# Note. Occurrence of *Listeria* spp. in salmon-trout (*Onchorhyncus mykiss*) and salmon (*Salmo salar*)

# Nota. Presencia de *Listeria* spp. en trucha asalmonada (*Onchorhyncus mykiss*) y salmón (*Salmo salar*)

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Salmon-trout (*Onchorhyncus mykiss*) and salmon (*Salmo salar*) are the main raw materials in the cold-smoked fish industry. It is important to prevent the contamination of these ready-to-eat products with *Listeria monocytogenes* and other (*Listeria* spp.) because the temperature used in the cold-smoking process is not sufficient to inactivate these organisms. The presence of *Listeria* spp. and *L. monocytogenes* in the cold-smoked salmon and salmon-trout processing chains of three Portuguese factories examined was already confirmed in previous studies. Thus, it was important to ascertain the possible sources of contamination, the raw material being the most important one. All the Portuguese cold-smoking fish factories use fresh salmon-trout from two trout farms in the north of Portugal and Norwegian salmon which arrives by lorry every week under refrigeration, imported always by the same company; 88 samples of salmon and salmon-trout were analysed; 67 environmental samples from the two trout farms were also examined. The overall frequency ($n = 40$) of *Listeria* spp. and *L. monocytogenes* in salmon was 12 and 0% respectively. The overall frequency ($n = 48$) of *Listeria* spp. and *L. monocytogenes* in salmon-trout was 6.3 and 2.1% respectively. *Listeria* was not found in the environmental samples.

*Keywords*: Listeria, Listeria monocytogenes, trout, Onchorhyncus mykiss, salmon, Salmo salar

La trucha asalmonada (*Onchorhyncus mykiss*) y el salmón (*Salmo salar*) son la materia prima principal de la industria del pescado ahumado en frío. Como tal, es importante prevenir la contaminación con *Listeria* spp. y *Listeria monocytogenes* en estos productos listos pare comer, si se tiene en cuenta que la temperatura usada en el processo de ahumado en frío no es suficiente para destruir estos organismos. La presencia de *Listeria* spp. y *Listeria monocytogenes* en las cadenas de producción de salmón y trucha asalmonada ahumados de tres industrias portuguesas ya se confirmó en trabajos anteriores. Por tanto, es importante determinar las posibles fuentes de contaminación, donde la materia prima es la más importante. Todas las industrias portuguesas de pescado ahumado en frío utilizan materia prima proveniente de dos piscifactorías en el norte de Portugal y salmón noruego importado y transportado en condiciones de refrigeración. Se analizaron 88 muestras de salmón y trucha asalmonada junto con 67 muestras ambientales de las dos piscifactorías produtoras de trucha. La incidencia total ($n = 40$) de *Listeria* spp. y *Listeria monocytogenes* en el salmón fue 12% y 0% respectivamente. La incidencia ($n = 48$) de las mismas especies en la trucha asalmonada fue así mismo del 6,3% y 2,1%. En las muestras ambientales ($n = 67$) no se detectó la presencia de *Listeria*.

*Palabras clave*: Listeria. Listeria monocytogenes, trucha, Onchorhyncus mykiss, salmón, Salmo salar

# INTRODUCTION

*Listeria* spp. are regular short rod-shaped bacteria; they are Gram positive, can grow both as aerobes and as facultative anaerobes, are non-spore formers, and have an optimum growth temperature of 30–37 °C. Their growth is restricted to 1–45 °C and they do not survive heating at 60 °C for 30 min (Seeliger and Jones, 1986). The genus includes seven species: *L. monocytogenes, L. innocua, L. seeligeri, L. ivanovii, L. grayi* and *L. murrayi* (Lovett, 1990). Although *L. monocytogenes* has been found occasionally in foods, it is only within the last few years that it has become fully established as a foodborne pathogen (Bracket, 1988). The ingestion of *L. monocytogenes* in foods can pose a significant health risk, with a relatively high mortality for specific sections of the population, such as pregnant women, foetuses and immunocompromised people.

