

ENVIRONMENTAL PREVALENCE AND PERSISTENCE OF LISTERIA MONOCYTOGENES IN COLD-SMOKED TROUT PROCESSING PLANTS

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The presence of Listeria monocytogenes on the surfaces of equipment and worker's hands during different production stages, as well as on fish skin and meat during processing and storage of cold-smoked trout, was investigated. Listeria monocytogenes was recovered from 10 (6.06%) of a total 165 cotton-swabbed samples collected from the surfaces of equipment and worker's hands at two separate processing facilities. Of 105 samples collected from fish skin and meat during various production steps in both processing plants, 14 (13.33%) were confirmed culture-positive for L. monocytogenes, with recovery being most frequently in samples collected in the area before vacuum packaging. Recovery rates at two different Serbian processing plants did not differ ($p < 0.05$), but suggested that different L. monocytogenes serotypes appeared to be resident within each processing plant and may have contributed to the final product contamination. From all smoked trout samples collected during 7, 14, 21 and 28 days of storage at 4°C only two were culture-positive for L. monocytogenes serotype 1/2a and both of these were collected on the 7th day of storage. Conversely, 4, 3 and 1 sample(s) were contaminated with L. monocytogenes serotypes 1/2a and 1/2b after 7, 14 and 21 days storage at 10°C. Listeria monocytogenes was not recovered from smoked trout stored 28 days at 10°C. Results emphasize the importance of adhering to strict hygienic and quality control standards throughout the processing environment.

Key words: cold-smoked trout, Listeria monocytogenes, storage

INTRODUCTION

Listeria monocytogenes is an invasive food-borne pathogen capable of causing serious illness in immune compromised individuals and pregnant women (Gahan and Hill, 2005). In pregnant women, the infection may spread to the fetus thereby causing abortion or birth of a child with septicaemia (Aureli et al., 2000;

Crum, 2002; Frye *et al.*, 2002; Sim *et al.*, 2002). In the United States, about 2500 people experience serious illness due to *Listeria* infections and about 500 individuals die from the disease (CDC, 2009). In the European Union, 1439 cases of listeriosis were reported in the European Union during 2005 and recent infections rates are estimated at 0.3 cases per 100 000 individuals (Denny and McLauchlin, 2008; Nørrung and Buncic, 2008). *Listeria monocytogenes* has been isolated from many ready-to-eat seafood products, hot and cold-smoked fish, including trout (Brett *et al.*, 1998; Miettinen *et al.*, 1999; Farber *et al.*, 2000). The smoked salmon industry has been expanding constantly in the world and import regulations of some countries are very strict. For instance, absence of *L. monocytogenes* in a 25 g sample is required to comply with USA regulations (Klontz *et al.*, 2008). *Listeria monocytogenes* has been isolated from estuaries and seafood processing plants (Lundén *et al.*, 2000; Fønnesbech Vogel *et al.*, 2001) and since processing practices in the cold-smoked fish industry do not necessarily inactivate *L. monocytogenes* (Rørwick, 2000), risks of contamination exist during processing. Additionally, while vacuum packaging of the above mentioned products does help extend product shelf life, this may also provide extended opportunity for *L. monocytogenes* to grow which would further risk the safety of these foods that are usually consumed without further heating (Giménez and Dalgaard, 2004). The aim of this study was to evaluate the incidence of *L. monocytogenes* at two Serbian fish processing plants during various stages of fish processing and vacuum packaging of cold-smoked trout. Knowledge gained from these investigations may ultimately aid the implementation of necessary measures to prevent the production and distribution of contaminated cold-smoked trout, as RTE (Ready to eat) food.

MATERIALS AND METHODS

Sampling

Samples were collected from two fish processing plants over three visits conducted during a single year (March, June and October 2007). Sterile cotton swabs, moistened with 1.0% peptone water, were used to sample the surfaces of sedation pools, tables for sedated fish acceptance, evisceration stations, hands of the workers who performed the evisceration, salting dishes, draining tables, fish-smoking stations, filleting tables, hands of the workers at the filleting station, sorting tables and hands of the workers who performed the sorting. During each production cycle, each area was sampled three times. Samples of fish skin and meat ($n = 3$ fish each) were taken immediately after fish sedation, evisceration, salting, draining, smoking, cooling and filleting as well as after sorting, i.e. just before packing. Fillets were vacuum packaged, 150 g per package, and random samples were stored for 28 days at 4°C or 10°C. Samples of raw fish, beginning at the throat were taken from the collar and belly flap area of fresh, frozen or fish fillets. Finished product samples were taken from vacuum packaged cold-smoked fish. Environmental swabs were transported in 2.0 mL of Maximum Recovery Diluent (MRD) containing 0.85% NaCl. All other samples were transported in sterile stomacher bags (Seward Ltd., London, UK) to the laboratory

on ice. In the laboratory, all samples were processed within 24 hours after collection.

