

Modeling the Growth Characteristics of *Listeria monocytogenes* and Native Microflora in Smoked Salmon

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ABSTRACT: Smoked salmon contaminated with *Listeria monocytogenes* has been implicated in foodborne listeriosis. The objectives of this study were to model the growth characteristics and examine the growth relationship of *L. monocytogenes* and native microflora in smoked salmon. Smoked salmon samples with a native microflora count of 2.9 log₁₀ CFU/g were inoculated with a 6-strain mixture of *L. monocytogenes* to levels of log₁₀ 1.6 and log₁₀ 2.8 CFU/g, and stored at 4, 8, 12, and 16 °C. Growth characteristics (lag phase duration [LPD, h], growth rate [GR, log₁₀ CFU/h], and maximum population density [MPD, log₁₀ CFU/g]) of *L. monocytogenes* and native microflora were determined. At 4 to 16 °C, the LPD, GR, and MPD were 254 to 35 h, 0.0109 to 0.0538 log₁₀ CFU/h, and 4.9 to 6.9 log₁₀ CFU/g for *L. monocytogenes*, respectively, and were 257 to 29 h, 0.0102 to 0.0565 log₁₀ CFU/h, and 8.5 to 8.8 log₁₀ CFU/g for native microflora. The growth characteristics of *L. monocytogenes* or the native microflora were not significantly different ($P > 0.05$), regardless the initial levels of *L. monocytogenes*. Mathematical equations were developed to describe the LPD, GR, and MPD of *L. monocytogenes* and native microflora as a function of storage temperature. The growth relationship between *L. monocytogenes* and native microflora was modeled and showed that the LPD and GR of *L. monocytogenes* were similar to those of native microflora. These models can be used to estimate the growth characteristics of *L. monocytogenes* in smoked salmon, and thereby enhance the microbiological safety of the product.

Keywords: growth rate, lag phase, *Listeria monocytogenes*, native microflora, smoked salmon

Introduction

Smoked salmon is a ready-to-eat (RTE) product, and is commonly sold in vacuum packages with a refrigerated shelf life of 3 to 8 wk. The product is produced by salting, smoking, trimming, or slicing the fish, and vacuum-packaging the final product. Smoked salmon contains approximately 65% to 78% water, 2% to 8% water-phase salt, and 2 to 15 ppm phenol, with pH values of 5.9 to 6.3 and a_w of 0.95 to 0.98. The total microbial counts in cold-smoked salmon after packaging were approximately 10³⁻⁴ CFU/g, mostly lactic acid bacteria (Duffes 1999; Rorvik 2000; Gimenez and Dalgaard 2004). Lactic acid bacteria are able to grow in smoked salmon at refrigerated temperature, and are mainly responsible for the shelf life of smoked salmon (Jorgensen and others 2000; Leroi and others 2001). Potential for contamination with *Listeria monocytogenes* exists, if smoked salmon is not processed and handled properly. *L. monocytogenes* is a human pathogen that causes listeriosis and, with an ability to grow at low temperature, is a significant pathogen of concern in refrigerated RTE foods. The rates of contamination of *L. monocytogenes* in cold-smoked salmon or smoked fish have been reported to be at 10% (Embarek 1994), 9.2% to 13.8% (Cortesi and others 1997), 34% to 43% (Jorgensen and Huss 1998), 7.3% (Norton and others 2001), and 4.3% (Gombas and others 2003). The levels of *L. monocytogenes* recovered from contaminated smoked

salmon were generally low at < 10 CFU/g (Cortesi and others 1997; Gombas and others 2003). Smoked salmon and smoked fish contaminated with *L. monocytogenes* have been implicated with causing foodborne listeriosis. One listeriosis outbreak in Sweden was linked to eating smoked rainbow trout or salmon and resulted in 9 cases of listeriosis and 2 deaths (Ericsson and others 1997). Risk assessments indicated that consumption of smoked salmon contaminated with *L. monocytogenes* may cause an elevated risk of listeriosis (FDA/USDA/CDC 2003; FAO/WHO 2004).

