and salting, except for the total protein contents (%), which were lower ($p<0.05$) in the PMC samples than in the RMC samples of the same age. Conversely, after manufacturing of the fresh (day 1) unsalted PMC samples had higher protein contents while retaining less moisture than their RMC counterparts (Table 2). Moisture decreased progressively in all cheese samples during storage. The greatest moisture loss from initial values of 71-74 % down to 65-67 % occurred within the first 15 days of storage at 4 °C of all salted cheese samples, reflecting accelerating effects of salting on draining of fresh (day 1) cheese curds (*40*). Notably, although 1.5 % salt added in the curd was declared by the Xinotyri cheese manufacturer, the average salt content of both cheese products during storage was around 1 % in the presence of approx. 65 % water and 49.5 to 53.9 % fat on dry mass basis (dm) of the cheese samples (Table 2). Based on these results, and in general consideration of their manufacturing technologies, the soft Xinotyri cheese is more closely related to the PDO Pichtogalo Chanion cheese (maximum permitted moisture 65 %, minimum permitted fat content 50 % dm, 1 % salt added to the milk) (*18,24*) rather than to the PDO Galotyri cheese (maximum permitted moisture 75 %, minimum permitted fat content 40 % dm, 3-4 % salt traditionally or 1.8 % salt in commercial Galotyris) (*18,19*) or any other of the abovementioned Greek acid-curd cheese varietes (*18*). Lowering salt contents in traditional cheese products reflects the current consumer demands for a 'healthier', less salty diet; this, however, also causes quality changes in cheese, while under certain circumstances salt reductions may compromise cheese safety (*19,26*).

Quantification of the technological LAB microbiota in the soft Xinotyri cheese

The fresh (day 1) Xinotyri RMC and PMC samples analyzed microbiologically within 2 h after arrival in our laboratory did not differ significantly ($p>0.05$) in their total viable counts (TVC) enumerated on CASO agar at 30 °C, which were (8.58±0.04) and (8.74±0.09) log CFU/g, respectively (data not shown). Along with the acid pH values of both cheese varieties in Table 2, these TVCs reflected the total LAB populations, which were also well above the 8-log level in all fresh (day 1) samples regardless of aerobic or anaerobic incubation on the M17 and MRS agar plates at 37 or 30 °C, respectively (Fig. 1). Conversely, the populations of enterococci, which constitute an important part of the indigenous LAB microbiota in traditional raw or thermized milk cheese varieties made in Greece or other Mediterranean countries (*1,4,41*), were approximately 6 log CFU/g, and by 0.5 log units higher in the RMC than in PMC samples (Fig. 1). It was thus evident that the microbiota of fresh Xinotyri cheese curds before salting was dominated by mesophilic LAB, which had grown abundantly within the first 20 h of milk curdling at 18 to 20 °C, followed by another 10 h of draining of the curds at 16 °C, irrespective of the use of raw or pasteurized goat's milk for cheese making. Since no commercial or natural starter cultures were added to the pasteurized milk in particular, the prolific LAB growth in the fresh (day 1) PMC samples was apparently either because the traditional open-batch pasteurization (63 °C, 30 min) process was poorly monitored, or the PMC bulks were somehow contaminated naturally with LAB from the creamery environment or subjected to 'back-slope' inoculation (*19*) despite the fact that the local cheese processor denied the application of such technique. Nevertheless, whichever the sources of the technological LAB were during PMC processing, their prolific growth and good milk acidifying capacity in all fresh (day 1) curds were considered beneficial for the microbial quality, safety and preservation of soft Xinotyri cheese during ripening and cold storage, in accordance with previous studies on the microbial and safety qualities of other well-known Greek PDO acid-curd cheese varieties (*19-22,24,25*).

During storage at 4 °C, further small increases in the populations of the predominant mesophilic LAB occurred within the first two weeks, followed by constant decline within the last two weeks, which were generally greater in the PMC samples (Fig. 1). LAB populations of-

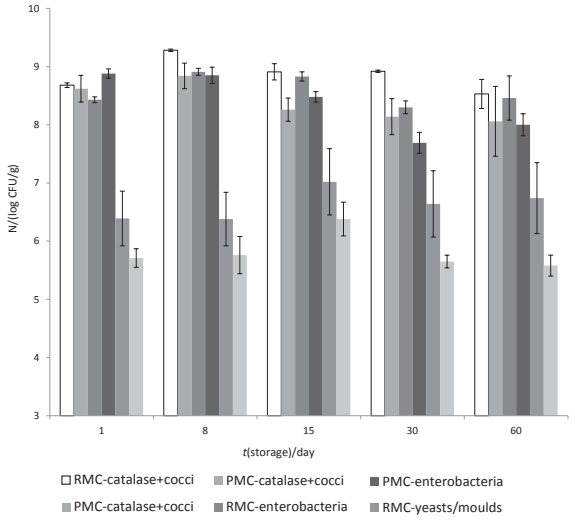

Fig. 1. Changes in the populations (log CFU/g) of total lactic acid bacteria (LAB) enumerated at 30 and 37 °C on MRS agar and M17 agar plates, respectively, and of enterococci in soft Xinotyri cheese samples made from raw (RMC) or pasteurized milk (PMC) during aerobic storage at 4 °C

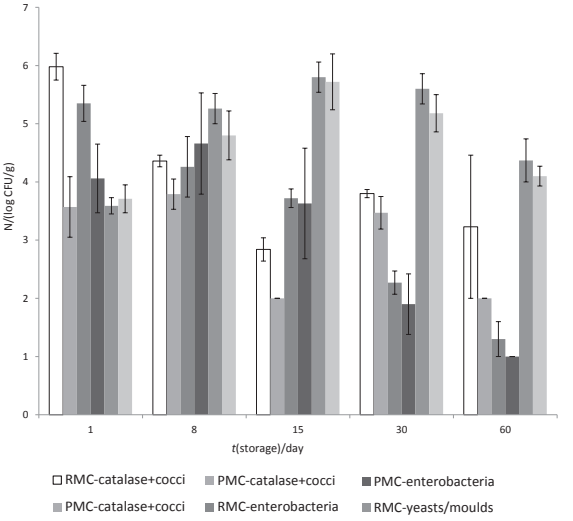

Fig. 2. Changes in the populations (log CFU/g) of Gram-positive, catalase-positive cocci, total enterobacteria and yeasts/moulds in soft Xinotyri cheese samples made from raw (RMC) or pasteurized milk (PMC) during aerobic storage at 4 °C

ten exceeded the 9 log level on CASO (data not shown) and MRS agar plates and generally remained above the 8 log level in the ripened cheese under refrigeration; most RMC samples contained LAB populations which were 0.5 to 1 log units higher ($p<0.05$) than those of the PMC samples (Fig. 1). The initial (day 1) populations of enterococci also increased by approx. 0.6 log CFU/g in the salted cheese products by mid of storage and decreased almost equally in all samples by the end of storage; their levels remained approx. 1 log unit higher ($p<0.05$) in the RMC than in the PMC samples throughout storage (Fig. 1).

Hygienic quality and safety of the soft Xinotyri cheeses

Compared to the fresh (day 1) RMC samples, and apart from enterococci (Fig. 1), which may also be considered as hygienic indicators, the PMC samples had significantly lower ($p<0.05$) populations of enterobacteria and catalase-positive bacteria, whereas yeasts were present at similar population levels in both cheese types (Fig. 2). Initial populations of aerobic Gram-negative (pseudomonad-like) bacteria were also at significantly lower ($p<0.05$) levels in PMC samples ((4.0±0.1) log CFU/g) than in RMC samples ((5.2±0.4) log CFU/g), whereas populations of total staphylococci on BP agar plates (data not shown in Fig. 2 for graph simplification) were at very similar levels to catalase-positive bacteria in fresh (day 1) cheese samples (Fig. 2). Based on latex agglutination testing of approx. 10 % of the lecithinase-positive colonies on countable BP agar plates, coagulase-positive staphylococci were estimated to be present at average levels of 5.4 log CFU/g in the fresh unsalted (day 1) RMC curds. In contrast, all PMC samples contained less than 2 log CFU/g of coagulase-positive staphylococci in the curd and throughout storage.

Although coagulase-positive staphylococci were reduced by 1.3 and 2.9 log units, and below the 2 log level, in the RMC samples after one, two and four weeks of storage at 4 °C, respectively (data not shown), their constant presence in the fresh unsalted RMC curds at levels above 5 log CFU/g was the greatest safety concern that arose during this study. This was the reason why comparative panel sensory evaluations between the soft Xinotyri cheese products during ripening at 4 °C were not conducted, in consideration of a previous alarming report on enterotoxin production by enterotoxinogenic *S. aureus* strains artificially contaminated in fresh acid-curd Galotyri cheese (*42*). Regulatory criteria specify that the population levels of coagulase--positive staphylococci should not exceed 4-5 log CFU/g in raw milk cheese and 1-2 log CFU/g in fresh nonripened soft cheeses from pasteurized milk (*38,43*). Also, staphylococcal populations sufficient to produce enterotoxins may be reached during the initial bacterial growth phase in milk or curd even though the counts may decrease to safe levels afterwards (*42,43*); thus regulations strictly specify the obligation to determine the potential presence of enterotoxins when populations of coagulase-positive staphylococci exceed 5 log CFU/g, which is the level above which cheese quality is considered defective (*38*). Since suspect colonies of staphylococci did exceed the 5 log units threshold in the Xinotyri RMC curds, additional research studies focused on standardization and strict hygienic control of the traditional manufacturing method of soft Xinotyri cheese with the emphasis on technological measures and/or interventions to prevent staphylococcal growth and enterotoxin production in RMC products are required.