Bacteriological analysis

Listeria monocytogenes strains were isolated according to the following protocol based on the ISO 11290-1:1996/Amd.1:2004 (E) standard method (ISO, 1996, 2004). Twenty-five-gram portions of raw, in-processed fish sample were homogenized in 225 mL of primary enrichment medium (Half Fraser broth, Oxoid, Basingstoke, UK) using a Stomacher 400 (Colworth, London, UK) for 2 min and then incubated 24 h at 30°C. Subsequently, 0.1 mL of broth was inoculated into a secondary enrichment medium (full-strength secondary liquid enrichment medium–Fraser Broth) and incubated for an additional 24-48 h at 35° to 37°C. After secondary enrichment, 0.1 mL of broth was streaked separately onto *Listeria* selective ALOA-Agar (Ottaviani *et al.*, 1997) and PALCAM Agar (Oxoid) and incubated for 24-48 h at 37°C. Colonies grown on ALOA or PALCAM agars were selected based on morphology and cultural criteria. The haemolytic activity of recovered strains was checked twice; after 24 and 48 h incubation on blood agar plates at 37°C.

All strains were identified biochemically using API-Listeria (BioMerieux, La Balme-les-Grottes, France) according to manufacturer's instructions using cultures from the agar plates. Prior to subsequent analyses all strains were subcultured 37°C for 24 h on Brain-Heart Infusion (BHI, Oxoid) agar. They were kept on BHI agar slants (2%) at 4°C before testing.

Serotyping of all strains of *L. monocytogenes* has been performed by slide agglutination tests for "O" and "H" antigens using standard commercially available *Listeria* Antisera products (Denka Seiken Co., Ltd., Tokyo, Japan).

Physicochemical and chemical analysis

Chemical composition of samples was analysed at day 0 of storage according to standard methods. Total salt content was determined using Volhard method (ISO, 1996). Salt content in the water phase (SWP) was calculated from corresponding total salt and water contents, using the equation: $SWP = \% \text{ salt} * 100 / (\% \text{ salt} + \% \text{ water})$. Water activity (a_w value) was calculated from the corresponding SWP value, using the equation: $a_w = 1 - 0.0052471 * SWP - 0.00012206 * SWP^2$, (Gimenéz and Dalgaard, 2004). In order to check the pH value in trout meat that originated from both groups kept at the two separate storage temperature regimes, samples were collected and measured at 0 and after 3 days of storage. The pH was measured using a 3310 WTW model 340i pH Meter.

Statistical analysis

Prevalence measurements were analyzed for potential differences due to sample origination, processing facility and storage temperatures using a Fisher's exact test appropriate for small sample sizes (Statistix9 Analytical Software, Tallahassee, FL, USA).

RESULTS

Listeria monocytogenes was recovered from a total of 7 of 99 (7.07%) and 3 of 66 (4.54%) swab samples collected from environmental surfaces and workers' hands at the first and second cold-smoked trout processing plants, respectively, and recovery rates did not differ ($p > 0.05$). At the first processing plant, *L. monocytogenes* was recovered from swabs collected at the evisceration area (2 samples), the filleting (2 samples) and sorting tables (2 samples) and from a swab (1 sample) of a worker's hands at the sorting table (Table 1). *Listeria monocytogenes* 1/2a was the predominant serotype recovered from the first processing plant although 2 of the 7 *L. monocytogenes* recovered were serotype 1/2b and both of these were recovered from the sorting environment (Table 1).

