Evaluation of *Listeria monocytogenes* Populations during the Manufacture and Vacuum-Packaged Storage of Kashar Cheese

F. CETINKAYA, G.E. SOYUTEMIZ

University of Uludag, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bursa,Turkey

Received January 9, 2006
Accepted September 5, 2006

Abstract

Cetinkaya F., G.E. Soyutemiz: Evaluation of *Listeria monocytogenes* Populations during the Manufacture and Vacuum-Packaged Storage of Kashar Cheese. Acta Vet Brno 76, 2007: 143-148.

Behaviour of *Listeria monocytogenes* was investigated during the manufacture and storage of Kashar cheese. For this purpose, pasteurized milk was inoculated at four different levels varying between 3 to 6 log cfu·ml^{-1} with the pathogen and its survival was determined in milk, coagulum, curd and cheese received from the production steps and at the 1st and 7th days of the storage. The heat treatment process applied after curd acidification step at 75 °C for 5 min was the most efficient barrier and greatly influenced the viability of the bacteria. Nevertheless this step was not enough to destroy all the bacteria especially when inoculated > 3 log cfu·ml^{-1} levels. For all the groups, this stage resulted in approximately 3 log decrease. Storage at 6 ± 1 °C had no influence on the viability and the count of pathogen remained nearly constant throughout storage. Results of this study indicate that neither production steps nor storage at 6 ± 1 °C for 7 days in vacuum packages was enough to eliminate *L. monocytogenes* in Kashar cheese particularly if the initial level was high. Thus, in addition to the pasteurization process, hygienic measures should be taken into account to avoid possible secondary contaminations.

Kashar cheese, inoculum, Listeria monocytogenes, survival

Kashar cheese is one of the most popular semi-hard cheeses produced in the Balkan countries since the 11th and 12th centuries (Topal 1991). It shows similarity with the 'Pasta Filata' type of cheese such as Mozzarella (Halkman K. and Halkman Z. 1991). It is produced from either sheep or cow's milk, or a mixture of both, and subjected to the stretching process at 75 °C during manufacture. It contains an average of 24.2% protein, 4.2% ash, 41.9% moisture, 25.1% fat, 4.6% salt (Tekinsen 2000); and its pH and acidity (LA%) are between 4.9 - 5.4 and 0.8 - 2.3, respectively (Cetinkaya 2000; Halkman et al. 1994). While Kashar is the second most popular cheese in Turkey with production of around 49.000 tons/year (Anonymous 2003), there is no standardized technique for its manufacture. It is produced either from raw milk according to traditional technology which is still employed in some dairies or from pasteurized milk in modern facilities. In traditional techniques, milk used in Kashar manufacture was not subjected to pasteurization process. Therefore the unique way to eliminate undesirable bacteria is the heat treatment applied at 75 °C during curd stretching (Halkman et al. 1994; Soyutemiz et al. 2000).

L. monocytogenes is a ubiquitous Gram-positive, facultative intracellular bacterium that causes severe infections in both humans and animals (Cabanes et al. 2002; Farber and Peterkin 1991). Several outbreaks and sporadic cases of listeriosis associated with the consumption of pasteurized milk, cheeses made from unpasteurized milk and other dairy products contaminated with *L. monocytogenes* have been reported (Dalton et al. 1997; Schuchat et al. 1997; Schlech 2000; Makino et al. 2005; Maijala et al. 2001). While particularly soft-ripened cheeses were the most frequently incriminated dairy products in regard to listeriosis, hard and semi-hard cheeses had also potential risk for public health

Address for correspondence:
Dr. Figen Cetinkaya
University of Uludag, Faculty of Veterinary Medicine,
Department of Food Hygiene and Technology, Gorukle, 16059 Bursa - TURKEY

Phone: +90 224 442 9200 ext. 278
Fax: +90 224 442 8025
E-mail: figcetinkaya@yahoo.com
http://www.vfu.cz/acta-vet/actavet.htm

