Journal of Food Protection, Vol. 67, No. 10, 2004, Pages 2189–2194

Occurrence of *Listeria monocytogenes* in Green Table Olives

CINZIA CAGGIA,[1]* CINZIA L. RANDAZZO,[1] MONIA DI SALVO,[1] FLORA ROMEO,[2] AND PAOLO GIUDICI[3]

[1]*Dipartimento di Orto Floro Arboricoltura e Tecnologie Agro-Alimentari, University of Catania, via Santa Sofia, 98-95123, Catania, Italy*
[2]*Dipartimento di Biotecnologie per il Monitoraggio Agro-Alimentare ed Ambientale, University of Reggio Calabria, Feo di Vito 89060, Reggio Calabria, Italy; and* [3]*Dipartimento di Scienze Agrarie, University of Modena and Reggio Emilia, viale Kennedy, 17-42100, Reggio Emilia, Italy*

MS 03-434: Received 29 September 2003/Accepted 21 March 2004

ABSTRACT

Microbiological safety of green table olives from different cultivars, prepared by both the Spanish-style and biological methods and fermented with starter cultures of lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus casei,* and *Lactobacillus pentosus*), was investigated. The fermentation process was monitored by measuring pH values, titratable acidities, and growth of lactic acid bacteria over time. During fermentation, lactic acid bacteria and yeasts were major microbial populations. Microbiological safety was evaluated by analysis for *Listeria monocytogenes* with the use of an enrichment method during storage (from 55 days to 18 months). Results demonstrated that *L. monocytogenes* can survive and grow in green table olives. *L. monocytogenes* was found in one of the commercial (thermally treated) samples analyzed and in all samples older than 2 months, irrespective of olive cultivar, lactic acid bacteria starter used, pH and titratable acidity of brine samples, or treatment applied.

The olive is the major fermented vegetable in western countries *(12).* The world production of green fermented olives is 1,180,000 metric tons (t) a year *(5),* with 290,000 t produced in the EU, mainly in the Mediterranean area. Traditionally, the production of green table olives is a spontaneous lactic acid fermentation based on an empirical process that relies on microorganisms present in the raw material and processing environment *(13).* Fermented green olives have an extended shelf life (between 1 and 2 years) and are consumed without cooking. Consuming food products without cooking makes the contamination from pathogens a public health concern, even for fermented products, in which many pathogens are able to survive *(6, 33).* Recently, the survival of *Escherichia coli* O157:H7 on green table olives was shown *(39),* but to our knowledge, no study has been reported on the natural occurrence of *Listeria monocytogenes* in olives.

The microorganism is firmly established as an important foodborne pathogen, with outbreaks and sporadic cases of listeriosis linked to a wide variety of contaminated foods of both animal and vegetable origin *(4, 34). L. monocytogenes* infections have been associated with fruits and vegetables such as celery and tomatoes *(20)* and cabbage, cucumbers, potatoes, and radishes *(17).* The microorganism can tolerate high acidity *(1, 2, 10, 35)* and survives longer under adverse environmental conditions than many other non–spore-forming bacteria of food safety concern. It can grow in foods with relatively low moisture content, with high salt concentration (up to 10% NaCl) and at refrigeration temperatures, unlike most foodborne pathogens *(43).* The usual sources of *L. monocytogenes* contamination are irrigation or wash waters, fertilizers of animal waste, infected operators, and operation of facilities with poor sanitation *(43).* A recent draft published by the U.S. Food and Drug Administration, U.S. Department of Agriculture (USDA), assessed the public health risk from *L. monocytogenes.* The study indicated that vegetables (lettuce, tomatoes, endive, carrots, cabbage, asparagus, broccoli, and cauliflower) were able to support *L. monocytogenes* growth *(9).* Thus, a degree of risk from green table olives could be present, even if no documented outbreaks of listeriosis have yet been directly attributed to their consumption.

The goal of this study was to evaluate the safety level of green olives fermented with lactic acid bacteria (LAB) by investigating *L. monocytogenes* occurrence at different storage times (up to 18 months).

MATERIALS AND METHODS

Samples. Samples of green table olives were experimentally prepared by the Spanish-style process with cv. Nocellara dell'Etna olives and by the biological method for cv. Cassanese and cv. Nocellara del Belice olives. All samples were obtained traditionally, without heat treatment.