Table 1. Prevalence of *L. monocytogenes* in environmental samples collected at first processing plant

Sampling site	Samples from work stations and workers' hands							
	First visit		Second visit		Third visit		All visits	
	n	Lm	n	Lm	n	Lm	n	Lm
Sedation pools	3	0	3	0	3	0	9	0
Sedated fish acceptance tables	3	0	3	0	3	0	9	0
Evisceration station	3	1 ^a	3	1 ^a	3	0	9	2
Hands of evisceration workers	3	0	3	0	3	0	9	0
Salting dish station	3	0	3	0	3	0	9	0
Draining tables (after salting)	3	0	3	0	3	0	9	0
Fish smoking station	3	0	3	0	3	0	9	0
Hands of filleting workers	3	0	3	0	3	0	9	0
Filleting tables	3	0	3	1 ^a	3	1 ^a	9	2
Sorting tables	3	0	3	1 ^a	3	1 ^b	9	2
Hands of sorting workers	3	0	3	0	3	1 ^b	9	1
Total	33	1	33	3	33	3	99	7

n – number of samples analysed; Lm – *L. monocytogenes* culture-positive. Recovery rates, as analyzed by Fischer's Exact test did not differ ($p > 0.05$). ^a– serotype 1/2a; ^b– serotype 1/2b

At the second production plant, *L. monocytogenes* serotype 1/2b was recovered from swabs collected from one sample obtained from the hands of a worker in the evisceration area and from one sample from the hands of a worker in the sorting area (Table 2).

The only other *L. monocytogenes* isolate obtained from this processing plant was serotype 1/2a which was recovered from the filleting table (Table 2).

Table 2. Prevalence of *L. monocytogenes* in environmental samples collected at second processing plant

Sampling site	Samples from work stations and workers' hands							
	First visit		Second visit		Third visit		All visits	
	n	Lm	n	Lm	n	Lm	n	Lm
Sedation pools	3	0	3	0	-	-	6	0
Sedated fish acceptance tables	3	0	3	0	-	-	6	0
Evisceration station	3	0	3	0	-	-	6	0
Hands of evisceration workers	3	0	3	1 ^b	-	-	6	1
Salting dish station	3	0	3	0	-	-	6	0
Draining tables (after salting)	3	0	3	0	-	-	6	0
Fish smoking station	3	0	3	0	-	-	6	0
Hands of filleting workers	3	0	3	0	-	-	6	0
Filleting tables	3	1 ^a	3	0	-	-	6	1
Sorting tables	3	0	3	0	-	-	6	0
Hands of sorting workers	3	0	3	1 ^b	-	-	6	1
Total	33	1	33	2	-	-	66	3

n – number of samples analysed; Lm – *L. monocytogenes* culture-positive. Recovery rates, as analyzed by Fischer's Exact test did not differ ($p < 0.05$). ^a– serotype 1/2a; ^b– serotype 1/2b

Listeria monocytogenes was recovered from a total of 9 of 63 (14.28%) and 5 of 42 (11.90%) fish skin and meat samples collected at various production and processing phases from the two separate processing plants, but again, recovery rates did not differ ($p > 0.05$). In the first production plant, *L. monocytogenes* was isolated from samples taken after evisceration (3 samples), draining (2 samples) and during filleting and sorting (4 samples) (Table 3). Serotype 1/2a was predominant in the first processing plant, being recovered from 7 of the 9 culture-positive samples and as observed with the environmental samples, the 2 serotype 1/2b isolates were recovered from fish skin and meat sampled from the filleting and sorting area (Table 3).

In the second production plant, serotype 1/2b was the predominant *L. monocytogenes* recovered, being isolated from one sample taken at the draining table, one sample after smoking and 2 of the 3 samples collected after filleting and assortment (but before vacuum packing), with the remaining isolate being identified as a serotype 1/2a strain (Table 4).

Upon examination of finished vacuum packaged cold-smoked trout produced at the first processing facility, *L. monocytogenes* was recovered from 1 (2.77%) of the 36 samples that had been stored at 4°C and from 3 (8.33%) of the 36 samples stored at 10°C (Table 5).

These recovery rates did not differ ($p > 0.05$). The single isolate recovered from the product stored at 4°C and 2 of the isolates recovered from the product stored at 10°C were obtained from samples stored for 7 days, the remaining

isolate recovered from the product stored at 10°C had been stored for 14 days. All 4 isolates obtained from the product that had been produced at the first processing facility were identified as serotype 1/2a.

Table 3. Prevalence of *L. monocytogenes* on the fish skin and meat at first sampled processing plant

Sampling site	Samples of fish skin and meat							
	First visit		Second visit		Third visit		All visits	
	n	Lm	n	Lm	n	Lm	n	Lm
Sedation pools	3	0	3	0	3	0	9	0
Evisceration station	3	1 ^a	3	1 ^a	3	1 ^a	9	3
Salting dish station	3	0	3	0	3	0	9	0
Draining tables (after salting)	3	1 ^a	3	0	3	1 ^a	9	2
Fish smoking station	3	0	3	0	3	0	9	0
Cooling station	3	0	3	0	3	0	9	0
Filleting and sorting tables	3	0	3	2 ^{a,a}	3	2 ^{b,b}	9	4
Total	21	2	21	3	21	4	63	9

n – number of samples analysed; Lm – *L. monocytogenes* culture-positive. Recovery rates, as analyzed by Fischer's Exact test did not differ ($p > 0.05$). ^a– serotype 1/2a; ^b– serotype 1/2b