Total staphylococci and enterobacteria were also reduced by approx. 3-4 log units in RMC samples, whereas their respective populations in PMC samples decreased below 2 and 1 log CFU/g by the end (day 60) of storage (Fig. 2). Aerobic Gram-negative bacteria proved to be the most sensitive microbial contaminants in the acidic Xinotyri cheese environment because their count fell below 2 log CFU/g in all samples after the first week of storage at 4 °C (data not shown). Conversely, the initial (day 1) populations of yeasts increased by approx. 2 log CFU/g during the first two weeks (day 15), while they decreased ($p<0.05$)

quite unexpectedly in all cheese samples after eight weeks (day 60) of aerobic storage at 4 °C. All RMC and PMC batches were free of *Salmonella* and *Listeria* species in 25 g cheese samples on day 1, and remained free of these pathogens upon their final testing on day 60 of storage (data not shown).

Evolution of proteolysis during cold storage of ripened soft Xinotyri cheese

Proteolysis is the most important phenomenon which determines texture and flavour development in fully ripened cheese as well as in fresh acid-curd cheese varieties that normally undergo slower rate ripening processes during storage at temperatures below 10 °C (*5,17*). Nitrogen fractions (WSN, TCA-SN and PTA-SN) were the first set of parameters used in this study to determine the extent of proteolysis of the soft Xinotyri cheese samples during storage at 4 °C (Table 3). The percentages of total nitrogen (TN) (also expressed as percentage of protein) in cheese as well as the fraction of WSN, TCA-SN and PTA-SN, expressed as percentage of TN, increased significantly during storage, in agreement with the results obtained for other cheese types including acid-curd cheese varieties (*17,44-46*). Since no commercial starters were used, the evolution of nitrogen fractions during storage was attributed to the peptidase activity of native microbiota. There were no statistical differences ($p>0.05$) between the TN, WSN, TCA-SN and PTA-SN contents of the RMC and PMC samples (Table 3). In agreement, no differences in soluble fractions of raw and pasteurized cheese were found by other authors (*2,14*).

Primary hydrolysis of cheese proteins is mainly the result of the action of indigenous milk proteinases and the residual coagulant when rennet is applied. Proteinases from starter LAB and non-starter microorganisms, however, are also active in the degradation of cheese proteins. Primary proteolysis in cheese may be defined as the changes in caseins (CN) and peptides detected by electrophoretic methods (*5*). Urea-PAGE electrophoretic patterns of soft Xinotyri RMC and PMC samples at different days of storage are shown in Fig. 3. Hydrolysis of individual CN fractions was expressed as residual mass fraction of the corresponding casein present in the fresh (day 1) cheese (Table 4). Similar protein degradation patterns were observed in both cheese types during storage (Fig. 1). The residual α_s-CN and β-CN decreased progressively in both cheeses during storage at 4 °C ($p<0.05$), but these decreases were low, indicating slight proteolysis of both CN fractions under refrigeration (Table 4). The rate of degradation of α_s-CN was higher than that of β-CN; there was however no difference ($p>0.05$) between RMC and PCM samples in the degradation of intact α_s- or β-CN (Table 4). While this result is in accordance with the findings of Lau *et al.* (*13*) and Moatsou *et al.* (*46*), opposite results were reported by Gaya *et al.* (*47*) and Gomez *et al.* (*48*) for ovine Manchego cheese. Such discrepancies are probably due to the numerous different factors affecting cheese manufacturing, including the type of milk, the technology used and mainly the type of cheese product in relation to the ripening conditions; proteolysis is accelerated in fully ripened cheese at elevated temperatures (*47*). This was not the case in the soft Xinotyri cheese, which apparently ripened more slowly at during storage at 4 °C.

Changes in the peptide profiles of the soft Xinotyri cheese products during storage at 4 °C are shown in Fig. 4. At 214 nm, the total area under the peaks on the HPLC chromatograms represents the light absorbed by aromatic amino acids and peptide bonds present in the water-soluble fraction of cheese. It can be observed that as the age of the cold-stored cheese increased, new peptide peaks appeared, while those peaks that existed at the onset of cold storage increased or decreased in size (Fig. 4). Differences between elution profiles of the WSN fraction of RMC and PCM Xinotyri samples were qualitative and quantitative for the same time of cheese storage (Fig. 4). The changes of hydrophilic (HL), hydrophobic (HB) peptides (expressed

Table 3. Changes of nitrogenous fractions of soft Xinotyri cheese made from raw (RMC) or pasteurized (PMC) milk during aerobic storage at 4 °C

t/day	Type of milk	*w*(TN)/%	*w*(WSN in TN)/%	*w*(TCA-SN in TN)/%	*w*(PTA-SN in TN)/ %
1	RMC	$(1.94\pm0.08)^{aA}$	$(6.1\pm0.2)^{aA}$	$(2.28\pm0.08)^{aA}$	$(0.77\pm0.07)^{aA}$
	PMC	$(2.01\pm0.01^{aA}$	$(6.8\pm1.0)^{aA}$	$(3.1\pm0.7)^{aA}$	$(0.8\pm0.24)^{aA}$
8	RMC	$(2.29\pm0.05)^{aB}$	$(7.8\pm0.6)^{aAB}$	$(3.7\pm0.4)^{aAB}$	$(1.04\pm0.01)^{bB}$
	PMC	$(2.12\pm0.07)^{aAB}$	$(6.87\pm1.42)^{aA}$	$(3.1\pm1.2)^{aA}$	$(0.85\pm0.02)^{cA}$
15	RMC	$(2.3\pm0.1)^{aB}$	$(8.12\pm0.67)^{aB}$	$(4.06\pm0.04)^{aB}$	$(1.0\pm0.3)^{aB}$
	PMC	$(2.19\pm0.08)^{aAB}$	$(7.13\pm0.52)^{aAB}$	$(3.9\pm0.8)^{aA}$	$(1.2\pm0.2)^{aB}$
30	RMC	$(2.17\pm0.04)^{aAB}$	$(8.23\pm0.90)^{aB}$	$(4.1\pm0.2)^{aB}$	$(1.0\pm0.2)^{aB}$
	PMC	$(2.19\pm0.04)^{aAB}$	$(8.78\pm0.76)^{aB}$	$(4.4\pm0.5^{aB}$	$(1.0\pm0.2)^{aB}$
60	RMC	$(2.4\pm0.1)^{aB}$	$(8.62\pm0.16)^{aB}$	$(4.0\pm1.0)^{aB}$	$(1.0\pm0.3)^{aB}$
	PMC	$(2.23\pm0.03)^{aB}$	$(8.67\pm0.45)^{aB}$	$(4.84\pm0.03)^{aB}$	$(1.09\pm0.05)^{aB}$

Results represent mean values of six measurements (three cheese-makings and duplicate analyses)±standard error. Mean values of each parameter in the same column of the same day with different lower case letters (a-c) are significantly different ($p<0.05$). Mean values of each parameter in the same column of the same type of milk with different capital letters (A, B) are significantly different ($p<0.05$).
TN=total nitrogen, WSN=water soluble nitrogen, PTA-SN=nitrogen soluble in 5 % phosphotungstic acid, TCA-SN=nitrogen soluble in 12 % trichloroacetic acid

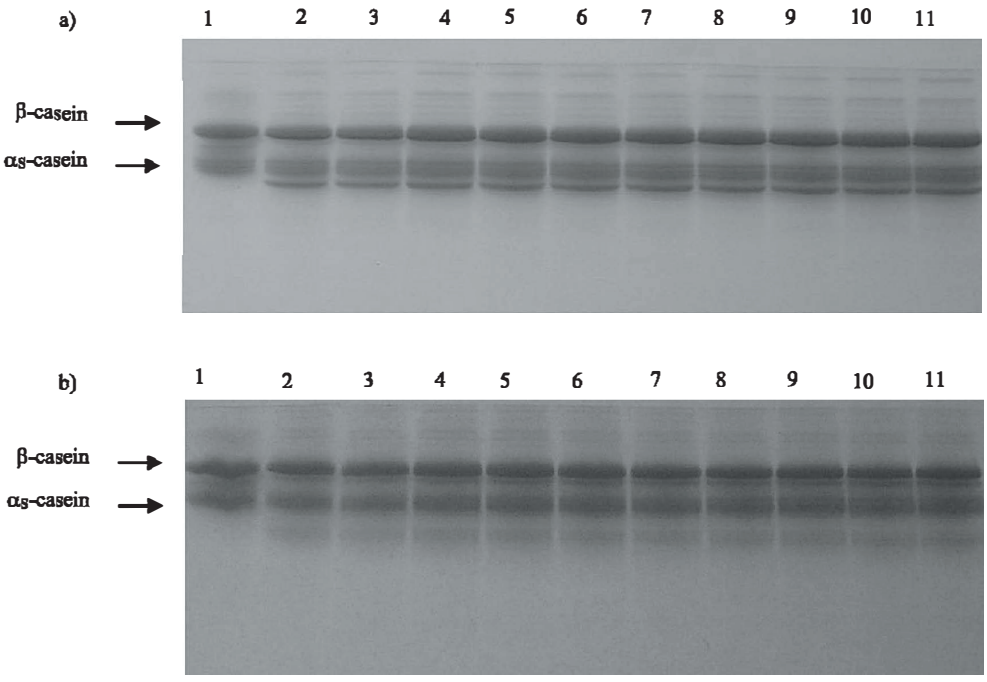

Fig. 3. Urea-polyacrylamide gel electrophoretograms of soft Xinotyri cheese made from: a) raw (RMC), or b) pasteurized milk (PMC) during aerobic storage at 4 °C. Lane 1=total goat casein, lanes 2–3=day 1 cheese samples, lanes 4–5=day 8 cheese samples, lanes 6–7=day 15 cheese samples, lanes 8–9=day 30 cheese samples, lanes 10–11=day 60 cheese samples

