A survey of soft cheeses in Greek retail outlets highlights a low prevalence of Listeria spp.

Apostolos Angelidis, Sofia Georgiadou, Zafeiropoulou, Emmanuel Velonakis, Demetrios Papageorgiou, Vatopoulos

Dairy Sci. & Technol. (2012) 92:189–201
DOI 10.1007/s13594-011-0049-y

NOTE

A survey of soft cheeses in Greek retail outlets highlights a low prevalence of *Listeria* spp.

Apostolos S. Angelidis · Sofia S. Georgiadou · Vasiliki Zafeiropoulou · Emmanuel N. Velonakis · Demetrios K. Papageorgiou · Alkiviadis Vatopoulos

Received: 25 May 2011 / Revised: 16 September 2011 / Accepted: 26 September 2011 /
Published online: 1 November 2011

Abstract Recently, the European Food Safety Authority proposed that each EU member state should conduct a survey of *Listeria monocytogenes* in soft cheeses for sale in retail outlets. Hence, retail samples of soft cheeses in Greece (n=137) were analyzed qualitatively and quantitatively for both *L. monocytogenes* and other *Listeria* spp., as well as for pH and a_w. None of the cheeses analyzed were found positive for *L. monocytogenes* (0%; 95% confidence interval (CI)=0.0–2.2%). Three samples (2.2%; 95% CI=0.5–6.3%) were positive for other *Listeria* spp., with populations ranging from <5 to 4.5×10^2 CFU g^{-1}. Although soft cheeses are regarded as foods that permit the proliferation of *L. monocytogenes*, 15.4% of the whey cheeses tested had pH values ≤4.4 and 29% of the interior mold-ripened cheeses had a_w values ≤0.92. Such low pH values for whey cheeses are unexpected, based on their manufacturing technology, and are associated with lower quality and reduced shelf life. Nonetheless, in the present survey, the percentage of whey cheeses in the Greek retail market exhibiting unusually low pH values or testing positive for *Listeria* spp. was much lower compared to the respective percentages reported from studies conducted in the 1990s. The absence of *L. monocytogenes* in the tested cheeses undoubtedly constitutes an encouraging result with respect to the safety of soft cheeses in the Greek retail market. However, the presence of other *Listeria* spp. in three of the tested samples implies that *L. monocytogenes* would,

A. S. Angelidis (✉) · S. S. Georgiadou · D. K. Papageorgiou
Laboratory of Milk Hygiene and Technology, Department of Food Hygiene and Technology,
Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece
e-mail: asangel@vet.auth.gr

S. S. Georgiadou · V. Zafeiropoulou · E. N. Velonakis · A. Vatopoulos
Department of Microbiology, National School of Public Health, 196, Alexandras Avenue,
11521, Athens, Greece

E. N. Velonakis · A. Vatopoulos
Central Public Health Laboratory, Hellenic Centre of Disease Control and Prevention,
34 A. Fleming Street, 16672, Vari, Attica, Greece

most likely, also be present in the food-processing or retail-handling environments of these products. Therefore, cheese manufacturers and retail handlers should continue or even intensify the application of all necessary measures with the aim of preventing food contamination.

希腊零售软质干酪李斯特菌的调查

摘要 近年来, 欧洲食品安全局(EFSA)要求每个欧盟成员国对零售的软质干酪中李斯特单胞菌(*Listeria monocytogenes*)进行抽样检查。本文对在希腊零售的软质干酪(n=137)中的李斯特单胞菌、李斯特菌属(*Listeria spp*)、pH和水分活度进行了定性和定量检测。在这些抽检的样品中, 李斯特单胞菌呈阳性的没有检出(0%; 95% 置信区间CI = 0.0-2.2%);但有3个样品的其它李斯特菌属检测呈阳性(2.2%; 95% 置信区间CI = 0.5-6.3%), 菌数小于5~4.5×102 CFU.g-1。虽然, 软质干酪有利于李斯特单胞菌的增殖, 但是测定结果发现15.4%的乳清干酪的pH≤ 4.4,29%的内部霉菌成熟干酪的水分活度≤ 0.92;根据乳清干酪的生产技术, 出现低pH的乳清干酪是未预料到的, 低pH会导致干酪品质的降低和减少产品的货架期。在目前的调查结果中, 尽管希腊零售市场中出现一定比例的低pH和李斯特菌属呈阳性的乳清干酪, 但还是低于文献报道的数据。然而, 在三个样品中检测到其它李斯特菌属则说明有可能在加工和零售贮藏过程中存在李斯特单胞菌的污染。因此, 干酪的生产者和零售管理者应该采取必要的措施来防止食品污染。

