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Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters

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The aim of the study was to assess the relationships between the remaining shelf-life (RSL) of cold-smoked salmon and various microbiological and physico-chemical parameters, using a multivariate data analysis in the form of stepwise forward multiple regression.

Methods and Results: Thirteen batches of French cold-smoked salmon were analysed weekly during vacuum-packed storage at 5°C for their lipid, water, salt, phenol, pH, total volatile basic nitrogen (TVBN) and trimethylamine contents, total psychrotrophic count, lactic acid bacteria, lactobacilli, B. thermosphacta, Enterobacteriaceae and yeast counts. At the sensory rejection time, the flora was dominated by lactobacilli, lactobacilli/Enterobacteriaceae or Carnobacteria/B. thermosphacta. Shelf-life was very variable (1->6 weeks) and was related to the initial Enterobacteriaceae load ($P < 0{\cdot}05$), depending on hygienic conditions in the smokehouse. High correlations existed between the RSL and lactobacilli count ($P < 0{\cdot}01$), yeast count ($P < 0{\cdot}05$) and TVBN concentration ($P < 0{\cdot}01$). A polynomial fitting the RSL as a function of those three factors was proposed ($R^2 = 0{\cdot}80$). Assuming that lactobacilli count could not exceed 109 cfu g-1, a minimum of 36 mg-N 100 g-1 was necessary for a product to be rejected, with a yeast count of 104 cfu g-1.

Conclusions: Estimation of cold-smoked salmon quality is possible by measuring three parameters: lactobacilli and yeast counts and TVBN concentration.

Significance and Impact of the Study: The technical content is important for the smoked salmon industry and for development of quality standards for cold-smoked salmon.

Keywords: cold-smoked salmon, regression, parameters, microbiological

INTRODUCTION

Vacuum-packed, sliced, cold-smoked salmon is a highly perishable product, because of light preservative treatments (salt on product ranging between 2.5 to 3.5 % w/w and phenol generally less than 0.5 mg 100 g^{-1}) and no other additives such as nitrate or nitrite allowed in France. The lifetime indicated by the producers is generally limited to 3-5 weeks at 4°C, due to early sensory deterioration (Leroi *et al*. 1996) and also to the possible hazard associated with the development of *Listeria monocytogenes* (Eklund *et al.* 1995 ; Huss *et al*. 1995 ; Cortesi *et al*. 1997 ; Jorgensen and Huss 1998).

Sensory damage of cold-smoked salmon is mainly caused by micro-organisms (Truelstrup Hansen 1995 ; Truelstrup Hansen *et al*. 1996, 1998 ; Joffraud *et al.* 1998 ; Leroi *et al*. 1998). However, the spoiling mechanisms are probably much more complex than in other fish products such as fish stored in ice or in vacuum or modified atmosphere for which a specific spoiling micro-flora can be related to each case (Gram and Huss 1996). Different studies indicate that various bacterial groups including lactic acid bacteria (*Lactobacillus* spp. and *Carnobacterium* spp.), marine vibrio/*Photobacterium* spp., Enterobacteriaceae and *Brochothrix thermosphacta* dominate the spoilage micro-flora (Truelstrup Hansen *et al*. 1995, 1998 ; Leroi *et al*. 1998 ; Lyhs *et al.* 1998 ; Paludan Müller *et al.* 1998 ; Truelstrup Hansen and Huss 1998 ; Leroi *et al*. 2000 ; Jorgensen *et al*. 2000). Among those bacterial groups, *Lact. sake*, *B. thermosphacta*, *Serratia liquefaciens* and *P. phosphoreum* have been identified as weak or strong spoilers, depending on the strains, when inoculated in pure culture in sterile cold-smoked salmon blocks (Leroi *et al.* 1999). These results explain the lack of correlation reported in the literature between total counts classically enumerated on cold-smoked salmon and sensory data (von Rakow 1977 ; Cann *et al*. 1984 ; von Hildebrandt and Herol 1988 ; Dodds *et al*. 1992 ; Truelstrup Hansen *et al*. 1996, 1998 ; Truelstrup Hansen and Huss 1998) and the inability to use an individual physico-chemical parameter as a quality indicator for cold-smoked salmon (Truelstrup Hansen *et al*. 1995). In the light of these considerations, it

appeared interesting to develop a multifactorial strategy. Recently, Jorgensen *et al*. (2000) established a multiple compound quality index for Danish cold-smoked salmon based on biogenic amines production and pH. Biogenic amines anabolism and changes in pH are the result of micro-organism activity and it seemed logical to assume that a multiple compound quality index based on microbiological counts and simple physico-chemical parameters could also be developed.

Thirteen vacuum-packed, cold-smoked salmon lots representative of the French production were analysed weekly during the storage at 5°C. Parameters linked to raw material and process *i.e.* lipid, water, salt and phenol contents were measured and also total psychrotrophic count (TPC), lactic acid bacteria (LAB), lactobacilli, *B. thermosphacta*, Enterobacteriaceae and yeast counts, pH, total volatile basic nitrogen (TVBN) and trimethylamine (TMA) contents. Shelf-life was estimated by a trained panel. The aim of the study was to assess the relationships between the remaining shelf-life (RSL) of cold-smoked salmon and the various microbial and physico-chemical parameters listed, using a multivariate data analysis in the form of stepwise forward multiple regression.

MATERIALS AND METHODS

Cold-smoked salmon

During 1998 and 1999, 13 batches of sliced, vacuum-packed, cold-smoked salmon (Atlantic *Salmo salar*) representative of the French traditional production were collected just after processing in 5 French smokehouses and transported to the laboratory in frozen conditions. Geographic origin of raw material and smokehouses are summarized in Table 1. Salmon were all treated according to the most common process in France, *i.e.* dry salted and traditionally smoked at temperatures in the range 20 to 26°C.

Three to five batches, studied in a work session, including 35 100-200-g bags for each batch, were thawed overnight and stored at 5°C for 5-6 weeks. Each week from week 0 until one week after sensory spoilage was evident, microbial, chemical and sensory analyses were made.

Table 1 : Characteristics of cold-smoked salmon lots linked with raw material and processing parameters and shelf-life during the vacuum storage at 5°C.

label	smokehouse	raw material	origin	shelf-life (week)	water (% w/w)	lipid (% w/w)	NaCl (% w/w)	phenol (mg $100g^{-1}$)	initial pH
F1	A	fresh (filleted)	Norway	4	61.1	14.4	2.87	0.78	6.09
F2	B	fresh	Norway	5	58.6	15.9	2.78	0.37	6.2
F3	C	fresh	Scotland	3	59.8	12.3	3.48	1.08	6.3
F4	D	fresh	Scotland	1	58.8	16.3	2.21	0.33	6.29
F5	B	fresh	Scotland	>5	58.7	14.5	2.55	0.48	6.27
F6	C	fresh	Norway	2	57.3	14.4	3.46	0.79	6.25
F7	A	fresh (filleted)	Norway	4	60.0	15.6	3.22	0.27	6.15
F8	A	fresh	Norway	5	59.7	13.2	4.29	0.74	6.13
F9	B	fresh	Ireland	>5	59.9	14.3	3.11	0.56	6.17
F10	D	fresh	Norway	2	57.5	16.9	3.12	0.73	6.13
F11	E	fresh	Norway	3	62.4	14.2	2.56	0.31	6.22
F12	E	frozen	Norway	>6	68.0	7.0	3.57	0.28	6.22
F13	E	fresh	Norway	>6	65.3	13.4	3.61	0.38	6.24
Average					60.5	14.0	3.1	0.55	6.20
95% confidence limit					1.8	1.4	0.3	0.15	0.04

Sensory analysis

Two to three bags per lot were opened and divided in 20-g portions in aluminum foil to keep the odours intact. 14 trained panelists smelled the samples and performed a profiling test, marking 14 spoilage attributes established by Leroi *et al.* (1999) on a non-structured 0.8 m line scale anchored at each end. The spoilage attributes were : amine, acid, grass, rancid, ham, plastic, sour, butter, rubber, feet, blue cheese/musty, hydrogen sulfide, cabbage and faecal. Results of the profiling test were transformed to adjust variations among assessors in their range of scoring. Sensory data were standardized using an isotropic scaling factor according to the procedure proposed by Kunert and Qannari (1999).

At the end of the profiling test, panelists classified each sample depending on their spoilage level as : 1 = no off-odour, 2 = weak off-odour, 3 = strong off odour. The sensory rejection time (SRT) was determined when 7 judges at least estimated that the product was in class 3. The RSL of a sample was the difference between the SRT in week (known at *posteriori*) and the week of analysis.

Microbiological analysis

At each sampling date, three bags per batch were opened and a 30-g portion of each bag representing all the slices was stomached in 120 ml of chilled diluant (0.85% NaCl and 0.1% peptone) for two min in a stomacher 400 (Lab. Blender, London, UK). After 30 min at room temperature for resuscitation, 10 ml of the three homogenates were pooled together to constitute the "mother" solution. Spread plates of modified Long and Hammer's medium (LH, van Spreekens 1974) incubated at 15°C for 5 days were used to determine the TPC. Total LAB were enumerated on spread plates of Nitrite Actidione Polymyxin agar (NAP, Davidson and Cronin 1973) at pH 6.8 and lactobacilli on Rogosa agar (ROG, Biokar, Beauvais, France) at pH 5.5 as suggested by Leroi *et al.* (2000). NAP and ROG plates were incubated at 20°C in

anaerobic conditions (Anaerocult A, Merck, Darmstadt, Germany). Yeasts were enumerated on Oxytetracycline Glucose Agar (OGA) made with OGA base (Biokar) and 0.01% oxytetracycline (Oxoid, Basingstoke, England). Enterobacteriaceae counts were determined in pour plates of CASO agar (Merck) overlaid by Violet Red Bile Glucose agar (Oxoid), incubated at 30°C for 2 days. Assuming that non typical colonies could also belong to the Enterobacteriaceae family (unpublished data), 2 counts were prepared : typical colonies (VRBG typical), corresponding to red colonies with diameter higher than $0.5\ 10^{-3}$ m, and total colonies (VRBG total), corresponding to all the colonies growing in the plate. *B. thermosphacta* was enumerated on spread plates of STAA (Gardner 1966) (2% peptone, 0.2% yeast extract, 0.1% KH_2PO_4, 0.1% $MgSO_4\ 7H_2O$, 1.5% glycerol, 1.3% agar, 0.05% streptomycin, 0.005% cycloheximide and 0.005% thallium acetate) incubated at 20°C, after examining the Gram, catalase and oxidase reactions of the colonies.

At the SRT and 2 weeks before, 30 and 15 colonies respectively were randomly picked from LH and NAP plates, purified on Brain Heart Infusion agar plates (BHI, Difco, Sparks, MD, USA) and partially identified with morphology and motility examination, KOH Gram reaction and catalase and oxidase activity, as described by Leroi *et al*. (1998).

Chemical analysis

The remainding flesh in the three bags opened for microbiological analysis was homogenized in a Waring Blendor (New Hartford, CO, USA). Each week, TVBN and TMA were measured in duplicate by the Conway micro-diffusion method (Conway and Byrne 1933). The pH value was measured in the five-fold-diluted flesh with a pH-meter Mettler Delta 320 (AES, Combourg, France). At week 0, lipids, dry matter, sodium chloride and total phenols were quantified by methods described by Leroi *et al*. (2000).

Statistical analysis

At the SRT or at the end of the experiment for samples that had not been rejected, the thirteen cold-smoked salmon samples were clustered on sensory descriptors, using the Ward's hierarchical clustering method with the Euclidean distance (Uniwin software, Uniwin Plus, version 3.01, Sigma Plus, Paris, France).

One-way variance analysis (ANOVA, Statgraphics Plus, version 4, Sigma Plus) was used to test differences between groups of samples having the same RSL using successively each microbiological or chemical index. Means were compared by the least significance difference (LSD) test at the 0.05 level of probability.

A polynomial fitting the RSL to the microbiological and chemical data was calculated using the stepwise forward multiple regression method (Statgraphics Plus). This method is preferable to classical multiple regression when correlation between factors is suspected.

RESULTS

Characterization of cold-smoked salmon samples

Technological parameters. Characteristics of cold-smoked salmons directly linked with the raw material composition and the processing parameters (lipid, water, NaCl, phenol and initial pH) are summarized in Table 1. The thirteen lots were relatively homogeneous in their lipid composition, with concentrations ranging between 12.3 and 16.9% (w/w) except F12 which was leaner (7.0%). Water content ranged from 57.3 to 68.0%, the higher value corresponding to sample F12. The salt concentrations for all samples were similar, with an average concentration of 3.1 ± 0.3% (w/w) (95% confidence limit calculated with Student $t = 2.179$), corresponding to 5.2 ± 0.5% in water phase. Conversely, a wide variation in phenol content was observed between the samples, with concentrations ranging between 0.27 and 1.08 mg

100 g^{-1} and an average value of 0.55 ± 0.15 mg 100 g^{-1}. The pH just after processing was fairly constant (6.20 ± 0.04).

Sensory analysis. Shelf-life observed by the panel ranged between 1 to more than 6 weeks (Table 1). The number of spoiled samples was identical for each sampling date (around 2 samples rejected each week), indicating the wide shelf-life variation among French cold-smoked salmon production. At the SRT or at the end of the experiment for samples which had not been rejected, the thirteen cold-smoked salmon lots could be divided in 3 groups by the Ward's hierarchical clustering method, each one characterized by some specific sensory descriptors. Group 1 consisted of samples 5, 9, 12 and 13 which had not been rejected by the panel. No specific descriptor could be attributed to this group. Group 2, including samples 1, 2, 3, 4, 7, 8 and 11, was mainly characterized by strong amine, sour and feet off-odours. Group 3 corresponded to samples 6 and 10 with strong H_2S, cabbage and feacal off-odours.

Microbiological composition. The microbiological composition of cold-smoked salmon samples at week 0 and at the SRT is presented in Table 2. Initial flora was very different from one sample to another. TPC in lots 2, 5, 9 and 13 were lower than 10^2 cfu g^{-1} while higher than 10^5 in lots 3, 6, 10, 11 and 12 and between those 2 values for lots 1, 7 and 8. During the vacuum storage at 5°C, TPC increased till its maximum level (10^7-10^9 cfu g^{-1}) more or less quickly depending of the samples and remained at this level until spoilage sometimes several weeks latter (data not shown). Variation in the composition of micro-flora between lots were very important and 3 scenarios could be distinguished. They are represented in Figures 1a, 1b and 1c corresponding to the growth patterns of the different micro-organisms in lots 3, 9 and 7 respectively. In scenario 1 (lots 3, 4, 6 and 10, Figure 1a), TPC reached $10^{8\text{-}9}$ cfu g^{-1} and total LAB and count on Rogosa agar were equal to TPC, indicating that lactobacilli were the

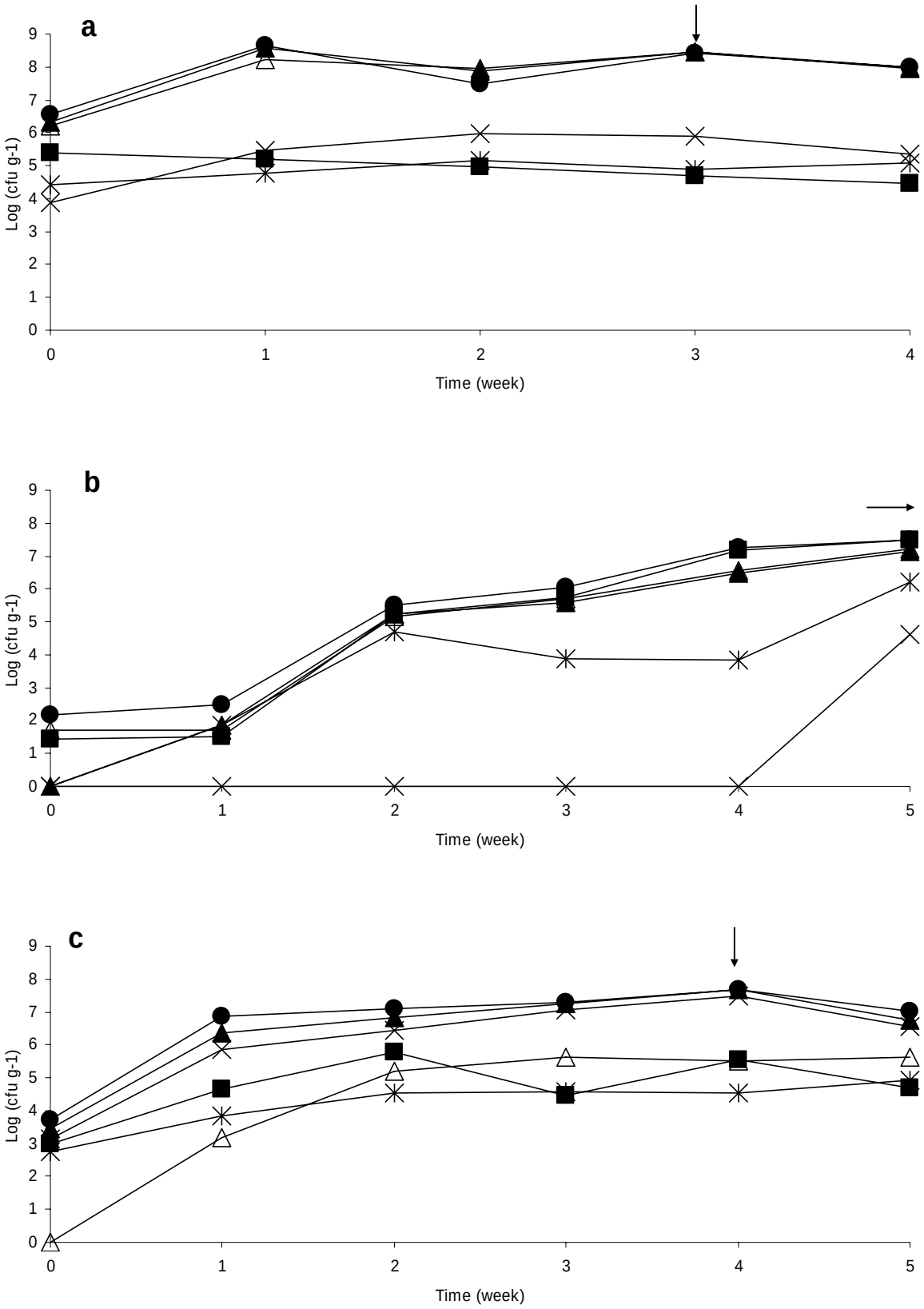
dominating flora. *B. thermosphacta*, yeasts and Enterobacteriaceae were in a minority, never exceeding 1% of TPC. In scenario 2, corresponding to lots 1, 2, 5 and 9 (Figure 1b), the spoilage micro-flora was mainly represented by lactobacilli and Enterobacteriaceae and to a lesser extent by yeasts. *B. thermosphacta* counts were generally below the detection threshold, except for lot 1 where it reached 10^{5-6} cfu g^{-1} (Table 2). In scenario 3 (lots 7 and 8, Figure 1c) TPC was dominated by total LAB and *B. thermosphacta*. According to Leroi *et al.*'s considerations (1998, 2000), LAB probably belonged to the *Carnobacterium* genus because the lactobacilli count on Rogosa agar was always 2 log lower than count on NAP, except for lot 8, for which lactobacilli became dominant at the end of the storage (Table 2). Yeasts and Enterobacteriaceae counts were low, ranging between 10^{4-5} cfu g^{-1}. The 3 samples 11, 12 and 13 could not be associated to any of the 3 scenarios because lactobacilli, *B. thermosphacta* and yeast counts were not determined. However, in the three samples, LAB were the dominating flora and Enterobacteriaceae count always remained 1 to 2 log lower than TPC, indicating that those samples could follow scenario 1 or 3.

Table 2 : Remaining shelf-life, microflora and chemical composition of cold-smoked salmon during the vacuum storage at 5°C

label	date of analysis*	remaining shelf-life*	pH	Total volatil basic nitrogen†	trymethylamine†	total psychrotrophic count‡	lactic acid bacteria‡	lactobacilli‡	*Brochothrix thermosphacta*‡	yeasts‡	Enterobacteriaceae (total colonies)‡	Enterobacteriaceae (typical colonies)‡
F1	0	4	6.09	14.1	1.3	3.1	0.0	0.0	2.1	2.2	2.3	1.5
F1	4	0	6.22	40.3	11.8	6.9	6.7	6.8	5.1	5.3	5.6	5.5
F2	0	5	6.20	18.3	1.3	2.0	0.0	0.0	0.0	0.0	0.0	0.0
F2	5	0	6.14	21.9	5.9	7.4	7.3	7.2	0.0	6.3	6.6	6.6
F3	0	3	6.30	19.0	1.3	6.6	6.3	6.2	3.9	4.4	5.4	5.4
F3	3	0	5.99	50.4	11.1	8.4	8.5	8.5	5.9	4.9	4.7	4.0
F4	0	1	6.29	16.4	2.6	5.6	4.2	4.2	2.9	2.7	4.6	4.6
F4	1	0	6.22	35.4	11.8	7.9	6.9	6.9	5.2	2.5	ND	3.7
F5	0	ND	6.27	15.7	1.3	1.7	0.0	0.0	0.0	0.0	0.0	0.0
F5	5	ND	6.19	27.5	3.9	8.5	7.8	7.9	0.0	7.9	8.4	8.4
F6	0	2	6.25	19.3	2.0	6.0	5.7	4.8	3.5	4.5	4.8	4.0
F6	2	0	5.90	21.3	3.3	7.0	8.4	8.1	5.8	5.0	4.6	3.2
F7	0	4	6.15	15.1	1.3	3.7	3.4	0.0	3.2	2.8	3.0	0.0
F7	4	0	6.24	27.2	2.6	7.7	7.7	5.5	7.5	4.5	5.5	4.5
F8	0	5	6.13	14.4	1.6	3.8	2.1	0.0	2.1	0.0	2.2	0.4
F8	5	0	6.21	27.8	4.6	6.7	6.3	6.4	6.4	4.6	4.3	0.0
F9	0	ND	6.17	13.8	1.3	2.2	0.0	1.7	0.0	0.0	1.4	0.4
F9	5	ND	6.22	17.7	1.3	7.5	7.2	7.2	4.6	6.2	7.5	7.5
F10	0	2	6.13	17.0	2.6	6.1	5.7	5.6	5.0	4.7	5.0	2.3
F10	2	0	6.01	33.7	8.2	9.0	9.1	9.0	5.3	3.5	4.9	4.9
F11	0	4	6.22	14.4	1.3	5.1	0.0	ND	ND	ND	2.1	1.0
F11	4	0	6.29	36.7	7.9	6.6	6.6	ND	ND	ND	5.4	4.9
F12	0	ND	6.22	17.0	2.0	5.0	5.0	ND	ND	ND	3.4	3.4
F12	6	ND	6.21	41.9	14.4	7.3	6.9	ND	ND	ND	4.6	4.5
F13	0	ND	6.24	15.1	1.3	2.0	2.4	ND	ND	ND	1.6	0.0
F13	6	ND	6.16	37.3	11.1	6.1	6.8	ND	ND	ND	5.8	4.2

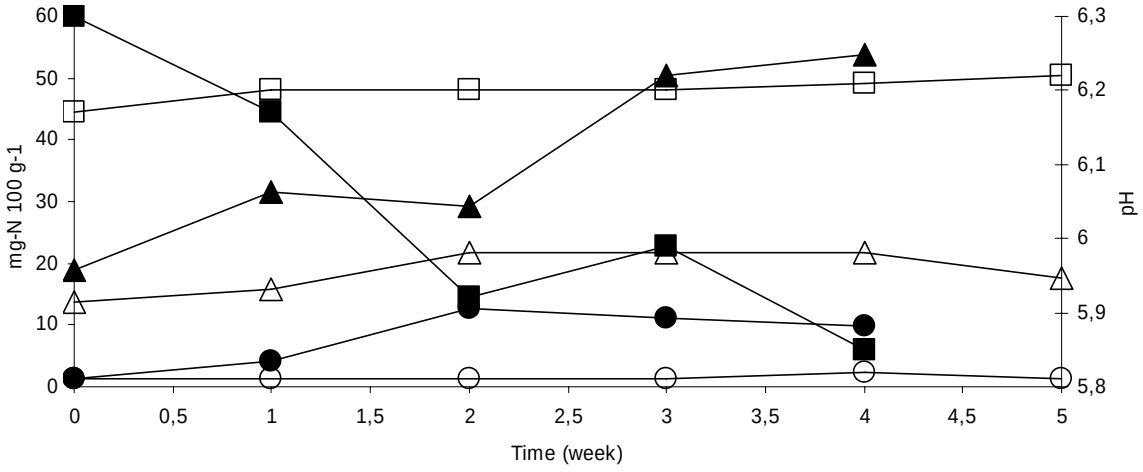
* week
† mg-N 100 g^{-1}
‡ cfu g^{-1}
ND : not determined

Figure 1 : Micro-flora evolution in a) sample 3, b) sample 9 and c) sample 7 during the vacuum storage at 5°C. ● : total psychrotrophic count ; ▲ : total lactic acid bacteria ; Δ : lactobacilli ; ■ : Enterobacteriaceae (VRBG total count) ; x : *Brochothrix thermosphacta* ; * : yeast. Arrow indicates the sensory rejection time.



Chemical analysis. Results of chemical analysis at week 0 and at the SRT are listed in Table 2. Just after the smoking process, TMA and TVBN concentrations were rather constant in all the lots, with average values of 1.6 ± 0.1 (95% confidence limit) and 16.1 ± 0.3 mg-N 100 g^{-1} respectively. During the vacuum storage at 5°C, 2 groups of samples could be distinguished : Group 1 (lots 2, 5, 7, 8, 9) in which TMA and TVBN never exceeded 6 and 30 mg-N 100 g^{-1} respectively, and group 2 (lots 1, 3, 4, 6, 10, 11, 12, 13) for which TMA and TVBN reached always concentrations higher than 11 and 37 mg-N 100 g^{-1} respectively. Figure 2 represents the kinetics of TMA and TVBN production during the vacuum storage at 5°C in samples 9 and 3 representing groups 1 and 2 respectively. The pH, initially equal to 6.20 ± 0.04, was rather constant during the storage of most of the samples except for samples 3, 6 and 10 in which a significant acidification to pH 5.9 was observed (Figure 2).

Figure 2 : Evolution of pH (■ □), trymethylamine (● ○) and total volatile basic nitrogen (▲ Δ) in samples 3 (close symbols) and 9 (open symbols) during the vacuum storage at 5°C.



Relationship between shelf-life and initial composition

Despite of the relatively homogeneous chemical composition of the thirteen samples, shelf-life ranged between 1 to more than 6 weeks. The results of fitting a multiple linear regression model to describe the relationship between shelf-life and initial pH, lipid, water, NaCl and phenol contents confirmed that there was no statistically significant correlation between the variables at the 90% or higher confidence level (data not shown). The data of Table 1 also indicate that shelf-life was not related to the raw material geographic origin. The relationships between shelf-life and initial microbiological load of the samples were investigated. Results of the stepwise forward multiple regression showed that the shelf-life was mostly linked to the initial Enterobacteriaceae count ($P<0.05$), the higher initial total count on VRBG agar, the shorter the shelf-life. However, the low R-squared statistic (0.69) indicated that this measure could not be used alone to precisely predict the shelf-life. The initial level of Enterobacteriaceae seemed to be related to the smokehouse rather than to the raw material quality. Samples coming from plants C and D, which had the shorter shelf-life (1-3 weeks), had an initial Enterobacteriaceae load always higher than $10^{4.6}$ cfu g^{-1}, whatever the raw material processed in these plants, and samples from plants A, B and E, which had the longer shelf-life (4->6 weeks), had an initial load always lower than $10^{3.4}$ cfu g^{-1}.

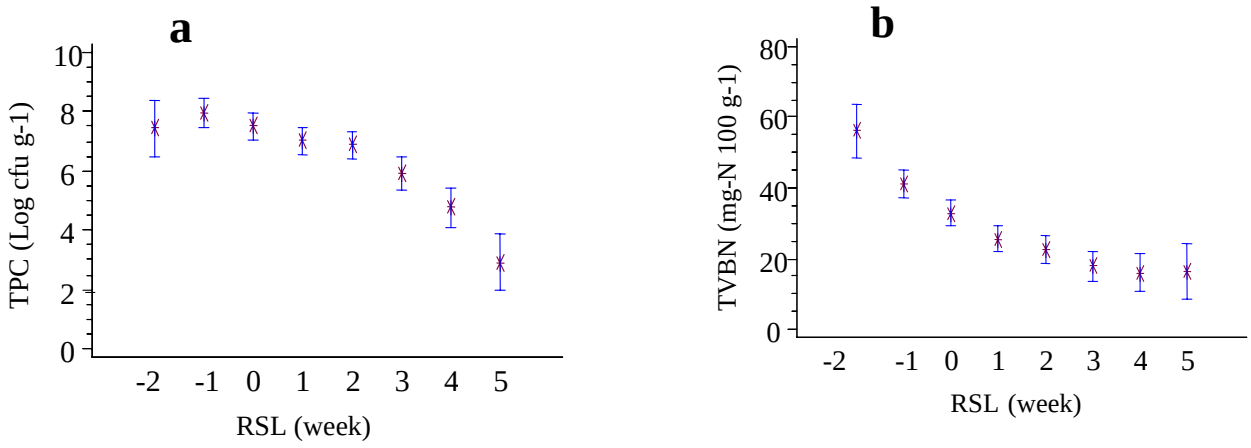
Relationship between remaining shelf-life and microbiological and chemical data

Simple compound quality index. When observing the microbial growth curves for the thirteen samples, it appeared difficult to find a single rule for prediction of shelf-life. In some cases, the product was rejected several weeks after all the enumerated micro-organisms had reached their maximum levels (Figure 1a, 1c) and in other cases (samples 4, 6 and 10, data not shown) very early during the exponential growth phase of the micro-organisms. Also we have seen that different micro-organisms dominated the spoilage micro-flora at the SRT. Lactobacilli,

lactobacilli/Enterobacteriaceae and carnobacteria/*B. thermosphacta* were in a majority of scenarios 1, 2 and 3 respectively, corresponding to samples with an associated shelf-life of 1-3, 4->5 and 4-5 weeks respectively. Results of the one-way ANOVA confirmed that there was no statistical difference between groups of samples that had reached their lifetime and samples that were not yet rejected by the panelists for any of the microbiological responses measured. As an example, Figure 3a shows the means plot and 95% LSD intervals for TPC. Although the average TPC was lower at the beginning of the storage period (RSL of 3 to 5 weeks), no significant difference could be observed between samples with RSL ranging between 2 and –2 weeks.

Chemical indices seemed to be of more value to estimate the shelf-life. All samples of group 1 with low TMA and TVBN values had a shelf-life longer than 4 weeks whereas 6 out of 8 samples of group 2 with high TMA and TVBN values had a shelf-life inferior to 4 weeks. One way ANOVA confirmed that there was a significant difference ($P<0.05$) in TVBN means between samples with a RSL of 1, 0, -1 and –2 weeks (Figure 3b). TMA was less discriminant as there was a difference between samples with a –2, 0 and 2 weeks RSL but not between samples with a –1, 0 and 1 weeks RSL (data not shown). The average TVBN and TMA concentrations for samples at the SRT were 32.7 ± 3.6 mg-N 100 g^{-1} and 7.4 ± 1.4 mg-N 100 g^{-1}. No statistical difference in the pH means was noticed. Although TVBN concentration in the flesh seemed to be of most value for estimation of cold-smoked salmon quality, it could not be used alone to precisely predict the shelf-life.

Figure 3 : Means plot and 95% intervals for (a) : total psychrotrophic count and (b) : total volatile basic nitrogen versus remaining shelf-life.



Multiple compounds quality index. