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Short communication

Distribution and behavior of *Listeria monocytogenes* in three lots of naturally-contaminated vacuum-packed smoked salmon stored at 2 and 10°C

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Abstract

The incidence, the number and the behavior of *L. monocytogenes* in three lots of naturally-contaminated vacuum-packed sliced smoked salmon, processed in different plants, were investigated during storage at 2 and 10°C. *L. monocytogenes* was isolated from 20 of the 100 packages stored at 2°C (16 from lot 1, 1 from lot 2 and 3 from lot 3) and from 12 of the 65 packages stored at 10°C (all from lot 1). The levels detected were 15, 20, 290, 1100 and >1100 cfu/g in 5 packages, all belonging to lot 1, and <10 cfu/g in the remaining samples. The high incidence of *L. monocytogenes* in lot 1 was assumed to be due mainly to the use of casual workers for the slicing and packing operations. © 1997 Elsevier Science B.V.

Keywords: Listeria monocytogenes; Smoked salmon; Storage; Temperature

1. Introduction

Listeria monocytogenes has been isolated often from smoked salmon. The overall incidence is approx. 10% (Guyer and Jemmi, 1990; Rørvik and Yndestad, 1991; Jemmi, 1993; Rørvik et al., 1995), although in some investigations the organism was found at higher rates (Farber, 1991) or not found at all (Hartemink and Georgsson, 1991; Valenti et al., 1991). Studies concerning the concentration of *L. monocytogenes* in smoked salmon have shown that the levels of the organism are generally low, i.e., less than 100 cfu/g (Jemmi, 1993) or less than 1 cfu/g (Guyer and Jemmi, 1990).

In inoculated smoked salmon, multiplication occurs during storage at 4, 5,and 10°C, (Farber, 1991; Guyer and Jemmi, 1991; Rørvik et al., 1991; Hudson and Mott, 1993), even with an inoculum of less than 6 cfu/g (Rørvik et al., 1991). In naturally contaminated foods, growth may follow different patterns. The aim of this study was to investigate the incidence, the number and the behavior of *L. monocytogenes* in three lots of naturally-contaminated vacuum-packed sliced smoked salmon processed in different plants and stored at 2 and 10°C.

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2. Materials and methods

Three lots of sliced cold-smoked salmon, packed under vacuum in 100 g portions, were provided by three different establishments, all meeting the requirements of Directive 91/493/EEC. The samples of lot 1 (55 packages) were taken from the store (2°C) of a processing plant one day after packaging; those of lot 2 (60 packages) were obtained from a wholesale warehouse (2–4°C) 6 days after packaging; those of lot 3 (50 packages) were taken from the store (2°C) of a processing plant 20 days after packaging. The shelf life indicated by the three producers was 60 days at 4°C. All samples were transported under refrigeration to our laboratory where they were immediately distributed in two refrigerators, as indicated in Table 1, and stored at 2 and 10°C until examination. The 165 packages (100 at 2°C and 65 at 10°C) were examined, at 10 d intervals, for the presence and number of *L. monocytogenes*, water activity, pH and sensory quality.

For the detection of *L. monocytogenes*, 25 g samples were homogogenized for 2–3′ in 225 ml of primary *Listeria* Enrichment Broth Base (LEB1) (CM863B and supplement, SR142E, Oxoid, UK) in a stomacher Lab-Blender 400 (pbi international, Milano, Italy). After incubation at 30°C for 24 h, 1 ml was transferred to secondary *Listeria* Enrichment Broth (LEB2) (CM863B and supplement, SR143E, Oxoid, UK) which was incubated at 30°C for 24 h. Isolation followed on Palcam agar (CM877B and supplement, SR150E, Oxoid, UK) incubated at 37°C for 24–48 h under microaerophilic conditions (BBL GasPack 150 Anaerobic System and Gas Generating Kits, BR 056 A, Oxoid, UK). Portions (1 ml) of broth from LEB1 and LEB2 were diluted in 4.5 ml of a sterile solution containing (w/v) 0.25% KOH and 2% NaCl and spread-plated onto Palcam agar, which was then incubated as previously described (Method A).

