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Salt and Smoke Simultaneously Affect Chemical and Sensory Quality of Cold-Smoked Salmon during 5°C Storage Predicted Using Factorial Design

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ABSTRACT

Simultaneous effect of salt and smoke on chemical indices of cold-smoked salmon and on its shelf life, estimated by sensory analysis, was investigated during vacuum-packed storage at 5°C. Salting salmon immediately decreased the pH in the flesh, probably due to the increase of the ionic force, then pH remained constant during storage. Total volatile base nitrogen and trimethylamine productions were mainly inhibited by the salt concentration in the flesh, whereas phenol had no effect. A highly synergistic effect between the two factors was observed on the shelf life response. When a high level of salt (5% wt/wt) or phenol (1 mg 100 g^{-1}) was added separately, shelf life did not exceed 1 week, whereas it could reach more than 10 weeks when salt and smoke were added simultaneously. Different combinations were examined for shelf life characteristics of the product. For instance, 2 and 3% (wt/wt) of salt with, respectively, 0.80 and 0.45 mg 100 g^{-1} of phenol were sufficient for a 4-week shelf life, satisfying most of French cold-smoked salmon producers and consumers. Correlation between microbiological responses measured in a previous study and chemical and sensory data were also established.

Smoking fish is an important tradition in France. Salting and smoking time parameters are often established empirically by the producers and are balanced to satisfy the French consumers' taste and to provide a sufficient product shelf life. Some studies have demonstrated that cold-smoked salmon shelf life was extended when increasing salt concentration in the flesh (*13, 14*), but no author has described its combined effect with smoke. Recently, Leroi et al. (*9*) quantified the simultaneous influence of salt and smoke level in the flesh on the natural flora contaminating cold-smoked salmon during vacuum-packed storage at 5°C. Total viable count was mainly inhibited by salt concentration (5% wt/wt) and effect of smoke was generally half that of salt, but behavior of the different flora varied among the different bacterial groups. Specific spoilage organisms have not yet been identified for cold-smoked salmon (*10, 11, 14*), and sensory analysis seems to be the best method to estimate the product shelf life. The aim of the present study was to estimate the effects of salt and smoke directly on the product shelf life, estimated by sensory analysis. Some chemical indices, such as total volatile base nitrogen (TVBN) and trimethylamine (TMA), were also added, because they are frequently measured for estimation of spoilage of fish and fish products (*7*). TMA results from the reduction of trimethylamine oxide (TMAO) by some aeroanaerobic microorganisms placed in low-oxygen conditions. TVBN includes TMA and other compounds such as ammoniac, which can be produced by deamination of urea or amino acids by laetic acid bacteria, for example.

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Correlation between microbiological responses measured in our previous study (*9*) and chemical and sensory data were also established.

MATERIALS AND METHODS

Experimental design and sample preparation. The two factors studied were the salt (NaCl) concentration in the flesh, ranging from 0 to 5 g 100 g^{-1} of finished product (noted %), and the total phenol content, ranging from 0 to 1 mg 100 g^{-1}. The total phenol concentration was used to estimate the smoke intensity, since it has been proved that phenolic compounds were active ingredients in smoke for microbial inhibition (*1, 6, 16*). The responses measured weekly from weeks 0 to 6 were concentrations of TVBN, TMA, and TMAO in the flesh, pH, and shelf life estimated by sensory analysis. A complete factorial design described by Leroi et al. (*9*) was used to investigate the individual and combined effect of each factor on those responses. The model fitting the different responses as a function of the factors was a quadratic polynome: $Y(t) = a0 + a1 \times (\text{salt}) + a2 \times (\text{phenol}) + a11 \times (\text{salt})^2 + a22 \times (\text{phenol})^2 + a12 \times (\text{salt}) \times (\text{phenol})$. The samples were prepared by salting the salmon fillets with dry salt and smoking with beech and oak shavings in the conditions described by Leroi et al. (*9*) to get the appropriate salt and phenol concentrations. Fillets were then machine sliced and vacuum-packed in 40- or 160-g portions. Packages were stored at 5°C for 6 weeks.

Chemical analysis. Each week the entire contents of three 40-g bags were homogenized in a Waring blender (New Hartford, Conn.). A 30-g portion was used for microbiological analysis (*9*), and the remainder was used for biochemical analysis. TVBN, TMA, and TMAO were determined in duplicate by the Conway microdiffusion method (*3*) and expressed in mg-N 100 g^{-1}. The pH value was measured directly in the minced flesh with a pH-meter Mettler Delta 320 (AES, Combourg, France).

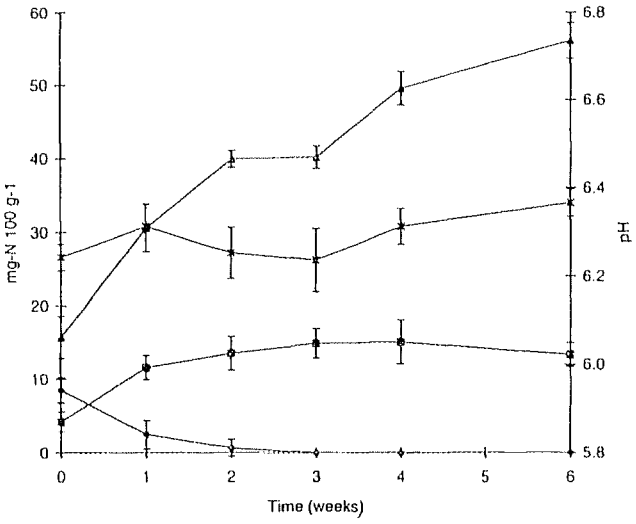

FIGURE 1. *Changes in TVBN (▲), TMA (▩), TMAO (♦), and pH (x) in lightly preserved cold-smoked salmon (1.7% NaCl and 0.46 mg 100 g^{-1} of phenol) during vacuum storage at 5°C. Means from experiments 6, 17, and 18.*

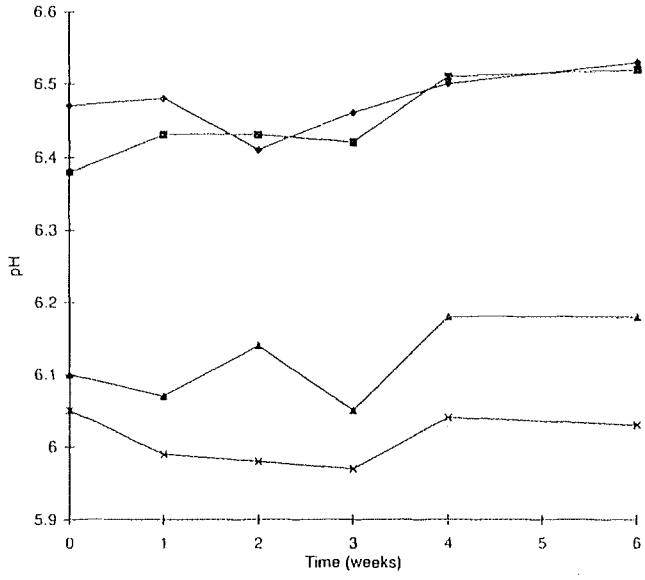

FIGURE 2. *pH evolution in salmon with no salt and no smoke (♦), 0% salt and 1 mg 100 g^{-1} of phenol (▩), 5% salt and 0 mg 100 g^{-1} of phenol (▲), and 5% salt and 1 mg 100 g^{-1} of phenol (x) during vacuum storage at 5°C.*

Sensory analysis. For each experiment, a 160-g package was opened weekly and aliquoted into 20-g portions, which were examined organoleptically (taste and odors) by eight trained panelists and classified as follows: 1 = no off-odor or taste, 2 = weak off-odor or taste, and 3 = strong off-odor or taste. Shelf life was determined when at least 4 panelists noted the sample was class 3.

Mathematical treatment. Effects of salt and phenol on the different responses and polynomial regression coefficients were estimated by the standard least-squares method (Statgraphics Plus, version 4, Sigma Plus, Paris, France).

