

Behavior of *Listeria monocytogenes* during Fabrication and Storage of Experimentally Contaminated Smoked Salmon

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Experiments were carried out to examine the behavior of *Listeria monocytogenes* in the course of fabrication and storage of smoked salmon. In three trials, raw salmon fillets were surface inoculated with *L. monocytogenes*, marinated, smoked at 26 to 30°C, and stored at 4 or 10°C for up to 30 days. At different times during the fabrication and storage, samples were taken and, by means of the three-tube most probable number (MPN) method, quantitatively analyzed for the concentration of *L. monocytogenes*. The initial *Listeria* levels in the raw fillets were 10⁴ MPN/g in trial 1, 10¹ MPN/g in trial 2, and 10² MPN/g in trial 3. During the fabrication, neither an increase nor a decrease of the inoculated quantities was observed. During the storage, however, a significant growth was measured in two of three trials; in trial 1, a 2.5 log₁₀ MPN/g increase and in trial 3, an increase of even 4.5 log₁₀ MPN/g. In the second trial, the *Listeria* level remained about the same. The results indicate the importance of preventing pre- and postprocessing contamination of *L. monocytogenes* in raw and smoked salmon. Because a significant increase of *L. monocytogenes* was measured during storage, there might be an increasing risk of infection for the consumer by storing such fish for a long time.

Listeria monocytogenes is an ubiquitous bacterium which can be found very often in food. It has been proven to be the causative agent in outbreaks of food-borne listeriosis in North America and Switzerland (2, 9, 13, 23). The lethality of these epidemics was very high (25 to 30%). Infections occurred mainly in pregnant women, their unborn children, and immunodeficient persons. The implicated foods were pasteurized milk, soft cheese, and vegetables. At present, there are no reports on epidemic outbreaks due to consumption of fish and meat products, although these products are often contaminated. In recent studies, however, a relationship between some sporadic cases of listeriosis and the consumption of contaminated meat products has been suspected or even proven (3, 5, 15, 25). *L. monocytogenes* has also been isolated from fish and seafood. Weagant et al. (29) found a contamination rate of 26% in frozen seafood products, and Caserio et al. (4) found a contamination rate of 25% in fish products. Jemmi (14) isolated *L. monocytogenes* in 24% of analyzed samples of smoked salmon. Lennon et al. (19) suggested that raw seafood may have played a part in some listeriosis cases in New Zealand. Little data are available on the incidence and behavior of *L. monocytogenes* during processing and storage of smoked fish.

In order to estimate the potential health hazard for the consumer eating contaminated salmon, the behavior of *L. monocytogenes* was studied during the fabrication and storage of artificially contaminated salmon.

MATERIALS AND METHODS

Preparation of bacterial inocula. For all experiments, *L. monocytogenes* serotype 1/2b was used. This serotype was most frequently found in fish and fish products (7, 14). In trials 1 and 2, a serological reference strain (SLCC 2755 from the Special *Listeria* Culture Collection, Würzburg, Ger-

many) was used, and in trial 3, a wild-type strain isolated from smoked salmon was used.

Inocula were prepared from cultures grown in trypticase soy broth (Becton Dickinson) for 18 h at 37°C and were diluted to provide concentrations of about 10⁴ to 10⁶ CFU/ml. Inoculum broths were enumerated by spread plating of serial (1:10) dilutions on tryptose phosphate agar plates (Becton Dickinson) incubated at 37°C for 24 h. The final concentrations in inoculum broths were 4 × 10⁶ CFU/ml in trial 1, 1.3 × 10⁴ CFU/ml in trial 2, and 2.9 × 10⁴ CFU/ml in trial 3.