Because of a high probability that smoked salmon may be contaminated with *L. monocytogenes*, the growth behavior of this pathogen in smoked salmon has been studied extensively. Dalgaard and Jorgensen (1998) reported that salt, moisture contents, pH of smoked salmon, and the prevailing storage temperatures were supportive to the growth of *L. monocytogenes*. Several studies have identified that salt, a_w , smoke compounds, liquid smoke (Vitt and others 2001), lactate and diacetate (Mejlholm and Dalgaard 2007), product pH, salt, phenolic compounds, and storage temperature (Membre and others 1997; Thurette and others 1998; Augustin and Carlier 2000; Lebois and others 2004; Cornu and others 2006; Hwang 2007) had effects on the growth of *L. monocytogenes* in vacuum-packed smoked salmon. There were also studies examined the growth of *L. monocytogenes* in smoked salmon with native microflora (Gimenez and Dalgaard 2004; Lappi and others 2004; Tome and others 2007) and added spoilage microorganisms (Gimenez and Dalgaard 2004) or lactic acid bacteria (Nilsson and others 1999; Pleasants and others 2001; Amezquita and Brashears 2002; Yamazaki and others 2003). However, these studies mainly reported the increase or decrease of cell counts of *L. monocytogenes* in smoked salmon during storage. Data on the growth characteristics of *L. monocytogenes* and the native

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microflora, and the growth relationship between these 2 microfloras in smoked salmon are limited. The objectives of this study were to examine the growth characteristics of *L. monocytogenes* and native microflora in smoked salmon at refrigerated and abuse temperatures, and develop models to describe the growth characteristics and the growth relationship between *L. monocytogenes* and native microflora.

Materials and Methods

L. monocytogenes and inoculum preparation

Six strains of *L. monocytogenes* (NFP7459; serotype 3b, NFP7533; serotype 4b, NFP7554; serotype 1/2b, NFP7712; serotype 1/2a, NFP7735; serotype 1/2a, and NFP7779; serotype 2/1a) from the Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, were used in this study. Each strain was transferred from -80°C stock culture into 10 mL brain heart infusion (BHI) broth (Difco, Becton, Dickinson and Co., Sparks, Md., U.S.A.) and incubated at 35°C overnight. A loopful of cell suspension of each strain was then transferred to fresh 10 mL BHI broth and incubated at 35°C for 24 h. One milliliter of cell suspension from each strain was mixed together, and the mixture was further diluted in sterile 0.1% peptone water (PW) to obtain inocula with populations of 10^{2-3} and 10^{3-4} CFU/mL.

Sample preparation and storage

To select a representative smoked salmon product for testing, 5 cold-smoked salmon products from different manufacturers were purchased from a local grocery store. Samples were analyzed by a commercial analytical laboratory (Microbac Laboratories Inc., Wilson, N.C., U.S.A.) to determine contents of moisture (forced-draft oven method), salt (AOAC method), fat (ether extract method), and total phenolic compounds (Folin-Ciocalteu method). A product that had the representative composition was used in this study within 3 d of purchase. The smoked salmon used in this study had a pH of 6.5, a_w 0.97, 65% moisture, 1.93% salt, 5.6% fat, and 4 ppm total phenolic compounds. Smoked salmon samples (3 g) were placed into 100-mL stomacher bags (Spiral Biotech Inc., Norwood, Mass., U.S.A.), and inoculated with 0.1 mL of the *L. monocytogenes* inoculum to achieve inoculum levels of 10^{1-2} and 10^{2-3} CFU/g. The bags were vacuum-sealed to 60 mbar using a Multivac A300 vacuum sealer (Multivac Inc., Kansas, Miss., U.S.A.). The samples were stored at 4, 8, 12, and 16°C for up to 6 wk. During the storage, duplicate samples were analyzed for counts of *L. monocytogenes* and native microflora. The experiment was performed in 2 separate trials with 2 samples prepared for each sampling interval for each trial.

Enumeration of *L. monocytogenes* and native microflora

To each bag of smoked salmon, 3 mL of sterile 0.1% PW was added and pumped in a stomacher at medium speed for 2 min. The sample homogenates were serially diluted in 0.1% PW. Appropriate dilutions (0.1 mL) were spread-plated onto PALCAM agar (Difco) in duplicate for *L. monocytogenes*, and plate count agar (PCA, Difco) for native microflora. All plates were incubated at 35°C for 24 to 48 h. In preliminary study, sample dilutions were also spread-plated on deMann Rogosa Sharpe (MRS, Difco) and incubated in a Bactron IV Anaerobic/Environmental Chamber (Sheldon Manufacturing Inc., Cornelius, Oreg., U.S.A.) with a gas composition of 10.1% carbon dioxide, 4.38% hydrogen, and balance nitrogen for 48 h at 37°C for lactic acid bacteria. The counts on PCA

and MRS were not significantly different, indicating the colonies appeared on PCA were mostly lactic acid bacteria.

