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Effect of Salt and Smoke on the Microbiological Quality of Cold-Smoked Salmon during Storage at 5°C as Estimated by the Factorial Design Method

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ABSTRACT

The simultaneous effect of salt and smoke on the natural flora of cold-smoked salmon was studied during 5 weeks of vacuum storage at 5°C. The quadratic polynomial, as a function of factors, was used to express total viable count (TVC), total lactic acid bacteria, lactobacilli numerated on Rogosa agar, H_2S-producing bacteria, and yeasts at different sampling times. TVC and total lactic acid bacteria were mainly inhibited by the salt concentration (5% wt/wt) in the meat and to a lesser extent by the phenol content. Inhibition was linearly proportional to salt and smoke content (the higher the concentration, the greater the inhibition). No synergistic effect on inhibition was observed between the two factors. In our working conditions, the TVC French standard ($<10^6$ CFU g^{-1}) was maintained during 4 weeks of storage at 5°C, with a minimum concentration of 2.4% (wt/wt) of salt in meat and smoking treatment corresponding to 0.6 mg 100 g^{-1} of phenol. When the salt level was higher than 3%, the TVC standard was maintained, regardless of phenol level. A negative interaction between the two factors was found for H_2S-producing bacteria and a positive one for yeasts.

Smoking is one of the oldest methods used to preserve fish. The preservative effects result from the three main processing steps: salting, dehydration, and smoking sensu stricto. Salting and dehydration (which occurs during the smoking step) decrease water activity (a_w) and thus prevent the development of bacteria and yeast, which generally cannot grow when a_w is lower than 0.90 *(13)*. Moreover, chloride ions are toxic for some microorganisms that inhibit enzymatic systems. The bacteriostatic and bactericidal properties of smoke are due to phenolic compounds *(1, 3, 6, 28)*, carbonyl compounds *(19)*, and organic acids *(23)*. During the hot-smoking process, the temperature rise (reaching 70 to 100°C) is responsible for the death of almost all the microbial vegetative cells in the raw material *(12)*, whereas temperatures remain low (20 to 30°C) during the cold-smoking process.

Various studies have demonstrated the inhibitory effect of salt or smoke on the growth and toxin production of pathogenic bacteria and on pure culture microorganisms inoculated either into model medium *(14, 18, 25)* or directly into smoked fish *(2, 17, 21)*. Other authors have reported the inhibitory effect of those factors in spoilage flora of Kenya fish *(7)* or cold-smoked salmon *(24, 27)*, but none of those studies have modeled the simultaneous incidence in situ of salting and smoking on fish spoilage flora.

The cold smoking of salmon is an economically important activity in France. Current processing methods involve only small amounts of total phenol (generally below 0.05 ppm) and salt (4 to 5% in water phase), which probably accounts for the problems of early sensory deterioration encountered in these products. The purpose of this study was to estimate the effect of salt and smoke and their interaction on the natural flora contaminating cold-smoked salmon during vacuum storage at 5°C to determine the best combination of these factors acceptable to consumer taste and providing satisfactory shelf life for the product.

MATERIALS AND METHODS

Experimental design. The two factors studied were salt concentration in meat and smoke intensity (as estimated by total phenol concentration). For each factor, four equidistant levels were chosen, according to the concentrations generally found in commercial French products: NaCl (g 100 g^{-1} of finished product [wt/wt]), 0, 1.7, 3.3, and 5%, and phenol (mg 100 g^{-1} of finished product), 0, 0.33, 0.66, and 1. A second-order polynomial model was postulated a priori to fit the different responses (Y) at each sampling time (t) as a function of the salt (%) and phenol (mg 100 g^{-1}) factors. The model took into account the main effect of each factor ($a1$, $a2$), the quadratic effect ($a11$, $a22$), and the interaction between the two factors ($a12$):

$$Y(t) = a0 + a1 \times (\text{salt}) + a2 \times (\text{phenol}) + a11 \times (\text{salt})^2 + a22 \times (\text{phenol})^2 + a12 \times (\text{salt}) \times (\text{phenol})$$

Microbiological responses measured were the total viable count (TVC) and numbers of H_2S-producing bacteria, lactic acid bacteria (LAB), camobacteria, and yeast (expressed in log CFU g^{-1}). These responses were measured weekly during 6 weeks of storage at 5°C.