For the Spanish-style process, fresh olives were treated with about a 2% (wt/vol) NaOH solution for 16 h, washed twice with tap water, and rinsed with water added to lactic acid. Next, the olives were brined in an 8% (wt/vol) NaCl solution and inoculated with *Lactobacillus plantarum* DSM 20174 and *Lactobacillus casei* wild strain (T19), the last strain previously isolated from naturally fermented green olives *(27).* The strains were added to brine olives to obtain a final concentration of about 10^7 CFU/ml of brine. Finally, the treated olives were divided into equal portions containing 10 olives (corresponding at about 70 g) dipped into 300 ml of brine in traditional 500-ml glass containers. The olives were kept at room temperature, and a single sample was taken for physicochemical and microbiological analyses.

For the biological method, fresh olives were calibrated, washed in potable tap water, and brined in 5-liter glass containers

* Author for correspondence. Tel: +39 095 7580218; Fax: +39 095 7141960; E-mail: ccaggia@unict.it; c.caggia@tiscali.it.

with 5% (wt/vol) NaCl sterilized solution that in 2 months was gradually upgraded to 7%. The olive/brine ratio was 1.0 to 1.5:1. After 3 days brining at room temperature, cv. Cassanese olives were inoculated with *L. plantarum* CIP 104442; a trial of cv. Nocellara del Belice olives was inoculated with *L. plantarum* DSM 20205; another trial with *L. plantarum* CIP 104442 and a third trial with a mix of *L. plantarum* DSM 20205 and *Lactobacillus pentosus* DSM 20314 (0.5% + 0.5%, wt/vol) was conducted. The LAB strains were inoculated to obtain a final concentration of 10^8 CFU/ml of brine. The samples were kept at room temperature, and pH was periodically measured and corrected by adding lactic acid solution to a final value of about 4.0.

In addition, 10 samples of thermally treated green table olives in cans, made by different producers, were purchased at supermarket and analyzed.

Physicochemical analyses of raw olives and olive brines during fermentation. Physicochemical characteristics of fermentation were monitored. Titratable acidity and pH of fermented brines of each trial were determined as described previously *(11)*. Chloride concentration was determined by titration with $AgNO_3$ and the Mohr method and total acidity by titration with NaOH. Reducing sugars were determined by the Fehling method.

Phenolic compounds were determined spectrophotometrically (on a Perkin Elmer λ2 spectrophotometer, Perkin Elmer, Wellesley, Mass.) with the use of Folin-Ciocalteu's reagent (Carlo Erba Reagents, Milan, Italy), and absorption of solution was measured at 725 nm. The results were expressed as milligrams per liter of gallic acid.

Polyphenols were extracted from olives following the method reported by Amiot and others *(3, 38)* and analyzed by high-performance liquid chromatography. The chromatographic system comprised a Waters 625 LC system, UV Waters 486 detector, and Nova-Pak C18 reversed-phase column (4 μm, 3.9 by 300 mm; Waters, Millford, Mass.). The eluates were detected at 280 nm, the mobile phase was 2% acetic acid in water and methanol, and the flow rate was 0.6 ml/min.

Microbiological analyses. The fermentation was monitored by enumeration of different microbial populations onto the following selective media: De Man Rogosa and Sharpe (CM359, Oxoid, Basingstoke, UK) with cycloheximide (Sigma, St. Louis, Mo.) at a final concentration of 0.05% and incubated at 32°C for 4 days in anaerobic conditions (LAB count); plate count agar (CM325, Oxoid) incubated at 32°C for 48 h (mesophilic viable count); and Sabouraud dextrose agar (CM41, Oxoid), with added chloramphenicol incubated at 25°C for 48 h (mold and yeast count). The analyses were done in triplicate, and the plates were subjected to microbiological enumeration (CFU per milliliter).

Isolation of *Listeria* spp. A total of 69 samples (34 biological, 25 Spanish style, and 10 thermally treated) were analyzed for *Listeria* spp. presence at different times during storage. Research was done from the 56th storage day until the 18th month of storage. The samples were analyzed according to the USDA method *(25)*. Brine (25 ml) was added with 225 ml of *Listeria* enrichment broth base (CM863, Oxoid) previously sterilized with added supplement (SR141E, Oxoid). The sample was blended in a Stomacher (BagMixer, Interscience, Saint Nom La Breteche, France) for 2 min, and the bag was closed and incubated at 30°C. After 4, 24, and 48 h and 7 days, 0.1-ml aliquots (or 10 μl for more highly contaminated samples) were selected from primary enrichment cultures and used for plate enumeration in *Listeria* selective agar base (CM856, Oxoid) with previously added supplement (SR140E, Oxoid) and incubated at 37°C for 24 h. Black colonies on selective agar base plates were considered presumptive *Listeria* colonies.

