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ORIGINAL RESEARCH

Microbiological quality of artisanal Sepet cheese

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Microbial diversity in milk and in cheese itself affects the biochemical and sensory characteristics of artisanal cheeses. In this study, the microflora of Sepet cheese, which is a traditional artisanal cheese in Turkey, was investigated. Average lactococci, lactobacilli, enterococci, yeast, mould, coliform, psychrotrophic and total aerobic bacteria, presumptive Staphylococcus aureus *counts were; 7.31 ± 1.08, 7.19 ± 1.02, 6.84 ± 0.92, 3.19 ± 1.40, 0.84 ± 0.89, 2.18 ± 1.81, 4.92 ± 1.15, 7.53 ± 1.13 and 1.25 ± 1.70 log cfu/g, respectively. Staphylococci, coliform and mould counts were less than 1.00 log cfu/g at the end of ripening, which was at around 6–8 °C for 3 months. According to phenotypic and genotypic identifications, isolates were closely related to* Lactobacillus plantarum, Weisella confusa, Weisella paramesenteroides, Pediococcus pentasaceous, Enterococcus casseliflavus, Enterococcus durans *and* Enterococcus faceium. *This study provides baseline data on the microflora of traditional artisanal Sepet cheese, which is a prerequisite for a successful scale up to industrial production.*

Keywords Sepet cheese, Microbiological count, Identification, Lactic acid bacteria.

INTRODUCTION

Milk is a nutritious food for humans, but milk is also a suitable growth medium for micro-organisms. Contaminated milk can spoil easily and may pose a health hazard if pathogens are present in milk. Therefore, various preservation processes are applied to raw milk to process it into microbiologically safe dairy products. Cheese-making is a traditional milk preservation process, and cheese has an important place in the food culture of rural regions (Dost *et al.* 2004). Kamber (2008b) stated that 60% of the cheeses produced in Turkey were estimated as White Cheese, 17% of them were Kashar Cheese, 12% of them were estimated as Tulum and Mihaliç Cheese, and 11% of production was estimated as other local cheeses. Sepet cheese (‘sepet’ in Turkish translates as ‘basket’ in English) is one of the traditional artisanal cheeses produced in the Aegean region in Turkey. In the production of Sepet cheese, raw goat milk is traditionally used. The artisanal process starts with heating the milk to 30–40 °C and the addition of rennet. After renneting, the heat is turned off and the milk is allowed to set for approximately 2 h. When the coagulum becomes firm, it is cut with a fork or a pine tree branch until no large lumps of curd remain (Kınık *et al.* 1999; Kamber 2008a). The curds are separated from the whey with a strainer, and approximately 1 kg of curd is placed into baskets, which are woven from stalks collected near rivers. The curd is left in the basket at ambient temperature (20–24 °C) to drain by itself, and the soft curd assumes the shape of the basket. The curd is turned upside down to enable even whey drainage and so that all surfaces take the shape of the basket (Kamber 2008a). The curd is then removed from the basket, and the upper and lower surfaces are sprinkled with fine salt (approximately 30 g salts/kg curd). Then, the salted curd is returned to the basket for further drainage at room temperature (20–24 °C) (Büke 1981; Kınık *et al.* 1999). Approximately 18 h later, when the curd has knitted together to form a block, it is taken out of the basket and placed in a brine (14% (w/w) NaCl) at around 6–8 °C for ripening and storage. The ripening time varies between 3 months to 12 months. The cheese could be stored in brine until consumption. Ercan *et al.* (2011) investigated the chemical, textural and sensorial characteristics of Sepet cheese and found that Sepet cheese is a

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semihard cheese according to IDF (1981). The average dry matter, fat-in-dry matter, protein, salt-in-dry matter, water activity, pH, titratable acidity, ripening and lipolysis indices of Sepet cheese were reported as 55.16%, 45.80%, 29.18%, 12.88%, 0.83, 5.50, 1.69%, 11.06 and 6.36, respectively (Ercan *et al.* 2011). Descriptors such as free fatty acid, animal like, sulphurous, creamy, cooked, whey aroma and salty basic taste have been used to describe the flavour of Sepet cheese. The most abundant volatile compounds in Sepet cheese were reported to be hexanoic, octanoic, decanoic and butyric acids, which were responsible for the cheesy, waxy and goaty odours (Ercan *et al.* 2011).

Microbial diversity in the raw milk and in cheese affects the flavour and texture of cheese. The micro-organisms may contribute directly through their metabolic activity or they may release enzymes, and those enzymes may cause changes in cheese texture or flavour (Beresford and Williams 2004). Mesophilic lactobacilli are reported as important nonstarter lactic acid bacteria in the maturation of cheeses because they can catabolise citrate and could be involved in proteolysis and in other enzymatic processes during cheese ripening (Crow *et al.* 2001; Terzic-Vidojevic *et al.* 2007). Moreover, nonstarter lactobacilli have lipase and esterase activities, which affect cheese flavour. *Enterococcus* spp. metabolise citrate and can form acetaldehyde, acetoin and diacetyl (Beresford and Williams 2004). *Enterococcus* spp. also contribute to cheese flavour by the hydrolysis of milk fat by lipases and esterases and the conversion of fatty acids into methyl ketones and thioesters (Tsakalidou *et al.* 1993; Giraffa 2003; Beresford and Williams 2004). Furthermore, some *Enterococcus* spp. are capable of producing a variety of bacteriocins, which can protect cheese against spoilage bacteria (Abdi *et al.* 2006). The natural microflora of cheese could become extinct, when artisanal cheesemaking is replaced by industrial scale processes. Therefore, it is important to document the natural biodiversity of artisanal cheese microflora. Some research on the microflora of traditional cheeses has been performed. For example; the natural microflora of artisanal Mexican Fresco and Manchego cheeses were investigated by Torres-Llanez *et al.* (2006) and Ballesteros *et al.* (2006). The increasing expansion of urbanisation and hence reduction in the rural population brings about a loss of expertise in traditional food-making technology such as cheesemaking. Many studies have shown that locally produced cheeses have different and typical microbial population dynamics related to the local production technology and environmental conditions (Boubekri and Ohta 1996; Terzic-Vidojevic *et al.* 2007). Pisano *et al.* (2006) and Williams and Withers (2010) conducted research to characterise Fiore Sardo, a traditional Sardinian cheese made from ewe's milk, and artisanal farmhouse cheeses manufactured in Scotland, respectively, to provide a better understanding of microbial diversity so that the typical characteristics of these cheeses can be retained, while at the same time, the microbiological quality can be improved.

The objectives of this study were (i) to investigate the lactic acid bacteria (LAB) and quality indicator micro-organisms in the microflora of traditional artisanal Sepet cheeses collected from 11 different towns in the Aegean region of Turkey, (ii) to isolate and identify representative lactic acid bacteria in Sepet cheese and (iii) to investigate the changes in the counts from milk to curd and during the ripening of Sepet cheeses made on two different dairy farms.

MATERIALS AND METHODS

Sampling

In the first part of the study, the microflora of fifty-two Sepet cheeses, which were ripened approximately for 3 months, were investigated. The cheese samples were collected from small dairy farms and local bazaars located in Çeşme ($n = 15$), Karaburun ($n = 7$), Zeytineli ($n = 3$), Gülbahçe ($n = 3$), Germiyan ($n = 3$), Ovacık ($n = 3$), Barbaros ($n = 3$), Üçkuyular ($n = 3$), Kemalpaşa Turgutlu ($n = 3$), Tire ($n = 3$) and Ayvalık ($n = 6$), which are the towns in Aegean region of Turkey. Samples were taken to the laboratory in an ice box on the same day. They were stored at 4 °C prior to analysis and analysed in 2 days.

In the second part of the study, Sepet cheeses were produced using goat milk in local small dairy farms located in Germiyan and Zeytineli towns. The only difference between the production methods of Germiyan and Zeytineli cheeses was the type of coagulant, which was used during production. Microbial rennet (Mayasan Co. Inc., Istanbul, Turkey) was used as a coagulant agent in cheeses produced in Germiyan. Artisanal rennet, produced from the fourth stomach of young goats in the farm, was used in cheeses produced in Zeytineli. After cheeses were placed into brine for ripening, the cheese samples were transferred to laboratory and ripened an incubator at 8 °C for 3 months. Milk, curd, 1 day, and 1-, 2- and 3-month-old cheeses were analysed to determine microbial counts and identify lactic acid bacterial species.

Microbiological analysis

Ten-mL samples of milk and 10-g samples of cheese or curd were homogenised into sterile 90-mL solutions of 2% (w/v) sodium citrate at 45 °C using a stomacher device (Interscience, Bagmixer 400W, Saint Dom, France), and decimal dilutions were prepared in sterile 0.1% (w/v) peptone water (Psoni *et al.* 2003). The decimal dilutions of homogenates were used for bacterial enumeration. The total bacterial count (TBC) was measured on skim milk plate count agar (Merck, Darmstadt, Germany) after incubation at 32 °C for 72 h. Lactococci were determined on M17 agar (Merck) after incubation at 37 °C for 48 h. Lactobacilli were counted on MRS agar (Fluka, Saint Louis, MO, USA) after anaerobic incubation at 37 °C for 48 h in sealed jars

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containing an Anaerogen sachet (Oxoid, Basingstoke. Hants, UK) (Mucchetti *et al.* 2008). Enterococci were counted on Kanamycin aesculin azide agar (Merck) after incubation at 37 °C for 48 h (Dolci *et al.* 2007). Presumptive coagulase positive *Staphylococcus aureus* (colonies with an opaque zone) were counted on Baird Parker agar (Difco, NJ, USA) supplemented with egg yolk tellurite medium plates after incubation at 37 °C for 48 h (Psoni *et al.* 2003). Yeast and mould were determined on yeast glucose chloramphenicol agar (Difco) plates after incubation at 25 °C for 5 days (Mucchetti *et al.* 2008). Coliforms were counted on double layer violet red bile agar (Difco) after incubation at 30 °C for 24 h (Psoni *et al.* 2003). Psychrotrophic bacteria were counted on skim milk plate count agar (Difco) after incubation at 7 °C for 10 days (Özdemir and Demirci 2006). Spread plate method was used for the enumeration of yeast, mould and presumptive coagulase positive *Staphylococcus aureus*. For the rest of the bacteria, pour plate method was used for enumeration. All analyses were carried out using duplicate plates at each dilution. After colony counting, the counts were expressed as log colony-forming unit (cfu) per gram of sample.

Isolation of bacteria

After incubation on MRS agar (pH 6.2–6.8) and Kanamycin esculin azide agar (pH 7.2), approximately 20 individual colonies from each plate having 30–300 colonies were selected (according to shape, size and colour) and transferred into sterile M17 (Merck) and MRS (Fluka) broth media. They were purified by two subsequent subcultures on Kanamycin esculin azide and MRS agars using the streak plate technique. Isolates that were purified were stored in MRS and M17 broth media, which contained 20% (v/v) glycerol as frozen stocks at −80 °C before being subjected to identification analysis. Glycerol stock samples were prepared by mixing 0.5 mL of overnight cultures, and 0.5 mL of 40% (v/v) glycerol.

Physiological and biochemical identification of isolates

The catalase test and Gram stain were carried out to make sure that all isolates were lactic acid bacteria (Gram-positive and lacked catalase). Cell shapes and arrangements (such as individual colonies, pairs or clusters) and the results of Gram staining were examined under an oil immersion lens by light microscopy (Olympus CX31, Tokyo, Japan).

Overnight cultures grown in 5-mL MRS broth at 30 °C were used for the identification procedures. For all tests, noninoculated media were used as a negative control. Tests for the ability to produce gas from glucose, growth at different temperatures (10, 40 and 45 °C), growth at different NaCl concentrations (20, 40 and 65 g/L), hydrolysis of arginine and gas production from citrate in Reddy broth were applied to cocci-shaped isolates for phenotypic identification. For the phenotypic identification of rod-shaped isolates, gas production from glucose, growth at 15 and 45 °C, growth in 65 g/L NaCl and arginine hydrolysis tests were performed. Then, carbohydrate fermentation tests were performed for species and subspecies level identification of isolates. Carbohydrate fermentation tests were performed in 96-well plates using the method described by Bulut (2003) and Bulut *et al.* (2005). The following 14 substrates; L (+)-arabinose, D(+)-galactose, lactose, maltose, D(+)-mannitol, raffinose, sucrose, D(−)-salicin, sorbitol, D(+)-trehalose, D(+)-xylose, glycerol, D(+)-mannose and D(−)-ribose were used for the carbohydrate fermentation tests. Samples with glucose and samples without sugar were used as positive and negative controls, respectively. After 24-h incubation at 30 °C, the carbohydrate fermentation abilities of isolates were measured at 615-nm absorbance using an automated microtiter plate reader (Thermo, Varioskan Flash, Finland). Sugar fermentation ability was determined based on turbidity and colour change from purple to yellow.

Genotypic identification of isolates

Two representative isolates from each group (Table 3) were chosen at random to obtain 16S rDNA sequences. DNA was extracted from the overnight cultures grown in 6-mL MRS broth at 37 °C. PureLink™ Genomic DNA Mini Kits (Catalogue Number K1820-02, Invitrogen™, Carlsbad, CA, USA) were used for purification of genomic DNA. After the DNA was purified by application of the method recommended by the manufacturer, the purified DNA was transferred to a new Eppendorf and stored at −20 °C.

Amplification of the 16S rDNA was performed in a 20-μL reaction mixture consisting of 5 μL of genomic DNA, 2 μL PCR buffer, 2 μL dNTP mix (2 mM each), 0.4 μL of bacterial universal primer 10 mM E334F (5′-CCAGACTCCTAC GGGAGGCAGC-3′), 0.4 μL of bacterial universal primer 10 mM E939R (5′-CTTGTGCGGGCCCCCGTCAATTC-3′), 0.1 μL Taq polymerase (5 U/μL), 1.2 μL $MgCl_2$ (25 mM) and sufficient water to make up the volume to 20 μL (Baker *et al.* 2003). The amplification reaction was performed in a PCR (Biorad, C100™ Thermo Cycler, Singapore) using the following conditions: predenaturation (3 min at 95 °C) followed by 25 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 62 °C and elongation for 1 min at 72 °C, and the final cycle was followed by an additional extension for 5 min at 72 °C (Giraffa *et al.* 2000).

The amplified products were purified using Sephadex™ columns (MN Receiver Column, 740522.250, Düren, Germany, and Sigma S6022 Sephadex, Steinheim, Germany) and were then sequenced using the ABI Prism BigDye Terminator v3.1 Cycle Sequencing Kit with forward primer (E 334) in a single direction using an Applied Biosystems 3130xl Genetic Analyzer (Foster City, CA, USA). The sequence chromatograms were analysed by the Finch TV 1.4.0. program and compared with the GenBank database to determine sequence homology (Tan *et al.* 2010).

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Statistical analysis

Mean values, standard deviations, maximum and minimum values were calculated for all the parameters determined. Analysis of variance was performed to investigate the differences ($P < 0.05$) in microbiological counts during production and ripening. The Student–Newman–Keuls range test was applied for comparison of the mean values. Principal component analysis (PCA) is a statistical method to reduce the dimensionality of the data, to calculate the components that best described the differences between samples and to identify clusters (Smith 2002). In this study, PCA was used to obtain the first two principal components from the microbiological counts of the cheese samples and to identify the potential grouping of samples using Statistica software (StatSoft Inc., Tulsa, OK, USA).

Table 1 Microbiological counts (log cfu/g) based on analysis of 52 Sepet cheeses

Microbiological analysis	*Mean*	*Maximum*	*Minimum*	*Standard deviation*	*Variance*
Total aerobic bacteria	7.53	9.03	5.52	1.13	1.28
Lactococci	7.31	8.89	5.49	1.08	1.16
Lactobacilli	7.19	8.74	5.30	1.02	1.05
Enterococci	6.84	8.87	5.44	0.92	0.85
Yeast	3.19	5.49	<1.00	1.40	1.96
Mould	0.84	2.58	<1.00	0.89	0.79
Coliform	2.18	5.51	<1.00	1.81	3.29
Psychrotrophic bacteria	4.92	7.23	3.10	1.15	1.33
Staphylococcus aureus	1.25	4.61	<1.00	1.70	2.87

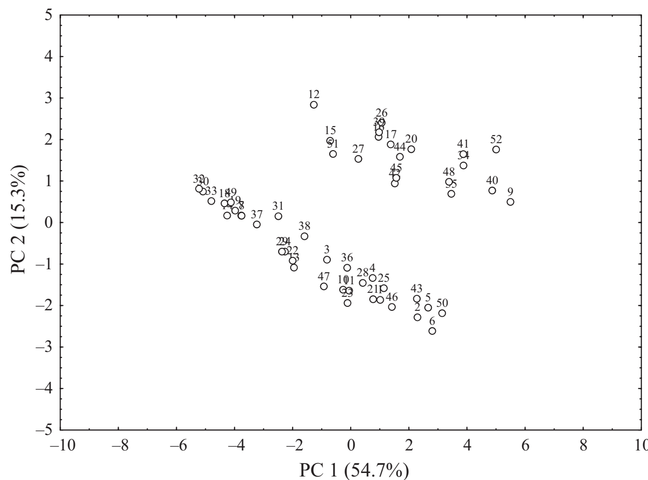

Figure 1 Score plot of the first two principal components of Sepet cheeses based on the microbiological counts.

Table 2 Microbiological counts (log cfu/g) in Sepet cheese from two cheesemaking locations at stages during production and ripening

Sample	*Location*	*Total aerobic bacteria*	*Lactococci*	*Lactobacilli*	*Enterococci*	*Yeast*	*Mould*	*Coliform*	*Psychrotrophic bacteria*	*Staphylococci*
Goat milk	Zeytineli	5.60 ± 0.05^{a}	5.29 ± 0.08^{a}	5.27 ± 0.02^{a}	5.35 ± 0.01^{a}	3.03 ± 0.01^{c}	1.39 ± 0.12^{b}	3.23 ± 0.07^{c}	5.56 ± 0.01^{b}	2.48 ± 0.04^{b}
Curd		6.70 ± 0.04^{b}	6.40 ± 0.07^{b}	6.15 ± 0.03^{b}	6.55 ± 0.06^{b}	3.98 ± 0.01^{d}	1.96 ± 0.17^{c}	3.56 ± 0.08^{d}	6.62 ± 0.09^{de}	2.62 ± 0.03^{c}
1st day cheese		7.74 ± 0.03^{e}	7.61 ± 0.01^{ef}	7.11 ± 0.01^{c}	6.71 ± 0.12^{b}	4.02 ± 0.03^{d}	2.22 ± 0.02^{d}	5.30 ± 0.05^{e}	6.54 ± 0.03^{d}	2.53 ± 0.02^{b}
1st month cheese		7.48 ± 0.01^{d}	7.51 ± 0.05^{e}	7.53 ± 0.02^{e}	7.39 ± 0.00^{d}	2.64 ± 0.05^{a}	1.74 ± 0.06^{c}	1.59 ± 0.16^{b}	5.01 ± 0.04^{a}	<1.00 ± 0.00^{a}
2nd month cheese		7.86 ± 0.03^{f}	7.69 ± 0.02^{f}	7.66 ± 0.02^{f}	7.64 ± 0.12^{e}	2.70 ± 0.07^{a}	1.74 ± 0.06^{c}	<1.00 ± 0.00^{a}	6.71 ± 0.05^{e}	<1.00 ± 0.00^{a}
3rd month cheese		7.23 ± 0.02^{c}	7.25 ± 0.01^{c}	7.17 ± 0.01^{d}	7.13 ± 0.03^{c}	2.90 ± 0.02^{b}	<1.00 ± 0.00^{a}	<1.00 ± 0.00^{a}	6.07 ± 0.03^{c}	<1.00 ± 0.00^{a}
Goat milk	Germiyan	6.23 ± 0.10^{b}	5.42 ± 0.05^{a}	5.00 ± 0.02^{a}	5.10 ± 0.01^{b}	2.70 ± 0.04^{b}	<1.00 ± 0.00^{a}	4.07 ± 0.02^{c}	6.12 ± 0.02^{d}	2.15 ± 0.15^{b}
Curd		6.47 ± 0.01^{c}	5.92 ± 0.03^{c}	6.02 ± 0.08^{c}	6.59 ± 0.01^{e}	3.05 ± 0.05^{c}	<1.00 ± 0.00^{a}	4.74 ± 0.02^{d}	6.97 ± 0.04^{e}	2.44 ± 0.09^{bc}
1st day cheese		6.70 ± 0.04^{d}	7.13 ± 0.02^{e}	7.27 ± 0.01^{e}	6.71 ± 0.25^{e}	3.79 ± 0.05^{d}	<1.00 ± 0.00^{a}	4.80 ± 0.17^{d}	6.06 ± 0.03^{cd}	2.32 ± 0.03^{c}
1st month cheese		6.62 ± 0.03^{d}	7.12 ± 0.02^{e}	6.71 ± 0.05^{d}	6.21 ± 0.02^{d}	<1.00 ± 0.00^{a}	<1.00 ± 0.00^{a}	1.39 ± 0.12^{b}	5.40 ± 0.02^{b}	<1.00 ± 0.00^{a}
2nd month cheese		6.46 ± 0.05^{c}	6.30 ± 0.00^{d}	6.07 ± 0.03^{c}	5.62 ± 0.07^{c}	<1.00 ± 0.00^{a}	<1.00 ± 0.00^{a}	<1.00 ± 0.00^{a}	6.02 ± 0.03^{c}	<1.00 ± 0.00^{a}
3rd month cheese		5.97 ± 0.03^{a}	5.70 ± 0.05^{b}	5.14 ± 0.03^{b}	4.79 ± 0.04^{a}	<1.00 ± 0.00^{a}	<1.00 ± 0.00^{a}	<1.00 ± 0.00^{a}	5.12 ± 0.03^{a}	<1.00 ± 0.00^{a}

(a–g) means within column are significantly different ($P < 0.05$) according to the Student–Newman–Keuls test.

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RESULTS AND DISCUSSION

Microbiological counts of Sepet cheeses

Variation in the microbiological counts of Sepet cheese samples is shown in Table 1. Maximum yeast, mould and coliform counts found were as 5.49, 2.58 and 5.51 log cfu/g, respectively, while yeast, mould and coliforms were absent in some of the Sepet cheese samples. Coliforms are used as a general indicator of the sanitary condition of food-processing environments (Feng *et al.* 2002). Coliforms

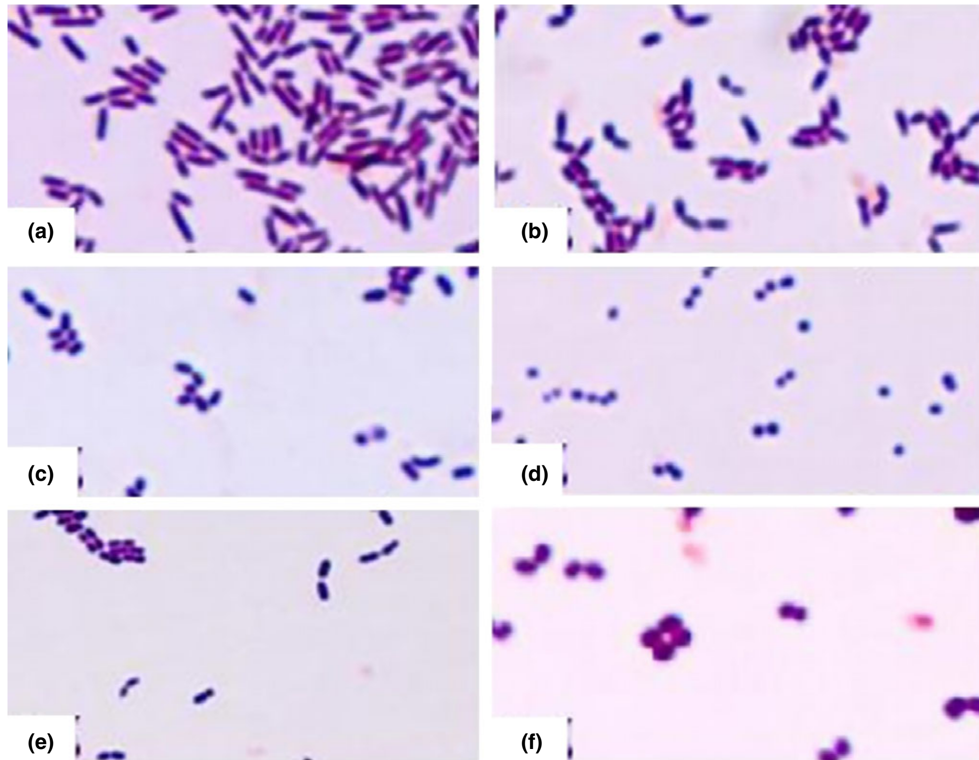

Figure 2 Cell morphology of isolated *Lactobacillus plantarum* (a), *Weisella confusa* (b), *Weisella paramesenteroides* (c), *Enterococcus durans/faecium* (d), *Enterococcus casseliflavus* (e) and *Pediococcus pentasaceous* (f).

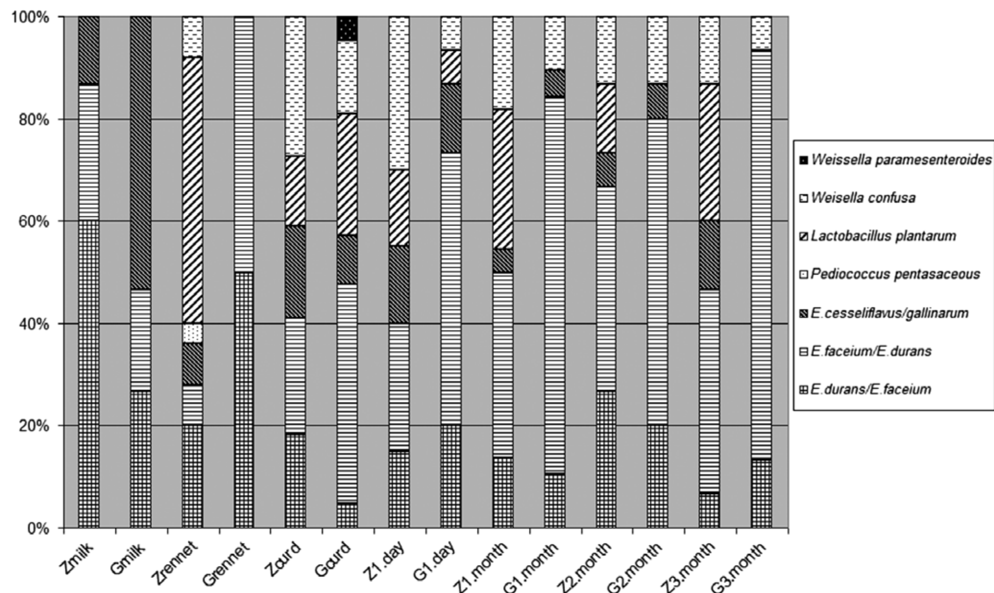

Figure 3 The percentages of isolated micro-organisms during production and ripening of Sepet cheeses. Z and G indicate the towns as Zeytineli and Germiyan, respectively.

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were detected in 35 of Sepet cheese samples. High coliform counts in Sepet cheese samples may be caused by the use of unpasteurised milk in the production, unsanitary production areas, postcontamination after production during marketing, etc. Nineteen Sepet cheese samples contained *Staphylococcus aureus,* and the maximum number counted was 4.61 log cfu/g. Hamid and Owni (2007) stated that high counts of *S. aureus* found in some cheese samples might be attributed to the high initial numbers of *S. aureus* in raw milk or contamination during processing. When the results are compared with previous artisanal cheese studies in Turkey, the coliform counts were lower than those reported for Örgü and Sıkma cheeses. Turkoglu *et al.* (2003) and Ceylan *et al.* (2003) reported 3.73 and 5.99 log cfu/g coliforms in Örgü and Sıkma cheeses, respectively. Kamber (2008b) reported that Mihaliç cheese had 3.30 log cfu/g coliforms, 5.9 log cfu/g lactococci and 7.69 log cfu/g total aerobic counts. The Sepet cheese samples had total aerobic bacteria counts similar to Mihaliç cheese. Kamber and Çelik (2007) investigated the microbiological characteristics of Gorcola cheese and found that Gorcola cheese had 1.9×10^7 cfu/g total aerobic mesophilic bacteria, 2.9×10^4 cfu/g lactic acid bacteria, 1.0×10^7 cfu/g lactococci, 1.5×10^4 cfu/g coliforms and 1.4×10^5 cfu/g yeast-mould count. Yeast and mould counts in Sepet cheeses were lower than the counts reported for Gorcola cheese. It was also observed that lactococci and total aerobic bacteria in Sepet cheese samples were higher than those reported for Gorcola cheese. The total aerobic bacteria counts for Sepet cheese samples found in this work were similar to those found by Kınık *et al.* (1999), but higher than those found by Karakaş and Korukluoğlu (2006). Moreover, Karakaş and Korukluoğlu (2006) found relatively higher yeast counts in their Sepet cheese samples.

The microbiological count data for fifty-two Sepet cheese samples were subjected to PCA. PCA is a tool to identify patterns in data to highlight their similarities and differences using an orthogonal transformation (Smith 2002). Figure 1 shows the score plot of the first two principal components (PC1 and PC2). These two PCs accounted for 70% of the total variance. Based on presumptive *S. aureus* counts, the cheese samples were separated into two clusters. The cheeses, which had higher *S. aureus* counts, were placed on the upper part of the plot. The cheeses, which had higher microbial loads, were placed on the right-hand side while the cheeses, which had lower microbial loads, were placed on the left-hand side of the plot in each cluster. The cheese samples (36% of total cheese samples), which had relatively higher presumptive *S. aureus* counts, were mostly collected from local bazaars. This may be related to unsanitary conditions in transportation and storage in the bazaars.

Changes in the microbiological counts during the production and ripening of Sepet cheese made at two sites were also investigated. The microbiological counts are shown in Table 2. All bacterial counts increased during cheese production ($P < 0.05$) due to concentration associated with cheesemaking. An exception was mould counts in the cheese made at the Germiyan site. The *Staphylococci*, coliforms and mould counts of Sepet cheeses were less than 1.00 log cfu/g at the end of ripening. Although yeast counts in Zeytineli cheese decreased until the 3rd month, their levels were higher than 1.00 log cfu/g at the end of the ripening period. Ercan *et al.* (2011) investigated the physicochemical changes during production and ripening and reported that the pH decreased during production and ripening. The decrease in pH could affect the population of staphylococci and coliforms. Since mould and yeast could grow at a wide range of pH and acidity, the most important reason for decreases in yeast and mould counts during the ripening of cheese may be anaerobic conditions (Jay 1996). The results reported here are similar to the results of Kılıç *et al.* (2004). They found a significant decrease in staphylococci and coliform counts during the ripening of fresh Turkish goat cheese. Similar trends were also found in the studies on Valdeteja raw goat's milk cheese and Manchego cheeses (Calleja *et al.* 2002; Cabezas *et al.* 2007).

Identification of isolates

Lactic acid bacteria are essential for the fermentation and are acceptable in very large numbers in traditional cheeses made with raw milk (Hamid and Owni 2007). Lactic acid bacteria in Sepet cheese during production and ripening for up to the 3 months were examined. Isolates were chosen for identification after subculturing, Gram staining and catalase tests were performed. The morphologies of isolated microorganisms are given in Figure 2. A total 240 isolates (approximately 20 colonies from each plate) were identified. According to the identification results, the percentages of cocci and rods in samples of milk and during cheese ripening fluctuated. The ratios of identified bacteria during cheese production and ripening are shown in Figure 3. The artisanal rennet used in Zeytineli had a more diverse bacterial population than the commercial rennet used in Germiyan. Thus, the artisanal rennet might be responsible for higher counts of total aerobic bacteria, lactococci, lactobacilli and enterococci found in Zeytineli cheese (Table 2).

The phenotypic identification results are given in Table 3. *Enterococcus* spp. were predominant during Sepet cheese production and ripening. Fox *et al.* (2000) stated that Enterococci have the ability to grow at the wide range of temperatures and salt contents found in Mediterranean cheeses. Based on the taxonomy of Devriese *et al.* (2006), 66.2% of total isolates in Sepet cheeses were closely related to *Enterococcus* species. Isolated enterococci were arginine hydrolyse positive, were able to grow at 6.5% NaCl and had coccoid shapes. Due to mannitol fermentation ability of

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Table 3 Phenotypic characteristics of micro-organisms isolated from Sepet cheeses

Shape	*Catalase*	*2% NaCl*	*4% NaCl*	*6.5% NaCl*	*10 °C*	*15 °C*	*40 °C*	*45 °C*	*Arginine hydrolysis*	*CO_2 in Reddy broth*	*CO_2 from glucose*	*Arab-inose*	*Gala-ctose*	*Lactose*	*Ribose*	*Sorbitol*
Cocci	–	+	+	+	+	ND	+	+	+	–	–	+	+	+	+	–
Cocci	–	+	+	+	+	ND	+	+	+	–	–	+	+	+	+	–
Cocci	–	+	+	+	+	ND	+	+	+	–	–	+	+	+	+	–
Cocci	–	+	+	+	+	ND	+	+	+	–	–	+	+	+	+	–
Cocci	–	+	+	+	+	ND	+	+	+	–	–	+	+	+	+	±
Cocci	–	+	+	+	+	ND	+	+	+	–	–	+	+	+	+	–
Cocci	–	+	+	+	+	ND	+	+	+	–	–	+	+	+	+	–
Group of four or two cocci	–	+	+	+	+	ND	+	+	+	–	–	+	+	+	+	–
Short rods	–	ND	ND	+	ND	+	ND	+	+	ND	+	–	+	+	+	–
Short rods	–	ND	ND	+	ND	+	ND	+	+	ND	+	±	+	+	+	–
Short rods	–	ND	ND	+	ND	+	ND	+	+	ND	+	±	+	+	+	+
Short rods	–	ND	ND	+	ND	+	ND	+	+	ND	+	–	+	+	+	±
Short rods	–	ND	ND	+	ND	+	ND	+	+	ND	+	–	+	+	+	–
Short rods	–	ND	ND	+	ND	+	ND	+	+	ND	+	±	+	+	+	–
Lenticular	–	ND	ND	+	ND	+	ND	–	–	ND	+	+	+	+	+	–
Rod	–	ND	ND	+	ND	+	ND	+	–	ND	–	–	+	+	+	+
Rod	–	ND	ND	+	ND	+	ND	+	–	ND	–	±	+	+	+	+
Rod	–	ND	ND	+	ND	+	ND	+	–	ND	–	±	+	+	+	+
Rod	–	ND	ND	+	ND	+	ND	+	–	ND	–	+	+	+	+	+
Rod	–	ND	ND	+	ND	+	ND	+	–	ND	–	+	+	+	+	+
Rod	–	ND	ND	+	ND	+	ND	+	–	ND	–	+	+	+	+	+
Rod	–	ND	ND	+	ND	+	ND	+	–	ND	–	±	+	+	+	+

ND indicates not determined. The sign of ±, +, – indicate weak, positive and negative reactions, respectively.

E. faceium, the isolates of *E. faceium* can be differentiated from the isolates of *E. durans* (Collins *et al.* 1984; Farrow and Collins 1985; Cai 1999; Klein 2003). *E. durans* is differentiated from *E. hirae* because the former cannot ferment raffinose (Collins *et al.* 1984; Farrow and Collins 1985; Cai 1999; Klein 2003). *E. casseliflavus* is different from *E. gallinarum* based on the fermentation of glycerol, and sorbitol. *E. casseliflavus* is also differentiated from other *Enterococcus* species based on the ability to ferment D-xylose (Collins *et al.* 1984; Cai 1999). Bulut *et al.* (2005), Torres-Llanez *et al.* (2006), Psoni *et al.* (2003) and Serhan *et al.* (2009) also isolated *E. durans* and *E. faceium* from artisanal raw milk cheeses.

Approximately 15% of total isolates were *Lactobacillus* spp. isolated during production and ripening of both cheeses. All *Lactobacillus* spp. were closely related to *L. plantarum*. *L. plantarum* is differentiated from *L. farmcinis* because it cannot hydrolyse arginine but it can ferment raffinose, mannitol and sorbitol. *L. plantarum* is differentiated from *L. fermentum* because it can ferment salicin, sorbitol and mannitol (Antara *et al.* 2002; Hammes and Hertel 2006 and Özkalp *et al.* 2009). Tserovska *et al.* (2002) also found *L. plantarum* in goat cheeses.

Based on the taxonomic data of Björkroth and Holzapfel (2006) and Björkroth *et al.* (2002), 13% of total isolates from Sepet cheese were closely related to *Weisella* spp.

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Salicin	*Raffinose*	*Mannitol*	*Mannose*	*Xylose*	*Maltose*	*Trehalose*	*Glycerol*	*Sucrose*	*Glucose*	*% Similarity with the microorganisms in the GenBank database with accession #*	*Species*
+	–	–	+	–	+	±	–	–	+	96, HE646381	*Enterococcus durans*
+	–	–	+	–	+	±	–	±	+	96, JQ366081	*Enterococcus durans*
+	–	±	+	–	+	+	–	+	+	96, GU904685	*Enterococcus faceium*
+	–	+	+	–	+	+	–	–	+	96, HQ616645	*Enterococcus faceium*
+	–	+	+	–	+	+	–	–	+	94, JQ712023	*Enterococcus faceium*
+	–	±	+	–	+	+	–	–	+	97, HE646396	*Enterococcus faceium*
+	+	+	+	+	+	+	–	±	+	96, JN645289	*Enterococcus casseliflavus*
+	–	–	+	–	+	+	–	–	+	96, JQ712016	*Pediococcus pentasaceous*
±	–	±	+	+	+	+	–	+	+	96, JQ801710	*Weissella confusa*
±	–	±	+	+	+	+	–	+	+	97, JF757231	*Weissella confusa*
+	–	+	+	+	+	+	–	+	+	95,HQ711354	*Weissella confusa*
±	–	±	+	+	+	+	–	+	+	92, AB494723	*Weissella confusa*
–	–	–	+	+	+	+	–	+	+	93, JQ801709	*Weissella confusa*
±	–	±	+	+	+	+	–	+	+	96, JQ801710	*Weissella confusa*
–	–	+	+	–	+	+	–	+	+	96, HQ721270	*Weissella paramesenteroides*
+	–	+	+	±	+	+	–	+	+	95, JN863662	*Lactobacillus plantarum*
+	–	+	+	±	+	+	–	+	+	95, HE646362	*Lactobacillus plantarum*
+	–	+	+	±	+	+	–	±	+	96, HE616213	*Lactobacillus plantarum*
+	±	+	+	+	+	+	–	+	+	96, JQ580990	*Lactobacillus plantarum*
+	–	+	+	+	+	+	–	+	+	97, HE646413	*Lactobacillus plantarum*
+	±	+	+	±	+	+	–	+	+	95, JN863682	*Lactobacillus plantarum*
+	±	+	+	±	+	+	±	+	+	97, HE616210	*Lactobacillus plantarum*

W. paramesenteroides has a lenticular shape. It cannot grow at 45 °C and cannot hydrolyse arginine. *W. paramesenteroides* is differentiated from *W. hellica* by the ability to ferment galactose and trehalose (Björkroth and Holzapfel 2006). *Weisella confusa (Lactobacillus confusus*) is differentiated from *W. ciberia* because it cannot ferment arabinose but has the ability to ferment ribose and galactose. *W. ciberia* and *W. confusa* are also differentiated from other *Weisella* species because they can ferment D-xylose (Björkroth *et al.* 2002; Björkroth and Holzapfel 2006).

Pediococcus spp. were only isolated from artisanal rennet. The isolates were closely related to *P. pentasaceous. P. pentasaceous* is differentiated from *Pediococcus acidilactici* because it can ferment arabinose, trehalose and maltose (Tserovska *et al.* 2002; Holzapfel *et al.* 2006).

Forty phenotypically identified isolates were selected for genotypic identification. The results of genotypic identification analysis were in agreement with the results of phenotypic identification analyses. The 16S rDNA sequences of micro-organisms isolated from Sepet cheese were similar to isolates found in Western Balkan traditional cheese, probiotic isolates from traditional dairy products of the Azarbayjan region, isolates from kefir, isolates from Altiplano Cundiboyacense (a traditional handmade double cream cheese), isolates from Caciocavallo Palermitano cheese and from artisanal Vlasina raw milk goat cheeses, which were

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reported in the GenBank database. The percentage similarity and the accession number of similar micro-organisms in GenBank database are given in Table 3.

CONCLUSIONS

In all Sepet cheese samples, lactococci, lactobacilli and enterococci were the predominant bacteria. Considerable variation was found in the microflora of Sepet cheese obtained from different sources in Aegean region of Turkey. Eight of the Sepet cheese samples did not contain mould and coliforms; this can be an indication of a good standard of hygiene during production and ripening. However, presumptive coagulase positive *S. aureus* was found in 36% of Sepet cheese samples, which is a public health concern.

According to phenotypic and genotypic identifications, two-thirds of isolates were closely related to *Enterococci* species including *E. casseliflavus, E. durans* and *E. faceium.* Other isolates were identified as *L. plantarum, W. confusa, W. paramesenteroides* and *P. pentasaceous*.

When a batch of cheese from each of two farms was monitored during production and ripening, it was observed that total aerobic bacteria, lactococci, lactobacilli and enterococci counts decreased after the 1st month ($P < 0.05$). Moreover, staphylococci and coliform counts decreased below 1.00 log cfu/g before the end of the ripening period. Thus, the traditional ripening process appears to contribute to microbiological and pathogen control.

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REFERENCES

Abdi R, Sheikh-Zeinoddin M and Soleimanian-Zad S (2006) Identification of lactic acid bacteria isolated from traditional Iranian lighvan cheese. *Pakistan Journal of Biological Sciences* **9** 99–103.

Antara N S, Sujaya I N, Yokota A, Asano K, Aryanta W R and Tomita F (2002) Identification and succession of lactic acid bacteria during fermentation of 'urutan', a Balinese indigenous fermented sausage. *World Journal of Microbiology & Biotechnology* **18** 255–262.

Baker G C, Smith J J and Cowan D A (2003) Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods* **55** 541–555.

Ballesteros C, Poveda J M, González-Viñas M A and Cabezas L (2006) Microbiological, biochemical and sensory characteristics of artisanal and industrial Manchego cheeses. *Food Control* **17** 249–255.

Beresford T and Williams A (2004) The Microbiology of Cheese Ripening. In *Cheese, Chemistry, Physics and Microbiology*, pp 287–317. Fox P F, McSweeney P L H, Cogan T M and Guinee T, eds. California: Elsevier Academic Press.

Björkroth J and Holzapfel W H (2006) Genera Leuconostoc, Oenococcus and Weissella. In *The Prokaryotes*, Vol **4**, pp 267–319. Dworkin M, ed. New York: Springer.

Björkroth K J, Schillinger U, Geisen R, Weiss N, Hoste B, Holzapfel W H, Korkeala H J and Vandamme P (2002) Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. *International Journal of Systematic and Evolutionary Microbiology* **52** 141–148.

Boubekri K and Ohta Y (1996) Identification of lactic acid bacteria from Algerian traditional cheese, El-Klila. *Journal of the Science of Food and Agriculture* **70** 501–505.

Büke E (1981) *Sepet Cheese*. Izmir: Ege University Agricultural Faculty Technology Department Graduation Thesis.

Bulut Ç (2003) *Isolation and Molecular Characterization of Lactic Acid Bacteria from Cheese*. İzmir: Master thesis in İzmir Institute of Technology.

Bulut C, Gunes H, Okuklu B, Harsa S, Kilic S, Coban H S and Yenidunya A F (2005) Homofermentative lactic acid bacteria of a traditional cheese, Comlek peyniri from Cappadocia region. *Journal of Dairy Research* **72** 19–24.

Cabezas L, Sánchez I, Poveda J M, Sesenã S and Palop M L (2007) Comparison of microflora, chemical and sensory characteristics of artisanal Manchego cheeses from two dairies. *Food Control* **18** 11–17.

Cai Y (1999) Identification and characterization of enterococcus species isolated from forage crops and their influence on silage fermentation. *Journal of Dairy Science* **82** 2466–2471.

Calleja A, Carballo J, Capita R, Bernardo A and Garcia-Lopez M L (2002) Changes in the microflora of Valdeteja raw goat's milk cheese throughout manufacturing and ripening. *Lebensmittel-Wissenschaft und-Technologie* **35** 222–232.

Ceylan Z G, Türkoğlu H and Dayısoylu K S (2003) The microbiological and chemical quality of sikma cheese produced in Turkey. *Pakistan Journal of Nutrition* **2** 95–97.

Collins M D, Jones D, Farrow J A E, Kilpper-Balz R and Schleifer K H (1984) *Enterococcus avium* nom. rev., comb. nov.; *E. casselipavus* norn. rev., comb. nov.; *E. durans* norn. rev., comb. nov.; *E. Gallinarum* comb. nov.; and *E. malodoratus* sp. nova. *International Journal of Systematic Bacteriology* **34** 220–223.

Crow V, Curry B and Hayes M (2001) The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. *International Dairy Journal* **11** 275–283.

Devriese L, Baele M and Butaye P (2006) The Genus Enterococcus: Taxonomy. In *The Prokaryotes*, Vol **4**, pp 163–174. Dworkin M, ed. New York: Springer.

Dolci P, Alessandria V, Rantsiou K, Rolle L, Zeppa G and Cocolin L (2007) Microbial dynamics of Castelmagno PDO, a traditional Italian cheese, with a focus on lactic acid bacteria ecology. *International Journal of Food Microbiology* **122** 302–311.