Keywords *Listeria monocytogenes* · *Listeria* spp. · Soft cheese · Prevalence · Greece

关键词 李斯特单胞菌 · 李斯特菌属 · 软质干酪 · 流行 · 希腊

1 Introduction

Listeria monocytogenes is a Gram-positive, foodborne pathogen that is widely distributed in the environment, including many animal hosts (Schlech et al. 1983; Weis and Seeliger 1975). The microorganism is also frequently isolated from the food-processing environment (Kornacki and Gurtler 2007). Therefore, contamination of foods can occur at various steps of food production and handling (Kousta et al. 2010). The pathogen has been isolated worldwide from foods of plant or animal origin and consumption of contaminated foods has led to sporadic or epidemic cases of illness (Farber and Peterkin 1991; Schlech et al. 1983). In 2008, 25 member states of the EU reported a total of 1,381 confirmed cases of human listeriosis and 134 deaths (EFSA 2010). Controlling *L. monocytogenes* growth in contaminated foods is problematic because the bacterium is resistant to low pH, low water activity, and low temperatures (Hado and Yousef 2007), conditions that constitute primary approaches for food preservation. Previous studies have documented the ability of *L. monocytogenes* to proliferate in soft cheeses under refrigeration (Back et al. 1993; Genigeorgis et al. 1991; Papageorgiou et al. 1996), a phenomenon aided largely through the pathogen's ability for intracellular accumulation of cryoprotective compounds from its environment (Angelidis et al. 2002).

In order to estimate the public health risk from consumption of different foods, quantitative data (i.e., numbers of listeriae in *Listeria*-contaminated foodstuffs) as well as data from different countries are needed (Rocourt et al. 2003; WHO/FAO 2004). Such data are also of major importance towards ongoing efforts for establishing appropriate microbiological criteria concerning the presence and populations of *L. monocytogenes* in foods (Dufour 2011; European Commission (EC) 2005, 2007).

Hence, a recent scientific report of the European Food Safety Authority (EFSA) proposed that each member state of the EU should conduct a survey on *L. monocytogenes* in selected categories of ready-to-eat (RTE) foods (including soft cheeses) at retail (EFSA 2009b). Therefore, the aim of this work was to estimate the prevalence and contamination levels of *L. monocytogenes* in soft cheeses in the Greek retail market according to the EFSA sampling and testing requirements for Greece. In addition, the prevalence and contamination levels of other *Listeria* spp. were also investigated in the same set of samples. Finally, the new microbiological and pH data for the Greek whey cheeses were compared to analogous data from previous surveys.

2 Materials and methods

2.1 Sampling

The EFSA scientific report concerning the EU member state surveys on *L. monocytogenes* in soft cheeses at retail specifies that a minimum of 60 to 68 samples are required to be sampled and analyzed from Greece, according to its human population (EFSA 2009b). Hence, in 2010, 137 samples of soft cheeses from 71 different manufacturers were collected from 18 retail stores (16 supermarkets, $n=128$; 1 minimarket, $n=5$; 1 flea market, $n=4$) located in and around the cities of Thessaloniki ($n=88$) and Athens ($n=49$). These two major cities account for more than 60% of the population of Greece. The sampled stores represent the 10 major supermarket chains in Greece. Based on sample origin, 67 cheeses were manufactured in Greece and 70 were imported from other EU member states. Overall, the 137 sampled items represented 13 different types of popular soft cheeses. Specifically, the sampled cheeses consisted of the whey cheeses Anthotyros, Manouri, “fresh” Myzithra (often also called “sweet” Myzithra to distinguish it from “dry” Myzithra or Xynomyzithra, which is a ripened variety of Myzithra), and Ricotta ($n=65$), the acid-curd cheeses Cottage and Katiki Domokou ($n=8$), the pasta-filata cheese Mozzarella ($n=10$), the surface mold-ripened cheeses Brie and Camembert ($n=16$), and the interior mold-ripened cheeses Blue, Gorgonzola, Kopanisti Tinou, and Roquefort ($n=38$) (Table 1). The samples were stored under refrigeration. The samples’ shelf life was quite variable, ranging from 1 month to over 1 year (Table 1). Based upon information listed on the packaging (when stated), 36 cheeses were made from pasteurized milk and 11 were made from raw milk (all 11 raw milk cheeses were Roquefort cheeses). Most of the samples ($n=74$) were cheeses sold in their original, consumer-type, intact, and sealed packaging (typically 200–500 g). These cheeses were sampled from refrigerated supermarket shelves, thereafter referred to as “shelf” samples. The remaining samples were obtained from cheeses packaged by the manufacturer in bulk quantities and stored in refrigerated supermarket cabinets ($n=63$). In this case, the consumer has the option to purchase a variable portion of the cheese by asking the supermarket employee to remove or cut out the desired weight/portion. The extracted cheese portion is subsequently weighed, wrapped in paper with an inner layer of plastic film, and sold to the consumer. These samples are thereafter designated as “counter” samples. All “counter” sampled items had a net weight of at least 200 g.

