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Effect of Salt, Smoke Compound, and Storage Temperature on the Growth of *Listeria monocytogenes* in Simulated Smoked Salmon†

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ABSTRACT

Smoked salmon can be contaminated with *Listeria monocytogenes*. It is important to identify the factors that are capable of controlling the growth of *L. monocytogenes* in smoked salmon so that control measures can be developed. The objective of this study was to determine the effect of salt, a smoke compound, storage temperature, and their interactions on *L. monocytogenes* in simulated smoked salmon. A six-strain mixture of *L. monocytogenes* (10^2 to 10^3 CFU/g) was inoculated into minced, cooked salmon containing 0 to 10% NaCl and 0 to 0.4% liquid smoke (0 to 34 ppm of phenol), and the samples were stored at temperatures from 0 to 25°C. Lag-phase duration (LPD; hour), growth rate (GR; log CFU per hour), and maximum population density (MPD; log CFU per gram) of *L. monocytogenes* in salmon, as affected by the concentrations of salt and phenol, storage temperature, and their interactions, were analyzed. Results showed that *L. monocytogenes* was able to grow in salmon containing the concentrations of salt and phenol commonly found in smoked salmon at the prevailing storage temperatures. The growth of *L. monocytogenes* was affected significantly ($P < 0.05$) by salt, phenol, storage temperature, and their interactions. As expected, higher concentrations of salt or lower storage temperatures extended the LPD and reduced the GR. Higher concentrations of phenol extended the LPD of *L. monocytogenes*, particularly at lower storage temperatures. However, its effect on reducing the GR of *L. monocytogenes* was observed only at higher salt concentrations (>6%) at refrigerated and mild abuse temperatures (<10°C). The MPD, which generally reached 7 to 8 log CFU/g in salmon that supported *L. monocytogenes* growth, was not affected by the salt, phenol, and storage temperature. Two models were developed to describe the LPD and GR of *L. monocytogenes* in salmon containing 0 to 8% salt, 0 to 34 ppm of phenol, and storage temperatures of 4 to 25°C. The data and models obtained from this study would be useful for estimating the behavior of *L. monocytogenes* in smoked salmon.

Smoked salmon products are a ready-to-eat fish product and are commonly sold in vacuum packages with a refrigerated shelf life of 3 to 8 weeks. Smoked salmon, whole or sliced, is produced by a process mainly consisting of salting, smoking, trimming, or slicing the fish and vacuum packaging the final product. Salting is commonly done by applying 2 to 4% dry salt to the fish or by submerging or injecting the fish with a 70 to 80% brine solution. After salting, the fish or fillets are smoked in a smoke chamber at temperatures of 20 to 30°C for 3 to 4 days (cold smoking) or at temperatures >60°C for 6 to 10 h (hot smoking), depending on the size of the salmon. The composition of smoked salmon varies because of the different formulations, ingredients, and processes used in producing the product. Generally, smoked salmon contains approximately 65 to 78% water, 8 to 14% lipids, 16 to 22% proteins, 2 to 4.6% salt in the water phase, and 0.2 to 1.5 mg of phenol per 100 g of flesh *(7)*. The concentrations of salt and phenol correspond to approximately 2.6 to 7.1% salt and 2 to 15 ppm of phenol in the product. The pH and water activity (a_w) of smoked salmon are in the range of pH 5.9 to 6.3 and a_w 0.95 to 0.98. The total microbial counts in cold-smoked salmon right after packaging were approximately 10^3 to 10^4 CFU/g and are predominantly gram-negative and lactic acid bacteria *(7)*.

If smoked salmon is not processed and handled properly, it can be contaminated with *Listeria monocytogenes*. Cold-smoked salmon products tested positive for *L. monocytogenes* in 9.2 to 13.8% of 165 retail samples collected in a study in Italy in 1997 *(4)*. A study in Denmark showed that 34 to 43% of 280 cold-smoked salmon samples collected from various production sites were positive for the presence of *L. monocytogenes*, and the prevalence differed greatly, ranging from <1.4 to 100%, among various production sites *(13)*. Levels of *L. monocytogenes* recovered from contaminated smoked salmon were generally low at <10 CFU/g and seldom exceeded 100 CFU/g, and the majority of the recovered strains were identified as serogroup 1/2 *(4, 18)*.