(Back et al. 1993; Genigeorgis et al. 1991). In some cases, the incidence was even found higher in hard and semi hard cheeses surface compared to soft cheeses (Pak et al. 2002). Guerra and Bernardo (1997) indicated that 17 of the 68 traditional hard and semi-hard cheeses (25%) manufactured in Alentejo were contaminated with *Listeria* spp. and eight of them (11.8%) were identified as *L. monocytogenes*. In studies conducted in Turkey on Tulum (Colak et al. 2006) and Herby cheeses (Sagun et al. 2001), two traditional semi hard cheeses, the incidence of the pathogen was found 4.8% and 3.93%, respectively.

To date, to our knowledge the survival of *L. monocytogenes* and the effects of special manufacturing factors used in the production of Kashar cheese have not been studied. The aim of the present study was to investigate the influence of special manufacturing steps and vacuum-packaged storage of Kashar cheese on the behaviour and survival of *L. monocytogenes,* particularly when inoculated at different levels.

Materials and Methods

Bacterial cultures and preparation of inoculum

L. monocytogenes strain SLCC 2375 serotype 4b used in this study was kindly provided by Dr. Ch. Jacquet, Institut Pasteur, Center National de Reference des *Listeria*, Paris, France. Stock cultures were maintained on Tryptone Soya Agar (TSA) (no. CM131, Oxoid, UK) slants at 4 °C and subcultured monthly. For intermediate cultures, a loopful of the stock cultures was transferred into Tryptone Soya Broth (TSB) (no. CM129, Oxoid, UK) and incubated at 35 °C for 24 h to obtain a cell density of about 10^3 to $10^6 \cdot ml^{-1}$, and then cultured on Oxford Agar (no. CM856, Oxoid, UK) followed by incubation at 35 °C for 24 h.

Starter cultures

Commercial cultures G_3Mix6 (Visbyvac, L 91115254) composed of *Lactococcus lactis* spp. *lactis and Lc. lactis* ssp. *cremoris* and SSK1 (Visbyvac, L 71812254) composed of *Streptococcus salivarius* spp. *thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus* and *Lb. helveticus* which were obtained from Peyma-Chr. Hansen (Istanbul, Turkey) were used as the starter culture. Test tubes containing 9 ml reconstituted sterile skim milk (11%) were inoculated with the activated starter stock culture. This culture was subcultured for 24 h at 30 °C before use in cheese making. Cheese making was performed with 1% of starter culture combination including 1:1 rate of G3 Mix 6 and SSK 1.

Cheese production

Kashar cheeses were prepared according to the protocol given in Fig. 1. The pasteurized cow milk (at 72 °C for 15 s) to be used in cheese making was firstly tested for the presence of *L. monocytogenes* before inoculation. Each of the cheeses was prepared by using 40 litres of pasteurized milk in a stainless steel cheese vat. $CaCl_2$ (0.025%) was added to the milk after cooling to 35 °C. *L. monocytogenes*, at the final concentration of approximately 3, 4, 5, and 6 log $cfu \cdot ml^{-1}$ for the groups 1, 2, 3 and 4, respectively and G_3Mix6 and SSK1 starter cultures combination (1%) grown 24 h at 30 °C were inoculated into milk. A 4 ml volume of calf rennet diluted in cold water was also added to induce coagulation. The coagulum (usually 45 - 60 min after rennet addition) was cut in nearly 4×4 cm cubic forms and remained quiescent for *ca* 15 min. The temperature of the vat content was gradually raised to 41 °C under continuous stirring. Thereafter, the coagulum was collected in cheesecloth and pressed for syneresis (2 - 4 h). Subsequently, the curd was cut into blocks (25 - 35 cm) and kept at room temperature for 6 - 7 h. At the end of this period, the curd took an elastic form and its pH reached 5.10 - 5.20. At this stage, heat treatment of curd in brine containing 6% NaCl at 75 ± 1 °C for 5 ± 0.5 min was performed. For this purpose, the curd was cut into long strips and placed in a water bath in a stainless steel bucket containing several holes (5 mm in diameter). After heat treatment, the curd was manually kneaded for 5 min to eliminate air bubbles and moulded in plastic moulds (10 cm in diameter and 6 cm in height). Cheese wheels were kept at room temperature for a period of 20 h and turned over once in every two hours at this time. After 20 h, the cheese wheels (approximately 600 g weight) were removed from the plastic moulds. Thereafter, they were vacuum packaged in polyethylene bags and stored at 6 ± 1°C for 7 day.