Table 4. Prevalence of *L. monocytogenes* on the fish skin and meat at second sampled processing plant

Sampling site	Samples of fish skin and meat							
	First visit		Second visit		Third visit		All visits	
	n	Lm	n	Lm	n	Lm	n	Lm
Sedation pools	3	0	3	0	-	-	6	0
Evisceration station	3	0	3	0	-	-	6	0
Salting dish station	3	0	3	0	-	-	6	0
Draining tables (after salting)	3	1 ^b	3	0	-	-	6	1
Fish smoking station	3	1 ^b	3	0	-	-	6	1
Cooling station	3	0	3	0	-	-	6	0
Filleting and sorting tables	3	2 ^{a,b}	3	1 ^b	-	-	6	3
Total	21	4	21	1	-	-	42	5

n – number of samples analysed; Lm – *L. monocytogenes* culture-positive. Recovery rates, as analyzed by Fischer's Exact test did not differ ($p > 0.05$). ^a– Serotype 1/2a; ^b– serotype 1/2b

For vacuum packaged cold-smoked trout that was produced at the second processing facility, a *L. monocytogenes* identified as serotype 1/2a was recovered from 1 (4.17%) of the 24 samples that had been stored at 4°C, again from a sample stored for 7 days (Table 6).

Table 5. Prevalence of *L. monocytogenes* in vacuum packaged cold-smoked trout produced in the first processing facility; effect of storage at 4° or 10°C

Days of storage	Visit number															
	First			Second			Third			Combined						
	n	Lm	n	Lm	n	Lm	n	Lm	n	Lm	n	Lm				
	Storage temperature															
	4°C						10°C									
7th day	3	1 ^a	3	0	3	0	3	1 ^a	3	3	1 ^a	3	0	9	2	
14th day	3	0	3	0	3	0	3	0	3	0	3	0	3	1 ^a	9	1
21st day	3	0	3	0	3	0	3	0	3	0	3	0	3	0	9	0
28th day	3	0	3	0	3	0	3	0	3	0	3	0	3	0	9	0
Total	12	1	12	0	12	0	36	1	12	1	12	1	12	1	36	3

n – Number of samples analysed; Lm – *L. monocytogenes* culture-positive. Recovery rates, as analyzed by Fischer's Exact test did not differ ($p > 0.05$). ^a – serotype 1/2a

Table 6. Prevalence of *L. monocytogenes* in vacuum packaged cold-smoked trout produced in the second processing facility; effect of storage at 4° or 10°C

Days of storage	Visit number														
	First			Second			Third			Combined					
	n	Lm	n	Lm	n	Lm	n	Lm	n	Lm	n	Lm			
	Storage temperature														
	4°C						10°C								
7th day	3	1 ^a	3	0	6	1	3	1 ^b	3	3	1 ^b	3	1 ^b	6	2
14th day	3	0	3	0	6	0	3	1 ^b	3	3	1 ^b	3	1 ^b	6	2
21st day	3	0	3	0	6	0	3	1 ^b	3	3	0	3	0	6	1
28th day	3	0	3	0	6	0	3	0	3	3	0	3	0	6	0
Total	12	1	12	0	24	1	12	3	12	3	2	12	2	24	5

n – number of samples analysed; Lm – *L. monocytogenes* culture-positive. Recovery rates, as analyzed by Fischer's Exact test did not differ ($p > 0.05$). ^a – serotype 1/2a; ^b – serotype 1/2b

For the product stored at 10°C; however, *L. monocytogenes* was recovered from a total of 5 (20.83%) of the 24 samples; 2 that had been stored for 7 days, 2 that had been stored for 14 days and 1 that had been stored for 21 days (Table 6). Recovery rates for these products produced at this second facility again did not differ ($P > 0.05$) when stored at different temperatures or from the rates observed with products produced and tested from the first processing facility. All strains recovered from vacuum packaged cold-smoked trout produced at the second processing facility and stored at 10°C were identified as serotype 1/2b.