Table 1 Classification and characteristics of the soft cheeses tested in this study

Cheese	Retail type	Manufacturer code	Milk origin	Milk type	Packaging atmosphere	Shelf life (days)[a]	Time to expiration (days)[b]	pH	a_w
Anthotyros	Counter	1	CO	–	N/A	–	18	5.61	0.978
		1	–	–	N/A	40	27	5.26	0.981
		2	BCO	P	N/A	–	50	4.64	0.986
		3	–	–	N/A	43	1	4.43	0.989
		3	–	–	N/A	30	9	5.40	0.989
		4	CO or BCO	–	N/A	21	0	5.45	0.986
		7	–	–	N/A	40	5	5.33	0.986
		8	–	–	N/A	30	6	5.25	0.985
		9	O	–	N/A	38	4	4.24	0.982
		12	CO	–	N/A	40	32	5.77	0.987
		13	CO	–	N/A	62	36	4.91	0.983
		16	–	–	N/A	–	–	6.14	0.996
		17[c]	–	–	N/A	30	15	5.92	0.952
		18	–	–	N/A	30	15	5.70	0.949
		21	–	–	N/A	31	2	4.65	0.984
	Shelf	2	BCO	P	V	86	46	4.96	0.985
		4	CO or BCO	–	MA	–	13	5.95	0.982
		4	CO or BCO	–	MA	–	2	5.90	0.948
		5	CO or B	–	V	45	15	6.39	0.983
		6	CO	–	V	30	5	5.20	0.992
		10	CO or B	–	V	90	59	6.09	0.978
		11	CO	–	V	39	26	5.13	0.985
		14	CO	P	V	62	26	4.18	0.982
		14	CO	P	V	61	34	4.90	0.981
		15	BCO	–	V	60	54	6.35	0.986
		16	–	–	V	39	25	5.80	0.992
		19	CO or BCO	–	V	51	31	4.87	0.959
		20	–	–	V	31	18	5.58	0.955
								(5.36±0.61)[d]	(0.979±0.013)[d]
Manouri	Counter	9	O	–	N/A	364	250	4.33	0.981
		9	O	–	N/A	364	247	4.30	0.997
		12	–	–	N/A	365	241	4.02	0.981
		15	–	–	N/A	–	259	3.76	0.968
		16	–	–	N/A	365	164	4.01	0.968
		22	O	–	N/A	365	264	6.48	0.960
		22	O	–	N/A	365	260	6.30	0.946
		24	–	–	N/A	365	322	6.05	0.984
		25	–	–	N/A	–	–	5.89	0.979
		26	CO	–	N/A	270	227	5.80	0.980
		27	–	–	N/A	365	261	4.59	0.972
		28	O	–	N/A	304	141	6.43	0.972
		28	O	–	N/A	366	254	6.50	0.946
		29	CO	–	N/A	365	312	5.09	0.977

Table 1 (continued)

