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Development and validation of a stochastic model for potential growth of *Listeria monocytogenes* in naturally contaminated lightly preserved seafood

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ABSTRACT

A new stochastic model for the simultaneous growth of *Listeria monocytogenes* and lactic acid bacteria (LAB) was developed and validated on data from naturally contaminated samples of cold-smoked Greenland halibut (CSGH) and cold-smoked salmon (CSS). During industrial processing these samples were added acetic and/or lactic acids. The stochastic model was developed from an existing deterministic model including the effect of 12 environmental parameters and microbial interaction (O. Mejlholm and P. Dalgaard, *Food Microbiology*, submitted for publication). Observed maximum population density (MPD) values of *L. monocytogenes* in naturally contaminated samples of CSGH and CSS were accurately predicted by the stochastic model based on measured variability in product characteristics and storage conditions. Results comparable to those from the stochastic model were obtained, when product characteristics of the least and most preserved sample of CSGH and CSS were used as input for the existing deterministic model. For both modelling approaches, it was shown that lag time and the effect of microbial interaction needs to be included to accurately predict MPD values of *L. monocytogenes*. Addition of organic acids to CSGH and CSS was confirmed as a suitable mitigation strategy against the risk of growth by *L. monocytogenes* as both types of products were in compliance with the EU regulation on ready-to-eat foods.

1. Introduction

Occurrence and growth of *Listeria monocytogenes* in ready-to-eat (RTE) seafood remain a challenge both in Europe and worldwide. The EU critical limits for *L. monocytogenes* in RTE foods are well established (EC, 2005; EC, 2007). However, for fishery products in Europe a high percentage of non-compliance has been observed both for absence in 25 g at the processing plant and for less than 100 cfu/g at retail (EFSA, 2013). Concentrations of *L. monocytogenes* higher than 100 cfu/g are potentially critical as dose–response studies suggest a relation between the intake of cells and the probability of listeriosis (Hoelzer et al., 2013).

Predictive models can describe growth in food during storage and importantly they can be used to estimate concentrations of *L. monocytogenes* in food at the time of consumption (McMeekin et al., 2006). Several factors influence growth of *L. monocytogenes* including (i) initial contamination with variability in concentration, physiological state and between strains, (ii) processing, (iii) product characteristics, (iv) storage conditions, and (v) microbial interaction during storage. Numerous deterministic *L. monocytogenes* growth models are available (Mejlholm et al., 2010; Ross and Dalgaard, 2004) and several stochastic models have been used in quantitative microbiological risk (or exposure) assessments (Delignette-Muller et al., 2006; FAO/WHO, 2004; Garrido et al., 2010; Pouillot et al., 2007; Ross et al., 2009a; Tenenhaus-Aziza et al., 2014). Stochastic models are important to describe variability and uncertainty. Stochastic *L. monocytogenes* growth models, however, remain incomplete and insufficiently validated. As an example, growth of *L. monocytogenes* is damped in different lightly preserved seafood (e.g. cold-smoked salmon) by the simultaneous growth of LAB to high concentrations. This Jameson effect has been included in complex deterministic growth models that take into account the effect of relevant product characteristics and storage conditions for these products (Mejlholm and Dalgaard, 2007a, submitted for

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publication). The Jameson effect has also been included in stochastic models but they do not include several of the environmental parameters known to influence growth of *L. monocytogenes* in lightly preserved seafood (FAO/WHO, 2004; Delignette-Muller et al., 2006; Pouillot et al., 2007; Couvert et al., 2010). Another limitation of available stochastic *L. monocytogenes* models is that they, to a very limited extent, have been validated by comparison of predicted growth responses with growth observed in naturally contaminated products (Couvert et al., 2010).

The objective of the present study was to develop and validate an extensive stochastic model for growth of *L. monocytogenes* in naturally contaminated lightly preserved seafood. The new stochastic model was based on an existing deterministic model for the simultaneous growth of *L. monocytogenes* and lactic acid bacteria (LAB) including the effect of 12 environmental parameters (Mejlholm and Dalgaard, 2007a, 2007b, submitted for publication). The main examined sources of variability were product characteristics and storage conditions. New data for growth of *L. monocytogenes* in naturally contaminated cold-smoked Greenland halibut (CSGH) and cold-smoked salmon (CSS) was generated and used to validate the performance of the new stochastic model. This validation included measured variability in product characteristics of CSGH and CSS collected by a Danish seafood processor during a period of 15 months.