Identification of *Listeria* spp. From the selective agar base, 150 colonies were recovered and streaked on tryptic soy agar medium (CM129, Oxoid) containing 0.6% yeast extract (L21, Oxoid) and 1.7% bacteriological agar. The plates were incubated at 37°C for 24 h. Presumptive *Listeria* isolates were confirmed and identified at the genus level on the basis of Gram stain catalase reaction, as described previously *(36, 37)*. Among the 150 strains studied, 115 showed typical nonpigmented colonies (1 to 2 mm diameter) with a characteristic caramel or sour buttery smell that were gram positive, short, rod shaped, and catalase positive. In order to identify the isolates, the presumptive *Listeria* were tested for sugar fermentation *(15)*, tumbling motility *(18)*, hemolytic reaction *(37)*, and growth at different salt concentrations (up to a final concentration of 20%). The sugar utilization test was carried in purple broth base (peptone, 10 g/liter; NaCl, 5 g/liter; phenol red, 4 mg/liter) at pH 7.6. Sugars (D-glucose, D-mannitol, L-rhamnose, D-xylose) were filter sterilized and added to the broth to give a final concentration of 1% (wt/vol). Tubes were incubated at 37°C for 48 h, and gas formation and acidification were recorded. The tumbling motility was detected microscopically on microbial suspensions of strains 24 h old after growth in brain heart infusion broth (CM225, Oxoid) with 0.5% bacteriological agar. Motility at 25°C and the loss of motility at 37°C was considered a distinctive response *(19)*. In order to distinguish between *L. monocytogenes*, *Listeria seeligeri*, and *Listeria ivanovii* and *Listeria innocua*, *Listeria welshimeri*, and *Listeria grayi*, all cultures were grown on tryptic soy agar layered with about 5 ml of blood agar base (CM55, Oxoid) plus 5% sheep blood (SR0051, Oxoid). The plates were incubated at 37°C for 24 h, and the hemolytic reaction was identified. The presumptive *Listeria* isolates also were tested for the ability to grow in nutrient agar (1810, Biolife) supplemented with different NaCl concentrations (8, 10, 12, 15, 28, and 20%). The plates were incubated at 37°C for 48 h. The isolates showing β-hemolytic reaction, motility at 37 but not 25°C, and production of acid from glucose was identified by the API *Listeria* identification kit (BioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions.

Enumeration of *L. monocytogenes* by the most probable number method. The enumeration of *L. monocytogenes* was carried out by the procedure cited in Italian legislation *(14)*. The most-probable-number (MPN) three-tube test procedure was followed. Brine was diluted in Ringer solution, and the dilution was transferred to enrichment broth with supplement (SR 141E, Oxoid). Tubes were incubated for 48 h at 37°C. After 24 h, 10 μl of suspension from each tube was transferred into a plate of selective agar base incubated at 37°C for 24 h.

The MPN count was determined with the MPN values showed in Table 1 *(9)*. Tubes were considered positive for growth on the selective medium if colonies showed appropriate gram and catalase reactions, β-hemolytic reaction, and motility at 25 but not 37°C.

RESULTS AND DISCUSSION

Fermentation process assessment. Table 2 shows the physicochemical characteristics of raw olives and pH, titratable acidity, and percent NaCl values determined in brine samples during the fermentation.

The LAB counts showed a constant trend, reaching the highest value on the 60th day of fermentation for cv. Nocellara dell'Etna and cv. Cassanese (Figs. 1 and 2) and on

TABLE 1. *The MPN counts and 95% confidence intervals (CIs) for three tubes, each with 0.10, 0.01, and 0.001 g inocula (appendix table 11 (9))*

No. of positive tubes			MPN	CI		No. of positive tubes			MPN	CI	
0.10	0.01	0.001	(cells/g)	Low	High	0.10	0.01	0.001	(cells/g)	Low	High
0	0	0	<3.0	—	9.5	2	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3.0	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1,000
2	0	2	20	4.5	42	3	3	0	240	42	1,000
2	1	0	15	3.7	42	3	3	1	460	90	2,000
2	1	1	20	4.5	42	3	3	2	1,100	180	4,100
2	1	2	27	8.7	94	3	3	3	>1,100	420	—

the 45th day for cv. Nocellara del Belice (Fig. 3). These results are evidence of the success of the fermentation processes. The lower LAB value, together with higher mesophilic viable count and yeast values in cv. Nocellara dell'Etna olives, could be a result of the brine composition, high pH, alkali treatment, higher salt concentration, and higher polyphenol content of this cultivar (Table 2), restrictive for LAB growth *(26, 29–31).* The mesophilic viable count values in olives fermented by the biological method were generally lower (Figs. 2 and 3) for all periods, probably because of adjustments in pH by adding lactic acid (Table 2). The high number of yeast and LAB during fermentation is in agreement with results reported in the literature *(24, 32, 39).*