Although often contaminated with *L. monocytogenes*, products of smoked salmon, or smoked fish in general, are rarely implicated in foodborne listeriosis. One listeriosis outbreak between August 1994 and June 1995 in Sweden was linked to eating smoked rainbow trout or salmon. This

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outbreak resulted in nine cases of listeriosis and two deaths *(22)*. Risk assessments that evaluated the risk of *L. monocytogenes* in sliced and vacuum-packed cold-smoked salmon indicated that there was an elevated risk of listeriosis caused by consuming smoked salmon that had been contaminated with *L. monocytogenes (8, 24)*. Because of the high possibility of smoked salmon contamination with *L. monocytogenes,* the potential growth of this pathogen in this product has been the subject of many studies: in naturally contaminated or inoculated smoked salmon during storage at refrigerated and mild abuse temperatures *(10, 13, 20, 25)*. After reviewing 26 challenge and 13 storage trials examining the growth of *L. monocytogenes* in cold-smoked salmon, Dalgaard and Jorgensen *(5)* concluded that the salt and moisture contents, pH of smoked salmon, and prevailing storage temperatures for this product were supportive to the growth of *L. monocytogenes* in smoked salmon. Studies have also been conducted to evaluate and model the effect of salt, a_w, smoke compounds, pH, lactate, lactic acid bacteria, native microflora, and storage temperatures on the growth of *L. monocytogenes* in microbiological cultural media and smoked salmon *(1, 2, 14, 16, 17, 23)*. Membre et al. *(17)* examined the growth, survival, and death of *L. monocytogenes* as a function of storage temperature (4, 8, and 12°C), salt concentration (2, 3, and 4% [wt/vol]), and phenol concentration (5, 12.5, and 20 ppm) in *Listeria* enrichment broth (pH 6.2) and reported a significant inactivation of *L. monocytogenes* (a ~2.5-log CFU/ml reduction in 300 h) with 4% salt and 20 ppm of phenol at temperatures from 4 to 12°C. Also with *Listeria* enrichment broth, Thurette et al. *(23)* reported that the growth of *L. monocytogenes* was inhibited by smoke compounds and an interaction between smoke compounds and storage temperature. Gimenez and Dalgaard *(9)* reported that salt and a_w, smoke compounds, lactic acid bacteria, storage temperature, and to a lesser extent, lactate and product pH controlled the growth of *L. monocytogenes* in vacuum-packed cold-smoked salmon. However, there were variations in lag-phase durations (LPDs) and growth rates (GRs) of *L. monocytogenes* in smoked salmon reported by various studies. Hudson and Mott *(11)* observed different lag and generation times between those obtained in their study and those of Rorvik et al. *(19)* and attributed the differences to the variation of *L. monocytogenes* strains, the composition of the smoked salmon, or the incubation temperatures used in these studies. Similarly, Cornu et al. *(3)* examined the effect of salt and smoke compounds on the GRs of *L. monocytogenes* in cold-smoked salmon in challenge tests and compared the data with several models that considered salt and phenol contents, pH, lactate, and dissolved CO_2. They reported that there were significant variations between the reported and predicted growth of *L. monocytogenes* and attributed the variations to the differences in product properties (salt and a_w, smoke compounds, and pH), test strains of *L. monocytogenes,* and native microflora in the samples.

Although studies have examined the effect of salt and smoke compounds on *L. monocytogenes,* the concentrations of salt and smoke compounds examined were limited because of sample availability, or the studies were conducted in microbiological cultural media. The objective of this study was to examine the effect of a wider range of concentrations of salt, smoke compounds, storage temperature, and their interactions on the growth of *L. monocytogenes* in a simulated smoked salmon. This study used cooked salmon with added salt and liquid smoke to obtain a wider range and more precise concentrations of both additives for study. The storage temperatures in this study were from 0 to 25°C to include the normal refrigerated storage temperatures for smoked salmon and abuse temperatures that the products were likely to be exposed to during distribution and storage.

MATERIALS AND METHODS

***L. monocytogenes* and inoculum preparation.** Seven strains of *L. monocytogenes* (NFP7459, serotype 3b; NFP7533, serotype 4b; NFP7554, serotype 1/2b; NFP7712, serotype 1/2a; NFP7735, serotype 1/2a; NFP7821, serotype 1/2b; and NFP7779, serotype 2/1a) from the culture collection of the Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, were used in this study. These strains were isolated from smoked seafood in a survey study conducted by the Food Processors Association *(10)*. Each strain was transferred from −80°C stock culture into 10 ml of brain heart infusion broth (Difco, Becton Dickinson, Sparks, Md.), and the broth was incubated at 37°C for 6 h without shaking. A loopful of cell suspension of each strain was then transferred to 10 ml of fresh brain heart infusion broth and incubated at 37°C for 24 h. One milliliter of cell suspension from each strain was mixed together. The mixture was then further diluted with sterile 0.1% (wt/vol) peptone water to an *L. monocytogenes* level that was approximately 10^4 to 10^5 CFU/ml for use as an inoculum.

Experimental design. A central composite design was used to select the concentrations of salt and liquid smoke (phenol) and the storage temperatures for testing. The concentrations for salt and phenol were 0 to 10% and 0 to 34 ppm (0 to 0.4% liquid smoke), respectively, in cooked salmon, and the storage temperatures were between 0 and 25°C. A total of 15 combinations (treatments 1 to 15, Table 1) were selected by the experimental design. In addition, three treatments at 4, 8, and 12°C (treatments 16 to 18, Table 1) were examined to obtain additional data for storage temperatures at refrigerated and mild abuse temperatures. The experiment was performed in two separate trials, with two samples prepared for each sampling interval during each trial.