For the enumeration of *L. monocytogenes*, a three tube MPN technique (Gazzetta Ufficiale della Repubblica Italiana n. 291, 13/12/1993) was used. Ten gram samples were homogenized as described above in 90 ml of buffered peptone water. For each dilution, (10^{-1}, 10^{-2} and 10^{-3}) 1 ml portions were added to each of three tubes containing 9 ml of Tryptone Soya Broth with 6% yeast extract (CM129B and LP021B, Oxoid, UK). The tubes were incubated at 32°C for 18–20 h and then transferred to *Listeria* Fraser Broth Base containing the Fraser selective supplement (CM895B and SR156E, Oxoid, UK). Tubes showing blackening after 24–48 h at 32°C were streaked onto *Listeria* Selective Agar Base (Oxford) containing the *Listeria* selective supplement (Oxford) (CM856B and SR140E, Oxoid, UK) and then incubated at 37°C for 24–48 h (Method B).

At least five suspect colonies from both Palcam and Oxford plates were tested for Gram staining, catalase and beta-hemolysis and then identified using API-*Listeria* (cod. 10 300 bioMérieux, Firenze, Italy).

The a_w was estimated at room temperature using a Rotronic-Hygroscop DT instrument (pbi international, Milano, Italy) following the instructions of the manufacturer. Determinations of pH were performed using pH-indicator strips (Merck) inserted between

Table 1
Distribution and sampling plan of 165 packages of vacuum-packed smoked salmon stored at 2 and 10°C

Lot no.	Temp. (C°)	Time (days) after packaging									Total no. of packages
		1	10	20	30	40	50	60	70	80	
1*	2	5**	5	5	5	5	5	5			35
	10		5	5	5	5					20
2*	2		5	5	5	5	5	5			30
	10		5	5	5	5	5	5			30
3*	2			5	5	5	5	5	5	5	35
	10					5	5	5			15
Total		5	20	25	25	30	25	25	5	5	165

* Before distribution at 2 and 10°C, samples from lot 1 were stored 1 d at 2°C at the processing plant; samples from lot 2 were stored 6 d at 2–4°C at a wholesale warehouse; samples from lot 3 were stored 20 d at 2°C at the processing plant.

** Numbers represent total numbers of packages.

two slices of salmon at different places in the package. The sensory qualities (colour, odour and taste) were evaluated by a panel of six people.

A sample pooled from each group of 5 packages was used for the enumeration of aerobic bacteria, by using Plate Count Agar (Difco Laboratories, Detroit, Michigan, USA), and the lactobacilli, by using MRS Broth (Difco) containing 1.5% agar. Both media were incubated 2 d at 37°C.

3. Results and discussion

With method A, *L. monocytogenes* was isolated from 11 packages (11%) stored at 2°C and from 9 packages (13.8%) stored at 10°C. With method B, 12 packages (12%) stored at 2°C and 6 (9,2%) stored at 10°C were positive and the levels of the organism were >1100 cfu/g in one package stored at 2°C, 15, 20, 290 and 1100 cfu/g in four packages stored at 10°C, all belonging to lot 1, and <10 cfu/g in the remaining samples. In 12 packages (9 at 2°C and 3 at 10°C) *L. monocytogenes* was detected only by using method B. Therefore, a total of 32 packages, i.e., 20 packages (20%) stored at 2°C (16 from lot 1, 1 from lot 2 and 3 from lot 3) and 12 packages (18.4%) stored at 10°C (all from lot 1) were found to contain *L. monocytogenes* (Table 2).

The a_w values did not show any variation during storage. The mean values ($\pm$SD) for samples stored at 2 and 10°C respectively were 0.96 ($\pm$0.005) and 0.97 ($\pm$0.005) for lot 1; 0.95 ($\pm$0.002) and 0.95 ($\pm$0.004) for lot 2; 0.96 ($\pm$0.009) and 0.96 ($\pm$0.007) for lot 3.

A pH-value of around 6 was obtained in most of the samples (results not shown). Lower values, of about pH 5,8, were more often observed in the samples stored at 2°C after day 40. Higher values, up to pH 7, were noted in lot 1 samples stored at 10°C, together with noticeable changes of the sensory qualities, on day 40.

The sensory qualities remained excellent or good until day 40 for salmon stored at 2°C. On day 50 a slight sour smell, which later became more remarkable or unpleasant, was observed in some packages. At 10°C, the samples from lots 1 and 2 were judged acceptable until day 20. Salmon from lot 3, which had been stored at 2°C for 20 days at the processing plant before being put at 2 and 10°C in our laboratory, showed noticeable changes of the sensory qualities on day 40. A sour, sharp or unpleasant smell was noted in some packages, and subsequently in all samples. At both temperatures, at the end of the storage period, slices sometimes showed a shiny or greasy surface, but modifications of the colour and presence of juice or gas inside the packages were never observed.