Occurrence of *L. monocytogenes* in green table olives. The genus *Listeria* comprises six species: *L. monocytogenes, L. innocua, L. ivanovii, L. welshimeri, L. seeli-*

TABLE 2. *Physicochemical characteristics of raw olives and brine samples*

	Physicochemical characteristics of raw olives			Physicochemical characteristics of brine during fermentation at time that *L. monocytogenes* was found				
Olive cultivar	Reducing sugars (%)	Titratable acidity (g of lactic acid/100 g of fruit flesh)	Polyphenol content (ppm gallic acid)	Brining period	Starter	pH	Titratable acidity (g of lactic acid/100 ml of brine)	NaCl (%)
Spanish style								
Nocellara dell'Etna	3.29	0.920	7,266	55 days	*L. plantarum* DSM 20174 + *L. casei* T9	4.18	0.282	6.2
				63 days		4.21	0.326	7.5
				70 days		4.23	0.333	7.2
				2.5 mo		4.28	0.334	7.0
				3 mo		4.44	0.357	7.0
Biological method								
Cassanese	2.96	0.606	6,187	5 mo	*L. plantarum* CIP 104442	3.76	0.450	6.88
					No starter	3.97	0.423	6.74
Nocellara del Belice	3.00	0.374	6,183	17 mo	*L. plantarum* DSM 2020	3.95	0.342	6.88
					L. plantarum + *L. pentosus*	4.34	0.207	6.87
				18 mo	*L. plantarum* CIP 104442	5.75	0.126	6.85
					No starter	4.07	0.297	7.00

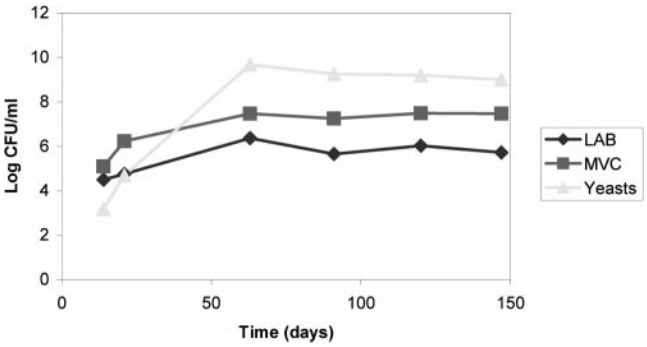

FIGURE 1. *Enumeration of microbial groups during fermentation in green table olives (cv. Nocellara dell'Etna) obtained by Spanish-style method. The values are reported as mean value for the three replicate experiments. (LAB, lactic acid bacteria; MCV, mesophilic viable count).*

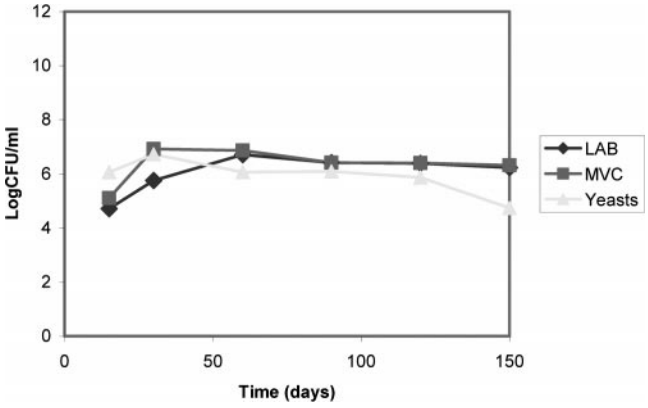

FIGURE 2. *Enumeration of microbial groups during fermentation in green table olives (cv. Cassanese) obtained by biological method. The values are reported as mean value for the three replicate experiments. (LAB, lactic acid bacteria; MCV, mesophilic viable count).*

geri, and *L. grayi (28).* Only *L. monocytogenes* is considered pathogenic to humans, although the presence of any *Listeria* species in food can be an indicator of poor hygiene. Results of the isolation and identification of presumptive *Listeria* at different storage times from different kinds of green table olives are reported in Table 3. *L. monocytogenes* was isolated from samples belonging to the three cultivars and fermented with different LAB starters. *L. monocytogenes* was isolated from all samples older than 2 months and from 1 of 10 thermally treated samples.