To estimate regression coefficients, experiments were run using a complete factorial design containing the 16 experiments summarized in Table 1. Two repetitions of experiment 6 were added (experiments 17 and 18) to estimate experimental error. Two points of the experimental domain (experiments 19 and 20)

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TABLE 1. *Experimental design levels of NaCl (% wt/wt) and phenol (mg 100 g^{-1})*

	Theoretical concentrations		Real concentrations[a]	
Experiment	NaCl	Phenol	NaCl	Phenol
1	0.00	0.00	0.20	0.00
2	0.00	0.33	0.20	0.46
3	0.00	0.67	0.20	0.77
4	0.00	1.00	0.20	1.18
5	1.67	0.00	1.65	0.00
6	1.67	0.33	1.57	0.45
7	1.67	0.67	1.72	0.67
8	1.67	1.00	1.75	0.92
9	3.33	0.00	2.91	0.00
10	3.33	0.33	3.56	0.25
11	3.33	0.67	3.42	0.38
12	3.33	1.00	3.44	0.65
13	5.00	0.00	4.72	0.00
14	5.00	0.33	5.02	0.22
15	5.00	0.67	5.52	0.41
16	5.00	1.00	4.81	0.73
17	1.67	0.33	1.87	0.47
18	1.67	0.33	1.74	0.46
19	2.50	0.50	2.90	0.37
20	0.90	0.90	1.44	0.81

[a] Real concentrations are the mean of six measurements at weeks 0, 1, 2, 3, 4, and 6.

not used for the estimation of polynomial coefficients were run to validate the model.

Sample preparation. Twenty-seven fresh-farmed salmons from Norway weighing 3 to 4 kg each were purchased from the market (Vives Eaux, Nantes, France). After filleting, they were analyzed for their lipid content and frozen at −20°C. After 1 week, 40 fillets corresponding to 20 salmons that were relatively homogeneous in their lipid content (10.2 ± 0.9%) were thawed overnight at 4°C, salted with dry salt at 4°C, dried at 22°C with a relative humidity of 70%, and smoked at 22°C with an relative humidity of 70% in a Thirode smoking kiln, with beech and oak shavings. Salting and smoking times had been adjusted in previous experiments to obtain the required salt and phenol levels. The salting times were 0, 1, 2, 6, 10, and 24 h to reach 0, 0.90, 1.67, 2.50, 3.33, and 5.00% (wt/wt), respectively, of salt, and the smoking times were 0, 2, 3, 4, 5, and 6 h to reach 0, 0.33, 0.50, 0.67, 0.90, and 1.00 mg 100 g^{-1}, respectively, of phenol. Fillets were machine sliced (2 mm thick), and 40 g of slices was placed on 0.2 by 0.11-m cardboard plates with a polypropylene metallic surface (tray-compact plates, Ateliers des Landes, Quintin, France). Samples were vacuum-packed (99.8% vacuum) in PA/PE 20/70 polyamide polyethylene bags (Bourdeau, St. Etienne de Montluc, France). At 23°C and 75% relative humidity, the PA/PE film had an oxygen transmission rate of 40 to 50 cm^3/m^2/24 h/1 atm (101.29 kPa) and a CO_2 transmission rate of 146 cm^3/m^2/24 h/1 atm (101.29 kPa). Packages were stored at 5°C for 6 weeks.

Microbiological analysis. Each week, three 40-g bags were opened for each of the 20 experiments. All slices from the three bags were chopped in a Waring blender (New Hartford, Conn.) at high speed for 1 min. A 30-g portion of minced meat was sampled in a sterile plastic bag and blended at high speed for 2 min with 120 ml of physiological water (0.85% NaCl) in a stomacher (Lab.

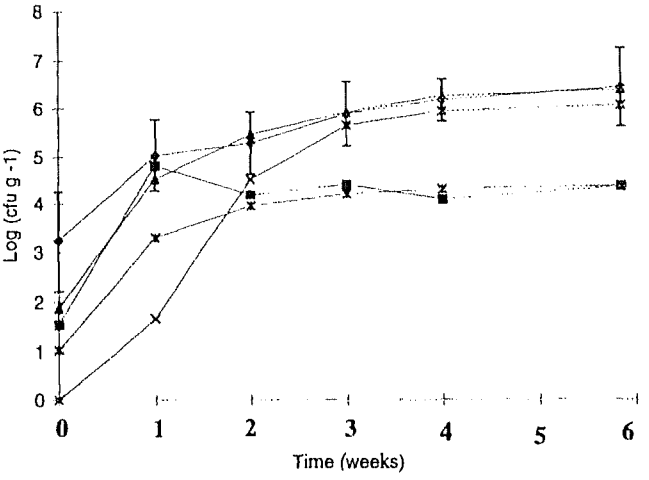

FIGURE 1. *Changes in total viable count (◆), total lactic acid bacteria count (▲), H_2S-producing bacteria (▩), yeasts (*), and lactobacilli (X) in lightly preserved cold-smoked salmon (NaCl = 1.7%, phenol = 0.46 mg 100 g^{-1}) during vacuum storage at 5°C. Means are from the three experiments 6, 17, and 18.*