Analysis of the vacuum-smoked trout fillets samples revealed that the salt content in the water phase of the tested product was 4.58 ± 1.42 and within the range of that typically used in brine solutions (Gram, 2001). The a_w value was 0.98. The average pH value of product tested at the beginning of the storage period was 6.07. After three days of storage, the pH in product that had been stored at 4° or 10°C was 5.96 and 5.79, respectively.

DISCUSSION

Listeria monocytogenes is a foodborne pathogen of particular importance to ready-to-eat foods such as cold-smoked fish. Current safety regulations necessitate very strict hygiene during processing and packaging. Results from the present study revealed that despite a high level of hygiene and biosecurity at two separate Serbian fish processing facilities, *L. monocytogenes* was present in between 7 of 99 (7.07%) and 3 of 66 (4.54%) of tested environmental samples such as the surfaces of processing equipment and the hands of the workers. Moreover, *L. monocytogenes* was present on 9 of 63 (14.28%) and 5 of 42 (11.90%) of tested skin and fish meat samples collected during processing at the first and second processing facility, respectively. These prevalence rates, however, were not altogether different than those reported in other trout processing facilities, which ranged from 0 to approximately 11% for comparable fish meat and skin samples and 0 to 30% for comparable environmental samples (Autio *et al.*, 1999). Whereas *L. monocytogenes* was recovered in the present study slightly more frequently from samples from the first processing plant than from the second plant, the proportions were not significantly different ($p > 0.05$). This finding is not surprising as we had no reason to expect the similarly managed plants to yield substantially different results. Nevertheless, *L. monocytogenes* was recovered earlier in the processing environment in the first processing plant than in the second plant, being recovered from fish skin and meat, as well as from environmental swabs collected at the evisceration station, and this may have affected recovery rates in subsequent samplings. The prevalence of *L. monocytogenes* can vary considerably in trout that have been raised at different locations and can be influenced by weather conditions and other factors that may enhance flow of *Listeria*-laden agricultural runoff into rearing ponds (Miettinen and Wirtanen, 2005; 2006). Whether or not such factors may have introduced incoming fish carrying *L. monocytogenes* to the first processing plant is indiscernible from the present data set as we were provided with no information pertaining to the trout prior to their arrival at the processing facilities. Other

scientists have suggested that *L. monocytogenes* strains sequestered within biofilms associated with uneven contact surfaces may be the most important factors for product contamination (Norton *et al.*, 2001; Lundén *et al.*, 2002; 2003). These "in house" *L. monocytogenes* biotypes, those resident to specific processing environments, are thus more generally thought to be the main contributors to contamination during processing of fresh catfish (Chen *et al.*, 2010) and smoked salmon and sea trout (Fonnesbech Vogel *et al.*, 2001; Medrala *et al.*, 2003). Likewise, Autio and colleagues (1999) found <2% prevalence of *L. monocytogenes* in raw trout entering a cold-smoked processing facility and found that recovery rates from fish clearly rose after the brining (salting) stage, due at least in part to the use of re-circulated *L. monocytogenes*-contaminated brine solution. Brine solutions containing 3.5 to 5% salt in the water phase are ineffective in killing halotolerant *L. monocytogenes* (Gram, 2001). Whereas we did not recover *L. monocytogenes* from the salting area or from fish meat or skin collected from the salting area, we did recover *L. monocytogenes* soon afterwards, whether from fish meat and skin samples at the draining tables, smoking station (plant two only) or from fish at the filleting and sorting tables. This latter observation suggests that manipulative mechanisms associated with filleting and sorting may have promoted transfer of the bacterium from contaminated surfaces to the fish product. Sikorski and Kalodziejska (2000) showed that a critical point, as far as hygiene is concerned, is the manipulation of product after smoking. Others also, as reviewed by Gram (2001), have reported that the incidence of *L. monocytogenes* in processed fish increases after cold smoking. In the case of the second processing plant sampled in the present study, the recovery of *L. monocytogenes* from workers' hands at various stages of processing implicates that hands of these workers could serve as a potential transfer mechanism of *L. monocytogenes* to fish during processing.