Sample preparation. Raw fresh salmon fillets were purchased from a local grocery store. To obtain moisture content similar to that of cold-smoked salmon, the fillets were frozen at −80°C for 3 days and thawed at refrigerated temperature. The liquid that came out of the fillet during the thawing was drained. Fillets were then cut into pieces, placed in stomacher bags (Spiral Biotech, Inc., Norwood, Mass.), and cooked in a water bath to a product temperature of 63°C and held for 30 min to inactivate the native microflora. Cooked salmon pieces were drained, cooled at 4°C overnight, and then flaked in the bags to near puree. According to the estimated sampling frequency for each storage temperature, appropriate amounts (100 to 200 g) of salmon puree were placed in stomacher bags and mixed with the amounts of salt and liquid smoke (Charsol-PN-9, Red Arrow Products Co., Manitowoc, Wis.), as indicated in Table 1, and then the bags were stomached for 2 min. The pH of the samples was 6.3 to 6.6, and the a_w was 0.91 to 0.97. Five grams of sample was placed into 100-

TABLE 1. *Treatments for examining the growth of* L. monocytogenes *in simulated smoked salmon containing 0 to 10% salt and 0 to 34 ppm of phenol and stored at 0 to 25°C*

Treatment no.	Storage temp (°C)	Salt (%)	Phenol (ppm)
1	0	5	17.0
2	5.1	2	6.9
3	5.1	8	6.9
4	5.1	2	27.1
5	5.1	8	27.1
6	12.5	5	0
7	12.5	5	34.0
8	12.5	0	17.0
9	12.5	10	17.0
10[a]	12.5	5	17.0
11	19.9	2	6.9
12	19.9	8	6.9
13	19.9	2	27.1
14	19.9	8	27.1
15	25	5	17.0
16	4	5	17.0
17	8	5	17.0
18	12	8	27.1

[a] The central point of the central composite design. This treatment was repeated six times.

ml stomacher bags (Spiral Biotech), and the bags were inoculated with 50 μl of the *L. monocytogenes* inoculum to achieve an inoculum level of 10^2 to 10^3 CFU/g. The bags were vacuum sealed to −98,000 Pa with a Multivac A300 vacuum sealer (Multivac Inc., Kansas City, Mo.) and stored at the selected storage temperatures (Table 1) for up to 6 weeks.

Enumeration of *L. monocytogenes*. Throughout the studies, levels of *L. monocytogenes* in samples were enumerated. To each bag, 5 ml of sterile 0.1% peptone water was added, and the bags were stomached for 1 min. Additional dilutions were prepared in sterile 0.1% peptone water. Duplicate sample dilutions (50 μl) were spread plated onto modified Oxford agar (Oxoid Ltd., Hampshire, UK) plates. Plates were incubated at 37°C for 48 h, and black colonies surrounded by black precipitation were counted. If needed, colonies recovered from samples were confirmed with an API test strip (bioMérieux, Marcy l'Etoile, France). At each sampling time, two samples from each treatment were enumerated.

Estimation of LPD, GR, and MPD of *L. monocytogenes*. Growth curves of *L. monocytogenes* (log CFU per gram of *L. monocytogenes* versus storage time) in samples were fitted with the DMFit curve-fitting software (http://www.ifr.ac.uk/Safety/DMfit/default.html) to estimate the lag-phase durations (LPDs; hour), growth rates (GRs; log CFU per hour), and maximum population densities (MPDs; log CFU per gram) of *L. monocytogenes*. Because *L. monocytogenes* was not able to grow in salmon containing 10% salt, the growth curves obtained from these samples were not analyzed. The GRs were transformed into square roots to stabilize the variance, as described by Devlieghere et al. *(6)*. The LPDs, square roots of GRs, and MPDs of *L. monocytogenes* in salmon samples of various treatments were analyzed by the General Linear Models procedure of the Statistical Analysis System (SAS) 9.1 for Windows (SAS Institute Inc., Cary, N.C.) as a function of concentrations of salt (percent) and smoke compounds (phenol, parts per million), storage temperature, and their interactions with the following quadratic response surface equation:

$$\begin{aligned}\text{LPD, } \sqrt{\text{GR}}\text{, or MPD} &= \alpha + \beta1(\text{temperature}) + \beta2(\text{phenol}) + \beta3(\text{salt\%}) \\ &+ \beta4(\text{temperature} \times \text{phenol}) + \beta5(\text{temperature} \times \text{salt\%}) \\ &+ \beta6(\text{phenol} \times \text{salt\%}) + \beta7(\text{temperature})^2 \\ &+ \beta8(\text{phenol})^2 + \beta9(\text{salt\%})^2.\end{aligned}$$

where α is the intercept, and β1 through β9 are the estimated coefficients for each parameter and their interactions.