To assess the correlation between microbiological counts obtained in the first part of our work *(9)* and chemical and sensory analysis obtained in the present study, all the data were summarized in a matrix containing 120 lines, the individuals, corresponding to the 120 samples (20 experiments checked weekly for 6 weeks), and 10 columns, the variables, corresponding to the 10 descriptors, which were total viable count, H_2S-producing bacteria, total lactic acid bacteria, lactobacilli, yeast, TMAO, TMA, TVBN, pH, and results of the sensory analysis. Results of the sensory analysis were expressed in weeks of remaining shelf life (observed shelf life minus its date of analysis). A positive remaining shelf life of x meant that the sample would be spoiled in x weeks, and a negative value (−x) meant it had passed the intended shelf life of x weeks. Data were treated by stepwise multiple regression analysis, performed on Statgraphics Plus, and by principal component analysis (PCA), performed on Uniwin software (Uniwin Plus, version 4, Sigma Plus). The aim of the PCA is to describe the differences and similarities between individuals and to estimate the correlation among variables. This method, using the relation among variables, allows the data to be summarized in a few dimensions (the principal components) while still explaining the differences among the individuals *(5)*.

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the physicochemical characteristics of a lightly processed salmon stored at 5°C for 6 weeks (mean of runs 6, 17, and 18, corresponding to NaCl of 1.7 ± 0.1% and phenol of 0.46 ± 0.01 mg 100 g^{-1}). The pH was fairly stable, ranging from 6.24 ± 0.03 to 6.37 ± 0.03. TVBN concentration increased from 15.7 ± 2.9 to 56.1 ± 2.5 mg-N 100 g^{-1}. TMA concentration increased from 4.1 ± 1.4 to 13.3 ± 1.6 mg-N 100 g^{-1}, corresponding to the consumption of the 8.5 ± 1.7 mg-N 100 g^{-1} of TMAO initially present in the flesh. Those results were consistent with previous results *(8, 10)*. The high initial TVBN and TMA values were explained by the fact that the initial time of analysis corresponded to around 2 days after filleting. Indeed, all the samples were stored in the same time-temperature conditions as the highest processed samples before analysis, approximately 24 h at 4°C for salting, 6 h at 22°C for smoking, and 24 h at 4°C for maturation before slicing. Only small fractions of the TVBN values could be attributed to production of TMA. Strains able to reduce TMAO to TMA, such as *Shewanella putrefaciens, Photobacterium* spp., and *Vibrio* spp., were present in the sample *(9)*. However, unpublished data indicate that those strains never produced more than 13 mg-N 100 g^{-1} of TMA, when inoculated at high levels in sterile cold-smoked salmon blocks, because of low concentration of TMAO in fresh *Salmo salar* flesh.

Effect of salt and smoke on pH. The pH was rather constant during the storage but varied considerably among experiments. Figure 2 shows pH patterns of 4 treatments. During the 6 weeks of storage, pH of fresh salmon (no salt and smoke) were between 6.41 and 6.53. Smoking the samples at 1 mg 100 g^{-1} of phenol had little effect (0.1 pH unit decrease), but salting at 5% NaCl greatly acidified the product to pH 6.05 to 6.18, and this difference persisted throughout storage. Combination of salt and smoke resulted in a pH varying between 5.97 and 6.05. Analysis of the combined effects of salt and smoke (data not shown) confirmed that salt had a highly significant linear negative effect on the pH, which was constant during the storage, whereas the effect of phenol was three- to fourfold less important. Just after the