Determination of lag phase duration, growth rate, and maximum population density

Growth curves of *L. monocytogenes* and native microflora (\log_{10} CFU/g against storage time) in smoked salmon during storage at 4, 8, 12, and 16°C were fitted with DMFit curve-fitting software (<http://www.ifr.ac.uk/Safety/DMFit/default.html>) to obtain the lag phase durations (LPD, h), growth rates (GR, \log_{10} CFU/h), and maximum population density (MPD, \log_{10} CFU/g). Means of GR and LPD of *L. monocytogenes* and native microflora at each storage temperature were compared using Tukey's mean comparison test (SAS 9.1, SAS Inst., Cary, N.C., U.S.A.) at a significance level of 95%. The LPD and GR of *L. monocytogenes* and native microflora at 4, 8, 12, and 16°C were fitted with a linear or polynomial equation as a function of storage temperature using the procedure of regression model or general linear model (GLM) of SAS 9.1 (SAS Inst.). Fitting results showed that LPD were better fitted with a polynomial equation, and GR, in square root transformation, and MPD were better fitted with a linear regression. The forms of the equations are:

$$\text{LPD} = \alpha + \beta_1 \times \text{temperature} + \beta_2 \times (\text{temperature})^2$$

$$\sqrt{\text{GR}} = \alpha + \beta_1 \times \text{temperature}$$

$$\text{MPD} = \alpha + \beta_1 \times \text{temperature.}$$

where α is the intercept, and β_1 and β_2 are estimated coefficients.

To describe the growth relationship between *L. monocytogenes* and native microflora in smoked salmon, the LPD and GR of *L. monocytogenes* as a function of those of native microflora were fitted with a linear regression:

$$\text{LPD}_{L. monocytogenes} = \alpha + \beta_1 \times (\text{LPD}_{\text{native microflora}})$$

$$\text{GR}_{L. monocytogenes} = \alpha + \beta_1 \times (\text{GR}_{\text{native microflora}}).$$

where α is the intercept, and β_1 is estimated coefficients.

Results and Discussion

Growth of *L. monocytogenes* and native microflora in smoked salmon at 4, 8, 12, and 16°C

The growth of *L. monocytogenes* and native microflora in smoked salmon at 4, 8, 12, and 16°C are shown in Figure 1A to 1D. The initial low-inoculum of *L. monocytogenes* was approximately $1.6 \log_{10}$ CFU/g. The counts increased to $4.6 \log_{10}$ after 35 d at 4°C , $5.4 \log_{10}$ CFU/g after 21 d at 8°C , $5.6 \log_{10}$ CFU/g after 15 d at 12°C , and $6.5 \log_{10}$ CFU/g after 7 d at 16°C . The initial high-inoculum of *L. monocytogenes* was approximately $2.8 \log_{10}$ CFU/g. The counts increased to $4.9 \log_{10}$ CFU/g after 35 d at 4°C , $5.6 \log_{10}$ CFU/g after 21 d at 8°C , $5.9 \log_{10}$ CFU/g after 15 d at 12°C , and $7.1 \log_{10}$ after 7 d at 16°C . The initial level of native microflora was approximately $2.9 \log_{10}$ CFU/g, and the counts increased to more than $8 \log_{10}$ CFU/g after 28 d at 4°C , 15 d at 8°C , 10 d at 12°C , and 5 d at 16°C , regardless of the initial inoculums of *L. monocytogenes*. The growth of *L. monocytogenes* in salmon observed in this study agreed with results reported by other studies that showed the ability of *L. monocytogenes* to grow in smoked salmon during storage at 4 to 10°C (Cortesi and others 1997; Yoon and others 2004; Cornu and others 2006). This study again demonstrates the risk of *L. monocytogenes*-contaminated smoked salmon stored under refrigerated or

abuse temperatures for an extended time. The growth of native microflora in smoked salmon reached the MPD of $> 8.5 \log_{10}$ CFU/g at all storage temperatures, whereas the *L. monocytogenes* reached $4.9 \log_{10}$ CFU/g at 4°C , $5.9 \log_{10}$ CFU/g at both 8°C and 12°C , and $6.9 \log_{10}$ CFU/g at 16°C . The MPD of *L. monocytogenes* appeared to be affected by the level of native microflora, in which the growth of *L. monocytogenes* was inhibited when the native microflora reached the MPD (Figure 1A to 1D). Gimenez and Dalgaard (2004) reported that *L. monocytogenes* grew to $8 \log_{10}$ CFU/g in vacuum-packed cold-smoked salmon stored at 5 to 25°C . With spoilage microorganisms, *L. monocytogenes* reached only 2 to $4 \log_{10}$ CFU/g, and the growth ceased when the spoilage microorganisms reached the MPD. The native microflora in smoked salmon in this study were mainly lactic acid bacteria (LAB) as evidenced by the similar counts on PCA plates and MRS plates (data not shown). LAB has been reported to inhibit the growth of *L. monocytogenes* in smoked salmon (Nilsson and others 1999; Jorgensen and others 2000; Katla and others 2001; Leroi and others 2001).