Cheese	Retail type	Manufacturer code	Milk origin	Milk type	Packaging atmosphere	Shelf life (days)[a]	Time to expiration (days)[b]	pH	a_w
	Shelf	5	–	–	V	162	64	5.04	0.981
		10	CO	–	V	120	93	4.49	0.971
		11	CO	–	V	169	79	4.50	0.983
		14	CO	P	V	184	149	4.84	0.987
		14	CO	P	V	183	141	6.34	0.952
		19	CO	–	V	–	–	4.38	0.985
		19	CO	–	V	67	7	4.71	0.982
		23	–	–	V	184	128	5.45	0.982
		28	O	–	V	155	59	5.96	0.941
								(5.19±0.92)[d]	(0.973±0.015)[d]
Myzithra	Counter	8	–	–	N/A	30	16	6.57	0.948
		9	–	–	N/A	364	261	4.68	0.964
		10	BCO	–	N/A	90	33	4.76	0.988
		10	BCO	–	N/A	90	41	5.01	0.984
		30	–	–	N/A	246	225	6.01	0.986
		31	C	–	N/A	35	18	4.51	0.984
		31	C	–	N/A	35	21	5.12	0.956
		32	–	–	N/A	184	163	6.10	0.987
	Shelf	2	BCO	–	MA	102	69	4.54	0.979
		10	BCO	–	V	90	38	5.04	0.983
		33	CO	–	V	432	103	4.08	0.981
		33	CO	–	V	428	300	3.90	0.945
								(5.03±0.82)[d]	(0.974±0.016)[d]
Ricotta	Counter	46	B	–	N/A	–	–	5.74	0.995
	Shelf	44	B	–	MA	–	1	6.92	0.973
								(6.33±0.83)[d]	(0.984±0.016)[d]
Cottage	Shelf	34	B	–	MA	36	17	4.33	0.988
		35	B	–	MA	–	17	4.55	0.987
		36	B	P	MA	–	21	5.03	0.988
		37	–	–	MA	210	107	4.65	0.985
		38	–	P	MA	–	67	4.31	0.988
		39	B	–	MA	–	7	4.82	0.989
								(4.62±0.28)[d]	(0.988±0.001)[d]
Katiki	Counter	40	CO	–	N/A	–	–	4.41	0.988
	Shelf	40	CO	–	MA	124	42	4.36	0.978
								(4.39±0.04)[d]	(0.983±0.007)[d]
Mozzarella	Shelf	41	B	P	MA	–	5	6.15	0.989
		42	B	–	MA	–	21	6.25	0.988
		43	B	P	MA	–	14	5.78	0.995
		43	B	P	MA	–	6	5.72	0.997
		43	B	P	MA	–	15	5.79	0.990
		44	B	P	MA	31	4	5.77	0.990
		44	B	P	MA	135	12	6.34	0.988

Table 1 (continued)

Cheese	Retail type	Manufacturer code	Milk origin	Milk type	Packaging atmosphere	Shelf life (days)[a]	Time to expiration (days)[b]	pH	a_w
		44	B	P	MA	–	19	6.42	0.995
		44	Bf	–	MA	28	10	5.17	0.998
		45	Bf	–	MA	–	–	5.53	0.994
								(5.89±0.39)[d]	(0.992±0.004)[d]
Brie	Counter	51	B	P	N/A	–	39	6.02	0.984
		52	B	–	N/A	89	13	7.59	0.947
		53[e]	B	–	N/A	–	25	5.87	0.955
	Shelf	47	B	P	APF	378	273	6.00	0.968
		48	B	P	APF	–	14	7.21	0.976
		49	B	–	APF	–	23	6.56	0.981
		50	B	P	APF	60	32	5.86	0.979
		51	B	P	APF	–	25	6.18	0.983
		52	B	–	APF	61	17	7.61	0.954
		52	B	–	APF	61	10	7.27	0.958
								(6.62±0.73)[d]	(0.969±0.014)[d]
Camembert	Shelf	47	B	P	APF	389	223	5.82	0.974
		52	B	P	APF	72	25	7.52	0.974
		52	B	P	APF	–	12	7.26	0.954
		54	B	P	APF	–	19	7.35	0.977
		54	B	P	APF	–	12	7.15	0.969
		55	B	P	APF	–	299	5.90	0.948
								(6.83±0.76)[d]	(0.966±0.012)[d]
Blue	Counter	56	B	–	N/A	–	–	5.27	0.920
		56	B	–	N/A	–	–	5.04	0.922
		56	B	–	N/A	365	176	5.13	0.920
		56	B	–	N/A	365	271	5.38	0.934
		57	B	–	N/A	–	–	5.25	0.934
		58	B	–	N/A	301	29	5.09	0.931
		59	B	–	N/A	576	142	4.94	0.914
		60	B	P	N/A	273	127	5.03	0.914
		60	B	P	N/A	285	257	5.98	0.942
		60	B	P	N/A	300	227	5.38	0.936
		60	B	P	N/A	294	214	5.63	0.933
		61	B	–	N/A	396	115	5.00	0.987
		62	B	P	N/A	–	45	7.59	0.946
	Shelf	56	B	–	MA	361	222	5.41	0.919
		56	B	–	MA	–	246	5.37	0.936
		56[f]	B	–	MA	–	312	5.40	0.922
		56	B	–	MA	–	310	5.30	0.926
		58	B	–	MA	240	96	5.27	0.923
		60	B	P	MA	269	122	5.01	0.928
		60	B	P	MA	198	158	5.12	0.940
		60	B	P	MA	215	148	5.07	0.931
		60	B	P	MA	269	151	5.02	0.927