All presumptive *Listeria* strains isolated from table olives at 5, 17, and 18 months of storage were identified as *L. monocytogenes.* From cv. Cassanese and cv. Nocellara del Belice samples, 34 presumptive *Listeria* were isolated, and 12 of them, chosen randomly, were identified as *L. monocytogenes.* Among the 14 presumptive colonies isolated from cv. Nocellara dell'Etna samples at 55 and 63 days of storage, 3 were chosen randomly and subjected to further tests. Two isolates did not correspond to any *Listeria* species, whereas one was identified as *L. innocua.* Among the seven presumptive *Listeria* isolates from cv. Nocellara dell'Etna at 70 days of storage, three strains were identified as *L. monocytogenes;* one was identified as *L. innocua,* and the remaining were not *Listeria* species.

TABLE 3. *Occurrence of* Listeria monocytogenes *in green table olives*

No. of samples analyzed	Olive cultivar	Storage time	Starter	No. of presumptive *Listeria* strains		
				Isolated	Identified	Identified as *L. monocytogenes*
Spanish style						
5	Nocellara dell'Etna	55 days	*L. plantarum* DSM 20174 + *L. casei* T9	4	2	None
5	Nocellara dell'Etna	63 days	*L. plantarum* DSM 20174 + *L. casei* T9	10	1	None
5	Nocellara dell'Etna	70 days	*L. plantarum* DSM 20174 + *L. casei* T9	27	7	3
5	Nocellara dell'Etna	2.5 mo	*L. plantarum* DSM 20174 + *L. casei* T9	18	6	5
5	Nocellara dell'Etna	3 mo	*L. plantarum* DSM 20174 + *L. casei* T9	19	4	2
Biological method						
6	Cassanese	5 mo	*L. plantarum* CIP 104442	6	2	2
2	Cassanese	5 mo	No starter	2	2	2
8	Nocellara del Belice	17 mo	*L. plantarum* DSM 20205	8	2	2
5	Nocellara del Belice	18 mo	*L. plantarum* + *L. pentosus*	5	3	3
6	Nocellara del Belice	18 mo	*L. plantarum* CIP 104442	6	2	2
7	Nocellara del Belice	18 mo	No starter	7	1	1
Commercial samples						
10	—	—	—	12[a]	4	4

[a] All from the same sample.

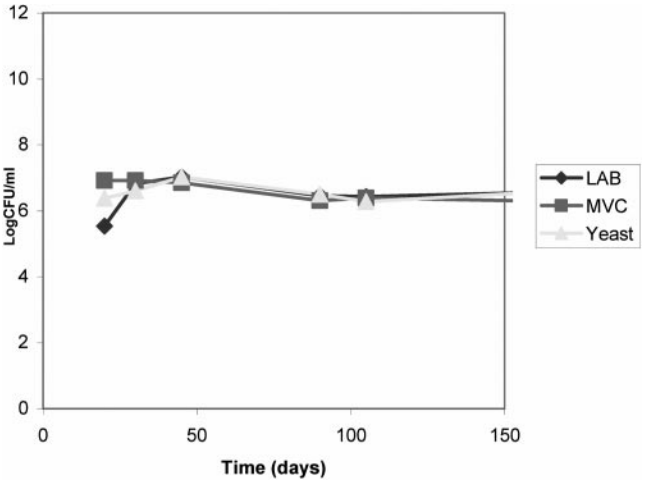

FIGURE 3. *Enumeration of microbial groups during fermentation in green table olives (cv. Nocellara del Belice) obtained by biological method. The values are reported as mean value for the three replicate experiments. (LAB, lactic acid bacteria; MCV, mesophilic viable count).*

The highest number of *L. monocytogenes* strains was isolated from 2.5-month-old cv. Nocellara dell'Etna. From this sample, 18 presumptive colonies were obtained and 6 were studied; 5 were *L. monocytogenes* and 1 was not identified. From the same olive samples after 3 months, among the 19 presumptive *Listeria*, 2 were identified as *L. monocytogenes* and 2 isolates were not identified. Among the 10 commercial thermally treated samples, only one showed typical presumptive *Listeria* colonies in selective agar plate. Twelve presumptive colonies were purified and four were selected and identified as *L. monocytogenes.*

Enumeration of *L. monocytogenes*. Results, obtained from cv. Nocellara dell'Etna at 7 months of storage through the MPN method, averaged 93 cells per ml up to >1,000 cells per ml (Table 4). The thermally treated sample had MPN < 3 cells per ml.