Growth characteristics of *L. monocytogenes* and native microflora

The growth characteristics of *L. monocytogenes* and native microflora in smoked salmon as indicated by LPD (h), GR (\log_{10}

CFU/h), and MPD (\log_{10} CFU/g) are shown in Table 1. The LPD and GR of *L. monocytogenes* in smoked salmon were in the range of 282 to 35 h, and 0.0109 to $0.0535 \log_{10}$ CFU/h, respectively, at 4 to 16°C , respectively. The LPD and GR of native microflora in smoked salmon were in the range of 257 to 29 h and 0.0102 to $0.0565 \log_{10}$ CFU/h at 4 to 16°C , respectively. In general, the LPD and GR values of *L. monocytogenes* and native microflora at low temperature were higher and lower, respectively, than those of the higher temperature. The LPD and GR values of *L. monocytogenes* and native microflora with low inoculum level were not significantly different ($P > 0.05$) from those of with high-inoculum level at each storage temperature (Table 1). This indicates that the growth (LPD and GR) of *L. monocytogenes* or native microflora in smoked salmon at storage temperatures of 4 to 16°C were similar and not affected by the initial population of *L. monocytogenes* in smoked salmon. This also indicates that the growth of *L. monocytogenes* or native microflora was not affected by the initial levels of *L. monocytogenes* in smoked salmon. The MPD of *L. monocytogenes* were 4.9 to $6.9 \log_{10}$ CFU/g at 4 to 16°C , whereas the MPD of native microflora was approximately $8.6 \log_{10}$ CFU/g. The MPD of *L. monocytogenes* was significantly higher at 16°C than those at 4 , 8 , and 12°C . The MPD reached by the native microflora were similar in all storage temperatures, whereas the MPD for *L. monocytogenes* were significantly