Table 1 (continued)

Cheese	Retail type	Manufacturer code	Milk origin	Milk type	Packaging atmosphere	Shelf life (days)[a]	Time to expiration (days)[b]	pH	a_w
		60	B	P	MA	235	160	5.09	0.931
		61	B	–	MA	396	166	5.18	0.932
								(5.33±0.53)[d]	(0.931±0.015)[d]
Gorgonzola	Counter	63	–	–	N/A	–	28	6.98	0.943
	Shelf	64	–	–	MA	–	10	6.05	0.965
								(6.52±0.66)[d]	(0.954±0.016)[d]
Kopanisti	Counter	65	–	–	N/A	–	–	5.17	0.938
Roquefort	Counter	66	O	R	N/A	–	32	6.77	0.909
		67	O	R	N/A	–	25	6.27	0.913
		68	O	R	N/A	–	57	5.93	0.923
		69	O	R	N/A	–	76	5.93	0.922
		70	O	R	N/A	–	91	6.14	0.932
		71	O	R	N/A	–	22	5.54	0.912
	Shelf	66	O	R	MA	–	18	6.00	0.904
		66	O	R	MA	–	18	6.02	0.927
		67	O	R	MA	154	62	5.80	0.909
		67	O	R	MA	154	84	5.75	0.918
		70	O	R	MA	–	441	6.56	0.924
								(6.06±0.36)[d]	(0.918±0.009)[d]

B cheese made with bovine milk, *Bf* cheese made with buffalo milk, *C* cheese made with caprine milk, *O* cheese made with ovine milk, *CO* cheese made with a mixture of caprine and ovine milk, *BCO* cheese made with a mixture of bovine, caprine, and ovine milk, *CO or B* cheese made with a mixture of caprine and ovine milk or with bovine milk, *CO or BCO* cheese made with a mixture of caprine and ovine milk or with a mixture of bovine, caprine, and ovine milk, – unspecified, *P* pasteurized milk, *R* raw milk, *MA* modified atmosphere (the assessment of MA packaging was done based on visual observation when packaging information was not specified), *V* vacuum, *APF* air-permeable film, *N/A* not applicable (information about the packaging atmosphere for "counter" samples was not available)

[a] Days from sample production to sample expiration date

[b] Days from sample examination to sample expiration date

[c] Sample contained 4.5×10^2 CFU g^{-1} *L. innocua*

[d] Average±standard deviation

[e] Sample contained *L. seeligeri* and *L. grayi* at levels below the enumeration limit (<5 CFU g^{-1})

[f] Sample contained 1.5×10^2 CFU g^{-1} *L. grayi*

The sampled cheeses consisted of 110 distinct products, i.e., different combinations of cheese type, name, manufacturer (brand), and retail packaging ("shelf" vs. "counter"). The remaining 27 consisted of replicate cheese samples (Table 1). Care was taken to ensure that replicate samples of the same cheese product belonged to different lots. After purchase, the samples were placed in isothermal containers (approximately 4 °C) and transported to one of two laboratories (one located in Thessaloniki, the other located in Athens) within a maximum of 2 h after purchase. The same two trained analysts tested the samples on the day of purchase.

