Available online at www.sciencedirect.com
ScienceDirect
International Journal of Food Microbiology 122 (2008) 287–295
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Influence of processing steps in cold-smoked salmon production on survival and growth of persistent and presumed non-persistent *Listeria monocytogenes* ☆

Cisse Hedegaard Porsby [a], Birte Fonnesbech Vogel [a], Mona Mohr [b], Lone Gram [a,*]

[a] *National Institute of Aquatic Resources, Technical University of Denmark, Søltofts Plads, bldg. 221, DK-2800 Kgs. Lyngby, Denmark*
[b] *Højmarklaboratoriet Adelvej 11, DK-6940 Lem St, Denmark*

Received 7 September 2007; received in revised form 3 January 2008; accepted 15 January 2008

Abstract

Cold-smoked salmon is a ready-to-eat product in which *Listeria monocytogenes* sometimes can grow to high numbers. The bacterium can colonize the processing environment and it is believed to survive or even grow during the processing steps. The purpose of the present study was to determine if the steps in the processing of cold-smoked salmon affect survival and subsequent growth of a persistent strain of *L. monocytogenes* to a lesser degree than presumed non-persistent strains. We used a sequence of experiments increasing in complexity: (i) small salmon blocks salted, smoked or dried under model conditions, (ii) fillets of salmon cold-smoked in a pilot plant and finally, (iii) assessment of the bacterial levels before and after processing during commercial scale production. *L. monocytogenes* proliferated on salmon blocks that were brined or dipped in liquid smoke and left at 25 °C in a humidity chamber for 24 h. However, combining brining and liquid smoke with a drying (25 °C) step reduced the bacterium 10–100 fold over a 24 h period. Non-salted, brine injected or dry salted salmon fillets were surface inoculated with *L. monocytogenes* and cold-smoked in a pilot plant. *L. monocytogenes* was reduced from 10^3 to $10–10^2$ CFU/cm^2 immediately after cold-smoking. The greatest reductions were observed in dry salted and brine injected fillets as compared to cold-smoking of non-salted fresh fillets. Levels of *L. monocytogenes* decreased further when the cold-smoked fish was vacuum-packed and stored at 5 °C. A similar decline was seen when inoculating brine injected fillets after cold-smoking. High phenol concentrations are a likely cause of this marked growth inhibition. In a commercial production facility, the total viable count of salmon fillets was reduced 10–1000 fold by salting, cold-smoking and process-freezing (a freezing step after smoking and before slicing). The prevalence of *L. monocytogenes* in the commercial production facility was too low to determine any quantitative effects, however, one of nine samples was positive before processing and none after. Taken together, the processing steps involved in cold-smoking of salmon are bactericidal and reduce, but do not eliminate *L. monocytogenes.* A persistent strain was no less sensitive to the processing steps than a clinical strain or strain EGD.

Keywords: Cold-smoking; Drying; *Listeria monocytogenes*; Persistence; Salmon; Salting

1. Introduction

Listeria monocytogenes is a human pathogen that can cause listeriosis, which is a food borne disease. Listeriosis is a very rare disease that has typically been caused by ready-to-eat (RTE) products in which *L. monocytogenes* can multiply to high numbers during extended refrigerated storage. *L. monocytogenes* is a ubiquitous bacterium, although its prevalence in the outdoor environment is not high (El Marrakchi et al., 2005; Frances et al., 1991; Luppi et al., 1988; Moshtaghi et al., 2003; Motes, 1991). It is potentially present on almost all types of raw materials for food processing and hence, processing steps that can eliminate the organism from the raw materials or intermediate product are important critical control points (CCPs).

☆ Part of the results have been presented at a poster session at the 93rd IAFP annual meeting in Calgary, Canada, August 13–16, 2006.

* Corresponding author. Tel.: +45 45 25 25 86; fax: +45 45 88 47 74.
E-mail address: gram@difres.dk (L. Gram).

0168-1605/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2008.01.010

Cold-smoked seafood has been linked to outbreaks of listeriosis (Brett et al., 1998; Miettinen et al., 1999), and has been categorized as a high risk food, as an estimated 6.2 cases of listeriosis occur per billion servings (FDA/FSIS, 2003). During processing of cold-smoked fish, the fish fillets are brined to 3–6% NaCl in the water phase of the final product and smoked and dried at 22–28 °C. The fillets are frozen to −8–10 °C (so-called process-freezing) to facilitate slicing after which they are vacuum-packed and distributed frozen or at refrigeration temperature. *L. monocytogenes* can multiply in a wide temperature range and in high levels of NaCl (Cole et al., 1990; Petran and Zottola, 1989) and taken as individual processing steps, salting or smoking temperature is not believed to reduce numbers of *L. monocytogenes*. Due to the low smoking temperature, one may even speculate that *L. monocytogenes* could proliferate during the smoke processing.

