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Microbiological and biochemical characteristics of Kashkaval cheese produced using pasteurised or raw milk

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ABSTRACT

Microbiological quality and biochemical changes of Kashkaval cheese manufactured using sheep's raw milk without starter addition or pasteurised milk with an added commercial starter were studied. Mature cheeses had pH values 5.0–5.3, salt content 2.1–2.7%, protein content 23.3–25.1%, moisture content 36.8–39.5%, fat content 28.0–32.2%, and ash content around 5.0%. In raw milk cheeses, mesophilic non-starter lactobacilli prevailed followed by enterococci. In pasteurised milk cheeses *Lactococcus lactis* starter prevailed. All cheeses were safe according to the criteria in Regulation (EC) 1441/2007. The proteolysis index was around 20%. Butyric, myristic, palmitic, stearic and oleic were the principal free fatty acids in both cheeses. Ketones were abundant in pasteurised milk cheeses and esters in mature raw milk cheeses. Pasteurisation did not affect ($P > 0.05$) the physicochemical composition and the proteolysis of cheeses. Raw milk cheeses showed higher levels ($P < 0.05$) of lipolysis than pasteurised milk cheeses.

1. Introduction

Pasta filata is a variety of cheeses characterised by a unique technology because the curd, acidified and kneaded in hot water, produces the plastic consistency characteristic of these types of cheese (Alichanidis & Polychroniadou, 2008; Kindstedt, Caric, & Milanovic, 2004). In Greece, in the traditional way of pasta filata cheese-making, shepherds coagulated and drained the cheese curd in the mountains to prolong the shelf-life of milk that would be otherwise reduced by the high temperature, especially during hot summers, the lack of refrigeration facilities and the difficulties of milk transportation. Then, the drained cheese curds were gathered all together and were transported to the cheese factories for further processing. During transportation, the pH of the drained curd was lowered to around 5.2 due to the indigenous microbiota of the milk; at this pH the curd was matured and ready to be kneaded in hot water to get the characteristic pasta filata texture. This step was essential because the pathogenic microbiota was either eliminated or controlled. Kasseri is the most typical Greek pasta filata cheese, denominated as Protected Designation of Origin (PDO) with very wide market popularity. Metsovone is another, PDO, pasta filata, smoked cheese produced in the region of Metsovo, Epirus. Another local, handcrafted pasta filata cheese is Kashkaval cheese, which is produced in specific places, such as the mountains of Pindos, using the traditional method. Its production has been handed down from generation to generation but nowadays its production is in danger due to urbanisation.

Traditional raw milk cheeses tend to display greater variability in comparison with their cheese counterparts made of pasteurised milk, and are characterised by strong and unique organoleptic and health-associated nutritional properties that are highly appreciated by the consumers (Beuvier et al., 1997; Montel et al., 2014). However, the use of raw milk in the production of traditional cheeses carries a potential health risk (Kousta, Mataragas, Skandamis, & Drosinos, 2010; Verraes et al., 2015). Therefore, milk pasteurisation prior to cheese manufacture is necessary since it eliminates the pathogenic or undesirable bacteria resulting in a safe product of constant quality. On the other hand, pasteurisation changes the biochemistry and microbiology of ripening as well as the flavour of the cheese (Beuvier et al., 1997). During the manufacturing process of pasta-filata cheeses, the heat treatment of the curd in hot water can have a pasteurising effect on the final product as it partially suppresses the undesirable microbial growth.

In the literature, there are many publications about Kashkaval cheese produced in many countries. In Greece, the traditional

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manufacturing process of Kashkaval cheese has been recorded since the beginning of the previous century (Dimitriadis, 1900; Liambeis, 1900; Polychroniadis, 1912); however, to our knowledge, no studies have dealt in depth with the microbiological and biochemical characteristics of this traditional type of cheese. Research-based characterisation of Kashkaval cheese would be useful to establish its identity, evaluate its quality and safety attributes and increase its market popularity. Therefore, this study was done to (i) describe the microbiological, compositional, biochemical and sensory characteristics of Kashkaval of Pindos cheese made using raw or pasteurised sheep's milk, during ripening and storage; (ii) evaluate the hygienic quality of the raw and pasteurised milk cheeses with the aim to assist small scale Greek manufacturers in producing safe Kashkaval cheeses in compliance with the current microbiological safety criteria in European Commission Regulation (EC) 1441/2007 (EC, 2007).

2. Materials and methods

2.1. Cheese manufacture and sampling

Three cheese-making trials were carried out in three consecutive days in the mountains of Pindos (>1000 m height), in Epirus, North Western part of Greece, using simple processing equipment. The drained cheese curds were carried to the pilot plant of the Dairy Research Department, Institute of Technology of Agricultural Products, for further processing.

Two cheeses were produced using either raw or pasteurised sheep's milk in each trial. Raw milk (32 kg) was heated under stirring at 33 °C. At that temperature, rennet (1:10.000 strength:-NATUREN Extra NB, Chr. Hansen, Hørsholm, Denmark) was added according to the manufacturer's instructions. Coagulation took place within 40 min. Then the coagulum was cut into pieces with size 6–8 mm, scalded-up to 42 °C within 30 min (rate 1 °C per 3 min) and held at this temperature, under gentle stirring, for 15 min. The pieces of the curd were ladled from the vat into pierced stainless steel moulds containing a cheese cloth. A metallic cover was placed on top of the mould and a weight of 4 kg was initially placed on the cove, to assist draining. After 30 min, the cheese curd was turned over and the weight was placed again. This was repeated three times; then the weight was removed and the curd was ready to be transported to the pilot plant of the Dairy Research Department at Ioannina for further processing.

Kashkaval cheese using pasteurised milk was manufactured as follows: milk (32 kg) was pasteurised in open-batch pasteurisation at 63 °C for 30 min and then cooled at 37 °C. A freeze-dried, direct to vat set (DVS) starter culture (RSF-736: Chr. Hansen) containing *Lactobacillus helveticus*, *Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Streptococcus thermophilus* was used following the supplier's instructions. To assist curdling of milk, $CaCl_2$ was added (20 g 100 kg^{-1}). All the other steps for the production of pasteurised Kashkaval cheese, including addition of the same type and amount of rennet at 33 °C, were performed as reported above.