2.2 Determination of a_w and pH

All samples were analyzed for a_w and pH. The a_w was measured at 25 °C using an Aqualab water activity meter (Aqualab, model series 3, Decagon Devices Inc., Pullman, WA, USA). The pH was measured after blending 25 g of cheese in quadruple volume (100 mL) of distilled and deionized water, using a Consort pH meter (Consort, model C830, Turnhout, Belgium) equipped with a Hanna FC-100 electrode (Hanna Instruments, Woonsocket, RI, USA).

2.3 Detection and enumeration of *L. monocytogenes* and other *Listeria* spp.

Unless otherwise specified, all media and reagents were purchased from Biolife (Biolife Italiana S.r.l., Milan, Italy). The sampled cheeses were analyzed with the concurrent application of the ISO 11290-1 and ISO 11290-2 protocols for the detection and enumeration of *L. monocytogenes*, respectively (ISO 2004a, b). Briefly, for each sample, 25-g portions representing segments from at least five different sites of the cheese were aseptically removed from their package and homogenized with 225 mL of half-Fraser broth base (HFBB) in 400-mL stomacher bags for 4 min in a BagMixer 400 stomacher (Interscience, St. Nom, France). After the homogenization step, the samples were stored at 20 °C for 1 h for resuscitation, and then 2 mL of the homogenate was spread-plated onto six, predried ALOA plates (enumeration limit=5 CFU g^{-1}). Volumes (0.1 mL) of the primary (10^{-1}) and secondary (10^{-2}) serial dilutions of the homogenate in quarter-strength Ringer's solution (Lab M, Bury, UK) were also plated in duplicate on ALOA to allow for the enumeration of up to 7.5×10^4 CFU g^{-1}. After conducting the initial plating step (enumeration), the appropriate selective agents were added to HFBB, as specified in the ISO 11290-2 method, to allow for sample enrichment. The remaining steps were conducted as described in the ISO 11290-1 and ISO 11290-2 protocols with the addition that suspect colonies displaying the morphological characteristics of either *L. monocytogenes* or other *Listeria* spp. (green colonies surrounded or not by an opaque halo) were also subjected to biochemical confirmation using the API *Listeria* strips (bioMérieux sa, Marcy l'Etoile, France).

2.4 Statistical analysis

The 95% confidence interval (CI) for the estimated proportion of *Listeria* spp.-positive samples was calculated using the exact probability method of Minitab (version 14, Minitab Inc., State College, PA, USA). The upper limit of the 95% CI for the proportion of *L. monocytogenes*-positive samples was calculated using the approach proposed by Hanley and Lippman-Hand (1983).

3 Results and discussion

3.1 Detection and enumeration of *L. monocytogenes* and other *Listeria* spp.

L. monocytogenes was not isolated from any of the samples examined (0/137). However, a 0 numerator should not be interpreted as "no risk" for contamination with *L. monocytogenes* in the "target" population of soft cheeses. Therefore, the approach of Hanley and Lippman-Hand (1983) was employed in order to estimate, with 95% confidence, the maximum value of the population proportion of the *L. monocytogenes*-positive cheeses that would be compatible with the observation of no positive results in a sample of 137. According to this approach, the upper limit of the 95% CI of a 0/*n* proportion (i.e., of the 0/137 proportion) can be estimated by solving the following equation:

$$(1 - \text{Maximum Risk})^n = 0.05$$

By substituting 137 for *n* in the above equation, the Maximum Risk, i.e., the upper limit of the 95% CI, is calculated to be 0.0216 or 2.16%. Hence, the 95% CI for the estimated population proportion of *L. monocytogenes*-positive soft cheeses in the Greek retail market is 0–0.0216, or 0–2.16 on a percent basis.