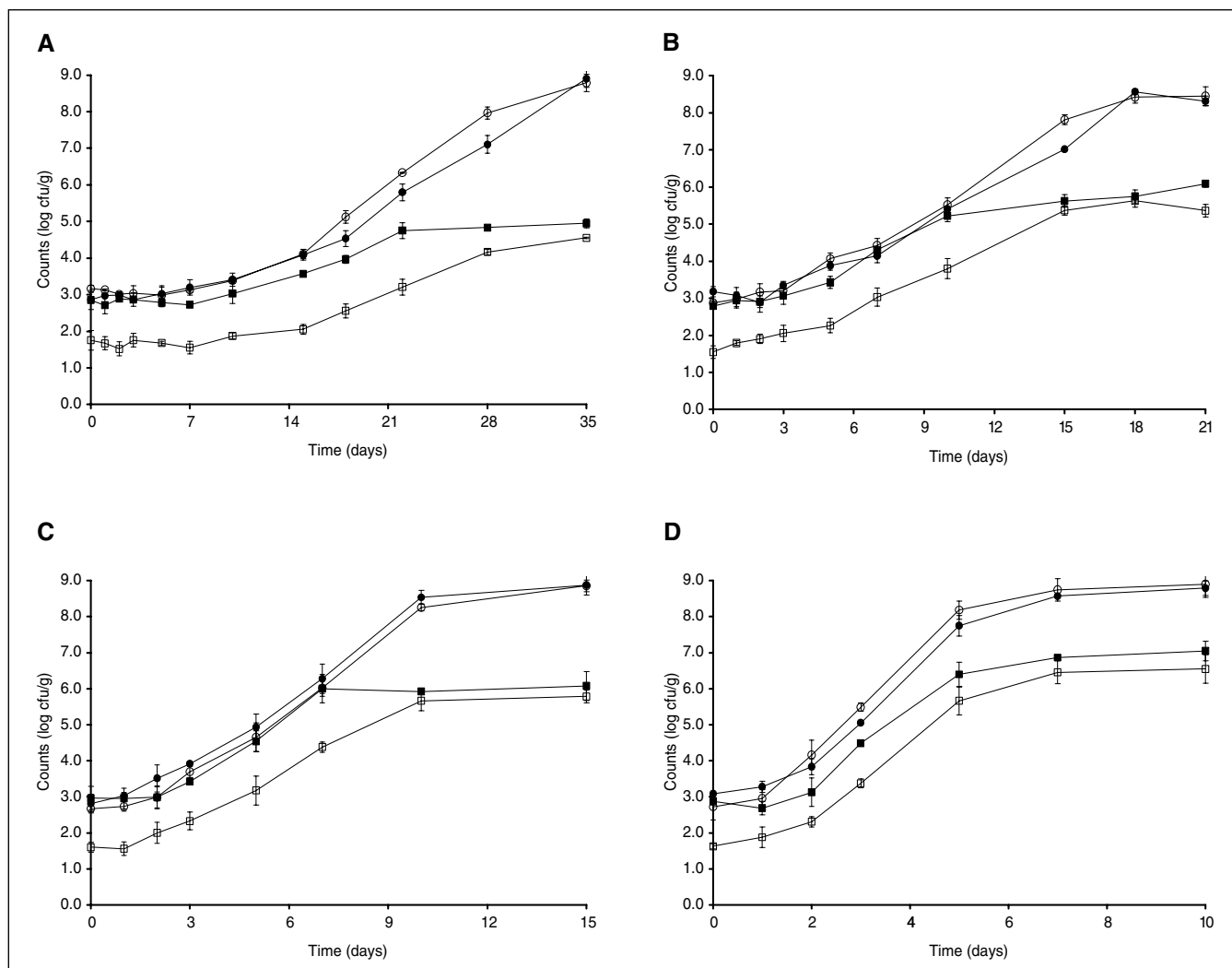


Figure 1—Growth of *L. monocytogenes* and native microflora in smoked salmon stored at 4°C (A), 8°C (B), 12°C (C), and 16°C (D); native microflora (\square) in samples inoculated with a low initial *L. monocytogenes* inoculum (\square), and native microflora (\bullet) in samples inoculated with a high initial *L. monocytogenes* inoculum (\blacksquare).

higher at higher storage temperatures. The growth of *L. monocytogenes* appeared to be more active at higher storage temperatures and be more competitive against the native microflora.

Modeling the growth characteristics and relation of *L. monocytogenes* and native microflora

Parameter coefficients for the fitted linear and quadratic equations describing the LPD, GR, and MPD of *L. monocytogenes* and native microflora as a function of storage temperature are presented in Table 2. For examples, the mathematical equations that describe the LPD and GR of *L. monocytogenes* at an initial level of 1.6 log₁₀ CFU/g in smoked salmon are:

$$\text{LPD} = 444.63 - 58.26 \times (\text{temperature}) + 2.09 \times (\text{temperature})^2$$

$$\sqrt{\text{GR}} = 0.0529 + 0.0110 \times (\text{temperature})$$

$$\text{MPD} = 3.6500 + 0.1800 \times (\text{temperature}).$$

The regression coefficients ($R^2 > 0.90$) indicate that storage temperature is a significant factor in influencing the LPD, GR, and MPD of *L. monocytogenes* and native microflora in smoked salmon (Table 2). The GR of *L. monocytogenes*, in square root transformation, in smoked salmon as a linear function of the growth temperatures is in the same form as the square root model proposed by Ratkowsky and others (1983). The linear relationship between the GR and growth temperatures allows predictions for temperatures below the maximum growth temperature, 40 °C (Ratkowsky and others 1983). These models describe the growth characteristics of *L. monocytogenes* and native microflora in smoked salmon as affected by storage temperatures at 4 to 16 °C, and enable the estimation of LPD, GR, and MPD of *L. monocytogenes* and native microflora (shelf life) in smoked salmon at temperature 4 to 16 °C. With the estimated LPD, GR, and MPD, the levels of *L. monocytogenes* or native microflora (N_t , log₁₀ CFU/g) in a smoked salmon after time t (h) can be estimated by the followings:

$$N_t = N_0, \text{ if } t \leq \text{LPD}, \text{ or}$$

$$N_t = N_0 + (t - \text{LPD}) \times \text{GR}, \text{ or}$$

$$N_t = \text{MPD}, \text{ if } N_t > \text{MPD}.$$

where N_0 = initial population levels, and N_t = population levels at time t (h).

Although the growth behavior of *L. monocytogenes* in smoked salmon have been studied and modeled, it is often difficult to compare data or models among different studies due to the variations in product formulations and characteristics (pH, a_w , pheno-

lic compounds, lactate, nitrite, dissolved CO₂), strains of *L. monocytogenes*, and the factors examined or different factors included in models (Cornu and others 2006). In addition, it is often difficult to determine the level of some factors, for example, dissolved CO₂, in smoked salmon and select results from applicable studies or models to estimate the growth behavior of *L. monocytogenes* in a particular smoked-salmon product. Therefore, instead of modeling the growth behaviors of *L. monocytogenes* in smoked salmon as a function of the product or environmental factors, models described the growth relationship between *L. monocytogenes* and native microflora were developed. Storage temperature, salt, a_w , pH, and smoke compound have been reported to affect the growth of *L. monocytogenes* and spoilage microorganisms in smoked salmon (Leroi and Joffraud 2000). Therefore, it is assumed that the product and/or environmental factors in a smoked salmon affect the growth of native microorganisms and *L. monocytogenes* in a similar pattern, and hence a growth relationship existed between *L. monocytogenes* and native microflora. In the present study, the LPD and GR of *L. monocytogenes* (L_m) as a function of those of native microflora (n_a) at storage temperatures 4 to 16 °C are:

$$\text{LPD}_{L_m} = 8.2 + 1.02 \times (\text{LPD}_{n_a}) (R^2 = 0.85)$$

$$\text{GR}_{L_m} = 0.01 + 0.96 \times (\text{GR}_{n_a}) (R^2 = 0.94)$$

The intercept parameter in both regressions is not significant ($P > 0.05$), while the coefficients for LPD_{na} and GR_{na} are significant. The models indicate that the LPD and GR of *L. monocytogenes* are a factor of 1.02 and 0.96, respectively, of those of the native microflora. The approximately 1:1 ratio in LPD and GR of *L. monocytogenes* to native microflora indicates that LPD and GR of *L. monocytogenes* are similar to those of native microflora in smoked salmon at 4 to 16 °C. Gimenez and Dalgaard (2004) examined the simultaneous growth of *L. monocytogenes* and native microorganisms in smoked salmon, and reported that the growth of *L. monocytogenes* was as fast as the spoilage microorganisms in vacuum-packed smoked salmon stored at 5 to 25 °C. Comparing the predicted GR of *L. monocytogenes* and native microflora (as a function of temperature) from this study to the observed values reported by Gimenez and Dalgaard (2004) (Table 3), the predicted GR are generally higher than the observed values for *L. monocytogenes* and native microflora at temperatures 2, 5, and 10 °C, and were lower at 17.5 °C. In Gimenez and Dalgaard's study, smoked salmon with a relatively high concentration of phenolic compounds (12.5 ppm) than those used in this study (4 ppm) was used. The lower concentration of phenolic compounds in smoked salmon used in this study may contribute to the higher observed and predicted GR of *L. monocytogenes* and native microflora in this study. Although different GR were reported by these 2 studies, the GR ratio of *L.*

Table 1 – Means of LPD, GR, and MPD (standard deviation) of *L. monocytogenes* and native microflora in smoked salmon stored at 4, 8, 12, and 16 °C.

Microorganisms	Temperature (°C)	LPD (h)		GR (log ₁₀ CFU/h)		MPD (log ₁₀ CFU/g)	
		Low ^a	High ^b	Low	High	Low	High
Native microflora	4	243 (75)	257 (3)	0.0108 (0.0012)	0.0102 (0.0003)	8.8 (0.1)	8.5 (0.1)
	8	88 (12)	72 (26)	0.0177 (0.001)	0.0164 (0.0011)	8.6 (0)	8.5 (0.1)
	12	69 (12)	50 (5)	0.0313 (0.0027)	0.0293 (0.0027)	8.8 (0.1)	8.8 (0.1)
	16	35 (4)	29 (13)	0.0562 (0.0013)	0.0565 (0.0024)	8.7 (0.1)	8.7 (0.1)
<i>L. monocytogenes</i>	4	254 (21)	282 (7)	0.0110 (0.0007)	0.0109 (0.0014)	5.2 (0.1)	4.9 (0)
	8	86 (16)	115 (22)	0.0163 (0.0005)	0.0189 (0.0031)	5.4 (0)	5.8 (0.1)
	12	71 (29)	63 (21)	0.0319 (0.0032)	0.0292 (0.0016)	5.5 (0.2)	5.9 (0.1)
	16	38 (14)	35 (1)	0.0535 (0.0018)	0.0504 (0.0002)	6.6 (0)	6.9 (0.1)

^aLow initial *L. monocytogenes* inoculum.

^bHigh initial *L. monocytogenes* inoculum.

Table 2 – Coefficients and regression coefficients (R^2) for the LPD, GR, and MPD regressions.

Microorganisms	Parameter coefficients			R^2
	Intercept	Temperature	Temperature ²	
LPD				
Native microflora (L ^a)	419.88	-53.73	1.88	0.85
<i>L. monocytogenes</i> (L)	444.63	-58.26	2.09	0.92
Native microflora (H ^b)	458.13	-61.98	2.24	0.90
<i>L. monocytogenes</i> (H)	495.50	-63.24	2.17	0.98
GR				
Native microflora (L)	0.0458	0.0120	-	0.96
<i>L. monocytogenes</i> (L)	0.0529	0.0110	-	0.96
Native microflora (H)	0.0462	0.0113	-	0.97
<i>L. monocytogenes</i> (H)	0.0605	0.0099	-	0.97
MPD^c				
<i>L. monocytogenes</i> (L)	3.6500	0.1800	-	0.96
<i>L. monocytogenes</i> (H)	4.5500	0.1337	-	0.91

^aLow initial *L. monocytogenes* inoculum.