Blender, London, England), and microbiological analysis was performed as described by Leroi et al. *(16)*. TVC was determined by spreading 0.1 ml of an appopriate dilution on plate count agar (PCA; Biokar, Beauvais, France), total LAB count was determined on Elliker broth (ELK; Biokar) with 1.5% agar, and numbers of H_2S-producing bacteria were determined in modified iron agar *(9)* and yeast on oxytetracycline glucose agar (Biokar) plus 0.01% oxytetracycline (Oxoid, Basingstoke, England). A count of lactobacilli on Rogosa agar (ROG; Biokar) at pH 5.5 was also done. The NaCl content of all media was adjusted to 0.5%. Petri dishes were incubated at 20°C for 3 days. ELK and ROG plates were placed in anaerobic jars with Anaerocult A (Merck, Darmstadt, Germany).

TABLE 2. *Total viable count (log CFU g^{-1}) in cold-smoked salmon samples during vacuum storage at 5°C*

	Vacuum storage at 5°C (week)					
Experiment	0	1	2	3	4	6
1	6.5	7.8	7.8	7.7	7.6	7.7
2	4.7	6.5	6.9	7.3	7.5	7.7
3	3.8	6.7	7.0	7.2	7.4	7.4
4	4.0	6.1	6.3	6.8	7.9	7.2
5	4.8	6.6	7.5	7.2	7.4	7.2
6	4.4	5.8	5.9	6.6	6.7	7.1
7	3.3	5.0	5.9	6.0	6.4	6.8
8	2.4	5.4	6.2	6.0	6.0	6.6
9	3.0	4.8	6.2	6.3	6.6	6.6
10	1.4	3.5	3.7	4.8	6.0	6.0
11	2.4	4.0	4.4	4.7	5.4	5.9
12	3.3	3.3	4.6	5.2	5.5	6.7
13	3.7	4.0	4.7	4.4	4.1	4.6
14	2.2	5.5	5.0	5.3	5.0	4.7
15	2.2	3.0	3.2	3.6	4.5	4.6
16	1.4	2.3	2.8	3.6	5.0	5.3
17	2.8	4.4	4.6	5.3	5.8	5.5
18	2.5	4.9	5.3	5.8	6.0	6.7
19	3.0	3.4	4.6	6.4	6.3	6.2
20	2.4	4.0	5.8	7.0	6.6	7.1

TABLE 3. *Effect of salt and phenol on total viable count in cold-smoked salmon during vacuum storage at 5°C*

	Vacuum storage at 5°C (week)					
Effects	0	1	2	3	4	6
Mean effect	2.5	4.1	4.6	5.0	5.7	6.3
NaCl	−2.5***[a]	−4.3***	−4.0***	−3.5***	−2.8***	−1.2**
Phenol	−1.5**	−2.2**	−1.3*	NS[b]	NS	+1.7*
(NaCl) × (phenol)	NS	NS	NS	NS	NS	+2.7***
$(NaCl)^2$	NS	NS	NS	NS	NS	NS
$(Phenol)^2$	NS	NS	NS	NS	NS	NS
R^2	0.76	0.84	0.90	0.88	0.83	0.83

[a] $*P < 0.1$; $**P < 0.05$; $***P < 0.01$.
[b] NS, not significant.

For samples analyzed immediately after the smoking process ($t = 0$) and after 2 weeks of storage ($t = 2$), 15 colonies were picked from PCA plates containing 30 to 300 colonies for each of the 20 experiments. The 600 isolates were purified three times on PCA plates and characterized by morphology and motility, potassium hydroxide gram reaction, catalase and oxidase activity, oxidative and fermentative glucose metabolism, H_2S production, and trimethylamine oxide reduction as described by Leroi et al. *(16)*.

Chemical analysis. Lipids were determined by a hexane extraction. Sodium chloride was measured with a Chloride Analyser 926 (Corning, Halstead, England), and total phenols were quantified by the method described in the French standard for smoked salmon *(20)*. The a_w was measured with a Thermoconstanter Novasina (type TH/RTD, Zurich, Switzerland) immediately after the smoking process for experiments 2, 6, 10, and 14, corresponding to the four salt levels.