Even though *L. monocytogenes* is a microorganism of public health concern, the minimum infective dose has not been established reliably. Published information on the number of *L. monocytogenes* in foods during outbreaks indicates that levels between 10^3/g and 10^4 per gram or milliliter are responsible for illness *(7, 23).* Recently, the International Commission on Microbiological Specifications for Food (ICMSF) has proposed a preventive scheme for managing microbial risk in foods, introducing the concept of the Food Safety Objective *(44)* that, regarding *L. monocytogenes* in ready-to-eat food, has been proposed by Szabo et al. *(40)* as all measures able to control the pathogen number lower than 100 CFU per gram or milliliter of product at consuming time.

Traditionally, researchers, regulators, and quality assurance specialists have been interested in determining the presence or absence of *Listeria* and *L. monocytogenes* in food products *(8, 16, 42).* However in recent years in many countries, zero tolerance already has been replaced by the natural occurrence tolerance; for this reason, the quantitative recovery and enumeration of *L. monocytogenes* has become of more and more interest. The MPN procedure is suggested by the U.S. Food and Drug Administration *(41),* and it traditionally has been used for determining this bacterium in food highly contaminated with other organisms, especially when a low number of *Listeria* are present (MPN ≤ 10 cells per g) *(8).* However, there is no specific MPN technique available. The Italian legislation *(14)* suggests the MPN technique for food products intended to be consumed after cooking (except milk and dairy products).

TABLE 4. *Enumeration by the MPN method of* Listeria monocytogenes *in green olive brine after 7 months of storage*

Sample analyzed	MPN/ml	95% confidence limit	
		Low	High
Nocellara dell'Etna	>1,000	420	—
Nocellara dell'Etna	150	37	420
Nocellara dell'Etna	93	18	420
Commercial sample	<3	—	9.5

In this study, we assayed the occurrence of *L. monocytogenes* in green table olives, demonstrating that the product can support *Listeria* survival, despite its low pH, low a_w, and high salt concentration, and that sufficient heat treatment must be applied to achieve a reduction in the *L. monocytogenes* count. Unfortunately, there is no international agreement on acceptable levels of *L. monocytogenes* in foods, and for risk assessment, several authors have pointed out that an increase of 2 log units constitutes a risk, even if, under normal conditions, the concentration in the raw food is very low. According to ICMSF *(22),* for people who do not have increased susceptibility, the presence of fewer than 100 CFU/g of *L. monocytogenes* does not constitute a risk. This criterion (<100 CFU/g) is considered more scientifically supportable and practical than zero tolerance *(21).*

The observations made in this work recommend the application of an appropriate heat treatment in order to guarantee the safety of such a popular food product. Furthermore, industry and regulatory agencies should identify process control and process verification systems to prevent recontamination of green table olives.

ACKNOWLEDGMENT

This work was supported by the Italian government (MIUR) through project no. 2002078159-005 (2002).

REFERENCES

1. Ahamad, N., and E. H. Marth. 1989. Behavior of *Listeria monocytogenes* at 7, 13, 21 and 35 °C in tryptose broth acidified with acetic, citric, or lactic acid. *J. Food Prot.* 52:688–695.
2. Ahamad, N., and E. H. Marth. 1990. Acid-injury of *Listeria monocytogenes. J. Food Prot.* 53:26–29.
3. Amiot, M. J., A. Fleuriette, and J. J. Macheix. 1986. Importance and evolution of phenolic compounds in olive during growth and maturation. *J. Agric. Food Chem.* 3:823–826.
4. Anonymous. 1995. Microbiology of food and animal stuffs. Horizontal method for the detection and enumeration of *L. monocytogenes.* Draft International Standard ISO/DIS 11290. ISO, Switzerland.