RESULTS

Effect of salt, phenol, and storage temperature on the growth of *L. monocytogenes*. The ability of *L. monocytogenes* to grow in salmon containing various concentrations of salt and phenol at various storage temperatures, as indicated by LPD (hour), GR (log CFU per hour), and MPD (log CFU per gram), is shown in Table 2. For *L. monocytogenes* that didn't grow in salmon samples, its LPD was reported as >1,000 h, the maximum storage time for the experiment; the GR was reported as 0 (zero); and the MPD was reported as none. The initial inoculum levels of *L. monocytogenes* in salmon were 2 to 3 log CFU/g. In samples that supported its growth, *L. monocytogenes* counts increased to 7 to 8 log CFU/g in all samples, except for those containing 8.0% salt and 6.9 ppm of phenol at 20°C, in which the MPD reached approximately 5.0 log CFU/g after 6 weeks. *L. monocytogenes* was not able to grow in salmon containing 5.0% salt and 17.0 ppm of phenol at 0°C, 8% salt and 27.1 ppm of phenol at 12.5 and 19.9°C, or 10% salt and 17.0 ppm of phenol at 12.5°C. *L. monocytogenes* was unable to grow in salmon containing 8% salt and 27.1 ppm of phenol at 12.5°C, whereas it was able to grow in salmon containing 8% salt and 6.9 ppm of phenol at 5.1°C. These data demonstrated an inhibitory effect of phenol on the growth of *L. monocytogenes*. Comparing the LPD and GR of *L. monocytogenes* in salmon containing 2% salt at 5.1°C, the increase of phenol concentrations from 6.9 to 27.1 ppm increased the LPD from 88 to 155 h and decreased the GR from 0.0177 to 0.0152 log CFU/h. The effect of higher phenol concentrations in extending LPD and reducing GR was also observed in samples containing 8% salt at 5.1°C. In salmon containing the same concentration of phenol (6.9 or 27.1 ppm), the increase of salt concentrations (from 2.0 to 8.0%) also increased the LPD and decreased the GR. At higher storage temperatures (12.5 or 19.9°C), the increase of salt or phenol concentrations in samples generally increased the LPD. However, the effect of higher salt or phenol concentrations in reducing the GR of *L. monocytogenes* as observed at 5.1°C was not observed in samples stored at 12.5 or 19.9°C. As expected, lower storage temperatures generally extended the LPD and reduced the GR of *L. monocytogenes* in salmon containing comparable amounts of salt and phenol.

Effect of salt, phenol, and storage temperature on the LPD of *L. monocytogenes*. The LPD in salmon of various treatments was fitted to the quadratic equation as previously described, and the effect of salt, phenol, storage temperature, and their interactions on the LPD of *L. mono-*

TABLE 2. *LPD, GR, and MPD of* L. monocytogenes *in salmon containing various concentrations of salt and smoked components (phenols) and stored at selected temperatures*

Temp (°C)	Salt (%)	Phenol (ppm)	*n*	LPD (h)	GR (log CFU/h)	MPD (log CFU/g)
0	5.0	17.0	2	>1,000	0	None
5.1	2.0	6.9	2	80.6 (6.5)	0.0177 (0.0006)	8.1 (0.0)
5.1	8.0	6.9	2	200.9 (22.2)	0.0104 (0.0054)	6.7 (1.8)
5.1	2.0	27.1	2	155.2 (0.8)	0.0152 (0.0001)	8.2 (0.1)
5.1	8.0	27.1	2	>1,000	0	None
12.5	5.0	0.0	2	11.9 (0.7)	0.0162 (0.0001)	8.0 (0.0)
12.5	5.0	34.0	2	68.7 (26.7)	0.0184 (0.0049)	7.9 (0.9)
12.5	0.0	17.0	2	37.7 (11.4)	0.0901 (0.0001)	8.0 (0.1)
12.5	5.0	17.0	6	108.5 (23.3)	0.0437 (0.0125)	7.6 (0.5)
12.5	10.0	17.0	2	>1,000	0	None
19.9	2.0	6.9	2	0.0 (0.0)	0.1029 (0.0018)	8.1 (0.1)
19.9	8.0	6.9	2	18.1 (1.9)	0.0324 (0.0038)	4.8 (0.0)
19.9	2.0	27.1	2	5.0 (1.6)	0.1189 (0.0031)	8.0 (0.1)
19.9	8.0	27.1	2	>1,000	0	None
25	5.0	17.0	2	24.9 (5.8)	0.2083 (0.0117)	7.4 (0.4)
4	5.0	17.0	2	210.8 (21.2)	0.0045 (0.0024)	7.8 (0.3)
8	5.0	17.0	2	99.8 (34)	0.1100 (0.0025)	7.6 (0.2)
12	8.0	27.1	2	>1,000	0	None

cytogenes was analyzed by the analysis of variance. The estimated coefficients and corresponding significance levels, as indicated by the *P* values, for each of the parameters in the equation are shown in Table 3. The LPD of *L. monocytogenes* from the experiment is described by the following quadratic equation:

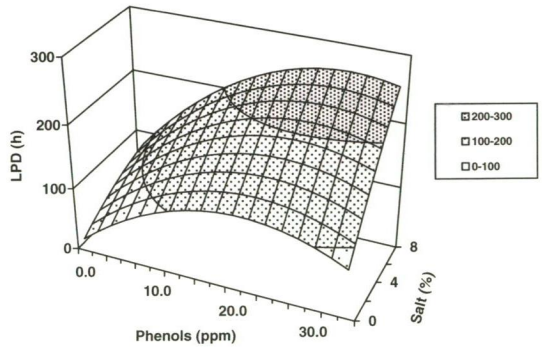
$$\begin{aligned}\text{LPD (h)} = {} & 61.35 - 11.66(\text{temperature}) + 10.16(\text{phenol}) \\ & + 14.75(\text{salt\%}) - 0.15(\text{temperature} \times \text{phenol}) \\ & - 0.86(\text{temperature} \times \text{salt\%}) \\ & + 0.42(\text{phenol} \times \text{salt\%}) \\ & + 0.32(\text{temperature})^2 \\ & - 0.23(\text{phenol})^2 + 0.49(\text{salt\%})^2\end{aligned}$$

The *P* value for the model is <0.001, indicating that at least one of the coefficients for the parameters is not zero; therefore, at least one of the parameters significantly affects the LPD. The correlation coefficient (R^2) for the model is 0.94, indicating that the variation of LPD is contributed largely (94%) by the variation in concentrations of salt and phenol and the storage temperature. Therefore, the model well describes the LPD as a function of the concentrations of salt and phenols, the storage temperature, and their interactions. As indicated by the significance levels (*P* values) for each of the parameters in the model, the concentrations of salt and phenol and the storage temperature significantly ($P < 0.05$) affected the LPD of *L. monocytogenes*. By the model, the estimated LPD of *L. monocytogenes* in salmon containing 12 ppm of phenol and 5% salt would increase 87% from 91 h at 4°C to 171 h at 8°C; the LPD would increase 25% from 146 to 182 h when salt is increased from 3 to 6% in salmon containing 12 ppm of phenol at 4°C; and the LPD would increase 76% from 125 to 220 h when phenol is increased from 5 to 20 ppm in salmon containing 5% salt at 4°C. In addition, the interactions between salt and temperature and the quadratic terms of temperature and phenol significantly affected the LPD. Response surface plots derived from the model to show the effects of salt, phenol, storage temperature, and their interactions on the LPD at three selected storage temperatures (4, 10, and

TABLE 3. *Coefficients and significance levels for each parameter in the LPD and GR equations*

	LPD regression		GR regression		MPD regression	
Parameter	Coefficient	Pr > |*t*|	Coefficient	Pr > |*t*|	Coefficient	Pr > |*t*|
Intercept	61.354	0.0764	−0.0639	0.3534	7.695212	<0.001
Temperature	−11.657	0.0014	0.0137	0.0327	−0.00057	0.9958
Phenol	10.159	<0.0001	0.0103	0.0285	−0.02625	0.7261
Salt	14.752	0.0427	0.0257	0.0105	0.594792	0.1058
Temperature × phenol	−0.147	0.0430	−0.0001	0.6772	0.000215	0.9427
Temperature × salt	−0.863	0.0063	−0.0018	0.0022	−0.01842	0.0826
Phenol × salt	0.423	0.0533	−0.0011	0.0072	0.000175	0.9853
Temperature^2	0.321	0.0059	0.0004	0.0605	0.001302	0.7142
Phenol^2	−0.231	<0.0001	−0.0002	0.1138	0.000686	0.6345
Salt^2	0.485	0.4542	−0.0011	0.3377	−0.07714	0.0145

A.



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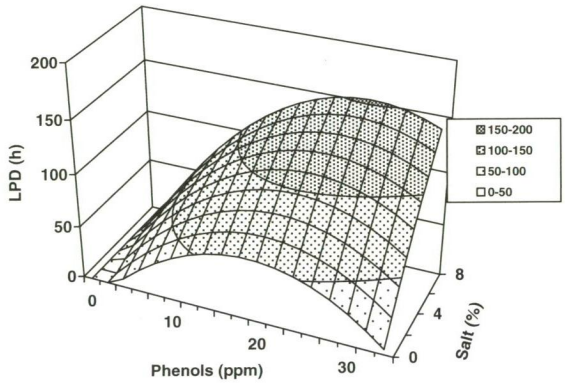


C.

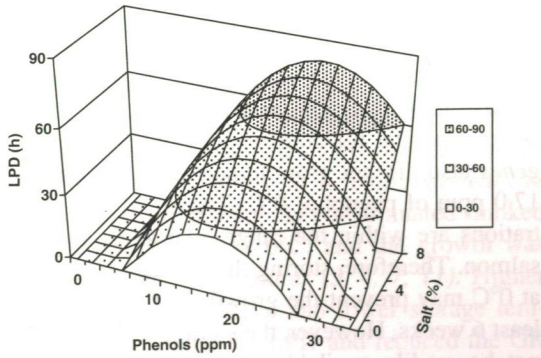


FIGURE 1. *Plots of response surfaces of the LPD model for* L. monocytogenes *in salmon containing 0 to 8% salt and 0 to 34 ppm of phenol and stored at 4 (A), 10 (B), and 15°C (C).*

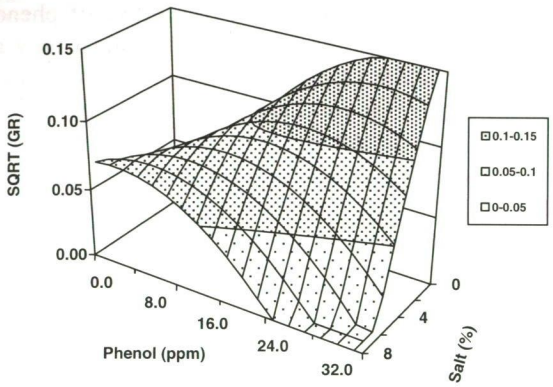
15°C) are shown in Figure 1A through 1C. The temperatures are selected to represent the normal refrigerated storage temperatures for smoked salmon and mild abuse temperatures that the product may be subjected to during distribution and storage. Comparing the LPD of *L. monocytogenes* in salmon containing the same concentrations of salt and phenol, lower storage temperatures extend the LPD. At each storage temperature, the increase of salt concentrations increases the LPD. The effect of phenol concentrations on extending the LPD is evident only at concentrations below 18 ppm. The increase of phenol concentrations above that concentration does not further increase the LPD. The model indicates that higher salt concentrations in salmon and lower storage temperatures extend the LPD of *L. monocytogenes* in smoked salmon, while the effect of higher concentrations of phenol on extending LPD is more significant at concentrations below 18 ppm.

Effect of salt, phenol, and storage temperature on the GR of *L. monocytogenes*. The GR of *L. monocytogenes* in salmon as a function of salt, phenol, storage temperature, and their interaction is described by the following quadratic equation:

$$\begin{aligned}\sqrt{\text{GR}}\ (\log \text{CFU/h}) = &-0.0639 + 0.0137(\text{temperature}) \\ &+ 0.0103(\text{phenol}) + 0.0257(\text{salt\%}) \\ &- 0.0001(\text{temperature} \times \text{phenol}) \\ &- 0.0018(\text{temperature} \times \text{salt\%}) \\ &- 0.0011(\text{phenol} \times \text{salt\%}) \\ &+ 0.0004(\text{temperature})^2 \\ &- 0.0002(\text{phenol})^2 - 0.0011(\text{salt\%})^2\end{aligned}$$

The estimated coefficients and significance levels for each of the parameters in the model are shown in Table 3. The model is significant ($P < 0.001$) and well describes ($R^2 = 0.91$) the GR of *L. monocytogenes* in salmon as affected by the concentrations of salt and phenol, the storage temperature, and their interactions. As indicated by the significance levels for each of the parameters in the model, the concentrations of salt and phenol and the storage temperature significantly ($P < 0.05$) affected the GR of *L. monocytogenes*. Estimated by the model, the GR of *L. monocytogenes* in salmon containing 12 ppm of phenol and 5% salt would decrease 63% from 0.0199 log CFU/h at 4°C to 0.0074 log CFU/h at 8°C; the GR would decrease 29% from 0.0088 to 0.0063 log CFU/h when the salt concentration is increased from 3 to 6% in salmon containing 12 ppm of phenol at 4°C; and the GR would decrease 31% from 0.0058 to 0.0040 log CFU/h when the phenol concentration is increased from 5 to 20 ppm in salmon containing 5% salt at 4°C. The interactions between salt and storage temperature and salt and phenol also significantly affected the GR. Response surface plots derived from the model to show the effects of salt, phenol, storage temperature, and their interactions on the GR at three selected storage temperatures (4, 10, and 15°C) are shown in Figure 2A through 2C. As expected, the increase of salt concentrations in salmon reduces the GR at each storage temperature, and lower storage temperatures reduce the GR in salmon containing the same concentrations of salt and phenol. The effect of phenol on GR is affected by salt concen-

A.



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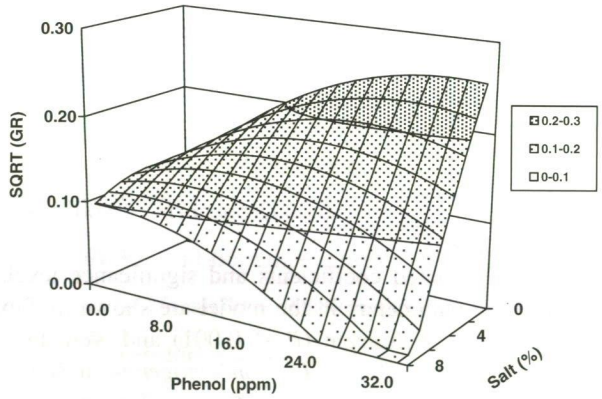

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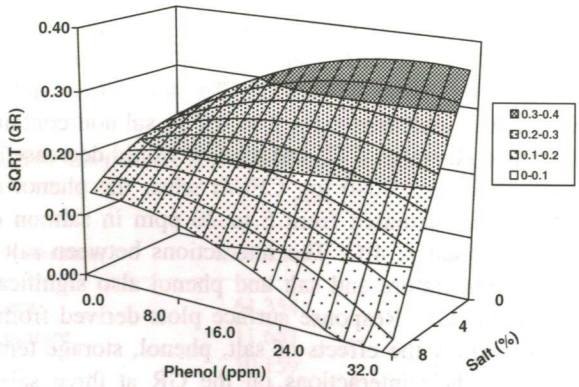

FIGURE 2. *Plots of response surfaces of the GR model for* L. monocytogenes *in salmon containing 0 to 8% salt and 0 to 34 ppm of phenol and stored at 4 (A), 10 (B), and 15°C (C).*

trations, as indicated by the significance of the interaction between phenol and salt. The increase of phenol concentrations in reducing the GR occurs only when the salt concentrations are >6%. At lower salt concentrations, the increase of phenol does not reduce the GR.

Effect of salt, phenol, and storage temperature on the MPD of *L. monocytogenes*. The fitting of the MPD of *L. monocytogenes* as a function of salt, phenol, storage temperature, and their interaction generated the following quadratic equation:

$$\begin{aligned}\text{MPD (log CFU/g)} &= 7.695 - 0.001(\text{temperature}) - 0.026(\text{phenol}) \\ &\quad + 0.595(\text{salt\%}) + 0.001(\text{temperature} \times \text{phenol}) \\ &\quad - 0.018(\text{temperature} \times \text{salt\%}) \\ &\quad + 0.001(\text{phenol} \times \text{salt\%}) + 0.001(\text{temperature})^2 \\ &\quad + 0.001(\text{phenol})^2 - 0.077(\text{salt\%})^2\end{aligned}$$

The estimated coefficients and their significance levels for each of the parameters in the model are shown in Table 3. They indicate that the model does not describe the MPD well. The concentrations of salt and phenol and the storage temperature were not significant in affecting the MPD of *L. monocytogenes*. The only significant parameter for the MPD is the quadratic term of salt ($P < 0.001$). The analysis of variance indicated that when *L. monocytogenes* was able to grow in salmon, regardless of the concentrations of salt and phenol and the storage temperature, its MPD would generally reach 7 to 8 log CFU/g, although the time to reach such levels would vary among treatments.

DISCUSSION

Results from this study showed that *L. monocytogenes* was not able to grow in salmon at a salt concentration of 10%, regardless of the storage temperature, which agreed with the reported growth-limiting salt concentration for *L. monocytogenes (12)*. The use of salt at concentrations >8% to prevent the growth of *L. monocytogenes* would be likely to have an undesirable sensory impact on the product and is not practical for commercial applications. *L. monocytogenes* also did not grow in salmon containing 5% salt and 17.0 ppm of phenol at 0°C. These salt and phenol concentrations are within the range commonly found in smoked salmon. Therefore, storing the product of such formulations at 0°C may prevent the growth of *L. monocytogenes* for at least 6 weeks. However, the storage temperature of 0°C may not be readily available in the product's distribution and storage channels. In this study, the minimum concentration of phenol that prevented the growth of *L. monocytogenes* was 27.1 ppm in salmon that contained 8% salt and was stored at 5.1 to 19.9°C. Because of the effect of salt, a phenol concentration of 27.1 ppm is not likely the minimum concentration that is capable of inhibiting the growth of *L. monocytogenes* in smoked salmon. Estimated minimum phenol concentrations that might prevent the growth of *L. monocytogenes* in smoked salmon have been reported.