Table 4. Residual α_s- and β-casein of soft Xinotyri cheese made from raw (RMC) or pasteurized (PMC) milk during aerobic storage at 4 °C

t/day	Type of milk	*w*(residual α_s-CN)/%	*w*(residual β-CN)/%
1	RMC	$(100.0)^{aA}$	$(100.0)^{aA}$
	PMC	$(100.0)^{aA}$	$(100.0)^{aA}$
8	RMC	$(98.7\pm0.3)^{aAB}$	$(96.3\pm0.6)^{aB}$
	PMC	$(99.5\pm0.3)^{aAB}$	$(96.9\pm1.8)^{aAB}$
15	RMC	$(96.8\pm0.2)^{aAB}$	$(92.0\pm1.8)^{aC}$
	PMC	$(96.7\pm1.6)^{aB}$	$(95.1\pm0.6)^{aBC}$
30	RMC	$(92.5\pm0.7)^{aAB}$	$(93.1\pm5.6)^{aD}$
	PMC	$(95.6\pm0.9)^{aC}$	$(91.3\pm0.2)^{aC}$
60	RMC	$(90.4\pm0.1)^{bB}$	$(86.5\pm1.3)^{aE}$
	PMC	$(94.9\pm0.5)^{cC}$	$(89.6\pm0.8)^{aC}$

Results present mean values of six measurements (three cheese-makings and duplicate analyses)±standard error, expressed as a percentage of the α_s- or β-casein content present in the fresh (day 1) cheese. Mean values of each parameter in the same column of the same day with different lower case letters (a-c) are significantly different ($p<0.05$). Mean values of each parameter in the same column of the same type of milk with different capital letters (A-E) are significantly different ($p<0.05$). CN=casein

as a percentage of the total area of the chromatograms) and their ratio (HB/HL) in the water-soluble fraction of the RMC and PMC samples during storage are summarized in Table 5. Based on the literature, hydrophilic peptides eluting in the front region of RP-HPLC profiles have molecular mass $M<3000$ Da (*49*). Hydrophobic peptides elute mainly in the rear region of RP-HPLC profiles and large-size peptides generally elute later than those with low molecular mass (although differences may exist in the retention time of medium- and large-size peptides with the same amino acid composition but slightly different sequences, due to conformational effects) (*50,51*). Results in Table 5 show that the hydrophilic peptide content of the PMC samples was similar ($p>0.05$) to that of the RMC samples, regardless of days of storage and the amount of hydrophobic peptides present in the water-soluble fraction of the soft PMC Xinotyri cheese. Also, the HB/HL ratio in PMC did not differ significantly from that of RMC during storage (Table 5). Therefore, milk pasteurization prior to cheese making did not significantly ($p>0.05$) affect the area of the peptides and the HB/HL ratio in the water-soluble fraction of soft Xinotyri cheese during storage. Similar results were reported by Trujillo *et al.* (*52*) for goat's cheese, Moatsou *et al.* (*46*) for Kasseri cheese, and Gomez *et al.* (*53*) for Manchego cheese. The results of this study showed that hydrophobic peptides decreased while hydrophilic peptides increased with cheese ageing during ripening and storage ($p<0.05$). Also, the ratio of hydrophobic to hydrophilic peptides decreased with cheese age ($p<0.05$). These results are in agreement with data reported by Gaya *et al.* (*54*). The decrease of the HB/HL ratio during Xinotyri storage could be attributed to the degradation of water-soluble HB peptides and the formation of HL peptides (*55*) as well as highly HB peptides that are no longer water soluble (*13,56*).

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 inter-conversion and degradation (*50*). The mass fractions of the different amino acids in 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 (*5*). The FAA

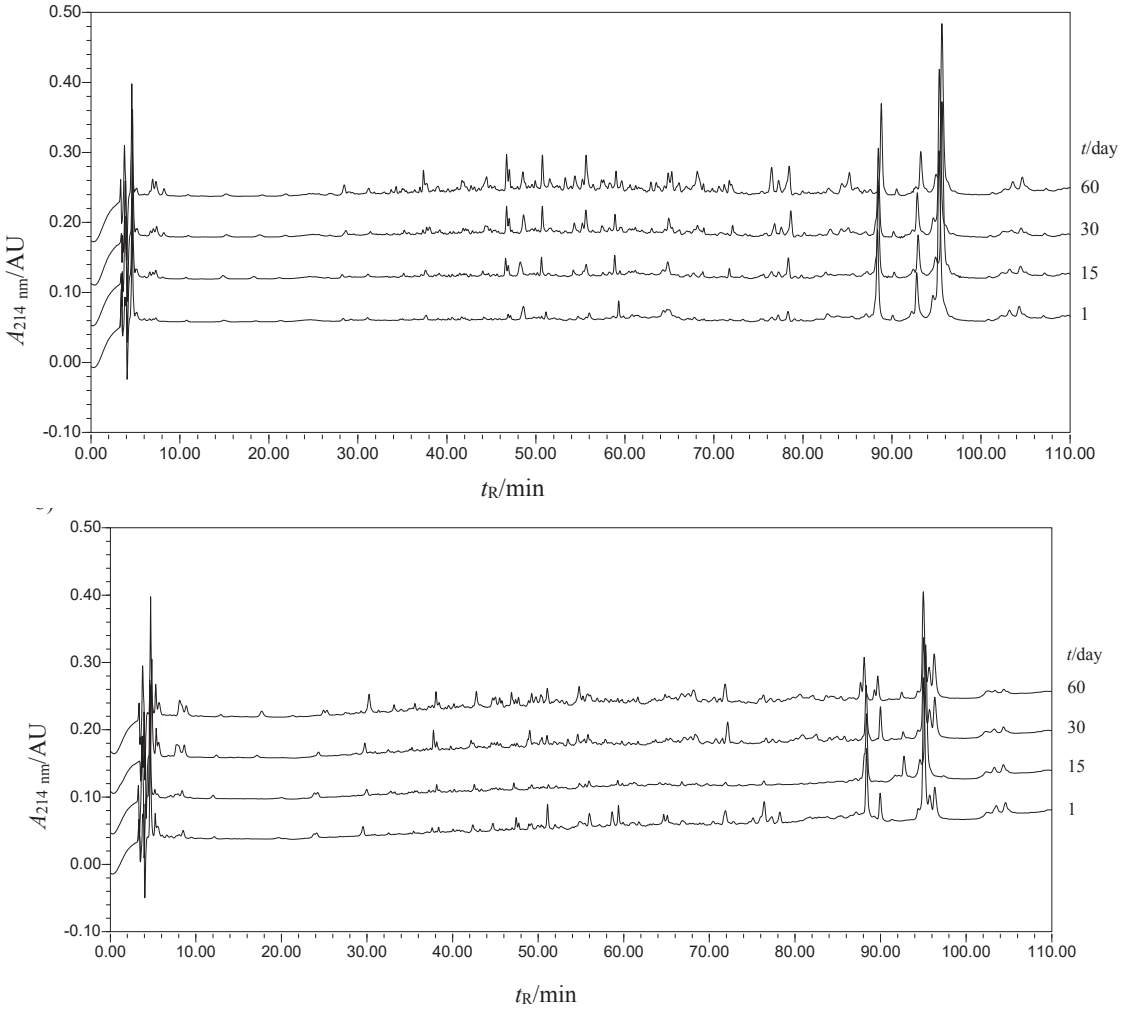

Fig. 4. Reversed-phase HPLC profiles of the water-soluble fraction of soft Xinotyri cheese samples made from: a) raw (RMC), or b) pasteurized milk (PMC) aged for 1, 15, 30 and 60 days of storage under aerobic refrigerated (4 °C) conditions. Absorbance was determined at λ=214 nm

Table 5. Hydrophobic (HB) and hydrophilic (HL) peptides in the water-soluble fraction of soft Xinotyri cheese made from raw (RMC) or pasteurized (PMC) milk during storage

t/day	Type of milk	*w*(HL)/%	*w*(HB)/%	HB/HL
1	RMC	$(53.2\pm1.8)^{aA}$	$(46.8\pm1.81)^{aA}$	$(0.89\pm0.07)^{aA}$
	PMC	$(52.9\pm0.5)^{aA}$	$(47.1\pm0.52)^{aA}$	$(0.89\pm0.02)^{aA}$
15	RMC	$(58.1\pm3.6)^{aAB}$	$(41.4\pm3.1)^{aAB}$	$(0.71\pm0.09)^{aAB}$
	PMC	$(64.1\pm0.6)^{aB}$	$(36.0\pm0.6)^{aB}$	$(0.57\pm0.02)^{aB}$
30	RMC	$(69.6\pm5.2)^{aBC}$	$(30.3\pm5.2)^{aBC}$	$(0.4\pm0.1)^{aBC}$
	PMC	$(63.5\pm0.2^{aB}$	$(36.5\pm0.2)^{aB}$	$(0.58\pm0.01)^{aB}$
60	RMC	$(71.4\pm0.9)^{aC}$	$(28.6\pm0.9^{aC}$	$(0.40\pm0.02)^{aC}$
	PMC	$(67.1\pm3.6)^{aB}$	$(32.8\pm3.6)^{aB}$	$(0.49\pm0.08)^{aB}$

Results present mean values of six measurements (three cheese-makings and duplicate analyses)±standard error expressed as a percentage of the total area of the chromatograms. Mean values of each parameter in the same column of the same day different lower case letters (a-c) are significantly different ($p<0.05$). Mean values of each parameter in the same column of the same type of milk with different capital letters (A-C) are significantly different ($p<0.05$)

composition of soft RMC and PMC Xinotyri samples during storage at 4 °C is shown in Table 6. The principal FAA in both cheese types at all stages of storage were threonine (Thr), alanine (Ala) and lysine (Lys) (Table 6). The γ-aminobutyric acid (γ-Aba) although found in high mass fraction (Table 6) does not originate from casein, but is accumulated as a metabolic product of microorganisms. Total FAA content per 100 g of dry mass of the RMC samples increased significantly from 141.0 mg in fresh (day 1) cheese to 274.0 mg by the end of storage (day 60). Conversely, in PMC samples, the total FAA content per 100 g of dry mass remained the same during storage, 179.6 and 168.0 mg on day 1 and day 60, respectively (Table 6). Milk pasteurization did not affect ($p>0.05$) the total FAA content of the soft Xinotyri cheese products. This finding is in accordance with the results found for the nitrogen soluble in 5 % PTA (Table 3). Generally, in other cheese varieties, RMC had higher amounts of total FAA than PMC (*54*). Soft RMC Xinotyri samples had higher ($p<0.05$) contents of phenalanine (Phe), valine (Val) and arginine (Arg) and lower contents of proline (Pro) than PMC samples. All other individual FAA were at similar levels ($p>0.05$) in both cheese types (Table 6).