The results of this study confirm the presence of *L. monocytogenes* in vacuum-packed smoked salmon. The microorganism was isolated from 19.3% of the samples but this rather high incidence resulted from the use of two different methods. If one considers the number of packages found positive with only one of the two techniques, the percentage positives are 9.2% and 13.8% and agree with those more often reported in the literature.

The incidence rates at the two storage temperatures did not differ markedly, being 20% at 2°C and 18.4% at 10°C. Large differences, however, were observed among the three lots. *L. monocytogenes* was isolated from 50.9% of lot 1 samples but only from 1.6% and 6% of the packages from lot 2 and 3. The results obtained may indicate an uneven distribution of the organism. In fact, in 12 packages, *L. monocytogenes* was isolated only with method B, i.e., the method with the smaller sample size.

In most packages the number of *L. monocytogenes* was <10 cfu/g. It is worth noting that higher levels of the organism were observed on day 60 in one package stored at 2°C and on days 30 and 40 in four packages stored at 10°C. These results point out that it is advisable, however, to apply low storage temperatures and to limit the shelf-life of this product, even if the sensory qualities may remain acceptable for rather long times.

If multiplication occurred it seemed to take place only following longer storage periods or higher storage temperature. The behavior of *L. monocytogenes* in naturally-contaminated smoked salmon appears, therefore, to be different from that shown in inoculated samples, where growth can be influenced by the type and the number of the strains used, the preparation of the cultures, the level of the inoculum and the associated microflora (Guyer and Jemmi, 1991; Rørvik et al., 1991). In low level inoculated samples Rørvik et al. (1991) found that *L. monocytogenes* grew faster in salmon of better hygienic quality. In our study samples with the highest levels

Table 2
Presence and levels of *L. monocytogenes* in vacuum-packed smoked salmon stored at 2 and 10°C

Time	Lot	2°C								10°C						
(days) after packaging	no.	*L. monocytogenes*						Aer. bac.[a] cfu/g	Lactob.[b] cfu/g	*L. monocytogenes*					Aer. bac.[a] cfu/g	Lactob.[b] cfu/g
1	1[c]	25g	pos.	pos.	neg.	neg.	pos.	$1.0x10^4$	$1.0x10^1$							
		MPN/g	—*	—	—	—	—									
10	1	25g	pos.	neg.	pos.	pos.	pos.	$4.3x10^4$	$2.6x10^5$	neg.	pos.	neg.	pos.	neg.	$1.0x10^5$	$2.6x10^6$
		MPN/g	—	7	—	4	—			—	4	—	—	—		
	2[d]	25g	neg.	neg.	neg.	neg.	neg.	$7.8x10^4$	$3.4x10^5$	neg.	neg.	neg.	neg.	neg.	$4.0x10^4$	$2.2x10^3$
		MPN/g	—	4	—	—	—			—	—	—	—	—		
20	1	25g	neg.	neg.	neg.	neg.	neg.	$2.6x10^6$	$3.3x10^7$	neg.	pos.	neg.	neg.	neg.	$2.2x10^5$	$2.6x10^8$
		MPN/g	—	—	—	—	—			—	—	—	—	—		
	2	25g	neg.	neg.	neg.	neg.	neg.	$4.6x10^5$	$1.1x10^6$	neg.	neg.	neg.	neg.	neg.	$3.8x10^6$	$1.8x10^4$
		MPN/g	—	—	—	—	—			—	—	—	—	—		
	3[e]	25g	neg.	neg.	neg.	neg.	neg.	n.e.°	n.e.							
		MPN/g	—	—	—	—	—									
30	1	25g	neg.	neg.	neg.	pos.	neg.	$1.4x10^8$	$2.0x10^8$	pos.	pos.	pos.	pos.	neg.	$5.6x10^8$	$6.9x10^8$
		MPN/g	—	—	—	4	—			—	—	15	7	—		
	2	25g	neg.	neg.	neg.	neg.	neg.	$5.5x10^6$	$4.7x10^6$	neg.	neg.	neg.	neg.	neg.	$4.3x10^7$	$4.5x10^5$
		MPN/g	—	—	—	—	—			—	—	—	—	—		
	3	25g	neg.	neg.	neg.	neg.	neg.	$1.2x10^5$	$2.4x10^2$							
		MPN/g	—	—	—	—	—									
40	1	25g	neg.	neg.	neg.	neg.	neg.	$6.0x10^8$	$4.0x10^8$	neg.	pos.	neg.	pos.	neg.	$6.9x10^8$	$5.2\ x10^8$
		MPN/g	—	—	4	—	—			20	—	1100	—	290		
	2	25g	neg.	neg.	neg.	neg.	neg.	$2.6x10^6$	$6.2x10^6$	neg.	neg.	neg.	neg.	neg.	$3.3x10^7$	$6.5\ x10^7$
		MPN/g	—	—	—	—	—			—	—	—	—	—		
	3	25g	neg.	pos.	pos.	neg.	neg.	$8.1x10^6$	$5.0x10^6$	neg.	neg.	neg.	neg.	neg.	$5.1x10^7$	$2.0\ x10^7$
		MPN/g	—	—	—	—	—			—	—	—	—	—		