Inoculation and preparation of smoked salmon. Fresh filleted salmon was obtained from a Swiss fish-smoking plant. Six fillets of about 800 g each were used per trial. The salmon fillets were laid in the respective inoculum broths for 5 min, removed, and left to dry off for 10 min at room temperature. Afterward, the fillets were laid for 24 h in a salt marinade containing 6% NaCl. Finally, the salmon was smoked at 26 to 30°C for 6 h. The finished products were stored under refrigeration—one portion at 4°C, the other at 10°C—for up to 30 days.

Sampling. At each sampling, a section of the fillet surface (4 by 4 cm) was marked and punched out. With a sterile knife, a 3-mm-thick slice weighing about 10 g was cut from this section. Duplicate samples were taken from the fresh fillets after inoculation, after marination, after smoking, and after freezing at -25°C (common in trade before slicing). Finally, the finished products were sampled after 10, 20, and 30 days of storage at 4 or 10°C.

Enumeration of *L. monocytogenes*. *L. monocytogenes* was enumerated by a three-tube most probable number (MPN) technique. The samples were macerated for 2 min in a stomacher with 90 ml of University of Vermont broth I and incubated for 24 h at 37°C (17, 20). A 0.1-ml volume of this broth was spread plated on PALCAM agar (28). The plates were incubated microaerobically for 48 h at 30°C. Colonies typical of *L. monocytogenes* were subcultured on sheep blood agar plates (5%) and confirmed by means of a Gram stain, catalase test, and beta-hemolysis.

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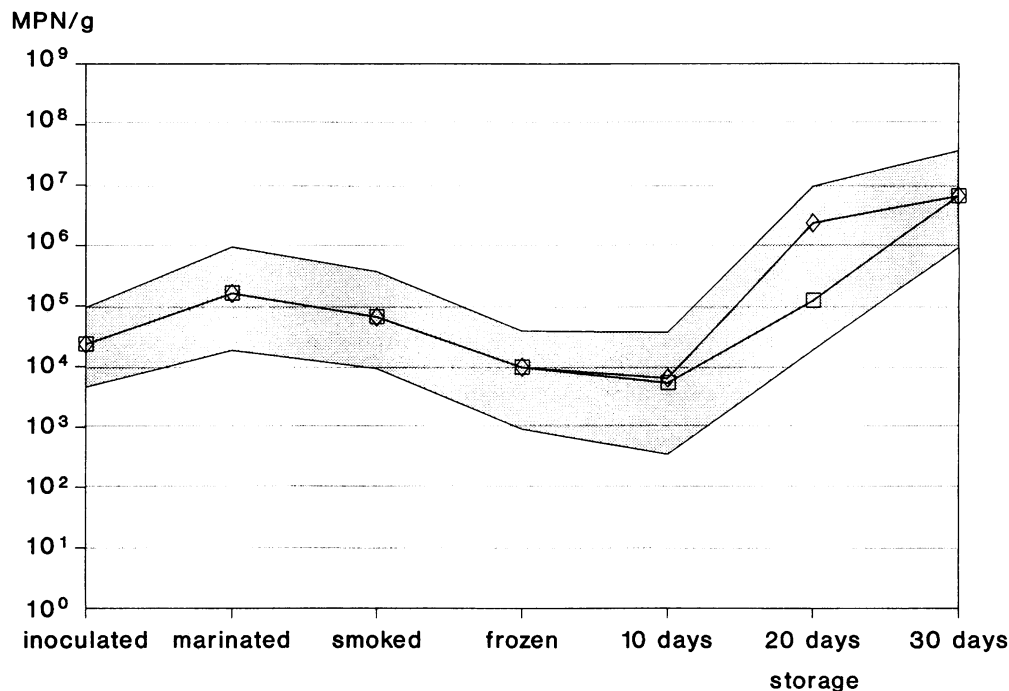


FIG. 1. Growth of *L. monocytogenes* SLCC 2755 during processing and storage of smoked salmon (trial 1). Symbols: \square , course of the *L. monocytogenes* concentration during processing; \circ , course of the *L. monocytogenes* concentration during storage at 4°C; \diamond , course of the *L. monocytogenes* concentration during storage at 10°C. Gray area, 95% confidence limits of the MPN values.