Statistical analysis. At each sampling time, regression coefficients of the fitting polynome (*a*0, *a*1, *a*2, *a*11, *a*22, *a*12) and effect of the factors were estimated by the standard least-squares method (Statgraphics Plus, version 3, Sigma Plus, Paris, France). The fitting polynome is used to predict a response for a chosen salt and smoke couple. Effects of the factors are used to classify the relative importance of each factors on the response. Effect of a factor represents the increase (or decrease if the value is negative) of the response when the factor varies from the lowest value of the experimental domain (i.e., 0% of salt and 0 mg 100 g^{-1} of phenol) to the highest value (i.e., 5% for salt and 1 mg 100 g^{-1} for phenol).

RESULTS AND DISCUSSION

For each experiment at each sampling time, salt and phenol contents were measured. The salt and phenol concentrations sometimes differed from theoretical values. Predictive models were calculated using the real concentrations at each sampling time (data not shown). For information, the mean concentrations for the six measurements for each experiment performed during the storage period are summarized in Table 1.

The a_w of samples 2, 6, 10, and 14, corresponding respectively to 0.2, 1.5, 3.2, and 4.8% of NaCl, was 0.987, 0.980, 0.957, and 0.928, which is concordant with the results of Himelbloom and Crapo *(11)*.

Figure 1 shows mean results for the different flora during storage of lightly preserved salmon at 5°C (experiments 6, 17, and 18, corresponding to NaCl of 1.7 ± 0.1% and phenol of 0.46 ± 0.01 mg 100 g^{-1}). These findings agree with our previous results *(16)*. TVC increased from 2×10^3 to 3×10^6 CFU g^{-1} in 4 weeks. Counts for H_2S-producing bacteria and yeasts increased during 1 or 2 weeks but always remained 2 log lower than TVC. Total LAB counts, which were initially low, reached the same level as total flora after 2 weeks. No colony was counted on ROG agar immediately after the smoking process. After 3 weeks, LAB enumerated on ROG became dominant, reaching 10^6 CFU g^{-1}. According to a previous identification of LAB isolated from cold-smoked salmon *(16)*, ROG was estimated to be selective for *Lactobacillus* enumeration, inhibiting *Carnobacterium* spp. Likewise, the carnobacteria count was estimated by the difference between ELK and ROG counts. These results indicate that LAB were probably represented by *Carnobacterium* spp. during the first 3 weeks and that *Lactobacillus* spp. then became dominant.

Effect of salt and smoke on TVC. The results for the TVC of the 20 experiments are summarized in Table 2, and those for the effects of NaCl and phenol are given in Table 3. Throughout the storage period, salting had a high linear negative effect on TVC, whereas that of smoking was also linear negative but to a lesser extent. The phenol effect was generally half that of salt; the effect was insignificant at

TABLE 4. *Predictive (P) and observed (O) total viable counts (log CFU g^{-1}) in cold-smoked salmon during storage at 5°C*

	Vacuum storage at 5°C (week)											
	0		1		2		3		4		6	
Experiment	P	O	P	O	P	O	P	O	P	O	P	O
19	2.3	3.0	4.0	3.4	5.0	4.6	5.6	6.4	5.9	6.3	6.0	6.2
20	3.0	2.4	4.8	4.0	5.8	5.8	6.0	7.0	6.6	6.6	6.8	7.2

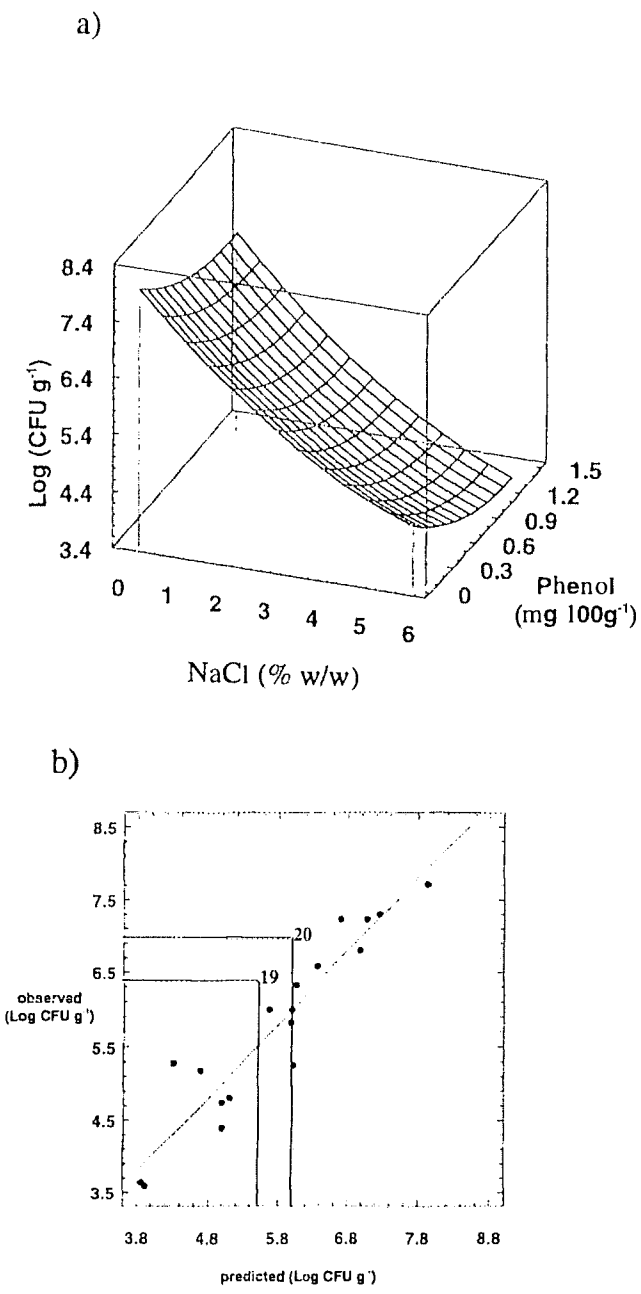

FIGURE 2. *(a) Response surface of total viable count (log CFU g⁻¹) versus salt and phenol concentrations in the meat of cold-smoked salmon after 3 weeks of vacuum storage at 5°C. (b) Predicted total viable count (log CFU g⁻¹) versus observed.*

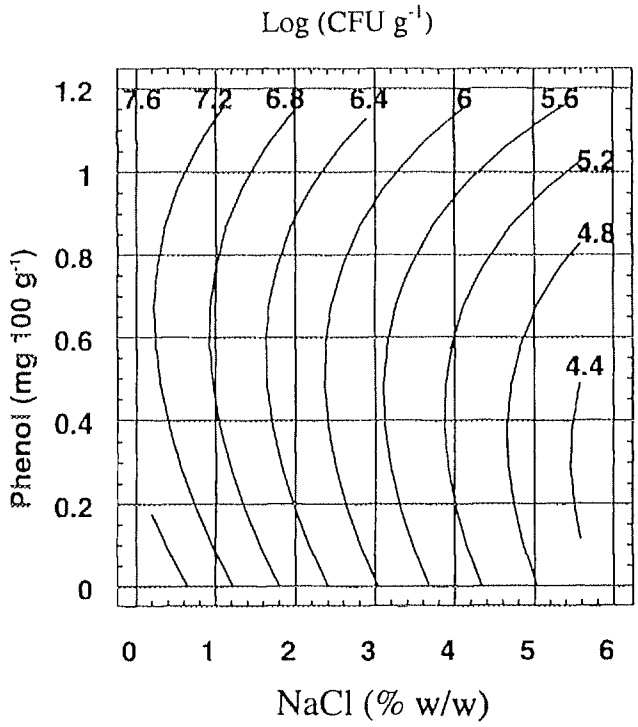

FIGURE 3. *Isoresponse curves for total viable count (log CFU g⁻¹) versus salt and phenol concentrations in the meat of cold-smoked salmon after 4 weeks of vacuum storage at 5°C.*

weeks 3 and 4 and slightly positive at week 6. There was no quadratic effect for salt and smoke and no significant interaction between the two factors. Thus, TVC was inhibited mainly by the salt concentration in meat and to a lesser extent by phenol content. This inhibition was linearly proportional to salt and smoke contents (the higher the concentration, the greater the inhibition). No synergistic effect on inhibition was observed between the two factors. The R^2 coefficient of determination of the fitting models ranged between 0.83 and 0.90 during weeks 1 to 6 (Table 3). At week 0 the R^2 value was 0.76 and was attributed to very low counts (sometimes under the detection threshold, which was 2.9 log units) on PCA plates (Table 2). Moreover, for the two additional experiments (19 and 20), the TVC predicted by the model was acceptable (less than 1-log difference) compared with observed counts (Table 4). These results enabled us to validate the models. For example, Figure 2a shows the predicted TVC in salmon meat after 3 weeks of storage versus NaCl and phenol concentrations, and Figure 2b shows the predicted versus observed values. The predictive model was as follows:

$$\begin{aligned}\text{TVC (log CFU g}^{-1}) &= 8.11 - 0.92 \times (\text{NaCl})_{\%} - 1.60 \times (\text{phenol})_{\text{mg 100 g}^{-1}} \\ &\quad + 0.05 \times (\text{NaCl})^2 + 0.67 \times (\text{phenol})^2 \\ &\quad - 0.02 \times (\text{NaCl}) \times (\text{phenol})\end{aligned}$$

TVC, which was predicted to reach 8.1 log (CFU g $^{-1}$) in fresh meat, was lowered by 3.3 log with 5% NaCl, 0.6 log with 1 mg 100 g $^{-1}$ of phenol, and 4.4 log with both NaCl and phenol.

To synthesize the kinetics according to biological characteristics (lag time, maximum growth rate, and maximum count), it would have been interesting to fit the TVC curves with well-described growth models such as Gompertz or logistic functions *(29)*. However, this was not possible, since only six points were measured for each curve, making the model unsuitable in some cases. Nevertheless, it could be determined that the effects of salt and smoke (Table 3) were maximal at the beginning of the storage period and then decreased, whereas the mean effects increased. For example, TVC in fresh salmon (no salt or smoke) decreased by 4.4 log with 5% NaCl and 1 mg 100 g $^{-1}$ of phenol to 3.2×10^6 and 1.2×10^2 CFU g $^{-1}$ immediately after the process but was 2.6 log lower after 4 weeks (1.2×10^8 and 3.2×10^5, respectively).

Figure 3 shows the isoresponse curves for TVC versus salt and phenol concentrations in meat after 4 weeks of vacuum storage at 5°C. French legislation specifies that TVC in cold-smoked salmon must be below 10^6 CFU g^{-1}. To obtain a product in accordance with this standard during 4 weeks of storage at 5°C, the minimum concentration of salt must be 2.4% and that of phenol must be 0.6 mg 100 g^{-1}. If the salt concentration is lower than 2.4%, TVC will reach 10^6 CFU g^{-1} regardless of the phenol concentration. Conversely, with a salt level above 3%, the TVC standard will be maintained regardless of the phenol level.

Effect of salt and smoke on LAB. Table 5 shows that the effects of salt and phenol on total LAB were similar to those observed on TVC, i.e., a high linear negative effect of salt throughout the storage period and a lower linear negative effect of phenol. R^2 values were higher than 0.82, except for weeks 0 and 1, when counts were very low. The results of experiments 19 and 20 (data not shown) enabled the models to be validated. The similarity of salt and smoke effects on TVC and LAB was not surprising, since TVC was mainly represented by LAB after 1 week of storage (data not shown).

It is noteworthy that LAB was always dominant at the end of the storage period, even for fresh salmon with no salt and smoke added (5.0×10^7 CFU g^{-1} for TVC and 4.0×10^7 for LAB after 6 weeks). These results appear to contradict those of Donald and Gibson *(4)*, who found that 90% of the strains isolated from vacuum-packed salmon steaks during ongoing spoilage at 5°C were gram negative, but their samples were analyzed during 10 days of storage.

The effects of salt and smoke on ROG counts, i.e., lactobacilli, were different (Table 5). Immediately after the smoking process and after 1 week of storage, all counts for lactobacilli were below the detection threshold, except for runs with no salt (no. 1, 2, 3, and 4) for which they appeared at week 1 (data not shown). At weeks 2 and 3, salting produced a marked linear inhibition of lactobacilli growth (the salt effect being greater than the mean effect). After 4 weeks of storage, there was a negative interaction between the two factors, i.e., the combined inhibitory effect of salt and phenol was higher. Conversely, a strong positive interaction was observed between the two factors after 6 weeks of storage, i.e., lactobacilli counts were enhanced by high levels of combined salt and smoke. This difference between the results at weeks 4 and 6 is difficult to explain.

Effect of salt and smoke on H_2S-producing bacteria. In fresh salmon slices, H_2S-producing bacteria increased rapidly for 1 week and then decreased slowly during the rest of the storage period (10^5, 3.8×10^7, 1.5×10^7, 2.8×10^6, 1.9×10^6, and 6.0×10^5 CFU g^{-1}, representing 3, 55, 24, 5, 4, and 1%, respectively, of TVC after 0, 1, 2, 3, 4, and 6 weeks, respectively, of storage).

The predictive models for the H_2S-producing bacteria count versus salt and phenol levels were validated up to 3 weeks of storage (data not shown). When salmon slices were only smoked, the count for H_2S-producing bacteria was not modified. Salt content produced a strong linear inhibition, which was even higher when salmon was both

TABLE 5. *Effect of salt and phenol on total lactic acid bacteria (Elliker count; ELK) and lactobacilli (Rogosa count; ROG) in cold-smoked salmon during vacuum storage at 5°C*

Effects	Vacuum storage at 5°C (week)											
	0		1		2		3		4		6	
	ELK	ROG	ELK	ROG	ELK	ROG	ELK	ROG	ELK	ROG	ELK	ROG
Mean effect	0.9	−0.2	3.7	1.8	4.3	3.7	4.9	4.1	5.8	5.2	6.7	7.0
NaCl	−3.8***[a]	NS[b]	−4.4***	−3.9**	−6.0***	−5.4***	−4.0***	−6.2**	−2.9***	−7.0***	NS	NS
Phenol	NS	0.9**	−2.3*	NS	−3.2***	NS	NS	NS	NS	−2.4*	3.3**	6.9***
(NaCl) × (phenol)	NS	NS	NS	NS	−2.3**	NS	NS	NS	NS	−3.9**	4.7***	7.5***
$(NaCl)^2$	−2.8**	0.8*	NS	NS	NS	NS	NS	NS	NS	−3.5**	NS	−4.0***
$(Phenol)^2$	NS	1.0*	NS	NS	NS	NS	NS	NS	NS	−2.6*	NS	NS
R^2	0.72	0.55	0.77	0.62	0.89	0.88	0.88	0.61	0.82	0.84	0.83	0.88

[a] $*P < 0.1$; $**P < 0.05$; $***P < 0.01$.
[b] NS, not significant.

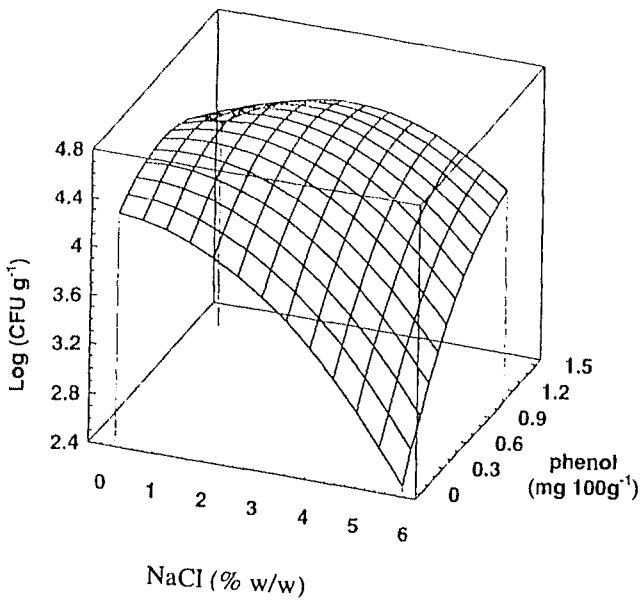

FIGURE 4. *Response surface of yeasts (log CFU g^{-1}) versus salt and phenol concentrations in the meat of cold-smoked salmon after 3 weeks of vacuum storage at 5°C.*

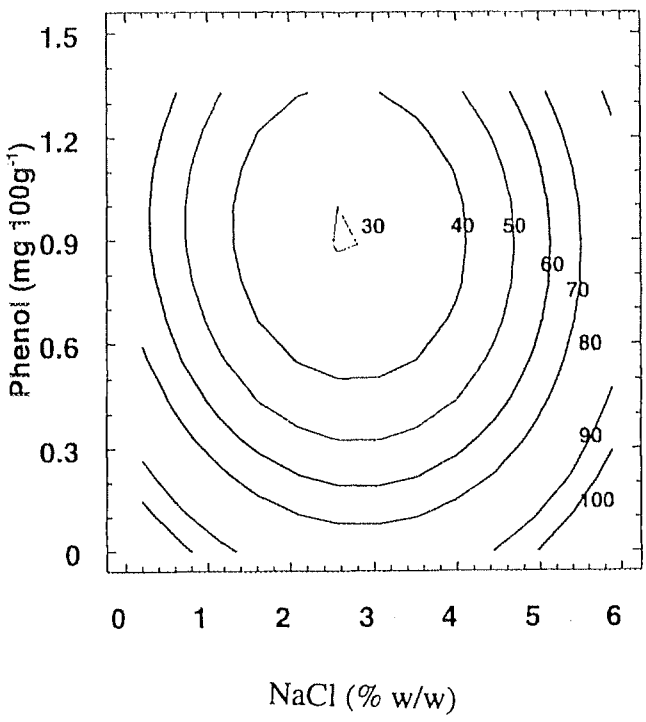

FIGURE 5. *Isoresponse curves for the percentage of carnobacteria within the total lactic acid bacteria population versus salt and phenol concentrations in cold-smoked salmon after 3 weeks of vacuum storage at 5°C.*

salted and smoked. The predicted logarithms for this count were 6.4, 6.1, 4.8, and 0.8, respectively, for fresh salmon, smoked salmon with 1 mg 100 g^{-1} of phenol, salted salmon with 5% salt, and salmon with both 1 mg 100 g^{-1} of phenol and 5% salt.

Effect of salt and smoke on yeasts. For the first 3 weeks of storage, the behavior of yeasts as affected by salt and smoke was similar to that observed for the ROG count after 6 weeks, i.e., no effect of smoke when applied alone, inhibition by salt when applied alone, but a protective effect of smoke when used simultaneously with salt. Figure 4 shows the predicted yeast count after 3 weeks of storage. Nevertheless, since yeast counts never exceeded 10^5 CFU g^{-1} in any of the samples analyzed, it seems unlikely that they could be responsible for smoked salmon spoilage.

Comparison of the behavior of the different flora affected by salt and smoke. Within the LAB population, different genera do not have the same spoilage potential. It has been clearly established that no correlation exists between sensory spoilage and total LAB counts *(8, 10, 16, 26)*. Carnobacteria do not seem to be involved in cold-smoked salmon spoilage, since no off-odor was detected in cold-smoked salmon inoculated with a high level of *Carnobacterium* spp. *(5, 15, 22)*, whereas *Lactobacillus sake* produced H_2S odors in this product *(26)*. This led us to compare the sensitivity of carnobacteria and lactobacilli to salt and smoke.

Accordingly, a new response, calculated from the two measured ELK and ROG counts, was studied, i.e., the percentage of *Carnobacterium* within the total LAB population: 100 × [1 − ROG (CFU g^{-1})/ELK (CFU g^{-1})]. Figure 5 shows the isoresponse curves for this percentage versus salt and phenol levels after 3 weeks of storage. LAB of fresh cold-smoked salmon was dominated by carnobacteria. The sensitivity of carnobacteria and lactobacilli to salt and smoke, when added separately, was identical, since the percentage of *Carnobacterium* within the LAB population remained unchanged (>80%) regardless of the concentration used. However, carnobacteria were more sensitive to a combination of low concentrations of salt and smoke (or lactobacilli may have been enhanced). With the classic levels found in French industry (NaCl, 3%; phenol, 0.5 mg 100 g^{-1}), the model predicted 40% of carnobacteria and 60% of lactobacilli, which is in agreement with our previous results *(16)* that show that various species of lactobacilli appeared after 2 weeks of storage.

The sensitivity of H_2S-producing bacteria to salt and smoke differed from that of LAB: the negative interaction between the two factors observed for the former population was not significant for the latter. To quantify this difference, we analyzed a new calculated response, i.e., the ratio of H_2S-producing bacteria to total LAB: iron agar (CFU g^{-1})/ELK (CFU g^{-1}). The difference in the behavior of H_2S-producing bacteria and LAB versus salt and smoke concentrations was not validated, since the predictive model was not suitable (R^2 = 0.38), probably because of the considerable variance of this response.

In an attempt to achieve greater precision, 600 colonies selected from the 20 experiments at weeks 0 and 2 were identified and classified into seven groups: LAB, *Brochothrix* spp., *Vibrio* spp., *Pseudomonas* spp., *Shewanella putrefaciens*, *Photobacterium* spp., and yeast (data not shown). Except for yeasts, the models predicting the percentage of each group versus salt and phenol concentrations were not validated so that no difference in sensitivity could be determined for the groups. However, the predictive model for

the response concerning the "percentage of gram-negative (or gram-positive) bacteria" was validated at both weeks 0 and 2 (data not shown). Immediately after the process, gram-negative bacteria were predominant (100%) in untreated products, which agrees with classic microbial data for cold-water fresh fish. Salt had a high inhibitory effect on the total flora, but gram-negative bacteria were more sensitive than gram-positive ones (the percentage of gram-negative bacteria decreased to 40% in salmon meat with 5% salt). At week 0, sensitivity to smoke did not differ between the gram-negative and gram-positive populations, since the initial percentage of gram-negative flora was not modified when smoke was added alone or with salt. After 2 weeks of storage, the model predicted 27% gram-negative bacteria in fresh salmon. This percentage increased when salt and smoking were combined, indicating that gram-positive bacteria were more sensitive to those conditions than gram-negative flora. For yeasts, the model was not validated at week 0 because yeast development was low. However, after 2 weeks, there was a highly significant positive interaction between salt and smoke. The percentage of yeasts, which was low in fresh salmon (5%) and unmodified with either salt or smoke alone, was enhanced when these two factors were combined, confirming results previously observed on oxytetracycline glucose agar petri dishes.

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