50	1	25g	neg.	neg.	neg.	neg.	neg.	$8.9x10^6$	$4.0x10^8$						
		MPN/g	—	9	4	4	—								
	2	25g	neg.	neg.	neg.	neg.	neg.	$2.9x10^6$	$1.7x10^6$	neg.	neg.	neg.	neg.	neg.	$5.0x10^7$	$3.5\ x10^7$
		MPN/g	—	—	—	—	—			—	—	—	—	—		
	3	25g	neg.	neg.	neg.	neg.	neg.	$1.1x10^7$	$2.9x10^7$	neg.	neg.	neg.	neg.	neg.	$9.5x10^7$	$4.9\ x10^7$
		MPN/g	—	—	—	—	—			—	—	—	—	—		
60	1	25g	pos.	neg.	neg.	neg.	neg.	$2.4x10^7$	$2.7x10^8$							
		MPN/g	4	7	—	>1100	—									
	2	25g	neg.	neg.	neg.	neg.	neg.	$4.1x10^6$	$3.3x10^7$	neg.	neg.	neg.	neg.	neg.	$8.9x10^8$	$6.6x10^7$
		MPN/g	—	—	—	—	—			—	—	—	—	—		
	3	25g	neg.	neg.	neg.	neg.	neg.	$7.8x10^7$	$5.9x10^7$	neg.	neg.	neg.	neg.	neg.	$2.1x10^8$	$1.6x10^8$
		MPN/g	—	—	—	—	—			—	—	—	—	—		
70	3	25g	neg.	neg.	neg.	neg.	neg.	$4.8x10^9$	$3.2x10^7$							
		MPN/g	—	—	—	—	—									
80	3	25g	neg.	neg.	neg.	neg.	neg.	$3.0x10^9$	$2.6x10^9$							
		MPN/g	6	—	—	—	—									

* not detected; ° not examined.

[a]Plate Count Agar, 37°C, 2 d.

[b]MRS agar, 37°C, 2 d.

[c]Samples were stored 1 d at 2°C at the processing plant and then distributed at 2 and 10°C.

[d]Samples were stored 6 d at 2–4°C at a wholesale warehouse and then distributed at 2 and 10°C.

[e]Samples were stored 20 d at 2°C at the processing plant and then distributed at 2 and 10°C.

of *L. monocytogenes* showed also high numbers of aerobic bacteria and lactobacilli (Table 2).

Different target levels have been established in many countries for ready-to-eat foods like smoked salmon, the target being generally the absence of *L. monocytogenes* in a 25 g sample (Jemmi, 1993; Huss et al., 1995). The minimum infective dose is not known and most aspects concerning the virulence of *L. monocytogenes* are unknown. The potential of low concentrations of *L. monocytogenes* to cause illness cannot be ignored, although there is little evidence that low numbers of *L. monocytogenes* in food will cause listeriosis, even in susceptible individuals (Harwig et al., 1991).

The absence of *L. monocytogenes* is difficult to attain in samples of ready-to-eat products which do not receive adequate lethal treatments or which may have been subsequently recontaminated. In the conclusions of the panel meeting held at the 10th International Congress on Listeriosis (Anonymous, 1989) it was recognized that it is impossible to avoid occasional low levels of *L. monocytogenes* in raw foods but it is also emphasized that application of hygienic measures during production and preparation can markedly reduce the numbers of *L. monocytogenes* in seafoods and that a risk analysis followed by design of GMP's is essential to control food-transmitted listeriosis. The examination of lots of smoked salmon from three different producers showed how different the incidence and the numbers of *L. monocytogenes* in foods processed in a similar way, but under different conditions, can be. Several authors have studied the sources and routes of *L. monocytogenes* contamination in smoked fish processing plants (Guyer and Jemmi, 1990; Eklund et al., 1995; Rørvik et al., 1995). The high level of contamination seen in lot 1 was assumed to be due mainly to the use of casual workers for the slicing and packing operations, caused by an increased processing activity. Further research has been planned to verify the sources of contamination, with a view to a better planning of production activity throughout the whole year.

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