5. Anonymous. 1999. El mercado internacional de las aceitunas de mesa. *Olivae* 79:27–29.
6. Brown, M. H., and I. R. Booth. 1991. Acidulants and low pH, p. 22–43. *In* N. J. Russell and G. W. Gould (ed.), Food preservatives. Blackie Academic Professional, London.
7. Buchanan, R. L., W. G. Damert, R. C. Whiting, and M. Van Schothorst. 1997. Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for *Listeria monocytogenes* levels and incidence of listeriosis. *J. Food Microbiol.* 60:918–922.
8. Capita, R., and C. Alonso-Calleja. 2003. Comparison of different most-probable number methods for enumeration of *Listeria* in poultry. *J. Food Prot.* 66:65–71.
9. Department of Health and Human Services, U.S. Food and Drug Administration, and Center for Food Safety and Applied Nutrition. 2001. Bacteriological analytical manual online. Appendix 2. Available at: http://vm.cfsan.fda.gov. Accessed 10 June 2002.
10. Farber, J. M., and F. Pagotto. 1992. The effect of acid shock on the heat resistance of *Listeria monocytogenes. Lett. Appl. Microbiol.* 15: 197–201.
11. Fernàndez Díez, M. J., R. de Castro Ramos, A. Garrido Fernàndez, F. Gonzàlez Cancho, F. Gonzàlez Pellissò, M. Nosti Vega, A. Heredia Moreno, M. I. Mìiguez Mosquera, L. Rejano Novarro, M. C. Duràn Quintana, F. Sànchez Roldàn, P. Garcìa Garcìa, and A. de Castro. 1985. Unidad estructural de investigación de Química, Microbiología y tecnología de aceitunas de mesa y otros productos vegetales, p. 51–123. *In* Consejo Superior de Investigaciones CIENTIFICAS (CSIC; ed.), Biotecnologìa de las Aceituna de Mesa. CSIC, Madrid.
12. Garrido-Fernàndez, A. 1997. Effect of processing conditions on lactic acid bacteria growth in table olive fermentations. Acetes Colloq. LACTIC 97 lactic acid bacteria, p. 277–316.
13. Garrido Fernández, A., P. Garcia Garcia, and M. Brenes Balbuena. 1995. Olive fermentation, p. 593–627. *In* H. J. Rhem and G. Reed (ed.), Biotechnology: enzymes, biomass, food and feed. VCH, New York.
14. Gazzetta Ufficiale n. 291 del 13 dicembre 1993. Ordinanza Ministeriale dell'11 ottobre 1978. Numerazione della *Listeria monocytogenes* mediante la tecnica dell'MPN in alimenti sfusi o preconfezionati destinati per loro natura ad essere consumati previa cottura o che rechino sulla confezione la dizione ''da consumarsi previa cottura'' (esclusi il latte e i derivati del latte).
15. Genigeorgis, C. A., D. Dutulescu, and J. Fernández-Garayábal. 1989. Prevalence of *Listeria* spp. in poultry meat at the supermarket slaughterhouse level. *J. Food Prot.* 52:618–624.
16. Gohil, V. S., M. A. Ahmed, R. Davies, and R. K. Robinson. 1995. The direct enumeration of *Listeria monocytogenes* in a food with a low background microflora. *Food Control* 6:365–369.
17. Heisick, J. E., D. E. Wagner, M. L. Nierman, and J. T. Peeler. 1989. *Listeria* spp. found on fresh market produce. *Appl. Environ. Microbiol.* 55:1925–1927.
18. Hitchins, A. D. 1998. FDA, p. 141–160. *In* G. J. Jackson (ed.), Bacteriological analytical manual, 7th ed. AOAC International, Arlington, Va.
19. Hitchins, A. D. 2001. *Listeria monocytogenes*, chap. 10. *In* FDA bacteriological manual on line. Available at: http://www.cfsan.fda.gov. Accessed 26 September 2002.
20. Ho, J. L., K. N. Shandes, G. Friedland, P. Ecking, and D. W. Fraser. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. *Arch. Int. Med.* 146: 520–524.
21. Huss, H. H., L. V. Jørgensen, and B. F. Vogel. 2000. Control options for *Listeria monocytogenes* in seafoods. *Int. J. Food Microbiol.* 62: 267–274.
22. International Commission on Microbial Specifications for Foods (ICMSF). 1994. Choice of sampling plan and criteria for *Listeria monocytogenes. Int. J. Food Microbiol.* 22:89–96.
23. Lorber, B. 1997. Listeriosis. *Clin. Infect. Dis.* 24:1–11.
24. Marsilio, V., B. Lanza, M. De Angelis, and N. Martinelli. 1995. Le tecnologie di trasformazione delle olive da tavola: il sistema sivigliano. *Riv. Frutticol.* 12:23–31.
25. McClain, D., and W. Lee. 1988. Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. *J. Assoc. Off. Anal. Chem.* 71:660–664.