2. Material and methods

2.1. Storage trials with naturally contaminated samples of cold-smoked Greenland halibut and cold-smoked salmon

2.1.1. Sample reception, preparation and packaging

Frozen samples of vacuum packed (VP) cold-smoked Greenland halibut (CSGH) and VP cold-smoked salmon (CSS) were supplied to the National Food Institute (DTU Food) by a Danish seafood processor, and kept at −20 °C until the start of the experiments. CSGH was supplied as a sliced product in packages of approx. 200 g, whereas CSS was supplied as whole sliced filets of 1000–1200 g. Samples of both CSGH and CSS originated from batches withheld by the seafood processor at the level of production due to detection of packages being positive for *L. monocytogenes*. Prior to the start of the storage trials, samples of CSGH and CSS were thawed overnight at 5 °C. No further preparation was carried out for samples of CSGH. Samples of CSS were divided into smaller portions of approx. 200 g, which were VP using a Multivac A 300/16 packaging machine (Multivac Ltd., Vejle, Denmark). Packaging film (NEN 40 HOB/LLPDE 75, Amcore Flexibles, Horsens, Denmark) with low gas permeability (0.45 ± 0.15 cm^3 m^{-2} atm^{-1} for O_2 and 1.8 ± 0.6 cm^3 m^{-2} atm^{-1} for CO_2) was used. During the handling of CSS, gloves as well as cleaned and disinfected cutting boards and knifes were used. For cleaning and disinfection purpose, Deconex® 15 PF-x (Borer Chemie AG, Zuchwil, Switzerland) and boiling water was used. Samples of CSGH and CSS were stored at 5, 8 and 12 °C for up to 44, 23 and 15 days, respectively (Table 1). 5 °C is the recommended storage temperature for marinated and cold-smoked seafood (EC, 2004), whereas 8 and 12 °C represented two different levels of temperature abuse. The storage temperature was recorded regularly throughout all the experiments using data loggers (TinytagPlus, Gemini Data Loggers Ltd., Chichester, UK).

2.1.2. Product characteristics

Product characteristics of CSGH and CSS were determined by analysis of five packages for each of the products at the start of the experiments. The concentration of dry matter and the pH were measured as previously described (Dalgaard et al., 1993). Salt

Table 1
Product characteristics and storage conditions of naturally contaminated cold-smoked Greenland halibut and cold-smoked salmon.

	Cold-smoked Greenland halibut (CSGH) ($n = 5$)[a]							Cold-smoked salmon (CSS) ($n = 5$)[b]						
	CSGH#1	CSGH#2	CSGH#3	CSGH#4	CSGH#5	AVG ± SD	Distribution[c]	CSS#1	CSS#2	CSS#3	CSS#4	CSS#5	AVG ± SD	Distribution[c]
pH	6.36	6.03	6.05	6.00	5.90	6.07 ± 0.17	Γ (1274.9, 0.004761)	6.09	6.12	6.15	6.17	6.19	6.14 ± 0.04	Γ (23,562, 0.000261)
Salt (%)[d]	2.78	3.83	3.15	3.20	3.61	3.31 ± 0.41	Γ (65.2, 0.0508)	3.00	3.10	2.53	3.51	2.94	3.01 ± 0.35	Γ (74.0, 0.0407)
Phenol (ppm)	9.8	10.4	9.5	9.5	11.3	10.1 ± 0.8	Γ (171.4, 0.0588)	<1	<1	<1	<1	<1	<1	Fixed[e]
Acetic acid (ppm)[d]	2524	3493	2714	2781	3542	3011 ± 472	Γ (40.7, 74.0)	1225	1458	3116	2049	2101	1990 ± 773	Γ (7.4, 270.0)
Lactic acid (ppm)[d]	6378	8923	6599	7710	8676	7657 ± 1162	Γ (43.3, 176.7)	8638	9467	14,980	10,006	10,583	10,735 ± 2479	Γ (18.8, 572.5)
Psi-value for *L. monocytogenes* at 5 °C[f]	0.66	1.30	1.00	1.15	1.68	–	–	0.75	0.78	1.07	0.82	0.80	–	–
Measured storage temperatures														
5 °C	–	–	–	–	–	5.17 ± 0.17	Γ (924.9, 0.00559)	–	–	–	–	–	3.74 ± 0.79	Γ (22.4, 0.167)
8 °C	–	–	–	–	–	7.30 ± 1.11	Γ (43.3, 0.169)	–	–	–	–	–	7.99 ± 0.21	Γ (1447.6, 0.005519)
12 °C	–	–	–	–	–	11.3 ± 0.73	Γ (239.2, 0.0472)	–	–	–	–	–	12.3 ± 0.29	Γ (1798.9, 0.006837)

[a] n, number of analysed samples identified as CSGH#1 to CSGH#5.
[b] n, number of analysed samples identified as CSS#1 to CSS#5.
[c] Γ (a, b), gamma distribution with shape parameter a and scale parameter b.
[d] In the water phase of the product.
[e] Fixed as 1.0 ppm phenol.
[f] Determined using the model of Mejlholm and Dalgaard (2009).