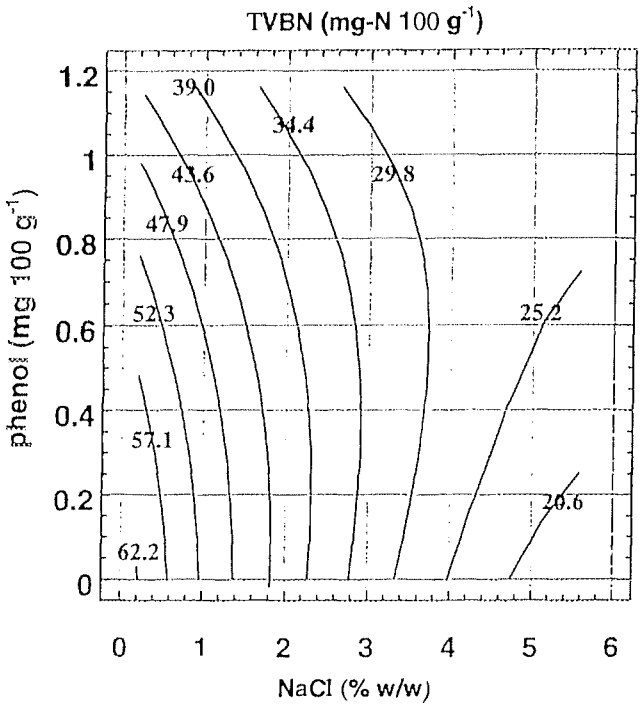

FIGURE 3. *Isoresponse curves for TVBN concentration versus salt and phenol concentration in the cold-smoked salmon flesh after 4 weeks of vacuum storage at 5°C.*

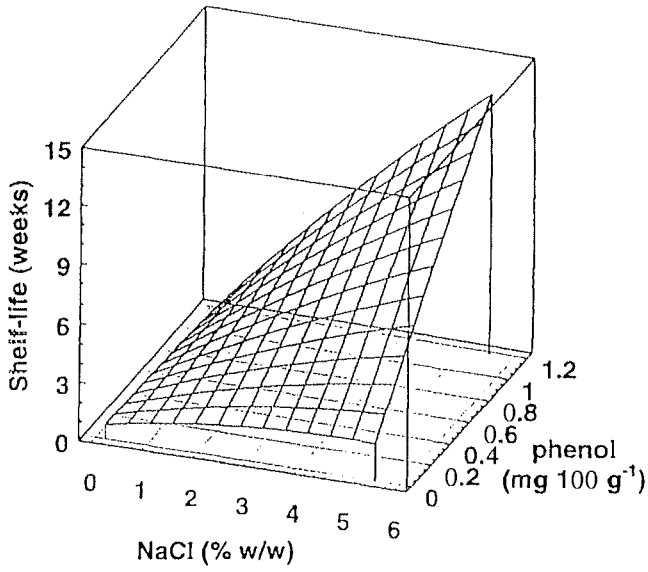

FIGURE 4. *Response surface of estimated shelf life as a function of salt and phenol concentrations in vacuum-packed cold-smoked salmon stored at 5°C.*

smoking process, the predicted pH was $6.51 - 0.13 \times (NaCl)_{\%\ w/wt} - 0.11 \times (phenol)_{mg\ 100\ g^{-1}} + 0.01 \times (NaCl)^2 + 0.03 \times (phenol)^2$. Observed values fit the model well ($R^2 = 0.96$). Differences between predicted and observed values for runs 19 and 20 were less than 0.08 pH unit. The pH decrease in the flesh when adding salt is explained by the increase of the ionic force *(2)*. In our previous study *(9)*, microbial growth inhibition from salting salmon was due to the water activity decrease. However, the pH decrease up to 0.5 pH unit could also contribute to this microbial inhibition. Further experiments, such as lowering the pH of fresh salmon flesh by addition of an acid, would be necessary to confirm this hypothesis.

Effect of salt and smoke on TVBN, TMA, and TMAO. Effects of salt and smoke on TVBN and TMA concentrations are summarized in Table 1. TVBN production in vacuum-packed fresh salmon in the absence of salt and smoke was high, increasing from 19.6 to 77.3 mg-N 100 g^{-1} after 6 weeks of vacuum storage. This concentration was mainly affected by the salt level, whereas phenol had no significant effect, except at the end of the storage when a weak interaction between the two factors was observed (Table 1). For instance, predicted TVBN concentrations after 4 weeks were 62.5, 49.5, 22.7, and 26.2 mg-N 100 g^{-1}, respectively, for fresh salmon, smoked salmon with 1 mg 100 g^{-1} of phenol, salted salmon with 5% salt, and salmon with both phenol and salt (Fig. 3). At the highest level of salt (with or without smoke), production of TVBN nearly ceased during the entire storage period. A concentration of 2.8% salt was necessary to maintain a TVBN level below 35 mg-N 100 g^{-1}, which corresponds to the European acceptability standard for fresh salmon.