*Listeria* spp. are ubiquitous organisms with many environmental niches and they can be readily identified in soil, water, plant materials, animals, sewage as well in a variety of uncooked and processed food products (Lovett and Twedt, 1988). *Listeria* spp. have been found in raw fish of fresh water and marine origins and *L. monocytogenes* was isolated from 62% of all water samples (Dillon and Patel, 1992). Farber (1991) reported the presence of *L. monocytogenes* in raw salmon from USA, Chile, Norway and Canada. *L. monocytogenes* was isolated from the thawing water, from the surface of the raw salmon, from the filleting table, from rinsing water and from product trimmings of a cold-smoking plant. They concluded that the initial source of contamination was the raw fish (Eklund *et al.*, 1995). This conclusion was in agreement with an investigation on three salmon-trout smoking plants in Switzerland where it was shown that the raw fish, in particular, was more contaminated than the finished products (Jemmi and Keush, 1994). According to Gibson (1992), as the organism is heat sensitive, cooked fish should pose no risk to consumers and has not been implicated as a vector of listeriosis even to invalids. However, currently, in Europe and North America, fish smoking is used simply as a means of imparting a desirable flavour and aroma, and today's smoking process is much lighter (a mild brine step and low smoking temperatures), and many smoked fish products keep in good condition for very little longer than the fresh fish from which they were made. If the cold-smoking process (temperature of the smoke below 30 °C) is favoured to the hot-smoking process (above 50 °C), the finished product does not undergo further listericidal steps and it might be a source of *Listeria monocytogenes*.

Once *L. monocytogenes* contaminates a food processing plant, it can survive for a long time if the temperature is low and the organism is protected by food components (Farber and Peterkin, 1991). Although some countries (Germany, UK, Denmark, Australia, Canada) considered *L. monocytogenes* pathogenic for specific segments at risk, the USA established the 'zero tolerance' for the organism which means that the process must conform to a level of 'no detectable *L. monocytogenes*' in the finished product (Ben Embarek, 1994).

As there is no step in the cold-smoking process that fully assures the absence of the pathogen in the finished product, it is probable that change will be necessary at the beginning of the process. Thus, this study was conducted to evaluate salmon and salmon-trout as potential sources of *Listeria* spp. in the cold-smoked fish.

# MATERIAL AND METHODS

During a 1-year period a total of 155 samples (67 environmental and 88 fish samples) were collected and analysed.

**Salmon-trout**

From trout farm 1, after a 48-h fast period, the fish were immediately transported from the lake cages into a container, placed in a tank and washed with spring water from a natural source and packed in polystyrene boxes covered with ice (two layers of fish and one of ice on the top) and transported to the factories in small insulated vans. From trout farm 2 after a 48-h fast period, the fish were transported from the lake in a water container to the packaging place, a distance of about 25 km, after which the same procedure as described above was carried out.

In the trout farms, environmental sampling was done at the following sites: containers, washing tanks and polystyrene boxes. The lake water in the fish cages, the ice and the three types of fish feed were also sampled.

**Salmon**

The Norwegian salmon (72 h travel) arrives by lorry in a chilled container with the temperature controlled between 0 and 4 °C inside polystyrene boxes (two layers of fish between two layers of ice) and is collected by the factories' small insulated vans. The fish are already eviscerated. Two sizes, 4–5 and 6–7 kg, are used by the smoking factories.

## Sampling procedure

Ten square centimetres of the fresh fish skin and surfaces were swabbed (five swabs per each sample) and placed in bottles with 25 ml of sterile peptone water 0.1% (w/v). Because the salmon had been eviscerated, both skin and belly cavity were swabbed. Water and ice samples were collected in 500 ml bottles for further filtration. Fish feed (25 g) was collected in sterile stomacher bags.