Cold-smoked fish can be eaten as a ready-to-eat (RTE) food and as such may be stored under abusive conditions (Baltic, 2009). Some of the potential storage conditions used by consumers may support growth of *L. monocytogenes*. Additionally, during the production of cold-smoked fish, if contaminated with *L. monocytogenes*, there are no production phases that would necessarily kill this bacterium and thus growth could occur during storage and distribution. That is why such products are recommended to be kept at 4°C or less. From all of the fish samples collected during 4°C storage of vacuum packages produced at these two Serbian processing plants, only two were positive, one from each processing plant in samples collected from the product stored for 7 days. Conversely, more *L. monocytogenes* were recovered from samples of the finished product stored at 10°C. For instance, *L. monocytogenes* was recovered from 3 of 36 finished product samples that were stored at 10°C, all identified as serotype 1/2a, with one obtained from the product stored for 7 days and other from product stored for 14 days. At the second processing plant, *L. monocytogenes* was recovered from 5 out of 24 finished product samples that were stored at 10°C, with all identified as serotype 1/2b, and were recovered from the product stored up to 21 days. This latter observation suggests, but does not prove, that product contaminated with serotype 1/2b may be more sensitive to

temperature abuse than the product contaminated with serotype 1/2a. Buncic *et al.* (2001) reported earlier that upon recovery from storage at 4°C, *L. monocytogenes* serotype 1/2a took longer to grow out of lag phase during culture at 37°C than serotype 4b. It is also probable that the *L. monocytogenes* serotype 1/2b may have been endemic to the second processing plant as this serotype was recovered much earlier and more frequently from the processing environment and from fish skin and meat during processing than serotype 1/2a. Conversely, serotype 1/2a was recovered earlier and more frequently from the first processing plant than serotype 1/2b.

CONCLUSIONS

Considerable research has been done with respect to *Listeria* contamination during the production of cold-smoked salmon (Fonnesbech Vogel *et al.*, 2001; Norton *et al.*, 2001; Hoffman *et al.*, 2003; Medrala *et al.*, 2003; Lappi *et al.*, 2004; Thimothe *et al.*, 2004; Gudmundsdóttir *et al.*, 2005) but less has been reported with respect to *L. monocytogenes* contamination during processing of cold-smoked trout (Autio *et al.*, 1999). Results from the present study reveal that while *L. monocytogenes* recovery rates at two different Serbian processing plants did not differ significantly, different *L. monocytogenes* strains appeared to be resident within each processing plant and may have contributed to the final product contamination. Moreover, our results revealed that improper storage of the final product (at 10°C) served to put product at greatest risk to potential consumers. Additionally, as noted in earlier reports with cold-smoked salmon (Medrala *et al.*, 2003; Gudmundsdóttir *et al.*, 2005) and trout (Miettinen and Wirtanen, 2006), incoming fish cannot be excluded as a potential source of product contamination, particularly with the first of the two plants sampled in this report. These results emphasize the importance of adhering to strict hygienic and quality control standards throughout the processing environment.

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NALAZ I PREŽIVLJAVANJE *LISTERIA MONOCYTOGENES* U POGONIMA ZA PROIZVODNJU HLADNO DIMLJENE PASTRMKE

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SADRŽAJ

U ovom radu je ispitivan nalaz *Listeria monocytogenes* na površini opreme, rukama radnika, kao i koži i mesu ribe tokom različitih faza proizvodnje hladno dimljene pastrmke. *Listeria monocytogenes* je utvrđena kod 10 (6,06%) od ukupno 165 briseva sa površine opreme i ruku radnika iz dva proizvodna pogona. Od

105 uzoraka mesa ribe sa kožom, uzetih tokom proizvodnje u oba proizvodna pogona, 14 (13,33%) je bilo pozitivno na *L. monocytogenes*, pri čemu je nalaz i preživljavanje ove bakterije bio najčešći u uzorcima uzetih tokom proizvodnih procesa pre vakuum pakovanja. Stepem preživljavanja bakterije se nije značajno razlikovao u dva proizvodna pogona ($P > 0.05$), ali se može zaključiti da su pojedini serotipovi *L. monocytogenes* otporniji unutar pogona i mogu dovesti do kontaminacije finalnog proizvoda. Od svih uzoraka iz oba proizvodna pogona, skladištenih tokom 7, 14, 21 i 28 dana pri temperaturi od 4°C, dva su uzorka, sedmog dana skladištenja, bila kontaminirana *L. monocytogenes* serotipom 1/2a. Nasuprot tome, pri temperaturi od 10°C, nalaz *L. monocytogenes* serotipa 1/2a i 1/2b ustanovljen je kod 4 uzorka sedmog dana skladištenja, 3 uzorka četrnaestog dana i jednog uzorka tokom dvadesetprvog dana skladištenja. *Listeria monocytogenes* nije preživela u uzorcima nakon skladištenja tokom 28 dana. Rezultati ukazuju na značaj primene strogih higijenskih mera, kao i standarda kontrole kvaliteta tokom proizvodnje hladno dimljene ribe.