Effect of salt and smoke on TMA concentrations was almost similar to those observed with TVBN; salt had a high linear negative effect during the storage (Table 1), i.e., inhibition of TMA production was proportional to the salt level in the flesh. These results confirmed those previously established on the microbiological responses *(9)*, i.e., increasing salt concentration reduced microbiological growth and consequently lowered the excretion of volatile metabolites.

Effect of salt and smoke on shelf life. Fresh salmon (no salt and smoke) was rejected by the taste panelists after 1 week of vacuum storage at 5°C. This shelf life was shorter than those observed by Donald and Gibson *(4)* and Pastoriza et al. *(12)* in similar products. As mentioned previously, fresh salmon fillets were stored for 2 days under similar conditions as samples that were processed and accelerating bacterial spoilage.

The model predicting the shelf life of a product based on salt and smoke concentrations was as follows: shelf life (week) $= 0.63 + 0.53 \times (salt)_{\%\ w/w} - 0.44 \times (phenol)_{mg\ 100\ g^{-1}} - 0.05 \times (salt)^2 - 0.21 \times (phenol)^2 + 1.86 \times (salt) \times (phenol)$. Figure 4 presents the predicted shelf life of smoked salmon at various salt and phenol concentrations. Adding salt alone increased the shelf life by a maximum of 1 week at the highest salt concentration. Similarly, smoking salmon that had not been salted did not modify the shelf life. Conversely, a strong interaction between the two factors was observed, and adding a combination of salt and smoke, even at low concentrations, greatly extended the shelf life. The predicted shelf life was around 3 weeks with 2% salt and 0.5 mg 100 g^{-1} of phenol and more than 10 weeks with 5% salt and 1 mg 100 g^{-1} of phenol. For a fixed concentration of phenol (>0.2 mg 100 g^{-1}), increasing the salt concentration increased the shelf life of the product. This is consistent with previous studies *(13, 14)* that have shown that cold-smoked salmon

TABLE 1. *Effect of salt and phenol on TVBN and TMA concentrations in cold-smoked salmon during vacuum storage at 5°C*

	Time (week)											
	0		1		2		3		4		6	
	TVBN	TMA	TVBN	TMA	TVBN	TMA	TVBN	TMA	TVBN	TMA	TVBN	TMA
Mean effect	16.8	3.8	23.4	6.3	26.8	7.2	28.3	8.5	34.1	9.6	42.5	10.1
NaCl	NS[a]	NS	−21.3[b]	−12.4[b]	−25.9[b]	−13.0[b]	−27.6[b]	−11.3	−31.7[b]	−13.3[b]	−34.1[b]	−8.0[b]
Phenol	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
(NaCl) × (phenol)	NS	NS	NS	NS	NS	NS	NS	NS	13.9[c]	NS	28.8[c]	9.8[c]
$(NaCl)^2$	NS	NS	NS	NS	12.4[d]	NS	14.9	NS	12.0[c]	NS	NS	7.6[c]
$(Phenol)^2$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R^2	0.35	0.24	0.79	0.82	0.87	0.78	0.90	0.80	0.90	0.87	0.87	0.83

[a] NS, not significant.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $P < 0.1$.