The samples were transported to the laboratory inside portable insulated boxes, placed in the fridge at temperatures between 2 and 4 °C and analysed on the following day (the trout farms are 200 km from the laboratory).

## Isolation, confirmation and identification of *Listeria* spp.

The 25 ml of peptone water from the fresh fish and surfaces samples were placed in 225 ml of UVM I (Merck). The water and ice samples were filtered (membrane filters of 0.45 μm, Gelman Science 47 mm) and the filters were placed in 20 ml of UVM I. The 25 g of fish feed were placed in 225 ml of UVM II (UVM I + 0.013 g of acriflavine) and homogenized in the Stomacher (Seward 400) for 2 min. All the UVM I samples were incubated at 30 °C for 24 h; 0.1 ml of the UVM I samples were transferred to 10 ml of the two secondary enrichment broths UVM II and Fraser (Merck) and incubated at 30 °C for, respectively, 24 h and 24/48 h. In parallel, 0.05 ml of the UVM I samples were added to 2 ml of *Listeria* electrical detection medium (LED) (Rodrigues *et al.*, 1995) prewarmed to 30 °C in the Bactometer module wells. The Vitek-Bactometer (Model 120 SC, BioMèrieux) was set to monitor capacitance for a test time of 48 h at 30 °C. The LED medium was made from a base of 20 g/L of tryptone (Lab M), 20 g/L of yeast extract (Lab M), 17.5 g/L of LiCl (Oxoid), 4 g/L glucose (BDH) and 1 g/L esculin (Sigma). After sterilization of the base (at 121 °C for 15 min) four vials of Oxford Selective Supplements (Merck) and 20 mg of nalidixic acid (Sigma) filter sterilized stock solution were added to each 1L LED base medium. The pH was then adjusted aseptically to 7.2 ± 0,1.

All the samples showing growth (turbidity in UVM II, esculin reaction in Fraser, more than 30% change in capacitance within 30 h) were subcultured on to Oxford (Merck) and PALCAM (Merck) agar media and incubated at 30 °C for 48 h. All the presumptive colonies were streaked on TSA (Lab M) with 0.6% yeast extract (Lab M) and incubated at 37 °C for 24 h, for further confirmatory tests. All the isolates were confirmed to the genus level by Gram staining, catalase and oxidase tests, umbrella shape motility and to the species level by API 10300, BioMèrieux and CAMP test with *Staphylococcus aureus* ATCC 25923 and *Rhodococcus equi* NCTC 1691 in sheep blood agar plates (BioMèrieux).

# RESULTS AND DISCUSSION

The overall frequency of *Listeria* spp. in fish samples was 9% (8/88). *Listeria* was not found in the environmental samples.

### Salmon-trout samples

The frequencies of *Listeria* spp. and *L. monocytogenes* in salmon-trout samples were 6.3% (3/48) and 2.1% (1/48), respectively; 2.1% of the salmon trout samples were contaminated with *L innocua* and *L. welshimeri*.

In previous studies, the occurrence of *Listeria* spp. and *L. monocytogenes* along the processing chain of Portuguese cold smoked fish (after filleting, washing, brining, smoking, slicing and vacuum packaging) was surveyed. All of the raw salmon-trout used by the factories was supplied by the trout farms 1 and 2. The overall frequencies of *Listeria* and *L. monocytogenes* found in salmon-trout samples from the processing chain of the analysed smokeries, were 47.8% and 30.4%, respectively (Duaert *et al.*, 1995).

*Listeria* spp. were found in the salmon-trout samples from trout farm 2. However, the supply of trout from trout farm 2 was interrupted from July 1996 due to repair work on the dam, which lowered the water level of the lake. The number of samples that could be collected from this farm was thereby reduced (nine fish samples and 13 environmental samples). Since then all trout used in all of the smoking factories were from trout farm 1, where *Listeria* was never found during the period of this survey. Actually, on reviewing the results of the previous study by Duarte *et al.* (1995), it appears that *Listeria* was not be found in the salmon-trout smoking processing chain, because the supply of fish from trout farm 2 was discontinued. Fortunately, both farms are owned by the same company, and after these findings, they were made aware that in the future they should sell only the product of trout farm 1 for smoking purposes.