Table 6. Free amino acids (FAA) of soft Xinotyri cheese made from raw (RMC) or pasteurized (PMC) milk during aerobic storage at 4 °C

FAA	Type of milk	*t*/day			
		1	15	30	60
		w(FAA)/%			
Asp	RMC	(1.9±0.2)^{aA}	(2.6±0.9)^{aA}	(2.9±0.8)^{aA}	(2.6±0.3)^{aA}
	PMC	(4.8±0.9)^{aA}	(4.2±1.6)^{aA}	(5.5±2.2)^{aA}	(1.8±0.1)^{aA}
Glu	RMC	(0.8±0.3)^{aA}	(1.8±1.1)^{aA}	(1.1±0.4)^{aA}	(2.2±0.2)^{bA}
	PMC	(4.3±1.2)^{aA}	(2.1±0.6)^{aA}	(2.3±1.3)^{aA}	(0.8±0.1)^{cA}
Ser	RMC	(4.7±0.7)^{aAB}	(2.6±0.5)^{aA}	(4.5±2.1)^{aAB}	(7.6±0.3)^{aB}
	PMC	(6.1±0.5)^{aA}	(2.3±0.3)^{aB}	(4.6±0.5)^{aAB}	(4.5±1.4)^{aAB}
Gly	RMC	(2.7±1.7)^{aA}	(2.2±0.7)^{aA}	(2.9±1.5)^{aA}	(2.9±0.2)^{aA}
	PMC	(5.0±1.0)^{aA}	(1.1±0.1)^{aB}	(1.7±0.6)^{aB}	(1.6±0.5)^{aB}
Gln	RMC	(3.4±1.2)^{aA}	(5.3±0.8)^{aAB}	(8.1±1.8)^{aB}	(6.0±0.1^{aAB}
	PMC	(4.4±1.2)^{aA}	(4.9±0.2)^{aA}	(8.3±2.0)^{aA}	(9.4±1.8)^{aA}
β-Ala	RMC	(0.4±0.2)^{aA}	(0.1±0.0)^{aA}	(0.2±0.1)^{aA}	(0.4±0.2)^{aA}
	PMC	(2.9±0.6)^{aA}	(0.5±0.2)^{aB}	(0.4±0.1)^{aB}	(0.3±0.1)^{aB}
Tau	RMC	(13.5±1.8)^{aA}	(5.7±0.9)^{aB}	(7.2±1.9)^{aB}	(4.3±1.1)^{aB}
	PMC	(16.4±2.0)^{aA}	(4.8±0.2^{aB}	(4.8±1.3)^{aB}	(4.1±0.2)^{aB}
His+ γ-Aba	RMC	(11.8±0.1)^{aA}	(13.7±0.9)^{aAB}	(20.1±2.5)^{aB}	(33.5±3.3)^{bC}
	PMC	(12.6±0.9)^{aA}	(10.9±1.7)^{aA}	(13.2±1.4)^{aA}	(7.9±1.4)^{cA}
Cit	RMC	(2.0±0.0)^{aAB}	(0.7±0.2)^{aA}	(2.0±0.5)^{aAB}	(3.8±1.4)^{aB}
	PMC	(3.6±1.9)^{aA}	(3.5±0.7)^{aA}	(1.9±1.3)^{aA}	(2.8±1.4)^{aA}
Thr	RMC	(9.1±1.7)^{bA}	(43.3±1.3)^{aB}	(47.9±6.1)^{aB}	(45.8±5.0)^{aB}
	PMC	(46.4±11.3)^{cA}	(46.3±5.7)^{aA}	(48.9±6.6)^{aA}	(43.5±6.6)^{aA}
Ala	RMC	(5.4±0.6)^{bA}	(17.1±3.9)^{aB}	(19.4±0.1)^{bB}	(28.3±1.0)^{bC}
	PMC	(10.1±0.4)$^{c\,A}$	(16.7±3.1)^{aA}	(13.7±0.2)$^{c\,A}$	(10.7±1.3)^{cA}
Arg	RMC	(8.7±1.1)^{bA}	(7.6±0.5)^{aA}	(3.7±0.9)^{aB}	(3.3±1.1)^{aB}
	PMC	(1.5±0.7)^{cA}	(3.1±1.3)^{aA}	(0.5±0.2)^{aA}	(3.1±1.7)^{aA}
Pro	RMC	(1.1±0.8)^{aA}	(1.5±0.3)^{aA}	(4.6±0.4)^{aB}	(4.0±0.2)^{aB}
	PMC	(4.5±1.9)^{aA}	(8.2±1.7)^{aA}	(6.0±1.9)^{aA}	(5.6±3.4)^{aA}
Aab	RMC	(33.6±2.6)^{aA}	(16.1±1.6)^{aB}	(22.6±3.3)^{aB}	(17.5±0.2)^{B}
	PMC	(23.9±2.8)^{aA}	(16.0±0.2)^{aA}	(21.4±3.9)^{aA}	(18.5±0.1)^{cA}
Tyr	RMC	(1.0±0.3)^{bA}	(8.4±2.1)^{aB}	(7.3±0.5)^{aB}	(2.2±0.5)^{aA}
	PMC	(3.2±0.3)^{cA}	(6.8±2.8)^{aA}	(8.7±0.9)^{aA}	(6.7±3.7)^{aA}
Val	RMC	(7.1±2.9)^{aA}	(6.7±0.2)^{bA}	(8.9±1.8)^{bA}	(13.9±2.3)^{bA}
	PMC	(2.5±1.2)^{aA}	(2.3±0.6)^{cA}	(3.6±0.9)^{cA}	(8.1±0.8)$^{c\,B}$
Met	RMC	(1.6±0.3)^{aA}	(0.6±0.1 ^{aA}	(2.3±0.9)^{aA}	(5.5±0.7)^{aA}
	PMC	(1.4±0.4)^{aA}	(0.8±0.3)^{aA}	(0.8±0.5)^{aA}	(2.9±0.9)^{aB}
Ile	RMC	(5.2±2.7) ^{aA}	(0.6±0.1)^{aA}	(0.7±0.5)^{aA}	(2.9±0.1)^{bA}
	PMC	(0.9±0.3) ^{aA}	(0.3±0.1)^{aA}	(0.4±0.3)^{aA}	(0.6±0.4)^{cA}
Leu	RMC	(5.7±0.4)^{aA}	(9.1±1.5)^{aA}	(19.3±1.2)^{bAB}	(43.0±3.2) $^{b\,B}$
	PMC	(4.2±0.8)^{aA}	(4.4±1.6)^{aA}	(7.5±2.6)^{cA}	(11.2±4.5) ^{cA}
Phe	RMC	(7.2±2.1)^{aA}	(5.0±1.1)^{aA}	(9.3±0.3)^{bA}	(14.8±1.1)^{bB}
	PMC	(5.3±1.9)^{aA}	(6.0±3.8)^{aA}	(3.9±0.5)^{cA}	(5.3±1.4)^{cA}
Trp+ Orn	RMC	(5.6±0.7)^{aA}	(5.7±0.2)^{aA}	(9.3±0.9)^{bA}	(11.5±3.8)^{bA}
	PMC	(7.9±4.5)^{aA}	(5.1±1.3)^{aA}	(5.0±1.1)^{cA}	(7.2±1.8)^{cA}
Lys	RMC	(8.8±0.9)^{aAB}	(5.4±0.5)^{aA}	(9.8±0.3)^{aB}	(18.5±1.6)^{aC}
	PMC	(8.3±1.0)^{aA}	(5.0±0.1)^{aA}	(7.3±1.1)^{aA}	(11.9±4.4)^{aA}
Tfaa	RMC	(141.0±6.1)^{aA}	(160.9±6.1)^{aAB}	(213.6±13.9)^{aB}	(274±22.1)^{aC}
	PMC	(179.6±6.7)^{aA}	(154.9±17.8)^{aA}	(170.0±0.8)^{aA}	(168.0±16.2)^{aA}

Results present mean values of six measurements (three cheese-makings and duplicate analyses)±standard error, epressed as mg per 100 g of dry matter. Mean values of each parameter in the same column of the same day with different lower case letters (a-c) are significantly different ($p<0.05$). Mean values of each parameter in the same row of the same type of milk with different capital letters (A-C) are significantly different ($p<0.05$). Asp=aspartic acid, Glu=glutamic acid, Ser=serine, Gly=glycine, Gln=glutamine, β-ALA=β-aminobutyric acid, Tau=taurine, His=histidine, γ-Aba=γ-aminobutyric acid, Cit=citrulline, Thr=threonine, Ala=alanine, Arg=arginine, Pro=proline, Aab=α-aminobutyric acid, Tyr=tyrosine, Val=valine, Met=methionine, Ile=isoleucine, Leu=leucine, Phe=phenylalanine, Trp=tryptophan, Orn=ornithine, Lys=lysine, Tfaa=total free amino acids

Evolution of lipolysis during ripening and cold storage of soft Xinotyri cheese

The evolution of total FFAs and the changes in individual FFAs and acetic acid of soft RMC and PCM Xinotyri samples during storage at 4 °C are summarized in Table 7. Acetic acid was included because it is a major volatile acid extracted with FFAs and it contributes greatly to the final flavour of cheese. Acetic acid is not a product of lipolysis but it is mainly a product of other biochemical pathways, such as carbohydrate breakdown by heterofermentative LAB or the fermentation of lactate or the metabolism of amino acids (*2*). In general, no significant differences ($p>0.05$) were found in individual and total FFA content of soft RMC and PMC samples during storage (Table 7). The

Table 7. Changes of free fatty acid (FFA) content (mg per kg of cheese) in raw (RMC) or pasteurized milk (PMC) soft Xinotyri cheese at different days of aerobic storage at 4 °C

FFA	*t*/day							
	1		15		30		60	
	w(FFA)/(mg/kg)							
	RMC	PMC	RMC	PMC	RMC	PMC	RMC	PMC
C2	(111.6±15.0)^{aA}	(102.7±22.4)^{aA}	(119.2±11.5)^{aA}	(72.9±4.0)^{aB}	(74.7±9.8)^{aB}	(62.1±13.8)^{aB}	(54.6±16.0)^{aB}	(67.9±10.0)^{aB}
C6	(87.0±9.5)^{aA}	(94.9±3.1)^{aA}	(91.7±0.1)^{aA}	(92.7±1.8)^{aA}	(107.7±15.7)^{aA}	(94.413±1.2)^{aA}	(134.1±18.0)^{aA}	(83.1±11.0)^{aA}
C8	(99.6±4.54)^{aA}	(85.7±0.2)^{aA}	(102.3±7.3)^{aA}	(79.7±1.2)^{aA}	(196.6±82.6)^{aA}	(86.8±3.3)^{aA}	(300.4±86.8)^{aA}	(133.3±49.0)^{aA}
C10	(98.2±7.24)^{aA}	(87.2±2.0)^{aA}	(107.5±14.3)^{aA}	(84.1±1.8)^{aA}	(186.8±73.7)^{aA}	(90.2±0.9)^{A}	(290.4±74.8)^{A}	(135.0±51.5)^{A}
C12	(123.1±10.26)^{aAB}	(70.6±7)^{aA}	(109.3±6.2)^{bA}	(70.6±1.2)^{cA}	(152.8±42.8)^{aAB}	(72.9±0.6)^{A}	(228.9±37.7)^{B}	(137.4±24.6)^{B}
C14	(185.6±24.73)^{aA}	(118.3±2.2)^{A}	(182.1±36.6)^{aA}	(110.9±4.1)^{aA}	(220.8±67.4)^{aA}	(116.7±7.3)^{A}	(298.5±25.0)^{A}	(156.2±29.2)^{A}
C16	(556.7±217.51)^{aA}	(201.5±22.5)^{aA}	(561.9±257.3)^{aA}	(207.3±26.9)^{A}	(668.2±338.771)^{aA}	(229.6±40.0)^{A}	(888.1±66.3)^{bA}	(366.29±67.1)^{cA}
C18	(251.8±38.1)^{aA}	(143.1±19.9)^{aA}	(238.1±67.7)^{aA}	(142.8±22.7)^{aA}	(266.0±96.5)^{aA}	(144.9±26.1)^{A}	(349.8±19.0)^{bA}	(195.7±1.4)^{cA}
C18:1	(641.7±251.9)^{aA}	(312.0±67.3)^{aA}	(623.3±238.3)^{aA}	(288.7±35.7)^{aA}	(1000.4±492.1)^{aA}	(437.6±42.1)^{A}	(2559.6±855.1)^{A}	(487. 8±89.3)^{A}
C18:2	(181.4±41.1)^{aA}	(125.7±29.5)^{aA}	(163.6±45.8)^{aA}	(137.0±23.8)^{aA}	(272.1±141.7)^{aA}	(163.7±22.2)^{A}	(417.0±83.6)^{A}	(229.2±52.6)^{A}
C18:3	(92.3±13.8)^{aA}	(77.6±1.2)^{aA}	(87.6±9.8)^{aA}	(76.7±2.4)^{aA}	(130.2±51.7)^{aA}	(79.2±0.7)^{A}	(175.0±25.9)^{A}	(83.7±6.6)^{A}
SCFFA	(396.4±14.7)^{aA}	(370.4±0.8)^{aA}	(420.7±21.4)^{aA}	(329.4±2.4)^{aA}	(565.8±172.0)^{aA}	(333.5±3.7)^{A}	(779.5±179.5)^{A}	(419.2±89.4)^{A}
MCFFA	(308.7±14.5)^{bA}	(188.8±8.9)^{cAB}	(291.3±42.8)^{aA}	(181.5±2.9)^{aA}	(373.6±110.3)^{aA}	(189.6±8.0)^{AB}	(527.4±62.7)^{A}	(293.7±53.8)^{B}
LCFFA	(1723.5±462.4)^{A}	(860.0±140.5)^{aA}	(1674.5±618.8)^{aA}	(852.5±111.6)^{aA}	(2337.0±1120.8)^{aA}	(1055.1±129.7)^{A}	(4389.4±879.3)^{A}	(1362.8±217.1)^{A}
TFFA	(2429.1±606.6)^{aA}	(1419.3±127.8)^{aA}	(2386.5±694.3)^{aA}	(1363.46±108.1)^{aA}	(3276.3±1412.8)^{aA}	(1578.3±155.2)^{A}	(5696.4±1105.5)^{A}	(2075.7±350.3)^{A}

Results present mean values of six measurements (three cheese-makings and duplicate analyses)±standard error. Mean values of the same age and the same row with different lower case letters (a-c) are significantly different ($p<0.05$). Mean values of each parameter in the same row of the same type of milk with different capital letters (A, B) are significantly different ($p<0.05$). SCFFA=short-chain fatty acids (C2-C10), MCFFA=medium-chain fatty acids (C12-C14), LCFFA=long-chain fatty acids (C16-C18:3), TFFA=total free fatty acids

60-day of cold storage of ripened soft Xinotyri cheese might have been too short compared with that of typical ripened cheeses to promote great differences in lipolysis between RMC and PMC samples. However RMC samples had higher mass fractions of lauric acid (C12) than PMC samples by day 15 and of palmitic acid (C16) and stearic acid (C18) by the end of storage (Table 7). It is known that pasteurization of milk prior to cheese making affects the FFA content in cheese as it reduces the effect of enzymes from milk (*8*).

The mass fractions of individual FFA remained the same during storage ($p>0.05$), except for lauric acid (C12), which increased ($p<0.05$) on day 60 in both cheese types (Table 7). Moreover, the rate of increase of SCFFA (short-chain fatty acids, C2-C10), of MCFFA (medium--chain fatty acids, C12-C14) and LCFFA (long-chain fatty acids, C16-C18:3) was compared during storage, and the results showed that SCFFA and LCFFA remained constant ($p>0.05$), whereas MCFFA increased with storage ($p<0.05$). SCFFA including acetic acid (C2–C8) have a significant impact on the development of the characteristic aroma of the cheese (*57*), whereas MCFFA and LCFFA are not the main contributors to cheese flavour (*58*). The major fatty acids present in soft Xinotyri cheese, regardless of the used treatment of milk, were oleic (C18:1), palmitic (C16), myristic (C14), capric (C10) and caprylic (C8) acids (Table 7). Caprylic and capric acids are likely to contribute to the flavour of goat's cheese (*28*).

The low TFFA content indicates that soft Xinotyri cheese, regardless of the type of milk used, underwent very weak lipolysis during storage (Table 7). Masotti *et al.* (*59*) also found weak lipolytic activity in an acid curd cheese obtained from goat's milk and Kondyli *et al.* (*26*) in Galotyri-type cheese stored for 30 days. Fresh acid curd cheeses are subjective to few biochemical changes during storage due to the processing conditions and the short period of ripening (*17*).

Volatile composition of the soft Xinotyri cheese

Mean mass fractions of volatile compounds identified in soft RMC and PMC Xinotyri samples during storage at 4 °C are shown in Table 8 and in Fig. 5. A total of 36 and 30 volatile compounds were identified in RMC and PMC samples, respectively. These volatiles were classified in different biochemical groups, namely acids, ketones, esters, alcohols, aldehydes and various other compounds, and most of them had previously been reported in different dairy products (*9*); their formation pathways were reviewed elsewhere (*6*).

Esters were quantitatively the major group of volatile compounds (Fig. 5). Different families of esters were detected, especially in soft RMC Xinotyri samples. The main family comprised ethyl esters from acetate to dodecanoate. Some methyl, propyl, butyl and pentyl esters were also present in RMC, whereas only ethyl esters from

Table 8. Relative abundance (peak area·10^3/peak area of internal standard) of volatile compounds isolated from raw (RMC) or pasteurized milk (PMC) soft Xinotyri cheese at different days of aerobic storage at 4 °C