Three samples (2.19%; 95% CI=0.45–6.27%) were found positive for *Listeria* spp. other than *L. monocytogenes*. One sample contained *Listeria innocua* at levels of 4.5×10^2 CFU g^{-1} (Anthotyros, "counter" sample). According to the information listed on its package, on the day of sampling and examination, the cheese was 15 days old and had a remaining shelf life of 15 days (Table 1). The pH of the sample was 5.92 and its a_w was equal to 0.952. Anthotyros can support the growth of *L. monocytogenes* when the pathogen is introduced as a post-processing contaminant, even when the cheese is stored at 4 °C (Papageorgiou et al. 1996), and interventions such as irradiation or addition of nisin have been proposed for the inactivation or the control of *L. monocytogenes* proliferation in Anthotyros (Samelis et al. 2003; Tsiotsias et al. 2002). The second positive sample contained *Listeria grayi* at levels of 1.5×10^2 CFU g^{-1} (Blue cheese, "shelf" sample). According to packaging information, the cheese was made from bovine milk and had 312 days of remaining shelf life on the day of examination. Its pH was equal to 5.4 and its a_w was equal to 0.922 (Table 1). *L. monocytogenes* can multiply during the first hours of manufacture of Blue cheese, but growth ceases when the pH of the cheese drops below 5 (Papageorgiou and Marth 1989). The third positive sample (Brie, "counter" sample) contained both *Listeria seeligeri* and *L. grayi* at levels lower than the limit of enumeration (i.e., <5 CFU g^{-1}). This cheese was also made from bovine milk and had 25 days of remaining shelf life. Its pH was equal to 5.87 and its a_w was equal to 0.955 (Table 1). Two studies have documented the ability of *L. monocytogenes* to proliferate in artificially inoculated market samples of Brie cheese under refrigerated storage (Back et al. 1993; Genigeorgis et al. 1991) Previous research has shown that RTE foods that test positive for *L. monocytogenes* frequently also contain additional *Listeria* spp. (Angelidis and Koutsoumanis 2006; Vitas et al. 2004). Therefore, food manufacturers should also take appropriate corrective actions whenever *Listeria* species other than *L. monocytogenes* are isolated from foods.

In recent years, several investigators have studied the behavior of *L. monocytogenes* in Greek protected designation of origin (PDO) soft cheeses, as well as possible interventions that lead to the prevention of *L. monocytogenes* proliferation in contaminated products. Kagkli et al. (2009) showed that *L. monocytogenes* does not proliferate in Katiki when introduced as a post-processing contaminant. Rather, the pathogen is gradually inactivated during storage at 5, 10, 15, or even 20 °C. The investigators also demonstrated that the inactivation of *L. monocytogenes* during storage was temperature- and strain-dependent (Kagkli et al. 2009). Like Katiki, Galotyri is another acid-curd fresh Greek PDO cheese. Although Galotyri was not included in our study due to its limited availability in the sampled stores at the time of sampling, Samelis and Kakouri (2007) reported the presence of *L. monocytogenes* (<10 CFU g^{-1}) in 3 out of the 12 batches examined (1 industrial and 2 artisanal batches). However, unlike Anthotyros, Galotyri, because of its low pH, does not support the growth of *L. monocytogenes* under refrigeration (Papageorgiou et al. 1998; Rogga et al. 2005).

Contamination of cheeses with *L. monocytogenes* has led to numerous product recalls worldwide (Ryser 2007). In addition, soft cheeses have been associated with outbreaks of listeriosis, resulting in abortions, stillbirths, and deaths primarily in the young, the elderly, and in the immunocompromised (Büla et al. 1995; Goulet et al. 1995; Linan et al. 1988). According to the community summary reports in 2006 and 2007, soft cheeses were the food vehicle most often implicated in the limited number of reported foodborne listeriosis outbreaks (EFSA 2007, 2009a). The reported prevalence estimates of *L. monocytogenes* in soft and fresh cheeses in different countries are quite variable. Estimates range from 0% to values as high as 87%, with the majority of studies reporting prevalence estimates below 10% (Lianou and Sofos 2007; Ryser 2007). More recent data from the EU indicate a major improvement in the contamination status of soft and semisoft cheeses at retail. According to the 2008 community summary report, only 0.2% of the 2,116 soft and semisoft cheese samples tested (single retail units collected from 25 EU member states) were deemed as noncompliant, i.e., contained more than 100 CFU g^{-1} of *L. monocytogenes*, while the respective percentage for batch samples was 2.8%. Also, approximately 1.3% of the soft and semisoft cheeses (single or batch samples from either processing plants or retail) made from pasteurized milk were positive for *L. monocytogenes* (presence in 25 g) (EFSA 2010). Therefore, the prevalence estimate for *L. monocytogenes* in soft cheeses in the Greek retail market (0.0–2.2%) is very similar to the latest average EU estimate. The respective estimate for *Listeria* spp. was also found to be low (2.2%; 95% CI=0.45–6.27). However, both estimates are reasonably imprecise due to the relatively small sample size.

3.2 Physicochemical characteristics of soft cheeses

Generally, soft cheeses are considered as foods that support the growth of *L. monocytogenes*. Interestingly, however, several of the tested samples had values of pH or a_w that, according to the limits specified under Regulation 2073/2005 (European Commission (EC) 2005), classify these products as ones that are "automatically considered to belong to the category of RTE foods unable to support the growth of *L. monocytogenes*" (Table 1). Hence, 13 of the total samples (9.5%) and, in particular, 10 out of the 65 whey cheeses (i.e., 15.4% of the whey cheeses tested) had pH values ≤4.4. Whereas such low pH values are normal for acid-curd cheeses, they are unexpectedly low for whey cheeses. These 10 whey cheeses had no noticeable off-odors, texture, or appearance (color) abnormalities at the time of sampling. Normally, the pH of whey cheeses immediately upon their manufacture is close to 6. Occasionally, lactic acid bacteria (LAB), most likely originating from the dairy plant environment, contaminate the whey cheeses during the hooping, drainage, cutting, and packaging steps. As a result, high numbers of LAB are often enumerated, even in freshly produced whey cheeses (Kalogridou-Vassiliadou et al. 1994; Lioliou et al. 2001). Hence, the pH of such cheeses is gradually reduced during their shelf life. For instance, two of the Myzithra samples exhibiting very long shelf lives (of 432 and 428 days, respectively; Table 1) already had low pH values at the day of sampling and examination (4.08 and 3.90, respectively). Shelf life in cheeses is not governed by a specific regulation in the EU. Instead, cheese manufacturers are responsible for determining the appropriate shelf lives for their products. Nonetheless, very long shelf lives, such as those recorded in the packaging of some of the Greek whey cheeses (Table 1), may not be realistically attainable and this is an issue that the respective whey cheese manufacturers should attend to. Also, 11 out of the 38 interior mold-ripened cheeses (29%) had values of $a_w \leq 0.92$, i.e., at or below the regulation's lower a_w limit for *L. monocytogenes* growth. Similar variability in the pH and a_w between products of the same category and type was also noted previously for RTE meat products (Koutsoumanis and Angelidis 2007). Although the reason(s) for this observed variability in the physicochemical characteristics (particularly with respect to the pH values) are not known, temperature abuse of LAB-contaminated products during distribution and storage at retail could be the cause. It has been previously shown that the temperature of retail cabinets in Greece can vary considerably (Koutsoumanis and Angelidis 2007).

3.3 Comparisons with previous data on Greek whey cheeses

In 1998, the results of two surveys regarding the prevalence of *L. monocytogenes* and *Listeria* spp. in Greek whey cheeses were published (Theodoridis et al. 1998). The surveys were conducted in 1990 and 1996 and 167 commercial samples were tested in each survey. The reported percentages of positive samples for *Listeria* spp. and *L. monocytogenes* were 15% and 13.8% (1990) and 6% and 1.8% (1996), respectively. Although the number of Greek whey cheeses examined in the current study was relatively small ($n=63$), the results may indicate an improvement in their hygienic status with respect to both *L. monocytogenes* and *Listeria* spp.

As previously mentioned, low pH in whey cheeses is indicative of contamination with LAB from the dairy environment (after the heat treatment step) and is associated with lower quality and reduced shelf life. A greater percentage of Greek whey cheeses in the current study (59%) were found to have pH values >5.0, compared to approximately 46% and approximately 37% of the cheeses in the 1996 and 1990 surveys, respectively (Theodoridis et al. 1998). Therefore, the current data may also indicate an improvement for Greek whey cheeses in terms of quality.

4 Conclusions

The absence of *L. monocytogenes* in the tested cheeses undoubtedly constitutes an encouraging result with respect to food safety. However, the presence of other *Listeria* spp. in three of the tested samples implies that *L. monocytogenes* would, most likely, be also present in the food-processing or retail-handling environment of these products. Therefore, cheese manufacturers and retail handlers should continue or even intensify the application of all necessary measures aiming at preventing food contamination and minimizing pathogen proliferation during manufacture, distribution, and retail storage. The data generated in this survey fulfill EFSA's minimum sampling requirements for retail soft cheeses in Greece and will be useful for assessing the effectiveness of the implementation of the community *L. monocytogenes* safety criteria at the EU level.

Acknowledgement This research was supported by funds from the Ministry of Education and the Ministry of Health and Solidarity of Greece, as well as by personal expenses of the authors.

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