^bHigh initial *L. monocytogenes* inoculum.

^cThere was no fitted equation for MPD of native microflora due to the similar MPD levels reached at 4 to 16 °C.

Table 3 – Predicted and observed growth rates for *L. monocytogenes* and native microorganisms in smoked salmon.

Temperature (°C)	Growth rate (log ₁₀ CFU/h)			
	<i>L. monocytogenes</i>		Native microflora	
	Predicted	Observed ^a	Predicted	Observed ^a
2	0.0023	0.0015	0.0054	0.001
5	0.0073	0.0037	0.0121	0.0042
10	0.0219	0.0180	0.0291	0.016
17.5	0.0584	0.0710	0.0683	0.065

^aGimenez and Dalgaard (2004).

monocytogenes and native microflora in both studies were all at approximately 1:1, indicating that the GR of *L. monocytogenes* and native microflora in smoked salmon, regardless of product formulation, are similar. Therefore, the growth of *L. monocytogenes* in smoked salmon may be estimated from the growth of native microflora. This provides an additional approach in estimating the growth of *L. monocytogenes* in smoked salmon. The levels of native microflora in smoked salmon during storage determine the shelf life of the product; therefore, data regarding levels of native microflora in a particular smoked salmon product during prevailing storage conditions are generally available. The relationship models may allow the estimation of LPD and GR of *L. monocytogenes* in any smoked salmon, regardless of the product formulation and storage conditions, when the growth data of native microflora are known. Additional studies using smoked salmon with significantly different product formulation/characteristics to confirm the similar growth characteristics between *L. monocytogenes* and native microflora in smoked salmon are warranted.

Conclusions

Models describing the LPD and GR of *L. monocytogenes* and native microflora in smoked salmon stored at 4 to 16 °C were developed and may be used to estimate the growth of both microflora in smoked salmon with product characteristics similar to the one used in this study. The growth characteristics (LPD and GR) of *L. monocytogenes* in smoked salmon were similar to those of native microflora. The growth relationship between *L. monocytogenes* and native microflora may be used to estimate the growth of *L. monocytogenes*, regardless of product and/or environmental

factors, when the growth characteristics of native microflora are available.