Volatile compound	*t*/day					
	15		30		60	
	RMC	PMC	RMC	PMC	RMC	PMC
Esters						
Ethyl acetate	$(15.2\pm2.7)^{bA}$	$(1.4\pm0.4)^{c}$	$(4.8\pm1.9)^{B}$	ND	$(3.0\pm1.2)^{B}$	ND
Ethyl butanoate	$(22.6\pm6.2)^{bA}$	$(3.5\pm1.7)^{cA}$	$(16.0\pm4.2)^{bA}$	$(2.0\pm1.0)^{cA}$	$(7.7\pm1.3)^{aB}$	$(3.5\pm1.9)^{aA}$
Isoamyl acetate	$(7.1\pm1.6)^{A}$	ND	$(2.9\pm10.)^{B}$	ND	$(8.0\pm2.4)^{aA}$	ND
Ethyl pentanoate	$(0.52\pm0.01)^{aA}$	ND	$(0.17\pm0.08)^{A}$	ND	$(0.3\pm0.1)^{A}$	ND
Butyl butyrate	$(0.5\pm0.2)^{A}$	ND	$(0.37\pm0.08)^{A}$	ND	$(0.22\pm0.03)^{A}$	ND
Ethyl hexanoate	$(103.5\pm29.4)^{bA}$	$(7.2\pm2.2)^{cA}$	$(72.9\pm4.3)^{bB}$	$(3.2\pm1.1)^{cA}$	$(48.5\pm6.1)^{bB}$	$(9.6\pm1.3)^{cA}$
3-methylbutyl butanoate	$(8.1\pm3.0)^{A}$	ND	$(6.6\pm1.8)^{A}$	ND	$(5.6\pm1.8)^{A}$	ND
Ethyl heptanoate	$(2.1\pm1.2)^{A}$	ND	$(2.7\pm1.2)^{A}$	ND	$(0.8\pm0.1)^{A}$	ND
Ethyl octanoate	$(65.7\pm18.7)^{bAB}$	$(7.0\pm2.0)^{cA}$	$(142.3\pm37.8)^{bB}$	$(18.6\pm2.5)^{cA}$	$(29.5\pm3.8)^{aA}$	$(61.6\pm15.1)^{aB}$
Isopentyl hexanoate	$(4.8\pm2.0)^{A}$	ND	$(7.6\pm2.9)^{A}$	ND	$(5.3\pm2.0)^{A}$	ND
Ethyl decanoate	$(29.9\pm8.7)^{bA}$	$(6.3\pm2.1)^{cA}$	$(245.1\pm37.9)^{bB}$	$(4.6\pm1.3)^{cA}$	$(11.7\pm5.0)^{bA}$	$(71.4\pm13.1)^{cB}$
Phenylethyl acetate	$(0.63\pm0.08)^{A}$	ND	$(0.9\pm0.3)^{A}$	ND	$(0.56\pm0.07)^{A}$	ND
Ethyl dodecanoate	$(1.9\pm0.7)^{A}$	ND	$(2.3\pm1.9)^{A}$	ND	$(0.7\pm1.0)^{A}$	ND
Alcohols						
Ethanol	$(16.6\pm2.0)^{aA}$	$(26.4\pm3.73)^{aA}$	$(50.3\pm11.7)^{bB}$	$(4.6\pm1.1)^{cB}$	$(18.3\pm4.5)^{aA}$	$(11.1\pm2.713)^{aAB}$
2-methyl propanol	$(2.2\pm1.0)^{A}$	ND	ND	ND	$(1.3\pm0.1)^{A}$	ND
3-pentanol	ND	(0.77±0.09)	ND	ND	ND	ND
2-pentanol	$(0.44\pm0.03)^{A}$	ND	$(10.7\pm2.1)^{bB}$	$(0.37\pm0.02)^{cA}$	ND	$(0.42\pm0.08)^{A}$
1-butanol	$(0.26\pm0.07)^{aA}$	$(0.42\pm0.03)^{aA}$	$(0.3\pm0.1)^{A}$	ND	$(0.2\pm0.1)^{A}$	ND
3-methyl butanol	$(71.7\pm13.1)^{bA}$	$(2.2\pm0.8)^{cA}$	$(33.5\pm10.7)^{A}$	ND	$(46.0\pm13.3)^{bA}$	$(1.5\pm1.0)^{cA}$
1-pentanol	ND	$(7.1\pm1.8)^{A}$	ND	ND	ND	ND
2-heptanol	$(2.0\pm0.614)^{A}$	ND	$(18.7\pm6.7)^{aB}$	$(2.11\pm0.97)^{aA}$	$(1.4\pm0.9)^{aA}$	$(1.490\pm0.9)^{aA}$
1-hexanol	ND	$(47.6\pm12.3)^{A}$	ND	$(0.85\pm0.08)^{B}$	ND	$(1.26\pm0.09)^{B}$
3-octanol	$(0.35\pm0.01)^{A}$	ND	$(0.27\pm0.02)^{A}$	ND	$(0.3\pm0.1)^{A}$	ND
3-octenol	$(0.9\pm0.215)^{aA}$	$(3.2\pm0.8)^{aA}$	$(0.4\pm0.3)^{A}$	ND	$(0.5\pm0.2)^{aA}$	$(0.37\pm0.07)^{a}$
1-heptanol	$(1.4\pm0.5)^{aA}$	$(15.1\pm1.6)^{aA}$	ND	$(0.72\pm0.04)^{B}$	$(1.05\pm0.07)^{aA}$	$(0.51\pm0.03)^{aB}$
Phenylethyl alcohol	$(44.5\pm12.4)^{bA}$	$(2.3\pm0.9)^{cA}$	$(41.3\pm14.1)^{bA}$	$(5.62\pm2.78)^{cA}$	$(31.1\pm11.1)^{bA}$	$(3.692\pm1.1)^{cA}$
1-dodecanol	ND	ND	$(12.3\pm3.2)^{A}$	ND	$(0.35\pm0.03)^{B}$	ND
Ketones						
Acetone	$(6.3\pm1.9)^{aA}$	$(6.0\pm1.4)^{aA}$	$(14.5\pm2.2)^{aA}$	$(22.1\pm11.3)^{aA}$	$(6.8\pm2.182)^{bA}$	$(22.9\pm9.793)^{cA}$
2-pentanone	ND	ND	$(2.9\pm1.0)^{A}$	ND	$(0.17\pm0.02)^{A}$	ND
2-heptanone	ND	ND	$(4.7\pm1.9)^{A}$	ND	$(1.181\pm0.982)^{A}$	ND
2-nonanone	$(0.4\pm0.0)^{aA}$	$(0.4\pm0.042)^{A}$	$(9.4\pm2.0)^{bB}$	$(1.4\pm1.0)^{cA}$	$(0.32\pm0.02)^{aA}$	$(1.395\pm1.0)^{aA}$
Aldehydes						
Hexanal	$(0.1\pm0.1)^{a}$	$(1.845\pm0.645)^{aA}$	ND	ND	ND	$(0.4\pm0.2)^{A}$
Heptanal	ND	(17.545±3.244)	ND	ND	ND	ND
Octanal	ND	(6.644±2.043)	ND	ND	ND	ND
Nonanal	$(0.3\pm0.1)^{a}$	$(2.146\pm1.041)^{aA}$	ND	$(0.6\pm0.0)^{A}$	ND	$(0.52\pm0.05)^{A}$
Other compounds						
2-ethyl furan	ND	(5.9±2.392)	ND	ND	ND	ND
α-pinene	ND	$(0.53\pm0.04)^{A}$	ND	$(0.55\pm0.02)^{A}$	ND	$(1.0\pm0.5)^{A}$
D-limonene	$(2.80\pm0.98)^{A}$	ND	$(3.9\pm1.0)^{A}$	ND	$(2.4\pm1.0)^{A}$	ND
2-pentyl furan	ND	$(15.3\pm2.3)^{A}$	ND	ND	ND	$(1.1\pm0.9)^{B}$

Results present mean values of nine measurements (three cheese-makings and triplicate analyses)±standard error, expressed as relative abundance to internal standard. Mean values of the same age and the same row with different lower case letters (a-c) are significantly different ($p<0.05$). Mean values of each parameter in the same row of the same type of milk with different capital letters (A, B) are significantly different ($p<0.05$). ND=not detected

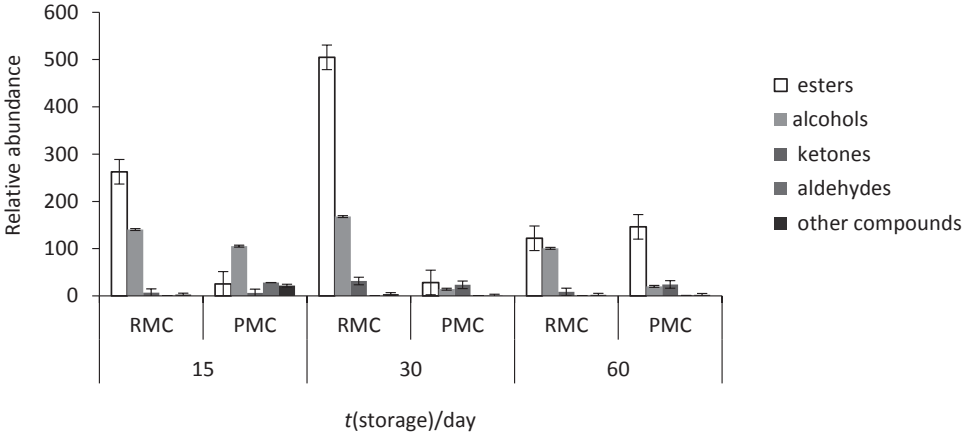

Fig. 5. Relative abundance of volatile compounds of soft Xinotyri cheese samples made from raw (RMC) or pasteurized milk (PMC) milk during aerobic storage at 4 °C

acetate to decanoate were identified in PMC. In general, the percentage of total esters was greater in RMC than in PMC samples (Fig. 5). The same trend was reported in other cheese varieties (*2,16*).

Alcohols constituted one of the main chemical families in the volatile fraction of both soft Xinotyri cheese types (Fig. 5); ethanol and phenylethyl alcohol were identified at all cheese ages. Phenylethyl alcohol compound is among the most odorous aromatic alcohols; it is identified in goat's cheese and it can be produced from phenylalanine by the action of yeasts (*7*). The mass fraction of alcohols was higher in the volatile fraction of RMC than in that of PMC samples (Fig. 3), which is in agreement with other findings (*15,60*). In general, RMC Xinotyri samples had higher mass fractions of 2-methyl propanol, phenylethyl alcohol, 3-methyl butanol, 2-heptanol and 3-octanol than PMC samples (Table 8).

Ketones were not one of the major groups of volatile compounds found in soft RMC or PMC Xinotyri cheese samples (Fig. 3). The identified ketones in both cheese products were acetone, 2-pentanone, 2-heptanone and 2-nonanone (Table 8). The mass fraction of acetone was higher in PMC than in RMC samples. This compound generally originates either from the milk or is produced from the thermal degradation of β-ketoacids (*61*). 2-Pentanone and 2-heptanone were not identified in PMC samples (Table 8).