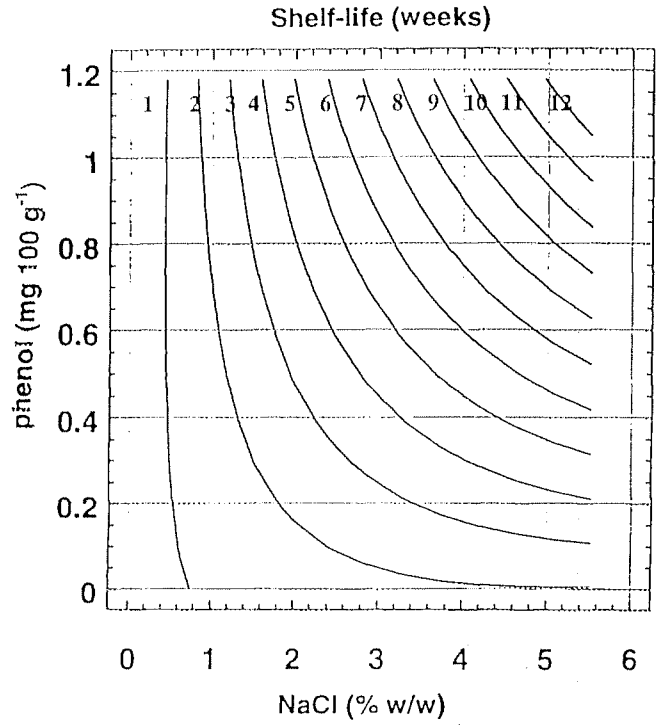

FIGURE 5. *Isoresponse curves for shelf life versus salt and phenol concentrations in vacuum-packed cold-smoked salmon stored at 5°C.*

shelf life was extended with increasing salt concentration in the flesh. However, Truelstrup Hansen et al. *(15)* observed no correlation between shelf life and salt content, but samples came from three different processing plants, which differed considerably on raw material and bacteria. Figure 5 represents the isoresponse shelf life curves versus salt and smoke levels in the flesh. Although different combinations of salt and smoke could be used to obtain a product with a shelf life of 4 weeks, this could not be obtained if salt and smoke were not used in combination. With the highest level of salt (5%), a minimum of 0.25 mg 100 g^{-1} of phenol was necessary to achieve this shelf life, and with the highest level of smoke (1 mg 100 g^{-1}), 1.8% salt was required. In France, salt concentration in cold-smoked salmon currently ranges from 2 to 3%, but smoke level varies considerably. It is common to observe concentrations lower than 0.2 mg 100 g^{-1}, which provides no more than 2 to 3 weeks of life to the product. Phenol (0.80 and 0.45 mg 100 g^{-1}) in combination with, respectively, 2 and 3% salt will be necessary to get the 4 weeks of shelf life generally indicated by the producers (Fig. 5).

Correlation between shelf life and microbiological or chemical indices. Data have been treated by PCA. The purpose of this study was to obtain a small number of linear combination of the 10 variables that account for most of the variability in the data. In our case, two components have been extracted; together, they account for 82% of the variability in the original data. Figure 6 plots the projection of the individuals (120 samples) and variables (chemical and microbial data) on the plan formed by the first two components of the PCA. The two groups (samples that have