26. Özay, G., and M. Borkakli. 1996. Effect of brine replacement and salt concentration on the fermentation of naturally black olives. *Food Res. Int.* 6:553–559.
27. Randazzo, C. L., C. Restuccia, A. D. Romano, and C. Caggia. 2003. *Lactobacillus casei*, dominant species in naturally fermented Sicilian green olives. *Int. J. Food Microbiol.* 2801:1–6.
28. Rocourt, J., and P. Cossart. 1997. *Listeria monocytogenes*, p. 337–352. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montiville (ed.), Food microbiology fundamentals and frontiers. ASM Press, Washington, D.C.
29. Rodriguez de la Borbolla y Alcalà, J. M., and L. Rejiano Navarro. 1979. Sobre la preparaciòn de las aceitunas estilo sevillano. La fermentation I. *Grasas Aceites* 30:175–185.
30. Rodriguez de la Borbolla y Alcalà, J. M., and L. Rejiano Navarro. 1981. Sobre la preparaciòn de las aceitunas estilo sevillano. La fermentation II. *Grasas Aceites* 30:103–113.
31. Ruiz-Barba, J. L., M. Brenes-Balbuena, R. Jmènez-Diaz, P. Garcìa-Garcìa, and A. Garrido-Fernandez. 1993. Inhibition of *Lactobacillus plantarum* by polyphenols extracted from two different kinds of olive brine. *J. Appl. Bacteriol.* 74:15–19.
32. Ruiz-Barba, J. L., and R. Jmènez-Diaz. 1995. Availability of essential B-group vitamins to *Lactobacillus plantarum* in green olive fermentation brines. *Appl. Environ. Microbiol.* 61:1294–1297.
33. Russel, J. B., and F. Diez-Gonzalez. 1998. The effects of fermentation acids on bacterial growth. Adv. Microbiol. Physiol. 39:205–234.
34. Ryser, E. T. 1999. Food borne listeriosis, p. 299–358. *In* E. T. Ryser, and E. H. Marth (ed.), *Listeria*, listeriosis and food safety. Marvel Dekker, New York.
35. Schik, W. van, C. G. M. Gahan, and C. Hill. 1999. Acid-adapted *Listeria monocytogenes* displays enhanced tolerance against the antibiotics nisin and lacticin 3147. *J. Food Prot.* 62:536–539.
36. Seeliger, H. P. R., and K. Höhne. 1979. Serotyping of *Listeria monocytogenes* and related species, p. 31–49. *In* T. Bergan and J. R. Norris (ed.), Methods in microbiology. Academic Press, London.
37. Seeliger, H. P. R., and D. Jones. 1986. Genus *Listeria*, p. 1235–1245. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharp, and J. G. Holt, (ed.), Bergey's manual of systematic bacteriology, vol. 2. Williams and Wilkins, Baltimore, Md.
38. Servili, M., M. Baldioli, R. Selvaggini, M. Macchioni, and G. Montedoro. 1999. Phenolic compounds of olive fruit: one and two dimensional nuclear magnetic resonance characterization of nüzhenide and its distribution in the constitutive part of fruits. *J. Agric. Food Chem.* 47:80–85.
39. Spyropoulou, K. E., N. G. Chorianopoulos, P. N. Skandamis, and G. J. E. Nychas. 2001. Survival of *Escherichia coli* O157:H7 during the fermentation of Spanish-style green table olives (conservolea variety) supplemented with different carbon sources. *Int. J. Food Microbiol.* 66:3–11.
40. Szabo, E. A., L. Simons, M. J. Coventry, and M. B. Cole. 2003. Assessment of control measures to achieve a food safety objective of less than 100 CFU of *Listeria monocytogenes* per gram at the point of consumption for fresh precut iceberg lettuce. *J. Food Prot.* 66:256–264.
41. U.S. Food and Drug Administration. 1992. FDA bacteriological analytical manual, 7th ed. AOAC International, Washington, D.C.
42. U.S. Food and Drug Administration. 1998. Fish and fishery products, hazard and critical control guide. Available at: http://vm.cfsan.fda.gov/~dms/haccp-2.html. Accessed 18 June 2002.
43. U.S. Food and Drug Administration, U.S. Department of Agriculture, and Centers for Disease Control and Prevention. 1998. Guidance for industry: guide to minimize microbial food safety hazards for fresh fruit and vegetables. Available at: http://vm.cfsan.fda.gov/~dms/prodguid.html. Accessed 26 October 1998.
44. Van Schothorst, M. 1998. Principles for the establishment of microbiological food safety objectives and related control measures. *Food Control* 9:379–385.