In goat's milk cheese the aldehyde content is generally low, perhaps because the enzyme aldolase is present in low quantities (*62*). Although acetaldehyde is one of the major aldehydes found in most cheese varieties, it was not found in the soft Xinotyri cheese samples in this study, probably due to the low quantities of enzyme aldolase, which is essential for acetaldehyde production (*63*). Tamine and Robinson (*64*) also found very low levels of acetaldehyde in fermented dairy products made from goat's milk such as yogurt and sour milk. Hexanal and nonenal were found in the RMC samples and hexanal, heptanal, octanal and nonenal were found in the PMC samples during refrigerated storage (Table 8).

A number of miscellaneous compounds were also detected in soft Xinotyri cheese samples (Table 8). Two terpenes, α-pinene and limonene, were present in the samples. Terpenes are mainly feed-derived compounds (*65*). The 2-ethyl furan was also found in Beaufort cheese (*66*). The 2-pentyl furan is believed to be derived mainly from the degradation of amino acids (*67*).

Conclusions

The evolution of the primary and secondary proteolysis and lipolysis of soft Xinotyri cheese was not affected by the use of raw or pasteurized goat's milk. Pasteurization of milk, however, affected the volatile profiles of soft Xinotyri cheese. The cheese samples produced from pasteurized milk contained significantly lower populations of enterobacteria, enterococci and mainly coagulase-positive staphylococci, which were detected at unsafe levels in raw milk cheese curds. Neither *Listeria monocytogenes* nor *Salmonella* contamination was detected in the cheese samples, which had a pH≤4.4. Thus, soft Xinotyri belongs to the group of Greek fresh acid curd cheese varieties (Galotyri, Katiki, Pichtogalo Chanion) that do not support *L. monocytogenes* growth, and so a maximum population of 100 CFU/g can be allowed in this cheese during its shelf life. Further research is required on the indigenous LAB species diversity and evolution, which was quantitatively similar in Xinotyri cheese samples obtained from raw or pasteurized goat's milk, without the use of LAB as starters. Additional experimental cheese trials employing in-plant supervision are also required to standardize the soft Xinotyri cheese production technology. This study suggested that an outgrowth of *Staphylococcus aureus* in the fresh curd would be prevented by pasteurization of the raw goat's milk. Addition of commercial or natural mesophilic starter cultures to pasteurized or thermized milk needs to be investigated to standardize and improve the total quality and safety of soft Xinotyri cheese.

Acknowledgements

The authors thank the Greek Dairy Organization for the financial support of this study.

References

1. Montel MC, Buchin S, Mallet A, Delbés-Paus C, Vuitton DA, Desmasures N, Berthier F. Traditional cheeses: Rich and diverse microbiota with associated benefits. Int J Food Microbiol. 2014;177:136-54. https://doi.org/10.1016/j.ijfoodmicro.2014.02.019
2. Beuvier E, Buchin S. Raw milk cheeses. In: Fox PF, McSweeney PLH, Cogan TM, Guinee P, editors. Cheese: Chemistry, physics and microbiology: General aspects, vol. 1. London, UK: Elsevier; 2004. pp. 319-46.
3. Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF, Cotter PD. The complex microbiota of raw milk. FEMS Microbiol Rev. 2013;37:664-98. https://doi.org/10.1111/1574-6976.12030
4. Gatti M, Bottari B, Lazzi C, Neviani E, Mucchetti G. Invited review: Microbial evolution in raw milk, long-ripened cheeses produced using undefined natural whey starters. J Dairy Sci. 2014;97:573-91. https://doi.org/10.3168/jds.2013-7187
5. Fox PF. Proteolysis during cheese manufacture and ripening. J Dairy Sci. 1989;72:1379–408. https://doi.org/10.3168/jds.S0022-0302(89)79246-8
6. McSweeney PLH, Sousa MJ. Biochemical pathways for the production of flavor compounds in cheeses during ripening: A review. Lait. 2000;80:293–324. https://doi.org/10.1051/lait:2000127
7. Le Quérè JL, Septier C, Demaizières D, Salles C. Identification and sensory evaluation of the character-impact compounds of goat cheese flavour. In: Taylor AJ, Mottram DS, editors. Flavour science: Recent developments. Proceedings of the 8. Weurman Flavour Research Symposium; 1996 July 23-26; Reading, UK. Cambridge, UK: The Royal Society of Chemistry; 1996. pp. 325-30. https://doi.org/10.1533/9781845698232.6.325
8. Urbach G. The flavour of milk and dairy product: II. Cheese: contribution of volatile compounds. Int. J Dairy Technol. 1997;50:79-89. https://doi.org/10.1111/j.1471-0307.1997.tb01743.x
9. Mariaca RG, Imhof MI, Bosset JO. Occurrence of volatile chiral compounds in dairy products, especially cheese- A review. Eur Food Res Technol. 2001;212: 253–61. https://doi.org/10.1007/s002170000250
10. Kousta M, Mataragas M, Skandamis P, Drosinos EH. Prevalence and sources of cheese contamination with pathogens at farm and processing levels. Food Cont. 2010;21:805-15. https://doi.org/10.1016/j.foodcont.2009.11.015
11. De Buyser ML, Dufour B, Maire M, Lafarge V. Implication of milk and milk products in food-borne diseases in France and different industrialised countries. Int J Food Microbiol. 2001;67:1-17. https://doi.org/10.1016/S0168-1605(01)00443-3
12. Verraes C, Vlaemynck G, Van Weyenberg S, De Zutter L, Daube G, Sindic M, et al. A review of the microbiological hazards of dairy products made from raw milk. Int Dairy J. 2015:50:32-44. https://doi.org/10.1016/j.idairyj.2015.05.011
13. Lau KY, Barbano DM, Rasmussen RR. Influence of pasteurization of milk on protein breakdown in Cheddar cheese during aging. J Dairy Sci. 1991;74:727-40. https://doi.org/10.3168/jds.S0022-0302(91)78218-0
14. McSweeney PLH, Fox PF, Lucey JA, Jordan KN, Cogan TM. Contribution of the indigenous microflora to the maturation of Cheddar cheese. Int Dairy J. 1993;3:61334. https://doi.org/10.1016/0958-6946(93)90104-8
15. Ortigosa M, Torre P, Izco JM. Effect of pasteurization of ewe's milk and use of native starter culture on the volatile components and sensory characteristics of Roncal cheese. J Dairy Sci. 2001;84:1320-30. https://doi.org/10.3168/jds.S0022-0302(01)70161-0
16. Fernández-García E, Carbonell M, Nuñez M. Volatile fraction and sensory characteristics of Manchego cheese. 1. Comparison of raw and pasteurized milk cheese. J Dairy Res. 2002;69:579-93. https://doi.org/10.1017/S0022029902005794
17. Guinee TP, Pudja PD, Farkye NY. Fresh acid-curd cheese varieties. In: Fox PF, editor. Cheese: Chemistry, physics and microbiology Vol. 2: Major cheese groups. London, UK: Chapman and Hall; 1993. pp. 363-419. https://doi.org/10.1007/978-1-4615-2648-3-13.
18. Cheeses of Protected Denomination of Origin. Hellenic Code of Food and Beverages, Article 83D; 2014. pp. 14-59.
19. Rogga KJ, Samelis J, Kakouri A, Katsiari MC, Savvaidis IN, Kontominas MG. Survival of Listeria monocytogenes in Galotyri, a traditional Greek soft acid-curd cheese, stored aerobically at 4 °C and 12 °C. Int Dairy J. 2005;15:59-67. https://doi.org 10.1016/j.idairyj.2004.05.002
20. Samelis J, Kakouri A. Microbial and safety qualities of PDO Galotyri cheese manufactured at the industrial or artisan scale in Epirus, Greece. Ital J Food Sci. 2007;19:81-90.
21. Hatzikamari M, Litopoulou-Tzanetaki E, Tzanetakis N. Microbiological characteristics of Anevato: a traditional Greek cheese. J Appl Microbiol. 1999;87:595-601. https://doi.org/10.1046/j.1365-2672.1999.00857.x
22. Panagou EZ. A radial basis function neural network approach to determine the survival of Listeria monocytogenes in Katiki, a traditional Greek soft cheese. J Food Prot. 2008;71:750-9. https://doi.org/10.4315/0362-028X-71.4.750
23. Karali F, Georgala A, Massouras T, Kaminarides S. Volatile compounds and lipolysis levels of Kopanisti, a traditional Greek raw milk cheese. J Sci Food Agric. 2013;93:1845-51. https://doi.org/10.1002/jsfa.5978
24. Papageorgiou DK, Abrahim A, Bori M, Doundounakis S. Chemical and bacteriological characteristics of Pichtogalo Chanion cheese and mesophilic starter cultures for its production. J Food Prot. 1998;61:688-92. https://doi.org/10.4315/0362-028X-61.6.688
25. Xanthopoulos V, Polychroniadou A, Litopoulou-Tzanetaki E, Tzanetakis N. Characteristics of Anevato cheese made from raw or heat-treated goat milk inoculated with a lactic starter. LWT -- Food Sci Technol. 2000;33:483-8. https://doi.org/ 10.1006/fstl.2000.0699
26. Kondyli E, Massouras T, Katsiari MC, Voutsinas LP. Lipolysis and volatile compounds of Galotyri-type cheese made using different procedures. Small Rumin Res. 2013;113:432–6. https://doi.org/10.1016/j.smallrumres.2013.04.006
27. Bontinis TG, Mallatou H, Alichanidis, E, Kakouri A, Samelis J. Physicochemical, microbiological and sensory changes during ripening and storage of Xinotyri, a traditional Greek cheese from raw goat's milk. Int J Dairy Technol. 2008;61:229-36. https://doi.org/10.1111/j.1471-0307.2008.00404.x
28. Bontinis TG, Mallatou H, Pappa EC, Massouras T, Alichanidis E. Study of proteolysis, lipolysis and volatile profile of a traditional Greek goat cheese (Xinotyri) during ripening. Small Rumin Res. 2012;105:193-201. https://doi.org/10.1016/j.smallrumres.2012.01.003
29. Ztaliou I, Tsakalidou E, Tzanetakis N, Kalantzopoulos G. Lactobacillus plantarum strains isolated from traditional Greek cheese. Taxonomic characterization and screening for enzymatic activities. Lait. 1996;76:209-16. https://doi.org/10.1051/lait:1996318
30. Mallatou H, Pappa EC, Boumba VA. Proteolysis in Teleme cheese made from ewes', goats' or a mixture of ewes' and goats' milk. Int Dairy J. 2004;14:977-87. https://doi.org 10.1016/j.idairyj.2004.03.009
31. Pappa EC, Sotirakoglou K. Changes of free amino acid content of Teleme cheese made with different types of milk and culture. Food Chem. 2008;111:606–15. https://doi.org/10.1016/j.foodchem.2008.04.027
32. De Jong C, Badings HT. Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up, and capillary gas chromatographic analysis. J High Res Chrom. 1990;13:94–8. https://doi.org/10.1002/jhrc.1240130204