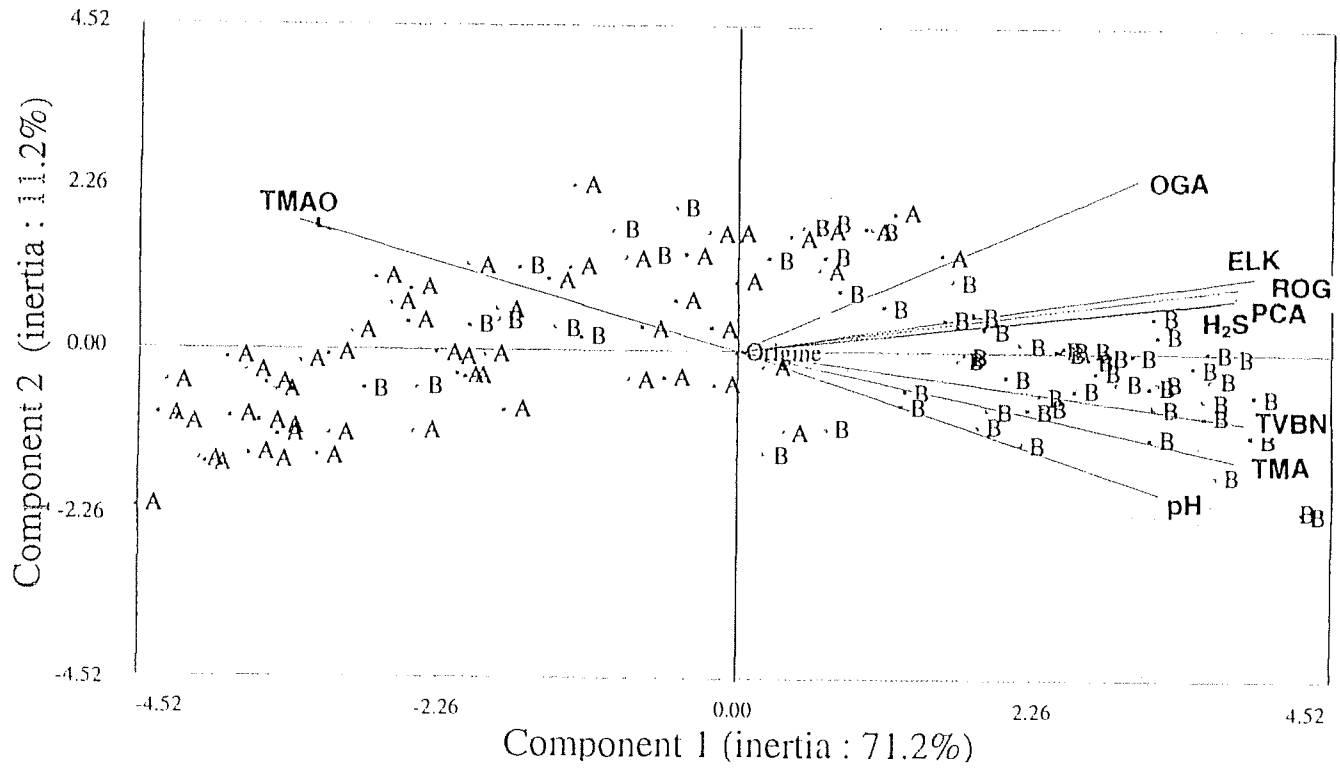

FIGURE 6. *Biplot of individuals (A: samples not rejected by the panel; B: samples rejected by the panel) and variables on the plane 1-2 of the principal component analysis. ELK, total lactic acid bacteria; ROG, lactobacilli; H_2S, H_2S-producing bacteria; OGA, yeast.*

not been rejected by the panelists and samples that have been rejected) were not perfectly discriminated. However, the first axis (71% of inertia) clearly appeared as a "spoilage axis," ascending from samples that have not been rejected by the panelists to samples that have reached the intended shelf life date or exceeded it. The latter samples were generally characterized by high levels of all microbiological counts and high concentrations of TMA and TVBN (in the right part of the graph, Fig. 6), even though some samples that had exceeded the intended shelf life had lower microbial counts and volatile compound concentrations (in the left part of the graph).

Data were also treated by forward stepwise multiple regression analysis to describe the relation between shelf life and the nine descriptors. Results showed that TVBN had the major influence on remaining shelf life and then, to a lesser extent, TVC. The equation of the fitted model was as follows: remaining shelf life (in weeks) = $7.4 - 0.10 \times (\text{TVBN})_{\text{mg-N 100 g}^{-1}} - 0.70 \times \log(\text{TVC})_{\text{CFU g}^{-1}}$. The R^2 statistic indicated that the model explained 63% of the variability in the remaining shelf life. The R^2 value was not significantly increased by adding the other microbial and chemical descriptors to the model. This meant that those other descriptors were either not good quality indices for smoked salmon or were highly correlated with TVBN or TVC without improving the quality of the model. In conclusion, microbiological and physicochemical indices are of limited value for estimating sensory quality of cold-smoked salmon. TVBN concentration in the flesh seemed to be of most value but could not be used alone to precisely predict the shelf life.

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