The cold-smoking step itself consists of evaporation of moisture from the fish, and addition of phenolic compounds from the smoke to the fish surface. This may be inhibitory to *L. monocytogenes* which was reduced during drying of beef jerky at 52–63 °C (Yoon et al., 2006), however, these temperatures are much higher than temperatures used during cold-smoking. *L. monocytogenes* can be inhibited or sometimes reduced by liquid smoke added to laboratory media or to fish and the degree of inhibition depends to some extent on the concentration of phenolic compounds (Faith et al., 1992; Membré et al., 1997; Neunlist et al., 2005; Suñen et al., 2003; Thurette et al., 1998). Only a few studies have been conducted on the effect of a commercial industrial cold-smoking on *L. monocytogenes* and the results are inconclusive. Both *L. monocytogenes* and *Listeria innocua* inoculated on the surface of salmon fillets were reduced by cold-smoking (Eklund et al., 1995; Sabanadesan et al., 2000), however, Guyer and Jemmi (1991) found no significant changes in the *L. monocytogenes* count during cold-smoking. Also of interest, is the effect of these processes on subsequent growth of the bacterium during distribution and storage, however, most experiments determining growth rate have inoculated the organism on smoked processed product. In a recent study, *L. monocytogenes* was inoculated on newly cold-smoked fish, and high concentration of phenols (up to 80 ppm) in combination with 4–5% NaCl inhibited growth even at 20 °C (Montero et al., 2007).

L. monocytogenes is able to colonize surfaces (Helke et al., 1993; Mafu et al., 1990; Moltz and Martin, 2005) and particular sub-types may persist in food production plants for several years. Many reports have linked food product contamination to processing environment contamination (Miettinen et al., 1999; Rørvik et al., 1995; Vogel et al., 2001). Hence, even when reduction steps are included in the food processing, great care must be taken to avoid post-process contamination. We have recently demonstrated using sub-typing by randomly amplified polymorphic DNA (RAPD) and amplified-fragment length polymorphism (AFLP) sub-typing that particular sub-types of *L. monocytogenes* dominate as persistent strains in several fish industries (Wulff et al., 2006). These sub-types are very uncommon in the outside environment (Hansen et al., 2006) and appear particularly suited for the processing environment. One may hypothesize that such persistent sub-types found in processing plants are more tolerant to the parameters used in processing steps than other, sporadic, strains.

The purpose of the present study was to examine the effect of different processing steps in the fish cold-smoking process on the level of different strains of *L. monocytogenes* to determine if the combined process can be viewed as a control point in a *Listeria* control program. As growth of *L. monocytogenes* is a very important risk factor, we also determined if growth of the pathogen in the product was affected by exposure to the salting, drying and cold-smoking steps.

2. Materials and methods

2.1. Preparation of bacterial inoculum for model and pilot plant experiments

Four *L. monocytogenes* strains of different origin were used. N53-1 represented a persistent RAPD-type that has been isolated from several fish slaughter and smoke houses for many years (Wulff et al., 2006). It is a sero-type 1/2 a and belongs to lineage II. The EGD strain, isolated from a rabbit in 1926, belongs to sero-type 1/2 a and lineage II (Goebel, University of Wurzburg, Germany). Strain 4446, which belongs to lineage I and sero-type 4 b, was isolated from a sporadic case of listeriosis in a human (State Serum Institute, Copenhagen, Denmark). Strain Br22 was isolated from the sediment at the bottom of a holding tank for deflavouring at a fresh water fish farm, and belongs to sero-type 1/2 a and lineage II (Hansen et al., 2006).

The cultures were maintained at −80 °C and grown in brain heart infusion (BHI) broth (Oxoid, CM225, Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) at 25 °C for 24 h. The cultures were inoculated (1% inoculum) in BHI broth supplemented with 3% NaCl to simulate the salting and smoking environment. After 24 h at 15 °C the cultures were OD_{450}-adjusted by individual standard curves relating colony counts to optical densities to approx. 1×10^5, 5×10^5 or 1×10^7 cells/ml.

2.2. Experimental design and set-up

The immediate effect on levels of *L. monocytogenes* strains of processes used in the cold-smoking process was studied at three levels and, also, the influence of these processes on subsequent growth during refrigerated storage of vacuum-packaged product was determined at two levels (Table 1). (i) A laboratory model consisting of salmon blocks (cut from fresh salmon) was salted, treated with liquid smoke and/or dried under laboratory conditions. (ii) In pilot plant scale, we determined the influence of the complete process on reduction and subsequent growth of the pathogen. (iii) Finally, we determined the overall bacteria-reducing effect of the cold-smoking process in a commercial operation.

2.3. Laboratory experiments with salmon blocks: treatment and inoculation

Fresh, non-salted salmon fillets were bought in the local supermarket for laboratory model experiments. Fillets were cut