From the MPN values of the duplicate samples, the arithmetic means were calculated. The 95% confidence limits were taken from the Swiss Food Manual (8). Significant increase or decrease of the *Listeria* levels was reached when the confidence intervals of the MPN values did not intersect.

Physicochemical analyses. a_w values were determined with a hygroscope (ROTRONIC-Hygroskop DT, Zürich, Switzerland), and pH values were determined with an electronic pH meter (type 11; Wintion, Gerzensee, Switzerland).

RESULTS

Trial 1 (Fig. 1). The *Listeria* inoculum of the salmon fillets was 2.3×10^4 MPN/g. The marination and smoking had no significant effect, since the concentrations still varied between 10^4 and 10^5 MPN/g. Also, after a storage of 10 days at either 4°C or 10°C, the quantities were still at the inoculum level. After 20 days, however, a significant increase of up to 2.3×10^6 MPN/g at the 10°C storage temperature could be measured, while at 4°C, the concentration was 1.2×10^5 MPN/g. Until the end of the storage time, the concentration of *L. monocytogenes* grew slightly (up to 6.5×10^6 MPN/g) at both temperatures. In fact, in this trial, a $2.5 \log_{10}$ MPN/g increase could be observed.

Trial 2 (Fig. 2). In this trial, the inoculum was quite small (23 MPN/g). During manufacture and storage of the fillets, neither an increase nor a decrease of the *L. monocytogenes* concentrations was ascertained. The different storage temperatures had no influence on the *Listeria* concentration.

Trial 3 (Fig. 3). The inoculum amounted to 6.5×10^2 MPN/g. During marination and smoking, the concentration

stayed about the same (0.65×10^3 to 2.2×10^3 MPN/g). During storage, the *Listeria* concentration increased distinctly; at 10°C, it increased from 9×10^6 MPN/g after 10 days to 1.6×10^7 MPN/g after 30 days, and at 4°C, it increased from 3.2×10^4 MPN/g to 2.5×10^7 MPN/g. The differences between the storage temperatures after 10 and 20 days are significant; the 95% confidence intervals did not intersect. At the end of the storage, after 30 days, the concentration of *L. monocytogenes* was about 10^7 MPN/g, and no difference between the storage temperatures could be stated. In fact, in this trial a $4.5 \log_{10}$ MPN/g increase was established.

Freezing. In all three trials, freezing of the finished products had no significant effect on the *Listeria* concentration.

Physicochemical analyses. The pH and a_w values varied between 5.8 to 6.3 and 0.93 to 0.96, respectively. These physicochemical properties had no significant influence on the behavior of *L. monocytogenes*.

DISCUSSION

During processing of smoked salmon, the *L. monocytogenes* concentration remained about the same. Therefore, technological parameters seemed to have no obvious influence.

(i) **NaCl.** *Listeria* species are considered to be very resistant to high salt concentrations and can easily survive in a marinade with 6% NaCl (27).

(ii) **Smoking.** Beltran et al. (1) showed in their investigations on sardine fillets that smoking had an antibacterial effect. Studies by Messina et al. (21) showed that liquid smoke preparations, used in manufacture of frankfurters,