On arrival at the pilot plant, raw and pasteurised curd was left at 17 °C to ripen until the pH fell to ~5.2, i.e., after 20 h. Afterwards, the acidified curd was cut into long thin slices. The slices were put in stainless steel containers with hot water (~80 °C) and manipulated with a wooden stick until a homogenous compact texture was obtained (pasta filata). Then, while still hot (55–58 °C), the elastic 'pasta filata' curd was transferred to a cheese-table, salted with 1.5% dry salt of fine grade, kneaded by hand and moulded in cylindrical moulds, and transferred to a ripening room (17 °C). After 24 h the moulds were removed; the raw and pasteurised Kashkaval cheeses were salted (15 g 10 kg^{-1} cheese) again. Cheese salting and turning lasted for ten days (four saltings took place) and then the cheeses were transferred to a lowered temperature ripening room (12 °C) for maturation until they were 90 days old. Then the cheeses were transferred at cold rooms 2–4 °C for storage.

Samples were taken immediately after the kneading and salting (day 1), at the end of the saltings (day 12), and after 30, 60, 90 and 180 days of ripening. All analyses were carried out in duplicate.

2.2. Gross composition

The pH of the cheese was measured with the micro-pH 2001 meter (Crison, Barcelona, Spain). The composition of the cheese (moisture, fat, protein, NaCl and ash content) was determined with established methods, as described by Pappa, Kandarakis, Anifantakis, and Zerfiridis (2006).

2.3. Microbiological analyses

Cheese samples (10 g each) were transferred aseptically to stomacher bags with 90 mL of sterile quarter strength Ringer solution (Lab M, Heywood, UK) and homogenised in a stomacher (Lab Blender 400; Seward, London, UK) for 60 s at room temperature. Each homogenate was serially diluted with Ringer solution, and duplicate 1 mL or 0.1 mL samples of appropriate dilutions were poured or spread on total or selective agar plates. Unless otherwise stated, all media and supplements were purchased from Lab M. The agar media, reagents and incubation conditions used were according to the procedures tabulated by Samelis et al. (2009), with slight modifications for improving certain microbial group quantifications (Noutsopoulos et al., 2017; Vandera, Tsirka, Kakouri, Koukkou, & Samelis, 2018).

Total viable counts (TVC) were enumerated on milk plate count agar (MPCA), incubated at 37 °C for 48 h, rechecked at 72 h; enumeration of TVC on MPCA at 37 °C was preferred over that on tryptic soy with 0.6% yeast extract (TSAYE) or CASO agars at 30 °C for 72 h (Bontinis, Mallatou, Alichanidis, Kakouri, & Samelis, 2008; Samelis et al., 2009) to enhance nonselective growth of all types of milk-specific (lactose-fermenting) bacteria expected to comprise the predominant total microbiota in raw and particularly pasteurised milk cheeses (Noutsopoulos et al., 2017).

Total mesophilic and thermophilic lactic acid bacteria (LAB) were enumerated on de Man, Rogosa, Sharpe (MRS) agar, incubated at 30 °C for 72 h under aerobic conditions and at 45 °C for 48 h under anaerobic conditions (Gas-Pack System, BBL, Becton Dickinson, Sparks, MD, USA), respectively; anaerobic incubation of the MRS agar plates at 45 °C was required to enhance recovery and growth of *Lb. helveticus* starter plus other thermophilic dairy *Lactobacillus* spp., whereas aerobic incubation of MRS at 30 °C has not been found to affect recovery and growth of mesophilic lactobacilli (mainly *Lactobacillus paracasei/casei* and *Lactobacillus plantarum*) and *Leuconostoc* in milk or traditional Greek cheese niches (Samelis et al., 2009, 2010).

Mesophilic cocci (presumptive lactococci) and thermophilic cocci (presumptive streptococci) were enumerated on M17 agar, incubated at 22 °C for 72 h and 42 °C for 48 h, respectively; enterococci on Slanetz & Bartley (SB) agar incubated at 37 °C for 48 h (Noutsopoulos et al., 2017; Vandera et al., 2018) instead of kanamycin aesculin azide agar we used in earlier dairy studies (Bontinis et al., 2008; Samelis et al., 2009, 2010); total staphylococci on Baird–Parker agar base with egg yolk tellurite (BP), incubated at 37 °C for 48 h; lactose-fermenting enterobacteria (coliforms) on double-layered violet red bile agar, incubated at 37 °C for 24 h; yeasts and moulds on rose bengal chloramphenicol agar, incubated at 25 °C for 5 days. The agar media electivity and the presence on BP agar of staphylococcal colonies that caused latex agglutination were checked with appropriate rapid tests according to Bontinis et al. (2008).

Finally, the presence of *Salmonella* sp. and *Listeria* sp./*Listeria monocytogenes* in 25 g samples of freshly prepared (day 1) and mature (day 90) cheeses was determined according to the culture enrichment procedures reported by Bontinis et al. (2008).

2.4. Proteolysis

Proteolysis was assessed by measuring different nitrogen fractions. Total nitrogen (TN), water-soluble nitrogen (WSN), nitrogen soluble in 5% phosphotungstic acid (PTA-N) and nitrogen soluble in 12% trichloroacetic acid (TCA-N) were determined as described by Mallatou, Pappa, and Boumba (2004).

2.5. Lipolysis

The method of De Jong and Badings (1990) was used for the extraction and isolation of free fatty acids (FFAs) of Kashkaval cheese samples. A Shimadzu model GC-17A gas chromatograph equipped with an on-column injector and a flame ionisation detector (FID) was used with a fused silica capillary column (length 15 m, inner diameter 0.53 mm) coated with free fatty acid phase OV-351 (bonded polyglycol-nitroterephthalic, film thickness 1.0 μm; Ohio Valley Capillaries, Marietta, OH, USA). Direct on-column injection took place at 60 °C; the injector temperature was raised from 60 °C to 230 °C, at a rate of 35 °C min^{-1}, and then held at 230 °C for 40 min. After a 2 min hold at 60 °C, the oven temperature was programmed from 60 °C to 70 °C at a rate of 1 °C min^{-1}, and then to 220 °C at a rate of 10 °C min^{-1} and then held at 220 °C for 18 min. The FID temperature was 225 °C. The flow rate of the carrier gas (helium) was 8.8 mL min^{-1} at 60 °C. The identification of the individual fatty acids of the cheese samples was based on a comparison of the retention times of the unknown FFAs with those obtained from known FFA standards (Sigma, Steinheim, Germany) under identical conditions. The quantification of the FFA levels of cheese samples was performed using the internal standardisation technique, i.e., with C9:0 as an internal standard and processing the chromatograms with the CLASS-VP TM Chromatography Laboratory Automated Software System (Shimadzu Scientific Instruments Inc., Columbia, MD, USA).

2.6. Volatile compounds

The volatile compounds of cheese samples at 1, 90 and 180 days of ripening and storage were studied by SPME-GC–MS analysis, as described by Kondyli, Pappa, and Svarnas (2016). Semi-quantification was performed by integrating the peak areas of total ion chromatograms (TIC) by the Shimadzu GCMS Solution software. The quantities of each compound were expressed as peak area $\times 10^{-6}$ (in arbitrary units).

2.7. Organoleptic evaluation

Samples of cheeses, cut in small cubes of ~2 cm side, were assessed organoleptically at 90 and 180 days by five trained panel members who were permanent staff of the Dairy Department; well experienced and familiar with pasta filata cheeses. The panel was asked to evaluate the appearance (exterior, interior), texture (body) and flavour (odour and taste) and to notice any defects (such as defective colour, sponge-like appearance; chalky, grainy or brittle texture; rancid, bitter, or mature flavour etc), according to the IDF (1987) guide for organoleptic evaluation of the cheese. Samples during evaluation were at ambient temperature (18 ± 2 °C).

2.8. Statistical analyses

The data were subjected to one-way analysis of variance (ANOVA) to test the differences (at $P < 0.05$) among the type of milk (raw or pasteurised) at each sampling day using the software Statgraphics Plus for Windows v. 5.2 (Manugistics Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Gross composition

The mean composition of the milk used for the manufacture of Kashkaval cheese was: fat 6.5% ± 0.2, protein 5.7% ± 0.0, lactose 4.5% ± 0.0, total solids 17.3% ± 0.2, and its pH was 6.6 ± 0.0.

The physicochemical characteristics of Kashkaval cheeses made from raw or pasteurised milk, during ripening and storage, are reported in Table 1. Mature Kashkaval cheese (over 60 days of ripening and storage), regardless the type of milk used (pasteurised or raw), had pH values 5.0–5.3, salt content 2.1–2.7%, protein content 23.3–25.1%, moisture content 36.8–39.5%, fat content 28.0–32.2%, and ash content around 5.0%. The gross composition of Kashkaval cheeses of this study was in agreement with the data reported by Alichanidis and Polychroniadou (2008), Caric (1993), and Kindstedt et al. (2004) for Greek pasta filata cheeses and fulfil the requirements of regulation No 1225/90 of European Economic Community (EEC, 1990).

There were no significant differences in mean pH, salt, protein and ash in all sampling days (Table 1). Furthermore, pasteurisation had no effect on moisture and fat composition, except in 60 days old Kashkaval cheeses, in which pasteurised samples had higher ($P < 0.05$) moisture and therefore lower fat content than raw milk cheese samples.

3.2. Microbiological characteristics of the cheeses

The changes in populations of the main microbial groups during ripening and storage of Kashkaval cheese are summarised in Table 2. The TVC of fresh (day 1) pasteurised milk cheeses ranged from 7.8 to 8.4 log cfu g^{-1}; thermophilic dairy cocci were the most numerous LAB, reflecting prevalence of the *S. thermophilus* starter. Conversely, the TVC of fresh raw milk cheeses ranged from 6.9 to 7.5 log cfu g^{-1} and consisted mainly of autochthonous mesophilic LAB. The most prominent ($P < 0.05$) difference between the two cheese types was the high occurrence of indigenous enterococci in the fresh raw milk cheeses which ranged from 6.2 to 6.8 log cfu g^{-1} (Table 2). In full contrast, enterococci were suppressed by 4 log units in the fresh pasteurised milk cheeses (Table 2), reflecting the detrimental effects of pasteurisation on them and the microbiota of raw milk overall (data not shown). Samelis et al. (2009) reported that even thermisation of raw ewes'/goats' milk mixtures at 60–67 °C for 30 s before traditional Greek hard cheese processing caused major reductions in all types of indigenous LAB. However, enterococci and streptococci resisted thermisation much better than lactococci, leuconostocs and other mesophilic LAB (Samelis et al., 2009).

In this study, the “pasta filata” process that involved immersion of the acidified (approximately pH 5.2) curd slices in hot water before kneading caused similar heat inactivation effects on the different LAB groups with those of milk thermisation (Samelis et al., 2009), thus favouring the prevalence of enterococci on all LAB-selective agar media after heating and stretching of raw milk cheese curds (Table 2). Conversely, following its rapid dominant growth (>8 to 9 log cfu g^{-1}) during acidification of the pasteurised milk curds within the first 24 h of fermentation (see also Beresford,

Table 1
Changes of physicochemical characteristics of Kashkaval cheese made from pasteurised (P) or raw (R) milk during ripening and storage.[a]

Parameter	Cheese type	Cheese age (days)					
		1	12	30	60	90	180
pH	P	5.1 ± 0.1[a]	5.0 ± 0.0[a]	5.1 ± 1.0[a]	5.1 ± 0.1[a]	5.2 ± 0.1[a]	5.0 ± 0.2[a]
	R	5.1 ± 0.1[a]	5.0 ± 0.0[a]	5.1 ± 0.0[a]	5.1 ± 0.0[a]	5.3 ± 0.1[a]	5.1 ± 0.1[a]
Moisture (%)	P	45.9 ± 1.0[a]	43.6 ± 0.3[a]	41.8 ± 0.4[a]	41.9 ± 0.7[a]	39.8 ± 0.4[a]	36.8 ± 0.6[a]
	R	45.3 ± 1.1[a]	42.4 ± 0.6[a]	40.5 ± 0.2[a]	39.5 ± 0.2[b]	38.7 ± 0.8[a]	36.9 ± 0.4[a]
Fat (%)	P	26.2 ± 1.0[a]	28.3 ± 0.3[a]	28.7 ± 0.4[a]	28.0 ± 0.0[a]	29.0 ± 0.4[a]	31.0 ± 0.6[a]
	R	26.8 ± 1.9[a]	27.6 ± 1.9[a]	30.0 ± 0.8[a]	30.4 ± 0.4[b]	30.9 ± 0.7[a]	32.2 ± 0.7[a]
Proteins (%)	P	23.8 ± 0.4[a]	23.8 ± 0.4[a]	24.2 ± 0.5[a]	23.3 ± 0.6[a]	24.6 ± 0.5[a]	25.1 ± 0.5[a]
	R	25.0 ± 1.2[a]	24.1 ± 0.3[a]	24.0 ± 0.3[a]	24.2 ± 0.4[a]	24.2 ± 0.5[a]	24.9 ± 0.4[a]
NaCl (%)	P	0.5 ± 0.3[a]	1.6 ± 0.3[a]	1.9 ± 0.2[a]	2.4 ± 0.2[a]	2.6 ± 0.3[a]	2.1 ± 0.4[a]
	R	0.5 ± 0.3[a]	1.4 ± 0.4[a]	2.0 ± 0.2[a]	2.1 ± 0.0[a]	2.7 ± 0.3[a]	2.5 ± 0.4[a]
Ash (%)	P	2.7 ± 0.2[a]	3.7 ± 0.2[a]	4.3 ± 0.2[a]	5.0 ± 0.2[a]	4.9 ± 0.2[a]	4.5 ± 0.2[a]
	R	2.8 ± 0.3[a]	3.5 ± 0.2[a]	4.3 ± 0.1[a]	4.6 ± 0.1[a]	5.1 ± 0.1[a]	5.0 ± 0.1[a]

[a] Values are the mean of three cheese-making trials ± standard error; means for each parameter in the same column with different superscript letters are significantly different ($P < 0.05$).

Table 2
Microbiological changes (log cfu g^{-1}) in Kashkaval cheese made from pasteurised (P) or raw (R) milk during ripening and storage.[a]

Microbial group	Cheese type	Cheese age (days)					
		1	12	30	60	90	180
Total viable bacteria (TVC)	P	8.1 ± 0.3[b]	6.7 ± 0.4[a]	6.2 ± 0.9[a]	6.0 ± 0.9[a]	6.8 ± 0.2[a]	4.4 ± 0.6[a]
	R	7.2 ± 0.3[a]	7.6 ± 0.4[a]	8.2 ± 0.4[b]	7.9 ± 0.4[b]	7.7 ± 0.3[a]	7.4 ± 0.2[b]
Mesophilic lactic acid bacteria	P	4.5 ± 0.5[a]	4.8 ± 1.3[a]	6.4 ± 1.4[a]	6.0 ± 1.8[a]	7.3 ± 1.1[a]	6.5 ± 1.3[a]
	R	6.5 ± 0.3[b]	8.3 ± 0.3[b]	8.7 ± 0.2[b]	8.9 ± 0.1[b]	8.5 ± 0.3[a]	7.9 ± 0.1[a]
Thermophilic lactic acid bacteria	P	5.2 ± 0.4[a]	3.0 ± 0.0[a]	2.4 ± 0.3[a]	2.5 ± 0.5[a]	<2.0[a]	NT
	R	6.0 ± 0.5[a]	6.8 ± 0.5[b]	6.6 ± 0.2[b]	7.0 ± 0.1[b]	6.1 ± 0.6[b]	NT
Mesophilic dairy cocci	P	6.8 ± 0.9[a]	4.9 ± 1.0[a]	6.7 ± 1.3[a]	6.2 ± 1.6[a]	7.1 ± 1.2[a]	NT
	R	7.2 ± 0.3[a]	8.1 ± 0.3[b]	8.6 ± 0.1[b]	8.1 ± 0.3[b]	7.9 ± 0.2[a]	7.4 ± 0.3
Thermophilic dairy cocci	P	7.9 ± 0.3[a]	6.1 ± 0.1[a]	6.7 ± 1.3[a]	4.4 ± 0.4[a]	4.3 ± 0.3[a]	NT
	R	7.2 ± 0.3[a]	7.5 ± 0.3[b]	8.6 ± 0.1[b]	7.4 ± 0.1[b]	7.1 ± 0.2[b]	6.7 ± 0.2
Enterococci	P	2.4 ± 0.3[a]	<2.0[a]	2.9 ± 0.5[a]	2.5 ± 0.3[a]	2.7 ± 0.2[a]	<2.0[a]
	R	6.5 ± 0.3[b]	7.2 ± 0.3[b]	6.3 ± 0.3[b]	6.3 ± 0.5[b]	6.1 ± 0.6[b]	4.9 ± 0.5[b]
Total staphylococci	P	<2.0[a]	3.9 ± 0.6[a]	3.7 ± 0.3[a]	2.7 ± 0.4[a]	3.3 ± 0.0[a]	2.4 ± 0.2[a]
	R	4.6 ± 0.6[b]	4.3 ± 0.5[a]	5.5 ± 0.4[b]	3.2 ± 0.1[a]	3.5 ± 0.7[a]	3.0 ± 0.4[a]
Enterobacteria (coliforms)	P	1.5 ± 0.4[a]	<1.0	<1.0	<1.0	<1.0	<1.0
	R	3.0 ± 0.8[b]	<2.0	<1.0	<1.0	<1.0	<1.0
Yeasts	P	<2.0	3.6 ± 0.6[a]	2.5 ± 0.3[a]	2.7 ± 0.2[a]	2.9 ± 0.2[a]	<2.0[a]
	R	<2.0	2.9 ± 0.5[a]	4.1 ± 0.2[b]	2.8 ± 0.3[a]	2.9 ± 0.5[a]	2.7 ± 0.5[b]

[a] Values are the mean of three cheese-making trials ± standard error; means for each microbial group in the same column with different superscript letters are significantly different ($P < 0.05$). NT, not tested.

Fitzsimons, Brennan, & Cogan, 2001; Noutsopoulos et al., 2017; Samelis & Kakouri, 2018), the *S. thermophilus* starter survived the acidified curd heating process better than the mixed mesophilic *Lc. lactis* starter strains (Table 2).

This finding is logical because following immersion of the curd slices at an approximate ratio of 1:1.5 (w/v) in 80 °C (measured) water, an immediate temperature reduction to around 72 °C followed by progressive reductions down to 65 °C occurred. Under the traditional cheese making conditions applied in this study, those temperature reductions neither could be avoided nor were recorded precisely because curd manipulation was more important at this critical processing step. Nevertheless, as mentioned, at the end of the 'pasta filata' process, the temperature of the still hot elastic cheese mass was 55–58 °C, indicating that major parts of the indigenous and starter LAB microbiota grown in the curds pre-heating could remain viable, especially dominant enterococci and *S. thermophilus* (Table 2). In agreement, Simov, Simova, and Beshkova (2006) found that hot-brining of Kashkaval curd at 72 °C for 2 min destroyed 91% and 84.5% of the selected mixed-LAB starter type A (*S. thermophilus, Lc. lactis, Lb. casei*) and type B (natural yoghurt starter; *S. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*), respectively. However, despite those major heat inactivation effects, both Kashkaval cheeses A and B started ripening with starter LAB counts above 8 log cfu g^{-1} and prevalence of thermophilic (69%) and mesophilic cocci (69%) over thermophilic (30%) and mesophilic (11.2%) lactobacilli, respectively (Simov et al., 2006).

During ripening of the salted Kashkaval cheeses, microbial recovery and growth generally were much more pronounced ($P < 0.05$) in the raw than the pasteurised milk cheeses (Table 2). Particularly the populations of thermoplilic dairy cocci (e.g., *S. thermophilus*) and thermophilic LAB (e.g., *Lb. helveticus*) declined after day-1 ($P < 0.05$), probably because growth of both salt-sensitive dairy starter species ceased in the salted 'pasta filata' cheese matrix during ripening. Hence, the TVC of pasteurised milk cheeses comprised mainly *Lc. lactis* starter strains that remained at the 7 log level after 90 days of ripening (Table 2). Conversely, in raw milk cheeses, TVC and mesophilic LAB counts exceeded the 8 log level on all media incubated aerobically at 22–42 °C by mid of ripening (day 30).

This result suggested that autochthonous mesophilic lactobacilli predominated in the salted raw milk cheeses after day 12; although this needs validation for Kashkaval by LAB identification, numerous previous reports on a progressive prevalence of *Lb. casei/paracasei* and *Lb. plantarum* genomic groups in traditionally ripened hard and semi-hard Balkan and other cheese types exist (Beresford et al., 2001; Montel et al., 2014). Particularly in mature traditional

Greek Graviera cheeses produced from thermised milk, these non-starter *Lactobacillus* groups comprised 83.8% of total LAB isolates, and were almost evenly isolated from all media except of MRS agar at 45 °C from which exclusively enterococci (approximately 7 log cfu g^{-1}) were isolated (Samelis et al., 2010). Enterococci also remained close to the 7 log level during ripening of Kashkaval raw milk cheeses (Table 2). All LAB groups tended to decline in both cheese types with prolonged ripening (day 60–90) and during storage (day 90–180), with the decline of *S. thermophilus* starter in the pasteurised milk cheeses after day 30 of age being the greatest (approximately 2.5 log units) (Table 2).

Simov and Ivanov (2005) found similarly low populations of *S. thermophilus* starter in mature low-salt Kashkaval cheeses in which, however, the symbiotic starter, *Lb. delbrueckii* spp. *bulgaricus*, reached 6 log cfu g^{-1}; this last finding is in full contradiction with the present *Lb. helveticus* starter, which failed to grow in pasteurised milk cheeses during ripening and was undetectable (<2 log units) at day 90 (Table 2). When, however, Simov et al. (2006) used *Lb. casei* RP5 as a starter, the ripening of Kashkaval cheese A occurred with the dominating presence of this strain from 64% at day 30 to 95.2% at day 90, in support of the preceding discussion on mature raw milk cheeses in Table 2.

Yeasts were below 100 cfu g^{-1} in all fresh cheese samples, and generally ranged below 3 log cfu g^{-1} in the cheeses during ripening, irrespective of the use of raw or pasteurised milk (Table 2). Mould colonies were very few (<50 cfu) to absent in all cheeses (data not shown).

3.3. Hygienic quality and safety of the cheeses

Although total staphylococci and enterobacteria are considered members of the beneficial ripening microbiota in cheese, they also include pathogenic species or serotypes of great concern for cheese hygiene and safety (Kousta et al., 2010; Verraes et al., 2015). In this study, however, total staphylococci and coliforms were at low levels and reduced ($P < 0.05$) in the fresh (day 1) pasteurised milk cheeses compared with their raw milk cheese counterparts (Table 2). Particularly the coliform bacteria, including *Escherichia coli*, appeared to be very sensitive to the 'pasta filata' cheese process since their counts declined below 10 cfu g^{-1} in all cheeses after day 12 (Table 2). Conversely, levels of staphylococci remained higher in raw than pasteurised milk cheeses; their highest level, 5.5 ± 0.4 log cfu g^{-1}, was found in the raw milk cheeses by day 30.

Notably, several (10–15%) of the staphylococcal colonies grown in the raw milk cheese samples from day 1 to day 30 showed lekithinase activity on BP agar, and about 50% of them further showed latex agglutination activity. Thus, potentially pathogenic staphylococci might have exceeded the 5 log threshold level, specified in Regulation (EC) 1441/2007, in the fresh raw milk cheese curds before their heating and stretching. Additional studies are in progress to assess the potential for major growth (>5 log) and enterotoxin production of pathogenic staphylococci in Kashkaval raw milk cheese during early steps of processing; this may be a safety risk despite the fact that total staphylococci were reduced below 4 log cfu g^{-1} in all ripened cheeses after day 30 (Table 2).

All fresh (day 1) and mature (day 90) cheeses were free of *Salmonella* and *Listeria* contamination in 25 g after culture enrichment, and overall, were safe according to microbiological criteria (EC, 2007). This positive finding for Kashkaval cheese safety was because either the raw milks did not contain salmonellae and listeriae, or pasteurisation of the milk, or curd heating during 'pasta filata' processing (also applied to raw milk cheeses) inactivated, if any, the above natural pathogen contaminants. Consistent with this, Alichanidis and Polychroniadou (2008) reported that despite the technology used (e.g., raw or pasteurised milk with or without starters, etc.) for Kashkaval production, heat treatment of the curd during texturising has a preservative effect on the final cheese product, enabling raw milk of poor microbiological quality and relatively high acidity to be processed.

3.4. Proteolysis

The nitrogenous fractions of Kashkaval cheese made using raw or pasteurised milk, during ripening and storage, are shown in Table 3. In general, the pasteurisation of cheese milk did not affect the soluble nitrogen fraction ($P > 0.05$); except at 30, 90 and 180 days, in which raw milk cheeses showed higher ($P < 0.05$) values of TCA and PTA fractions than pasteurised Kashkaval cheeses (Table 3). The proteolysis index (100× WSN/TN) of mature (90 days) Kashkaval cheese ranged from 16.6 to 22.9% in agreement with the results of Caric (1993) and Alichanidis and Polychroniadou (2008) who reported it to be around 20%. Similar levels (17.3–20.2%) of proteolysis of Kashkaval cheeses A and B, though obtained at shorter ripening times of 30 and 60 days, respectively, and attributed to the high proteolytic activity of *Lb. casei* and *Lb. delbrueckii* starters, were reported by Simov et al. (2006).

3.5. Organoleptic evaluation

The scores of the organoleptic evaluation are shown in Table 4. The increase in the total sensory characteristics followed as a result of the increase of the individual characteristic. Pasteurisation decreased ($P < 0.05$) flavour and total organoleptic scores at 180 days of storage of Kashkaval cheese (Table 4). In general, panellists described Kashkaval as a cheese with a yellowish colour and a pleasant, piquant, slightly salty taste. Kashkaval cheeses from raw milk were described with more complex flavours (such as fruity, floral and piquant) than the pasteurised milk cheeses, which were described not as piquant as the raw milk cheeses. The above results also show that pasteurisation did not seem to reduce the quality of the cheese.

3.6. Lipolysis

The evolution of total FFAs (TFFAs) and individual FFAs and acetic acid of Kashkaval cheese made from raw or pasteurised milk, at different sampling days are presented in Table 5. Milk pasteurisation significantly ($P < 0.05$) affected TFFA content; pasteurised milk cheeses showed lower values of TFFA content than raw milk cheeses, except at day 30 when no differences ($P > 0.05$) were observed (Table 5). Raw milk cheeses showed higher concentrations of individual FFAs than pasteurised milk cheeses. Pasteurisation of milk affects the FFA content in pasteurised cheese as it inactivates the lipolytic enzymes present naturally in raw milk (Urbach, 1997).

Lipolysis in Kashkaval cheese was not very intense. In 90 days old Kashkaval cheese the concentration of FFAs ranged between 0.586 and 2.160 g kg^{-1} (Table 5); similar values were reported by Caric (1993) for pasta filata cheeses. The major fatty acids present in raw and pasteurised milk cheeses (Table 5), at 180 days of storage, were butyric acid (C4:0), myristic (C14:0), palmitic acid (C16:0), stearic (C18:0) and oleic acid (C18:1) and this is in accordance with the results of Kindstedt et al. (2004). The results of this study also showed that acetic acid (C2) was present in high concentrations, although Kindstedt et al. (2004) reported it to be present in low amounts. This difference can be attributed to variations in curd composition, added starter cultures or other factors. It is also known that acetic acid is not a product of lipolysis; it is mainly a product of other biochemical pathways, such as the fermentation of lactic acid (Fox, Law, McSweeney, & Wallace, 1993).

Table 3
Changes of soluble nitrogenous fractions (% of total nitrogen, TN) of Kashkaval cheese made from pasteurised (P) or raw (R) milk during ripening and storage.[a]

Soluble nitrogenous fractions (%TN)	Cheese type	Cheese age (days)					
		1	12	30	60	90	180
WSN	P	3.4 ± 0.6[a]	7.2 ± 0.3[a]	9.7 ± 0.3[a]	13.9 ± 0.6[a]	22.9 ± 0.9[a]	42.8 ± 0.8[a]
	R	3.3 ± 0.5[a]	7.3 ± 0.6[a]	11.3 ± 0.6[a]	15.3 ± 1.4[a]	16.6 ± 1.0[a]	38.3 ± 5.2[a]
TCA	P	1.3 ± 0.0[a]	2.3 ± 0.1[a]	3.4 ± 0.1[a]	5.1 ± 0.1[a]	5.3 ± 0.2[a]	6.1 ± 0.4[a]
	R	1.5 ± 0.1[a]	2.7 ± 0.3[a]	4.6 ± 0.3[b]	8.1 ± 1.2[a]	7.8 ± 0.7[b]	8.9 ± 1.0[a]
PTA	P	0.4 ± 0.0[a]	0.6 ± 0.0[a]	1.2 ± 0.2[a]	1.1 ± 0.2[a]	1.0 ± 0.1[a]	1.1 ± 0.1[a]
	R	0.4 ± 0.1[a]	0.7 ± 0.1[a]	1.1 ± 0.1[a]	2.1 ± 0.4[a]	2.0 ± 0.3[a]	2.5 ± 0.1[b]

[a] Values are the mean of three cheese-making trials ± standard error; means for each parameter in the same column with different superscript letters are significantly different ($P < 0.05$). WSN, water-soluble nitrogen; TCA, nitrogen soluble in 12% trichloroacetic acid; PTA, nitrogen soluble in 5% phosphotungstic acid.

Table 4
Organoleptic evaluation of Kashkaval cheese made from pasteurised (P) or raw (R) milk during storage.[a]

Parameter (maximum score)	Cheese type	Cheese age (days)	
		90	180
Appearance (10)	P	9.2 ± 0.1[a]	9.3 ± 0.2[a]
	R	9.2 ± 0.0[a]	9.5 ± 0.3[a]
Body-Texture (40)	P	36.1 ± 0.1[a]	36.4 ± 0.2[a]
	R	35.6 ± 0.6[a]	37.6 ± 0.7[a]
Flavour (50)	P	44.4 ± 0.9[a]	43.9 ± 0.5[a]
	R	44.2 ± 0.8[a]	47.0 ± 0.3[b]
Total (100)	P	89.7 ± 1.0[a]	89.6 ± 0.8[a]
	R	89.0 ± 1.4[a]	93.7 ± 0.5[b]

[a] Values are the mean of three cheese-making trials ± standard error; means for each parameter in the same column with different superscript letters are significantly different ($P < 0.05$). Total (100) is the sum of values of appearance (10), body-texture (40) and flavour (50).

3.7. Volatile compounds

Twenty-one volatile compounds were identified in the volatile fraction of Kashkaval cheese including six ketones, six alcohols, five esters, four aldehydes and are listed in Table 6. Ketones were the major group found in pasteurised mature (90–180 days) milk cheese and esters in raw mature milk cheese. Ketones are intermediate compounds, which may be reduced to secondary alcohols. They have typical odours and low perception thresholds. Esters are mainly produced by enzymatic or chemical reaction of fatty acids with primary alcohols (Engels, Dekker, De Jong, Neeter, & Visser, 1997) and also by trans-esterification of partial glycerides to ethanol (Collins, Mc Sweeney, & Wilkinson, 2004). The major volatile compounds found in pasteurised mature milk cheese were acetoin, acetone, 2-pentanone and 2-heptanone whereas acetoin, 1 butanol 3 methyl, acetone and hexanoic acid ethyl ester in mature raw milk cheese (Table 6).