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Dost A, Yenikan H, Okumuş F and Işıklı N D (2004) *Bazı geleneksel peynirlerin üretim yöntemleri*. Van: Geleneksel Gıdalar Sempozyumu.

Ercan D, Korel F, Karagül Yüceer Y and Kınık Ö (2011) Physicochemical, textural, volatile, and sensory profiles of traditional Sepet cheese. *Journal of Dairy Science* **94** 4300–4312.

Farrow J A E and Collins M D (1985) *Enterococcus hirae,* a new species that includes amino acid assay strain NCDO 1258 and strains causing growth depression in young chickens. *International Journal of Systematic Bacteriology* **35** 73–75.

Feng P, Stephen D, Grant M A and Burkhardt W (2002) *BAM: Enumeration of Escherichia coli and the Coliform Bacteria*. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.htm

Fox P F, Guinee T P, Cogan T M and McSweeney P L H (2000) Pathogens and food-poisoning bacteria in cheese. In *Fundamentals of Cheese Science*, pp 500–501. Gaithersburg, Maryland: Aspen Publishers.

Giraffa G (2003) Functionality of enterococci in dairy products. *International Journal of Food Microbiology* **88** 215–222.

Giraffa G, Rossetti L and Neviani E (2000) An evaluation of chelex-based DNA purification protocols for the typing of lactic acid bacteria. *Journal of Microbiological Methods* **42** 175–184.

Hamid O I A and Owni O A O E (2007) Microbiological properties and sensory characteristics of white cheese (*Gibna bayda)* collected in Zalingei Area, West Darfur State. *Research Journal of Animal and Veterinary Sciences* **2** 61–65.

Hammes W P and Hertel C (2006) The Genera *Lactobacillus* and *Carnobacterium*. In *The Prokaryotes*, Vol **4**, pp 320–403. Dworkin M, ed. New York: Springer.

Holzapfel W H, Franz C M A P, Ludwig W, Back W and Dicks L M T (2006) The Genera *Pediococcus* and *Tetragenococcus*. In *The Prokaryotes*, Vol **4**, pp 229–266. Dworkin M, ed. New York: Springer.

IDF (1981) *International Dairy Federation Catalogue of Cheeses. International Dairy Federation Bull. Doc. 141*. Brussels, Belgium: International Dairy Federation.

Jay M J (1996) Intrinsic and extrinsic parameters of foods that affect microbial growth. In *Modern Food Microbiology*, pp 35–56. NY: Chapman & Hall.

Kamber U (2008a) The traditional cheeses of Turkey: the Aegean region. *Food Reviews International* **24** 39–61.

Kamber U (2008b) The traditional cheeses of Turkey: Marmara Region. *Food Reviews International* **24** 175–192.

Kamber U and Çelik T H (2007) Some microbiological and chemical characteristics of Gorcola cheese. *Yüzüncü Yıl University Veterinary Faculty Journal* **18** 87–92.

Karakaş R and Korukluoğlu M (2006) Geleneksel bir peynirimiz: sepet peynirinin kimyasal ve mikrobioyolojik özellikleri. *Gıda* **31** 169–172.

Kılıç S, Uysal H, Kavas G, Akbulut N and Kesenkaş H (2004) Manufacture and some properties of Turkish fresh goat cheese. *Pakistan Journal of Biological Science* **7** 1037–1039.

Kınık Ö, Ergüllü E and Akbulut N (1999) A study on the production and some characteristics of Sepet cheese. *Gıda* **24** 151–161.

Klein G (2003) Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastro-intestinal tract. *International Journal of Food Microbiology* **88** 123–131.

Mucchetti G, Remagni C M, Ghiglietti R *et al.* (2008) Influence of cheese-making technology on composition and microbiological characteristics of Vastedda cheese. *Food Control* **19** 119–125.

Özdemir C and Demirci M (2006) Selected microbiological properties of kashar cheese samples preserved with potassium sorbate. *International Journal of Food Properties* **9** 515–521.

Özkalp B, Aladağ M O, Ögel Z, Özcan M M and Çelik B (2009) Determination of some metallic antimicrobial activities and plasmid and DNA prophiles of Lactobacillus strains isolated from fermented Caper pickle. *World Applied Sciences Journal* **6** 347–354.

Pisano M B, Fadda M E, Deplano M, Corda A and Cosentino S (2006) Microbiological and chemical characterization of Fiore Sardo, a traditional Sardinian cheese made from ewe's milk. *International Journal of Dairy Technology* **59** 171–179.

Psoni L, Tzanetakis N and Litopoulou-Tzanetaki E (2003) Microbiological characteristics of Batzos, a traditional Greek cheese from raw goat's milk. *Food Microbiology* **20** 575–582.

Serhan M, Cailliez-Grimal C, Borges F, Revol-Junelles A-M, Hosri C and Fanni J (2009) Bacterial diversity of Darfiyeh, a Lebanese artisanal raw goat's milk cheese. *Food Microbiology* **26** 645–652.

Smith L I (2002) *A Tutorial on Principal Components Analysis*. http://www.cs.otago.ac.nz/cosc453/student_tutorials/principal_components.pdf.

Tan Z, Pang H, Duan Y, Qin G and Cai Y (2010) 16S ribosomal DNA analysis and characterization of lactic acid bacteria associated with traditional Tibetan Qula cheese made from yak milk. *Animal Science Journal* **81** 706–713.

Terzic-Vidojevic A, Vukasinovic M, Veljovic K, Ostojic M and Topisirovic L (2007) Characterization of microflora in homemade semi-hard white Zlatar cheese. *International Journal of Food Microbiology* **114** 36–42.

Torres-Llanez M J, Vallejo-Cordoba B, Díaz-Cinco M E, Mazorra-Manzano M A and González-Córdova A F (2006) Characterization of the natural microflora of artisanal Mexican Fresco cheese. *Food Control* **17** 683–690.

Tsakalidou E, Manolopoulou E, Tsilibari B, Georgalaki M and Kalantzopoulos G (1993) Esterolytic activities from *Enterococcus durans* and *Enterococcus faecium* strains isolated from Greek cheese. *Netherlands Milk Dairy Journal* **47** 145–150.

Tserovska L, Stefanova S and Yordanova T (2002) Identification of lactic acid bacteria isolated from katyk, goat's milk and cheese. *Journal of Culture Collections* **3** 48–52.

Turkoglu H, Ceylan Z G and Dayisoylu K S (2003) The microbiological and chemical quality of Orgu cheese produced in Turkey. *Pakistan Journal of Nutrition* **2** 92–94.

Williams A G and Withers S E (2010) Microbiological characterization of artisanal farmhouse cheeses manufactured in Scotland. *International Journal of Dairy Technology* **63** 356–369.

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