33. Mallatou H, Pappa E, Massouras T. Changes in free fatty acids during ripening of Teleme cheese made with ewes', goats', cows' or mixture of ewes' and goats' milk. Int Dairy J. 2003;13:211–9. https://doi.org/10.1016/S0958-6946(02)00153-X
34. CLASS-VP™ (Chromatography Laboratory Automated Software System), v. 4.2, Shimadzu Scientific Instruments Inc., Columbia, MD, USA; 2009.
35. GCMSsolution, v. 2.5, Shimadzu Corporation, Analytical and Measuring Instruments Division, Kyoto, Japan; 2012.
36. NIST Mass S spectral Library, v. 05, Gaithersburg, MD, USA: National Institute of Standards and Technology; 2014.
37. Statgraphics Plus for Windows v. 5.2. Statpoint (since April 25, 2005; i.e. Statgraphics Plus 5.1), Inc, Warrenton, VA, USA, 2017.
38. Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. Off J Eur. Union. 2007;L322:12-29.
39. Lekkas C, Kakouri A, Paleologos E, Voutsinas LP, Kontominas MG, Samelis J. Survival of Escherichia coli O157:H7 in Galotyri cheese stored at 4 and 12 °C. Food Microbiol. 2006;23:268-76. https://doi.org/10.1016/j.fm.2005.03.008
40. Hofi AA, Youssef EH, Ghoneim MA, Tawab GA. Ripening changes in Cephalotyre "RAS" cheese manufactured from raw and pasteurized milk with special reference to flavor. J Dairy Sci. 1970;53:1207-11. https://doi.org/ 10.3168/jds.S0022-0302(70)86369-X
41. Samelis J, Lianou A, Kakouri A, Delbés C, Rogelj I, Bogovič-Matijašić B, Montel MC. Changes in the microbial composition of raw milk induced by thermization treatments applied prior to traditional Greek hard cheese processing. J Food Prot. 2009;72:783-90. https://doi.org/10.4315/0362-028X-72.4.783
42. Pexara A, Solomakos N, Sergelidis D, Govaris A. Fate of enterotoxigenic Staphylococcus aureus and staphylococcal enterotoxins in Feta and Galotyri cheeses. J Dairy Res. 2012;79:405-13. https://doi.org/10.1017/S0022029912000325
43. Samelis J, Lianou A, Pappa EC, Bogovič-Matijašić B , Parapouli M, Kakouri A, Rogelj I. Behaviour of Staphylococcus aureus in culture broth, in raw and thermized milk, and during processing and storage of traditional Greek Graviera cheese in the presence or absence of Lactococcus lactis subsp. cremoris M104, a wild, novel nisin A-producing raw milk isolate. J Food Prot. 2014;77:1703-14. https://doi.org/10.4315/0362-028X.JFP-14-105
44. Ordóñez Al, lbáñez FC, Torre P, Barcina Y. Effect of ewe's milk pasteurization on the free amino acids in ldiazábal cheese. Int Dairy J. 1999;9:135-41. https://doi.org/10.1016/S0958-6946(99)00034-5
45. Güven M, Karaca OB. Proteolysis levels of white cheeses salted and ripened in brines prepared from various salts. Int J Dairy Technol. 2001;54:29-33. https://doi.org/10.1046/j.1471-0307.2001.00003.x
46. Moatsou G, Kandarakis I, Moschopoulou E, Anifantakis E, Alichanidis E. Effect of technological parameters on the characteristics of Kasseri cheese made from raw or pasteurized ewes' milk. Int J Dairy Technol. 2001;54:69-77. https://doi.org/10.1046/j.1471-0307.2001.00010.x
47. Gaya P, Medina M, Rodriquez-Marin MA, Nuñez M. Accelerated ripening of ewes' milk Manchego cheese: The effect of elevated ripening temperatures. J Dairy Sci. 1990;73:26-32. https://doi.org/10.3168/jds.S0022-0302(90)78641-9
48. Gomez MJ, Rodriguez E, Gaya P, Nuñez M, Medina M. Characteristics of Manchego cheese manufactured from raw and pasteurized ovine milk and with defined-strain or commercial mixed-strain starter cultures. J Dairy Sci. 1999;82:2300-7. https://doi.org 10.3168/jds.S0022-0302(99)75478-0
49. Kaiser KP, Belitz HD, Fritsch RJ. Monitoring Cheddar cheese ripening by chemical indices of proteolysis. 2: Peptide mapping of casein fragments by reverse-phase high-performance liquid chromatography. Z Lebensm Untersuch Forsch. 1992;195:8–14. https://doi.org/10.1007/BF01197831
50. Polo MC, Gonzalez de Llano D, Ramos M. Determination and liquid chromatographic separation of peptides. In: Nollet LML (editor). Food analysis by HPLC. New York, NY, USA: Marcel Dekker; 1992. pp. 117-40.
51. Lee KD, Warthesen JJ. Mobile phases in reverse-phase HPLC for the determination of bitter peptides in cheese. J Food Sci. 1996;61:291–4. https://doi.org/10.1111/j.1365-2621.1996.tb14178.x
52. Trujillo AJ, Buffa M, Casals I, Fernández, Guamis B. Proteolysis in goat cheese made from raw, pasteurized or pressure-treated milk. Innov Food Sci Emerg Technol. 2002;3:309-19. https://doi.org/10.1016/S1466-8564(02)00056-5
53. Gomez MJ, Garde S, Gaya P, Medina M, Nuñez M. Relationship between level of hydrophobic peptides and bitterness in cheese made from pasteurized and raw milk. J Dairy Res. 1997;64:289–97. https://doi.org/10.1017/S0022029996002129
54. Gaya P, Sánchez C, Nuñez M, Fernández-García E. Proteolysis during ripening of Manchego cheese made from raw or pasteurized ewes' milk. Seasonal variation. J Dairy Res. 2005;72:287–95. https://doi.org/10.1017/S0022029905001019
55. Engels WJM, Visser S. Isolation and comparative characterization of components that contribute to the flavour of different types of cheese. Neth Milk Dairy J. 1994;48:127–40.
56. Picon A, Gaya P, Medina M, Nuñez M. The effect of liposome encapsulation of chymosin derived by fermentation on Manchego cheese ripening. J Dairy Sci. 1994;77:16–23. https://doi.org/10.3168/jds.S0022-0302(94)76923-X
57. Baldwin RE, Cloninger MR, Lindsay RC. Flavor thresholds for fatty acids in buffered solutions. J Food Sci. 1973;38:528–30. https://doi.org/10.1111/j.1365-2621.1973.tb01473.x
58. Freitas AC, Malcata FX. Lipolysis in Picante cheese: Influence of milk type and ripening time on free fatty acid profile. Lait. 1998;78:251-8. https://doi.org/10.1051/lait:1998227
59. Masotti F, Battelli G, De Noni I. The evolution of chemical and microbiological properties of fresh goat milk cheese during its self life. J Dairy Sci. 2012;95:4760–7. https://doi.org/10.3168/jds.2011-5039
60. Rodríguez-Alonso P, Centeno JA, Garabal JI. Comparison of the volatile profiles of Arzúa-Ulloa and Tetilla cheeses manufactured from raw and pasteurized milk. LWT - Food Sci Technol. 2009;42:1722-8. https://doi.org/ 10.1016/j.lwt.2009.04.002
61. McGugan WA, Howsam SG, Elliott JA, Emmons DB, Reiter B, Sharpe ME. Neutral volatiles in cheddar cheese made aseptically with and without starter culture. J Dairy Res. 1968;35:237–45. https://doi.org/10.1017/S0022029900018951
62. Abrahamsen RK, Holmen TB. Goat's milk yoghurt from non-homogenized and homogenized milks, concentrated by different methods. J Dairy Res. 1981;48:457-63. https://doi.org/10.1017/S0022029900021932
63. Lees GJ, Jago GR. Acetaldehyde: an intermediate in the formation of ethanol from glucose by lactic acid bacteria. J Dairy Res. 1976;43:63-73. https://doi.org/10.1017/S0022029900015600
64. Tamine AY, Robinson RK. Microbiology of yoghurt starter cultures. In: Yoghurt: Science and technology, Oxford, Pergamon Press; UK: 1985. pp. 276-94.
65. Nogueira MCL, Lubachevsky G, Rankin SA. A study of the composition of Minas cheese. LWT - Food Sci Technol. 2005;38:555–63. https://doi.org/10.1016/j.lwt.2004.07.019
66. Bosset JO, Gauch R. Comparison of the volatile flavour compounds of six European 'AOC' cheeses by using a new dynamic headspace GC-MS method. Int Dairy J. 1993;3:359–77. https://doi.org/10.1016/0958-6946(93)90023-S
67. Vranová J, Ciesarová Z. Furan in food – A review. Czech J Food Sci, 2009;27:1–10.