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References

- Amezquita A, Brashears MM. 2002. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. *J Food Prot* 65:316–25.
- Augustin JC, Carlier V. 2000. Modeling the growth rate of *Listeria monocytogenes* with a multiplicative type model including interactions between environmental factors. *Int J Food Microbiol* 56:53–70.
- Cornu M, Beaufour A, Rudelle S, Laloux L, Bergis H, Miconnet N, Serot T, Delignette-Muller ML. 2006. Effect of temperature, water-phase salt and phenolic contents on *Listeria monocytogenes* growth rates on cold-smoked salmon and evaluation of secondary models. *Int J Food Microbiol* 106:159–68.
- Cortesi ML, Sarli T, Santoro A, Murru N, Pepe T. 1997. Distribution and behavior of *Listeria monocytogenes* in three lots of naturally contaminated vacuum-packed smoked salmon stored at 2 and 10 °C. *Int J Food Microbiol* 37:209–14.
- Dalgaard P, Jorgensen LV. 1998. Predicted and observed growth of *Listeria monocytogenes* in seafood challenge tests and in naturally contaminated cold-smoked salmon. *Int J Food Microbiol* 40:105–15.
- Duffes F. 1999. Improving the control of *Listeria monocytogenes* in cold smoked salmon. *Trend Food Sci Technol* 10:211–6.
- Embarek PKB. 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. *Int J Food Microbiol* 23:17–34.
- Ericsson H, Eklow A, Danielsson-Tham ML, Loncarevic S, Mentzing LO, Persson I, Unnerstad H, Tham W. 1997. An outbreak of listeriosis suspected to have been caused by rainbow trout. *J Clinical Microbiol* 35:2904–7.
- FAO/WHO. 2004. Risk Assessment of *Listeria monocytogenes* in ready-to-eat foods. Technical report. Microbiological risk assessment series 5. Food Safety Dept., Geneva, Switzerland: World Health Organization.
- [FDA/USDA/CDC] Food and Drug Administration/United States Department of Agriculture/Centers for Disease Control and Prevention. 2003. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Available from: <http://www.foodsafety.gov/~dms/lmr2-toc.html>.
- Gimenez B, Dalgaard P. 2004. Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage microorganisms in cold-smoked salmon. *J Appl Microbiol* 96:96–109.
- Gombas D, Chen Y, Rocelle SC, Scott VN. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. *J Food Prot* 66:559–69.
- Hwang CA. 2007. Effect of salt, smoke compound, and storage temperature on the growth of *Listeria monocytogenes* in simulated smoked salmon. *J Food Prot* 70:2321–8.
- Jorgensen LV, Huss HH. 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. *Int J Food Microbiol* 42:127–31.
- Jorgensen LV, Dalgaard P, Huss HH. 2000. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *J Agric Food Chem* 48:2448–53.
- Katla T, Moretro T, Aasen IM, Holck A, Axelsson L, Naterstad K. 2001. Inhibition of *Listeria monocytogenes* in cold smoked salmon by addition of sakacin P and/or live *Lactobacillus sakei* culture. *Food Microbiol* 18:431–9.
- Lappi VR, Ho A, Gall K, Wiedmann M. 2004. Prevalence and growth of *Listeria* on naturally contaminated smoked salmon over 28 days of storage at 4 °C. *J Food Prot* 67:1022–6.
- Lebois M, Conni N, Onno B, Prevost H, Doussat X. 2004. Effect of divercin V41 combined to NaCl content, phenol (liquid smoke) concentration and pH on *Listeria monocytogenes* Scott A growth in BHI broth by an experimental design approach. *J Appl Microbiol* 96:931–7.
- Leroi F, Joffraud JJ, Chevalier F, Cardinal M. 2001. Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. *J Appl Microbiol* 90:578–97.
- Leroi F, Joffraud JJ. 2000. Salt and smoke simultaneously affect chemical and sensory quality of cold-smoked salmon during 5 °C storage predicted using factorial design. *J Food Prot* 63:1222–7.
- Mejlholm O, Dalgaard P. 2007. Modeling and predicting the growth boundary of *Listeria monocytogenes* in lightly preserved seafood. *J Food Prot* 70:70–84.
- Membre JM, Thurette J, Cateau M. 1997. Modelling the growth, survival and death of *Listeria monocytogenes*. *J Appl Microbiol* 82:345–50.
- Nilsson L, Gram L, Huss HH. 1999. Growth control of *Listeria monocytogenes* on cold-smoked salmon using a competitive lactic acid bacteria flora. *J Food Prot* 62:336–42.
- Norton DM, McCamey MA, Gall KL, Scarlett JM, Boor KJ, Wiedmann M. 2001. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry. *Appl Environ Microbiol* 67:198–205.
- Pleasant A, Soboleva TK, Dykes GA, Jones RJ, Filippov AE. 2001. Modelling of the growth of populations of *Listeria monocytogenes* and a bacteriocin-producing strain of *Lactobacillus* in pure and mixed cultures. *Food Microbiol* 18:605–15.
- Ratkowsky DA, Lowry RK, McMeekin TA, Stoke AN, Chandler RE. 1983. Model for bacteria culture growth rate throughout the entire biokinetic temperature range. *J Bacteriol* 154:1222–6.
- Rorvik LM. 2000. *Listeria monocytogenes* in the smoked salmon industry. *Int J Food Microbiol* 62:183–90.
- Thurette J, Membre JM, Ching LH, Tailliez R, Cateau M. 1998. Behavior of *Listeria* spp. in smoked fish products affected by liquid smoke, NaCl concentration, and temperature. *J Food Prot* 61:1475–9.

- Tome E, Gibbs PA, Teixeira PC. 2007. Could modification of processing parameters enhance the growth and selection of lactic acid bacteria in cold-smoked salmon to improve preservation by natural means? *J Food Prot* 70:1607–14.
- Vitt SM, Himelbloom BH, Crapo CA. 2001. Inhibition of *Listeria innocua* and *Listeria monocytogenes* in a laboratory medium and cold-smoked salmon containing liquid smoke. *J Food Safety* 21:111–25.
- Yamazaki K, Inoue N, Montville TJ, Suzuki M, Kawai Y. 2003. Inhibition of *Listeria monocytogenes* in cold-smoked salmon by *Carnobacterium piscicola* CS526 isolated from frozen surimi. *J Food Prot* 66:1420–5.
- Yoon KS, Burnette CN, Abou-Zeid KA, Whiting RC. 2004. Control of growth and survival of *Listeria monocytogenes* on smoked salmon by combined potassium lactate and sodium diacetate and freezing stress during refrigeration and frozen storage. *J Food Prot* 67:2465–71.
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