As *Listeria* is an ubiquitous organism it was expected to be found in the freshwater fish or in the water of the lake in trout farm 1, which is surrounded by agricultural land and animals, but, surprisingly, this was not the case. This could not be due to the

method used in this study because the three different isolation methods used must have improved the recovery rate of *Listeria* from each sample. However, there are contradictory findings about the incidence of *Listeria* in freshwater reported in several studies. Ben Embarek (1994) suggested that *Listeria* spp. are likely to occur naturally in polluted freshwater or in freshwater with a high content of organic material, such as rivers or coastal water. Jemmi and Keusch (1994) analysed the water of three different salmon-trout farms (two using ground water and one using spring water) and *Listeria* spp. were found in only one of the three fish farms (4/36) but *L. monocytogenes* was not among them. However, in the same trout farm where no *L. monocytogenes* was isolated from the water, this organism was found in the skin and guts of the fish. In the present study the possible sources of *Listeria* in the fish could be either the water, the fish feed or the sediments, but in trout farm 2, where *L. monocytogenes* was isolated from the fish, it was never isolated from the water or from the three types of fish feed analysed. As the trout are feeding in cages of 5 m depth and the depth of the lake is about 30 m there was no point in sampling the sediments.

### Salmon samples

In salmon samples, the frequencies of *Listeria* spp. and *L. monocytogenes* were 12% (5/40) and 0%, respectively; 10%, 2.5% and 2.0% of the salmon samples contained, respectively, *L. innocua*, *L. welshimeri* and *L. seeligeri*.

In previous studies the overall frequencies of *Listeria* spp. and *L. monocytogenes* in the smoked-salmon samples of the portuguese smoking factories sampled, were 43.9% and 26.8%, respectively (Duarte *et al.*, 1995). The raw material of these smoking factories was the Norwegian salmon imported always by the same company and analysed in this study.

In spite of the fact that in this study *L. monocytogenes* had never been isolated from the salmon samples, the presence of other *Listeria* spp. in the samples is significant because it indicates the potential for growth of the pathogenic species in a similar environment (Perry and Donnelly, 1990). Eklund *et al.* (1995) reported the occurrence of *L. monocytogenes* in the slime, skin and belly cavity of salmon; *L. innocua* was also found in those samples; *L. seeligeri* was isolated from the skin and belly cavity; Rørvik *et al.* (1995) found a level of contamination of 17% in raw salmon.

As the analysed salmon was imported from Norway, no environmental samples could be taken and as the fish was already eviscerated the pre- and post-harvest handling process of this raw material was unknown. For example, if there was a washing step after evisceration it could lead either to suppression of the organism, if present, or to the contamination from other sources via the water.

Although the incidence of *Listeria* spp. was reported to be lower in marine waters compared to freshwater, 33% and 81% respectively (Colburn *et al.*, cited by Dillon and Patel, 1992), Farber (1991) reported the presence of *L. monocytogenes* in raw salmon from USA, Chile, Norway and Canada.

The results of this study together with those from the same authors (Duarte *et al.*, 1995), and also the information reported by several other authors, suggested that it is unlikely that a fish farm could assure the absence of *L. monocytogenes* in the fish. Thus, the need for minimizing the presence of this pathogen in the raw material is crucial. Unless a listericidal step is introduced in the cold-smoking process, it is difficult to avoid the presence of *L. monocytogenes*. As chemical treatments might damage the products after filleting, all the control measures to be taken must be focused on the raw material – the whole fish after evisceration – since the flesh of the fish is sterile, and only the exposed surfaces, such as skin and belly cavity, are likely to contain *Listeria*.

## ACKNOWLEDGEMENTS

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