Gimenez and Dalgaard *(9)* fitted 16 specific GRs with an extended Ratkowsky square-root model containing storage temperature, a_w, and phenol terms and reported a theoretic minimal growth-inhibitory phenol concentration of 28.1 ± 2.8 ppm. Through a model prediction, Augustin and Carlier *(1)* reported that the minimum concentration of phenol that inhibited the growth of *L. monocytogenes* in cold-smoked salmon was 12.6 ppm. In *Listeria* enrichment broth, *L. monocytogenes* was able to grow in the broth containing 12.5 ppm of phenol but was inactivated by 20 ppm of phenol *(23)*. Apparently, there is not an agreement on the minimal concentration of phenol for inhibiting the growth of *L. monocytogenes* in smoked salmon. Phenol is a major component in smoke compounds, and its concentrations are commonly used to indicate the concentration of smoke compounds on smoked food products. Phenol in smoke compounds consists of various phenolic compounds such as guaiacol, eugenol, and syrigol *(7)*. In addition to phenolic compounds, smoke compounds contain other volatile components such as organic acids, aldehydes, alcohols, and hydrocarbons *(7)*. The differences in the composition of smoke compounds and the ways that smoke compounds are applied to smoked fish affect its effectiveness against *L. monocytogenes*. Sunen *(21)* reported that smoke components derived from liquid or solid smoke had different effects on *L. monocytogenes* and that liquid smoke was more effective against *L. monocytogenes* than solid smoke because of its capacity to penetrate the flesh. The differences in the type of phenolic compounds and volatile compounds likely contribute to the different antimicrobial activity of smoke compounds. Although it is generally agreed that higher phenol concentrations were more inhibitory against *L. monocytogenes,* the concentrations that are capable of inhibiting the growth of *L. monocytogenes* in smoked salmon may be too high to have an acceptable sensory impact. Therefore, the types and concentrations of phenolic compounds in solid or liquid smoke that are effective in inhibiting the growth of *L. monocytogenes* merit further study.

Results from this study showed the growth of *L. monocytogenes* in salmon containing the concentrations of salt and smoke compounds commonly found in commercial smoked salmon. The growth of *L. monocytogenes* in salmon observed in this study confirmed results reported by other studies that showed the ability of *L. monocytogenes* to grow in naturally and experimentally contaminated smoked salmon during storage at 4 to 10°C, and the growth was slower at lower storage temperatures *(3, 4, 7, 11)*. Higher concentrations of salt and phenol and lower storage temperatures generally extended the LPD and reduced the GR of *L. monocytogenes*. The effects of salt and storage temperature on the GRs of *L. monocytogenes* in smoked salmon have also been reported by other researchers. For example, with predictive models, Gimenez and Dalgaard *(9)* reported that higher salt concentrations and lower storage temperatures reduced the GRs of *L. monocytogenes* in smoked salmon. This study also identified a combined (interaction) effect of salt and phenol in reducing the growth of *L. monocytogenes*. Researchers have also reported that salt and smoke compounds act synergistically to extend the microbiological shelf life of vacuum-packed cold-smoked salmon *(9, 14, 15)*.

The GRs of *L. monocytogenes* in salmon obtained in this study were higher than those reported in other studies *(3, 9, 18)* that used products with salt and phenol concentrations and storage temperatures that were comparable to this study. For example, the GR reported in the present study was 0.0104 log CFU/h in salmon containing 8.0% salt and 6.9 ppm of phenol stored at 5.1°C compared with 0.0013 log CFU/h in smoked salmon containing 7.4% salt and 12.6 ppm of phenols stored at 5°C, as reported by Gimenez and Dalgaard *(9)*. The difference in the GRs was likely due to the lack of native or spoilage microflora in samples used in the present study. Native microflora on smoked salmon, mainly lactic acid bacteria, inhibit the growth of *L. monocytogenes*. Gimenez and Dalgaard *(9)* reported that the growth of *L. monocytogenes* in smoked salmon was reduced by 4 to 6 log CFU/g in samples inoculated with spoilage microorganisms (lactic acid bacteria, *Enterobacteriaceae,* and *Photobacterium*) when compared with samples containing no spoilable microorganisms at storage temperatures in the range of 5 to 25°C. A study by Jorgensen and Huss *(13)* also showed that *L. monocytogenes* grew moderately in naturally contaminated cold-smoked fish, whereas it grew more rapidly in hot-smoked fish, because of the comparatively lower native microflora in hot-smoked fish. These results demonstrated the important role of native microflora in smoked salmon, in addition to salt, phenol, and storage temperature, in controlling the growth of *L. monocytogenes* in smoked salmon.

Several models have been proposed for predicting the growth of *L. monocytogenes* in smoked salmon of various product parameters. These models include a modified logistic model with a parameter to address the factor of competition between lactic acid bacteria and *L. monocytogenes* *(9)*; a cardinal model with parameters of storage temperature, a_w, undissociated lactate, and phenol *(1)*; a global model with parameters of storage temperature, a_w, pH, and their interactions *(2)*; and three expanded Ratkowsky square-root models with parameters of salt, pH, lactate, dissolved CO_2, phenol, and storage temperature *(6, 9, 20)*. These models mainly examined the effect of each individual parameter, while the effect of possible interactions between each parameter was not included. In addition, most of these models were developed from smoked salmon that contained high numbers of background microflora. Therefore, these models may not be suitable for smoked salmon with low numbers of background microflora, e.g., hot-smoked salmon. The main objective of the present study was to examine the effect of salt, phenol, and storage temperature, along with their interactions, on *L. monocytogenes* in cooked salmon that had physical, nutritional, and chemical properties similar to smoked salmon and without the interference of background microflora. Therefore, the LPD and GR models derived from this study may be used to estimate the behavior of *L. monocytogenes* in hot-smoked salmon.

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