Sampling

Duplicate samples were taken from the pasteurized milk, inoculated milk, coagulum, acidified curd, heat-treated curd and cheese after storage for 1 and 7 days.

Microbiological analysis

For enumeration of *L. monocytogenes*, the samples (10 ml or g) were transferred into individual sterile stomacher bags, mixed with 90 ml of 0.1% peptone water and homogenized in a Seward Stomacher 80 Lab System for ~ 2 min. Thereafter, a 0.1 ml portion of the homogenate was plated by spreading in duplicate on Oxford agar plates with supplement (no. SR140E, Oxoid, UK). To increase the detection level to 10 organisms/g, 0.2 ml of the initial 1 : 10 dilution was surface plated on each of 5 plates of Oxford agar, and resulting *Listeria* counts were combined.

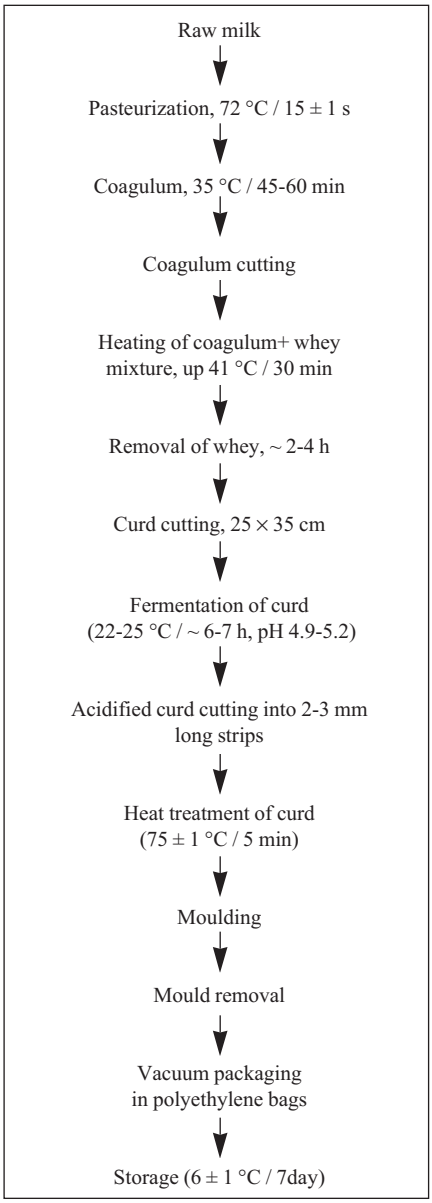

Fig. 1. Flow diagram of Kashar cheese production

All plates were incubated aerobically at 37 °C for 48 h and characteristic colonies (exhibiting a grey-black colour with a black halo) were counted. Representative colonies (five per counted plate) were confirmed as *L. monocytogenes*, based on morphology, catalase production, beta-haemolysis, tumbling motility, oxidase test, xylose, rhamnose and mannitol fermentation. For samples, in which *L. monocytogenes* was not detected by direct plating, enrichment procedure was applied. This included incubation of 25 g cheese samples in 225 ml Tryptone Soya Broth + 0.6% Yeast Extract (TSB, no. CM129, Oxoid, UK; YE, no. L21, Oxoid, UK) at 35 °C for 48 h. Enrichment culture was then plated on Oxford agar medium and plates were examined for typical *Listeria* colonies after 48 h incubation at 37 °C. Following above mentioned confirmation tests, the characteristic colonies were identified as *L. monocytogenes* (Hitchins 1995).

Determination of pH

The pH of each sample was measured with an Orion pH-meter (Ionalyzer model 399A/F, Cambridge, Mass., USA) at 25 ± 1 °C (Kosikowski and Mistry 1997).

Statistical analysis

The experiments were established in a completely random design with three replications. All data were subjected to analysis of variance using MSTAT-C (Version 2.1 - Michigan State University 1991) and MINITAB (University of Texas at Austin) software. The significance of differences between groups was determined at the 0.01 probability levels, by the F-test. The F-protected least significant difference (LSD) was calculated at the 0.01 probability level.

Results and Discussion

Influence of manufacturing steps and vacuum packaged storage of Kashar cheese produced from pasteurized milk that was inoculated with different *L. monocytogenes* levels was investigated. Data regarding the populations of *L. monocytogenes* during manufacturing stages and storage at 6 °C in vacuum-packages of Kashar cheese are shown in Table 1. Pasteurized milk samples were free (by direct plating and enrichment process) of *L. monocytogenes* before inoculation. It is a general consensus that *L. monocytogenes* does not survive at proper pasteurization (Rudolf and Scherer 2001; Farber and Peterkin 1991), nonetheless post-process contamination is possible. The numbers of pathogens at different stages or days were significantly different between the groups ($p < 0.01$). When the coagulum was heated at 41 °C for approximately 30 min, the numbers of *L. monocytogenes* remained nearly constant. This can be explained by the prolonged lag phase of the bacteria under these conditions. A previous study showed that *L. monocytogenes* has a lag phase of approximately 2 h when grown in sterile whole milk incubated at 35 °C (Rosenow and Marth 1987). Therefore, growth during the early stages of cheese manufacture would not be expected. Following

Table 1. *L. monocytogenes* counts ($\log_{10}$ cfu·ml^{-1} or·g^{-1})* during manufacture and storage of Kashar cheese

		1st group	2nd group	3rd group	4th group
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Manufacture stages	Inoculated milk	$3.25^{d} \pm 0.13$	$4.21^{c} \pm 0.08$	$5.52^{b} \pm 0.02$	$6.41^{a} \pm 0.04$
	Coagulum	$3.92^{d} \pm 0.09$	$4.95^{c} \pm 0.20$	$5.97^{b} \pm 0.24$	$6.95^{a} \pm 0.05$
	Acidified curd	$4.37^{c} \pm 0.25$	$5.47^{bc} \pm 0.26$	$6.49^{ab} \pm 0.21$	$7.41^{a} \pm 0.26$
	Heat-treated curd	$0.43^{b} \pm 0.43$	$2.35^{ab} \pm 0.56$	$3.07^{a} \pm 0.29$	$4.09^{a} \pm 0.51$
Storage (day)	1	ND^{c}	$2.37^{b} \pm 0.58$	$3.04^{ab} \pm 0.28$	$4.34^{a} \pm 0.33$
	7	ND^{b}	$2.74^{a} \pm 0.59$	$3.48^{a} \pm 0.40$	$4.53^{a} \pm 0.31$

a, b, c, d: Means having different letters within same row differ significantly ($P < 0.01$)
*: Data are based on means of three experiments with duplicate analyses
ND: *L. monocytogenes* not detected

syneresis, because of the retention of bacteria in curd matrix the population was about 0.97 - 1.26 log cfu·g^{-1} greater compared to the inoculated milk. Similar increase in the number of *L. monocytogenes* was also stated during the manufacture of Colby cheese (Yousef and Marth 1988), Mozzarella cheese (Buazzi et al. 1992) and Cameros cheese (Olarte et al. 2002). This increase would also have resulted from a combination of favourable conditions such as high moisture of curd, high temperature and the initial pH, which was around 6.0 at the beginning and decreased to 5.0 during curd acidification.

In the 1st, 2nd, 3rd and 4th group cheeses, the numbers of *L. monocytogenes* decreased by an average of 3.94, 3.12, 3.42 and 3.32 log units after the heat treatment process, respectively. In the first group, where the initial inoculation level was approximately 3 log cfu·ml^{-1}, almost totality of the bacteria was eliminated. However, this heat treatment was not sufficient to eliminate the pathogen where its final count varied between 2.35 - 4.09 $\log_{10}$ cfu·g^{-1} in the 2nd, 3rd and 4th group cheeses (*ca* 4, 5, 6 log cfu·ml^{-1}). This can be explained by the fact that even though the temperature of brine used in heat treatment of curd is quite high to kill all the bacteria, heat transfer towards the curd core might be impeded by the massive structure of the curd block. In studies with Mozzarella cheese, the authors reported that even at high inoculation levels *L. monocytogenes* could be eliminated during stretching process at 77 °C for 1 - 4 min (Buazzi et al. 1992; Kim et al. 1998). On the other hand, Villani et al. (1996) working on the elimination of *L. monocytogenes* during stretching of traditional Mozzarella cheese reported a decrease of about 2 log at 95 °C.

During the storage at 6 ± 1 °C for 7 days in vacuum packages, no bacteria was isolated on the 1st and 7th days in the 1st group cheese. On the other hand, there was no remarkable change in bacteria counts of the 2nd, 3rd and 4th groups after 7 days of storage. Previous studies reported the ability of *L. monocytogenes* to survive and even grow in the cheeses packed under modified atmosphere during refrigerated storage (Olarte et al. 2002; Whitley et al. 2000). Rogga et al. (2005) reported that *L. monocytogenes* cannot grow but may survive during storage at 4 °C and 12 °C in Galotyri cheese despite its low pH below 4.0. Contrarily, in a study realized on Cottage, Feta and Kasseri cheeses wrapped up by cellophane, Genigeorgis et al. (1991) observed that the pathogen numbers decreased to 1.87, 2.04 and 2.04 log cfu·g^{-1} after storage of 8, 8 and 6 days at + 4 °C, respectively.

Results of this work indicate that the heat treatment of curd at 75 °C for 5 min during Kashar cheese production was not sufficient to eliminate the pathogen especially when it is at > 3 log cfu·ml^{-1} level. Therefore, correlation between heating temperature and heating treatment time should be readjusted to inactivate *L. monocytogenes* if post-pasteurization contamination is at high levels (> 3 log cfu·ml^{-1}). Nevertheless, *L. monocytogenes* can survive in Kashar cheese stored in vacuum packed conditions. This demonstrates that if Kashar cheese is made from raw or heat treated milk containing *L. monocytogenes*, its

storage at 6 ± 1 °C for 7 days will not render the cheese free from viable pathogens. The presence of *L. monocytogenes* at levels of 2.74 - 4.53 log cfu·g^{-1} in Kashar cheeses at the end of storage indicates a possible public health hazard. According to the microbiological criteria for milk-based products in the European Union, *L. monocytogenes* should not be present in 25 g of cheeses (European Council Directive 1992). To avoid health risks and to offer fresh and safe Kashar cheese to the consumers, the use of raw milk with high hygienic quality is indispensable. Moreover, strict sanitary production steps and proper HACCP procedures including efficient pasteurization and post-pasteurization handlings constitute an important barrier to obtain good quality final products.

Vyhodnocení populací Listeria monocytogenes během výroby a skladování vakuově baleného sýra Kashar

Ve studii bylo zkoumáno chování *Listeria monocytogenes* během výroby a skladování sýra Kashar. Za tímto účelem bylo pasterizované mléko inokulováno čtyřmi různými dávkami patogenu, pohybujícími se mezi 3-6 log cfu·ml^{-1}. Množství patogenů bylo stanoveno ve vzorcích mléka, koagula, sýřeniny a sýra z jednotlivých kroků výroby, 1. až 7. den skladování. Proces tepelné úpravy při teplotě 75 °C po dobu 5 min provedený po kyselém srážení byl nejúčinnější bariérou a značně ovlivnil množství bakterií. Nicméně tento krok nestačil ke zničení všech bakterií, zejména při inokulaci koncentrací >3 log cfu·ml^{-1}. Tato úprava vedla přibližně ke 3 log snížení u všech skupin. Skladování při 6 ± 1 °C nemělo žádný vliv na životaschopnost patogenu a jeho množství zůstalo během skladování téměř konstantní. Výsledky této studie ukázaly, že postup výroby ani skladování při 6 ± 1 °C po dobu 7 dní ve vakouvaných baleních nepostačovalo k eliminaci *L. monocytogenes* v sýru Kashar, zvláště při vysoké počáteční koncentraci. Kromě procesu pasterizace by se proto měla brát v potaz i hygienická opatření k zamezení možné sekundární kontaminace.

References

ANONYMOUS 2003: Report of National Food and Nutrition Strategy Study Group. General Directorate of Economic Sectors and Coordination, Edition number DPT: 2670, Turkey.

BACK JP, LANGFORD SA, KROLL RG 1993: Growth of *Listeria monocytogenes* in Camembert and other soft cheeses at refrigeration temperatures. J Dairy Res **60**: 421-429

BUAZZI MM, JOHNSON ME, MARTH EH 1992: Fate of *Listeria monocytogenes* during the manufacture of Mozzarella cheese. J Food Protect **55**: 80-83

CABANES D, DEHOUX P, DUSSURGET O, FRANGEUL L, COSSART P 2002: Surface proteins and the pathogenic potential of *Listeria monocytogenes*. Trends Microbiol **10**: 238-245

CETINKAYA F 2000: Effect of manufacturing and ripening stages on *Listeria monocytogenes* in Kashar cheese. Doctorate Thesis, Department of Food Hygiene and Technology, Bursa, Turkey, pp. 119-123

COLAK H, HAMPIKYAN H, BINGOL EB, ULUSOY B 2006: Prevalence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese. Food Control, Volume 18, Issue 5, pp. 576-579, 2007

DALTON CB, AUSTIN CC, SOBEL J, HAYES PS, BIBB WF, GRAVES LM, SWAMINATHAN B, PROCTOR ME, GRIFFIN PM 1997: An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. N Engl J Med **336**: 100-105

EUROPEAN COUNCIL DIRECTIVE 1992: Laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. European Council Directive 92/46/EEC of 16 June 1992. Official Journal of The European Communities, L268, 14.9.1992, p.26.

FARBER JM, PETERKIN P 1991: *Listeria monocytogenes*, a foodborne pathogen. Microbiol Rev **55**: 476-511

GENIGEORGIS C, CARNICIU M, DUTULESCU D, FARVER TB 1991: Growth and survival of *Listeria monocytogenes* in market cheeses stored at 4 to 30°C. J Dairy Sci **54**: 662-668

GUERRA M, BERNARDO F 1997: Occurrence of *Listeria* spp. in traditional cheeses from Alentejo, Portugal. World Congress on Food Hygiene, The Hague, 214 p.

HALKMAN K, HALKMAN Z 1991: Studies on the different combinations of Kashar cheese starter cultures. Gida **16**: 99-105

HALKMAN AK, YETISMEYEN A, YILDIRIM M, YILDIRIM Z, HALKMAN Z, CAVUS A 1994: Investigation of Kashar cheese starter culture. Turk J Agric For **18**: 365-377

HITCHINS AD 1995: *Listeria monocytogenes*, FDA Bacteriological Analytical Manual. 8th edition, AOAC International, Gaithersburg, pp. 1-13

KIM J, SCHMIDT KA, PHEBUS RK, JEON IJ 1998: Time and temperature of stretching as critical control points for *Listeria monocytogenes* during production of Mozzarella cheese. J Food Protect **61**: 116-118
KOSIKOWSKI FV, MISTRY VV 1997: Analysis of Cheese and Fermented Milk Foods. In: KOSIKOWSKI FV (Ed.): Procedures and Analysis. Volume 2, L.L.C.1 Peters Lane, pp. 208-264
MAIJALA R, LYYTILCAINEN O, JOHANSSON T, AUTIO T, AALTO T, HAAVISTO L, HONKANEN-BUZALSKI T 2001: Exposure of *Listeria monocytogenes* within an epidemic caused by butter in Finland. Int J Food Microbiol **70**: 97-109
MAKINO SI, KAWAMOTO K, TAKESHI K, OKADA Y, YAMASAKI M, YAMAMOTO S, IGIMI S 2005: An outbreak of food-borne listeriosis due to cheese in Japan, during 2001. Int J Food Microbiol **104**: 189-196
OLARTE C, GONZALEZ-FANDOS M, GIMENEZ M, SANZ S, PORTU J 2002: The growth of *Listeria monocytogenes* in fresh goat cheese (cameros cheese) packaged under modified atmospheres. Food Microbiol **19**: 75-82
PAK S-II, SPAHR U, JEMMI T, SALMAN MD 2002: Risk factors for *L.monocytogenes* contamination of dairy products in Switzerland, 1990-1999. Prev Vet Med **53**: 55-65
ROGGA KJ, SAMELIS J, KAKOURI A, KATSIARI MC, SAVVAIDIS IN, KONTOMINAS MG 2005: Survival of *Listeria monocytogenes* in Galotyri, a traditional Greek soft acid-curd cheese, stored aerobically at 4 °C and 12 °C. Int Dairy J **15**: 59-67
ROSENOW EM, MARTH EH 1987: Growth of *Listeria monocytogenes* in skim, whole and chocolate milk, and in whipping cream during incubation at 4, 8, 13, 21 and 35 °C. J Food Protect **50**: 452-459
RUDOLF M, SCHERER S 2001: High incidence of *Listeria monocytogenes* in European and smear cheese. Int J Food Microbiol **63**: 91-98
SAGUN E, SANCAK YC, ISLEYICI Ö, EKICI K 2001: The presence and prevalence of *Listeria* species in milk and Herby cheese in and around Van. Turk J Vet Anim Sci **25**: 15-19
SCHLECH WF 2000: Foodborne listeriosis. Clin Infect Dis **31**: 770-775
SCHUCHAT A, ROBINSON K, WENGER JD, HARRISON LH, FARLEY M, REINGOLD AL, LEFKOWITZ L, PERKINS BA 1997: Bacterial meningitis in the United States in 1995. N Engl J Med **337**: 970-976
SOYUTEMIZ GE, ANAR S, CETINKAYA F 2000: Investigation on the microbiological and chemical changes of Kashar cheese at production stages. J Fac Vet Med **19**: 87-92
TEKINSEN OC 2000: Technology of Dairy Products. Selcuk Univ. Basimevi, Konya, Turkey, pp. 214-216.
TOPAL RS 1991: Effects of different packaging materials and techniques on the curing of Kashar cheese and on its surface moulds. Lebensm- Wiss u.- Technol **24**: 341-349
VILLANI F, PEPE O, MAURIELLO G, MOSCHETTI G, SANNINO L, COPPOLA S 1996: Behaviour of *Listeria monocytogenes* during the traditional manufacture of water-buffalo mozzarella cheese. Lett Appl Microbiol **22**: 357-360
WHITLEY E, MUIR D, WAITES WM 2000: The growth of *Listeria monocytogenes* in cheese under modified atmosphere. J Appl Microbiol **88**: 52-57
YOUSEF AE, MARTH EH 1988: Behavior of *Listeria monocytogenes* during the manufacture and storage of Colby cheese. J Food Protect **51**: 12-15