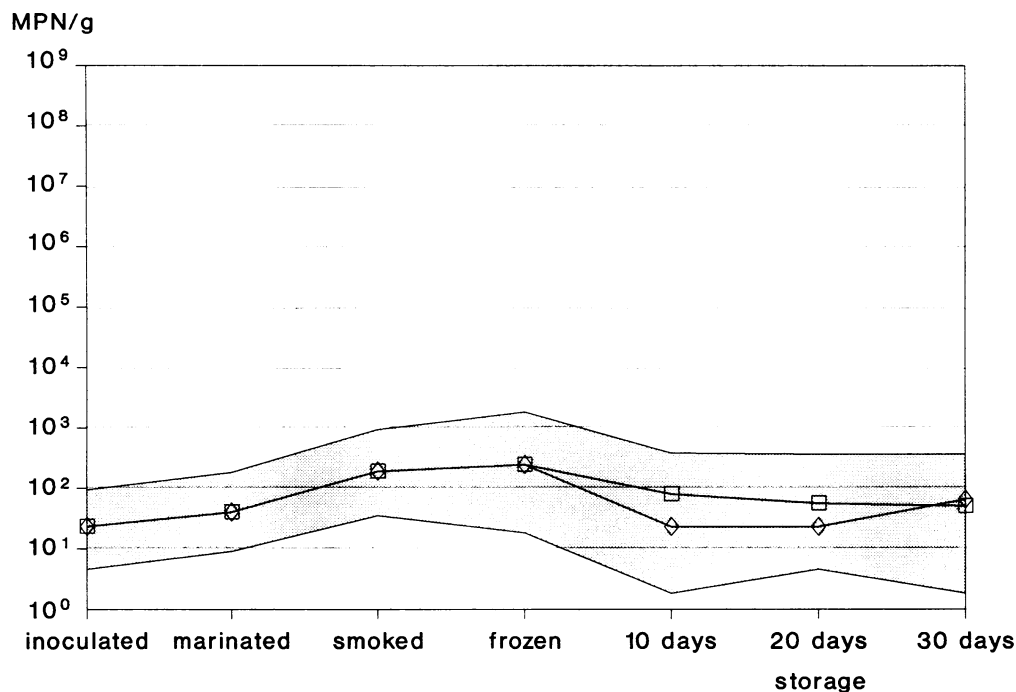


FIG. 2. Growth of *L. monocytogenes* SLCC 2755 during processing and storage of smoked salmon (trial 2). Symbols are the same as for Fig. 1.

had antimicrobial activity against *L. monocytogenes*. In the present study, these results could not be confirmed.

(iii) **Smoking temperature.** Cold smoked fishes are more frequently contaminated with *L. monocytogenes* than hot

smoked fishes (14). Smoking temperatures below 30°C (usual in the manufacture of smoked salmon) cannot eliminate *L. monocytogenes*. But obviously, the conditions during smoking prevented *L. monocytogenes* from growing.