Table 5
Changes of free fatty acids (FFAs) of Kashkaval cheese made from pasteurised (P) or raw (R) milk during ripening and storage.[a]

FFA (μg g^{-1})	Cheese type	Cheese age (days)				
		1	30	60	90	180
C2	P	142.7 ± 21.4[a]	192.6 ± 17.3[a]	188.0 ± 61.6[a]	292.9 ± 10.4[a]	330.7 ± 44.9[a]
	R	269.7 ± 66.0[a]	561.3 ± 148.8[a]	779.5 ± 76.5[b]	856.9 ± 12.7[b]	967.9 ± 13.2[b]
C4	P	3.9 ± 0.4[a]	12.0 ± 1.2[a]	26.5 ± 4.2[a]	47.9 ± 3.2[a]	98.4 ± 7.9[a]
	R	5.7 ± 0.5[b]	28.8 ± 11.9[a]	49.8 ± 8.3[a]	55.8 ± 2.2[a]	234.4 ± 18.2[b]
C4ISO	P	1.8 ± 0.4[a]	2.9 ± 0.3[a]	10.7 ± 2.5[a]	26.6 ± 10.9[a]	33.5 ± 10.4[a]
	R	4.7 ± 2.6[a]	8.0 ± 4.5[a]	18.5 ± 4.2[a]	50.1 ± 0.5[a]	61.5 ± 5.5[a]
C5ISO	P	4.4 ± 0.5[a]	7.0 ± 0.3[a]	8.4 ± 0.6[a]	13.6 ± 0.2[a]	20.1 ± 0.4[a]
	R	7.1 ± 1.4[a]	14.5 ± 4.2[a]	17.2 ± 1.5[b]	18.0 ± 0.4[b]	56.0 ± 4.9[b]
C6	P	27.7 ± 4.0[a]	31.7 ± 0.4[a]	29.6 ± 1.2[a]	26.3 ± 1.6[a]	30.7 ± 2.5[a]
	R	27.2 ± 1.0[a]	30.6 ± 3.8[a]	29.4 ± 2.1[a]	31.7 ± 3.1[a]	31.5 ± 2.8[a]
C8	P	1.9 ± 0.2[a]	2.8 ± 0.2[a]	3.5 ± 0.4[a]	4.2 ± 0.8[a]	4.7 ± 0.3[a]
	R	2.5 ± 0.1[b]	6.4 ± 1.9[a]	7.7 ± 0.7[b]	11.2 ± 2.8[a]	16.7 ± 2.1[b]
C10	P	7.3 ± 1.0[a]	15.5 ± 0.9[a]	20.1 ± 2.2[a]	20.2 ± 2.0[a]	31.5 ± 2.7[a]
	R	11.4 ± 1.4[a]	24.2 ± 5.7[a]	46.3 ± 4.2[b]	44.0 ± 9.6[a]	67.6 ± 5.7[b]
C12	P	17.5 ± 0.9[a]	25.8 ± 2.7[a]	34.7 ± 2.4[a]	32.10 ± 1.2[a]	30.3 ± 2.6[a]
	R	27.5 ± 1.4[b]	28.2 ± 4.3[a]	29.4 ± 1.6[a]	52.9 ± 6.7[b]	62.3 ± 7.9[b]
C14	P	14.9 ± 2.3[a]	47.1 ± 2.7[a]	65.2 ± 5.0[a]	66.2 ± 3.98[a]	89.1 ± 5.6[a]
	R	33.7 ± 3.4[b]	122.9 ± 30.3[a]	126.9 ± 7.7[b]	187.5 ± 29.93[a]	222.9 ± 21.9[b]
C16	P	80.8 ± 24.6[a]	125.6 ± 1.8[a]	163.2 ± 7.0[a]	148.6 ± 1.3[a]	201.7 ± 7.0[a]
	R	102.1 ± 9.6[a]	276.9 ± 64.3[a]	284.2 ± 9.5[b]	397.9 ± 55.6[b]	444.7 ± 25.7[b]
C16:1	P	5.4 ± 2.3[a]	7.4 ± 0.7[a]	10.3 ± 0.3[a]	9.8 ± 1.4[a]	12.5 ± 0.5[a]
	R	11.0 ± 1.3[a]	13.4 ± 3.3[a]	13.8 ± 1.0[b]	20.2 ± 2.2[a]	26.4 ± 0.9[b]
C18	P	31.2 ± 10.4[a]	54.3 ± 5.9[a]	54.0 ± 8.1[a]	58.1 ± 2.6[a]	74.3 ± 1.3[a]
	R	53.7 ± 5.8[a]	99.4 ± 21.3[a]	100.9 ± 9.5[b]	138.9 ± 13.4[b]	136.1 ± 0.5[b]
C18:1	P	42.4 ± 11.3[a]	76.6 ± 12.9[a]	113.6 ± 5.3[a]	106.5 ± 1.1[a]	147.1 ± 7.8[a]
	R	56.5 ± 8.6[a]	175.1 ± 37.0[a]	195.5 ± 19.0[b]	280.6 ± 27.2[b]	335.4 ± 1.2[b]
C18:2	P	0.5 ± 0.4[a]	3.2 ± 0.5[a]	4.6 ± 0.7[a]	7.0 ± 0.7[a]	8.4 ± 0.6[a]
	R	2.3 ± 0.3[b]	8.2 ± 2.1[a]	12.0 ± 1.4[b]	15.1 ± 1.2[b]	17.4 ± 0.9[b]
Total	P	351.9 ± 63.8[a]	604.4 ± 12.5[a]	650.0 ± 79.3[a]	586.2 ± 156.1[a]	887.0 ± 234.6[a]
	R	615.1 ± 35.8[b]	1387.8 ± 328.0[a]	1707.0 ± 65.5[b]	2160.6 ± 261.9[b]	2680.5 ± 36.9[b]

[a] Values are the mean of three cheese-making trials ± standard error; means for each parameter in the same column with different superscript letters are significantly different ($P < 0.05$).

Table 6
Relative abundance (peak area × 10^{-6}) of volatile of Kashkaval cheese made from pasteurised (P) or raw (R) milk during ripening and storage.[a]

Volatile compound	Day 1		Day 90		Day 180	
	P	R	P	R	P	R
Ketones						
Acetone	367.2 ± 41.7[a]	764.4 ± 43.9[b]	4116.8 ± 1021.5[a]	10784.5 ± 9.4[b]	46376.8 ± 39864.7[a]	18457.2 ± 166.4[a]
2 Pentanone	ND	ND	14656.5 ± 443.4[a]	4058.1 ± 1010.9[b]	26275.7 ± 12567.6[a]	568.0 ± 53.5[a]
2,3 Pentanedione	462.8 ± 16.3	ND	1005.8 ± 2.5	ND	ND	ND
2 Heptanone	ND	ND	7906.4 ± 1880.0	ND	26183.6 ± 12041.3	ND
Acetoin	53166.6 ± 400.1	ND	41542.5 ± 8403.6[a]	32214.0 ± 65.6[a]	16319.9 ± 3554.1[a]	4321.2 ± 273.0[a]
2- Nonanone	251.7 ± 26.1	ND	1350.7 ± 349.7	ND	10478.9 ± 2793.0[a]	1869.0 ± 219.5[a]
Total ketones	6398.5 ± 451.5[a]	764.5 ± 43.5[b]	70578.5 ± 11208.5[a]	47056.5 ± 1067.5[a]	125635.0 ± 65235.0[a]	25215.5 ± 59.5[a]
Alcohols						
Ethanol	4456.1 ± 226.8[a]	14276.4 ± 2847.2[a]	6414.2 ± 47.3[a]	7612.1 ± 106.6[b]	2904.5 ± 619.4[a]	5185.6 ± 3128.4[a]
1 Butanol 3 methyl	1770.1 ± 577.0[a]	17037.4 ± 5782.2[a]	1940.7 ± 228.1[a]	20734.1 ± 4376.2[a]	912.1 ± 161.1[a]	4685.7 ± 908.3[a]
1 Hexanol	248.3 ± 27.2[a]	718.0 ± 212.2[a]	1422.5 ± 899.4[a]	927.2 ± 454.1[a]	ND	ND
1 Heptanol	ND	ND	590.9 ± 9.1[a]	576.5 ± 55.3[a]	ND	ND
1-Octanol	ND	ND	ND	260.3 ± 22.4	ND	ND
Phenylethyl alcohol	ND	ND	ND	771.5 ± 65.5	ND	ND
Total alcohols	6474.5 ± 776.6[a]	32031.8 ± 8417.3[a]	11400.0 ± 720.9[a]	29849.8 ± 3870.8[b]	3816.6 ± 780.5[a]	9871.3 ± 2220.1[a]
Esters						
Butanoic acid ethyl ester	ND	ND	530.1 ± 7.0[a]	5957.4 ± 24.5[b]	695.5 ± 254.8[a]	1699.0 ± 766.8[a]
Hexanoic acid methyl ester	ND	ND	ND	45007.3 ± 1009.6	ND	53958.4 ± 7179.1
Hexanoic acid ethyl ester	ND	ND	559.7 ± 87.6[a]	3163.6 ± 18.1[b]	440.3 ± 2.9[a]	2427.5 ± 572.9[a]
Octanoic acid methyl ester	ND	ND	317.5 ± 50.0[a]	10725.3 ± 1864.8[b]	2023.5 ± 49.1[a]	2038.7 ± 776.5[a]
Octanoic acid ethyl ester	ND	ND	ND	1653.3 ± 1014.7	ND	424.1 ± 158.1
Total esters	ND	ND	1407.2 ± 30.7[a]	66506.9 ± 3895.5[b]	3159.3 ± 301.0[a]	60547.6 ± 9453.4[b]
Aldehydes						
Hexanal	3502.5 ± 67.6[a]	6005.2 ± 136.7[b]	7763.7 ± 31.5[a]	10661.0 ± 2395.1[a]	2544.2 ± 39.7[a]	1491.55 ± 42.3[b]
Heptanal	1247.1 ± 279.3[a]	1668.6 ± 23.9[a]	ND	ND	ND	ND
Octanal	157.5 ± 2.3[a]	320.4 ± 29.6[b]	435.2 ± 19.8[a]	530.2 ± 293.7[a]	ND	ND
Nonanal	490.3 ± 42.7[a]	738.1 ± 2.7[b]	712.9 ± 42.9[a]	944.4 ± 312.7[a]	ND	ND
Total aldehydes	5397.5 ± 387.5[a]	8732.5 ± 133.5[b]	8912.0 ± 54.0[a]	12135.5 ± 3001.5[a]	2544.5 ± 39.5[a]	1491.5 ± 42.5[b]
Total volatile compounds	18270.5 ± 62.4[a]	41528.8 ± 8594.3[a]	92297.7 ± 10572.3[a]	155549.0 ± 1958.7[b]	135155.0 ± 66355.9[a]	97125.9 ± 11775.5[a]

[a] Values are the mean of three cheese-making trials ± standard error; means for each parameter in the same row and sampling day with different superscript letters are significantly different ($P < 0.05$). ND, not detected.

Total ketones did not differ significantly in raw and pasteurised milk Kashkaval cheese, at any sampling day. Mature (90–180 days) pasteurised milk cheese showed higher levels of acetone and acetoin than raw milk cheese. Pasteurised milk cheese showed higher level of total alcohols content only at 90 days of ripening and storage. Total ester content was higher ($P < 0.05$) in raw milk cheese than in pasteurised milk cheese and hexanoic acid ethyl ester was found in abundance in raw mature Kashkaval milk cheese. Raw milk cheese showed higher levels of total aldehydes than pasteurised milk cheese. Hexanal was found in abundance in both cheeses.

4. Conclusions

Kashkaval of Pindos is a semi-hard, pasta-filata Greek cheese that is traditionally manufactured with raw milk. Nowadays, it is manufactured using pasteurised milk, for hygienic reasons. The results of this study showed that the traditional curd acidification and subsequent 'pasta filata' curd heating procedures were sufficient to deliver safe Kashkaval cheese products that were in compliance with the Regulation (EC) 1441/2007 microbiological safety criteria for cheese, irrespective of the use of raw or pasteurised milk. The only concern was the presence of potentially pathogenic staphylococci in raw milk cheeses before curd heating for stretching, which, however, was prevented by the open-batch pasteurisation of the milk before cheese making. Overall, the microbiological and biochemical results of the present study might contribute to Kashkaval cheese standardisation to make small scale producers capable of producing a safer cheese.

It can be concluded that revitalising the traditional production of Kashkaval of Pindos cheese while taking into account concern of consumers' safety, would result in improving its quality, preserve the cultural identity of the region of Pindos and support small family-run farms. Additionally, challenge studies are required to validate survival, growth and toxin formation of artificially contaminating pathogenic bacteria, mainly *L. monocytogenes, Staphylococcus aureus* and enterotoxigenic *E. coli* serotypes, during Kashkaval cheese processing, particularly when raw milk is used.

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