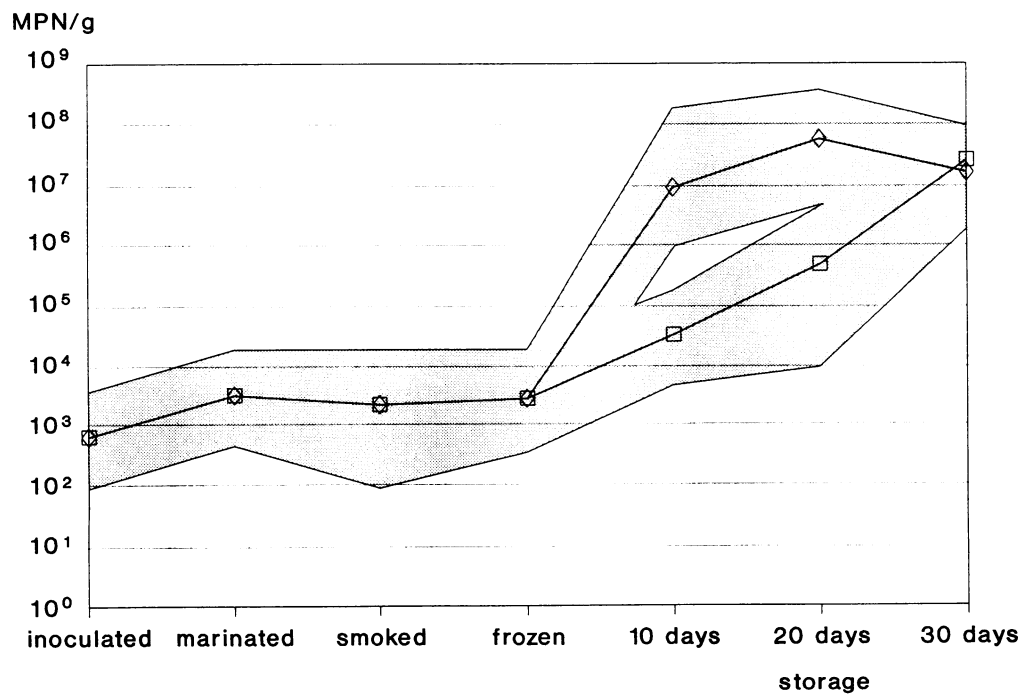


FIG. 3. Growth of *L. monocytogenes* 1/2b (wild-type strain) during processing and storage of smoked salmon (trial 3). Symbols are the same as for Fig. 1.

(iv) pH. pH values varied between 5.8 and 6.3; no influence on the *Listeria* concentration was observed. Seeliger (26) stated that *Listeria* species can multiply in a pH interval from 5.6 to 9.6, while Conner et al. (6) observed a multiplication even at a pH of 5.0.

(v) a_w . The a_w values were very stable (0.93 to 0.96) and did not prevent a multiplication of *L. monocytogenes*.

Freezing to temperatures of -25°C had no influence on the *Listeria* concentration, either. Kaya and Schmidt (16) had the same experiences with experimentally contaminated minced meat, which they stored at -18°C for more than 6 months. Bille and Glauser (2), however, noted a decrease of *L. monocytogenes* of about 10^1 to 10^2 in stored Vacherin Mont d'Or (a soft cheese implicated in the Swiss listeriosis outbreak).

During storage, a significant multiplication of *L. monocytogenes* was recorded for trials 1 and 3. These results show that refrigeration at 4 to 10°C will not prevent the growth of *L. monocytogenes* in smoked salmon. Other authors indicated that *L. monocytogenes* is able to grow also on various meat products at refrigeration temperatures (10, 24). Increases of 2.5 \log_{10} units and 4.5 \log_{10} units to values of approximately 10^7 cells per g, as observed in the present study, are very important and can be compared with those found in Vacherin Mont d'Or, which was contaminated with 10^5 to 10^7 cells per g of rind (2). In trial 2, however, there was no significant change of the inoculated numbers of *L. monocytogenes*. This could be due to the following reasons. (i) In trials 2 and 3, the inocula were somewhat low. A reference strain was used in the second trial, while in the third trial, a wild-type strain isolated from smoked salmon was inoculated. It seems probable that this wild-type strain can grow better on salmon than a reference strain. (ii) The different bacterial load of the raw materials seems to play an essential role in the further development of *Listeria* species in smoked salmon. The interactions between *Listeria* species and other bacteria are quite complex. If lactobacilli are added to heated meat, the *Listeria* growth is inhibited (18), while *Pseudomonas* spp., in combination with other bacteria such as *Escherichia coli* or *Micrococcus* spp., possibly even exert a stimulating influence on the *Listeria* growth (11, 18). The bacterial flora of stored smoked fish consists mainly of *Lactobacillus* spp. (12, 22). If at the beginning of the storage lactobacilli predominate, a multiplication of *L. monocytogenes* will probably be inhibited. The use of some specific *Lactobacillus* spp. as protective cultures should be tested.

These results indicate the importance of preventing an initial contamination of raw fish. Since the concentration of *L. monocytogenes* in the finished product depends on the initial bacterial load, it should be kept as low as possible. It should also be regarded as a strict rule to maintain the refrigeration chain during the whole manufacturing and storage process until the sale to the consumer. Recontamination should be prevented by means of appropriate application of hygiene and technology.

Because a significant increase of *L. monocytogenes* during the storage of smoked salmon was measured, there may be an increasing risk of infection for the consumer by storing such fish for a long time.

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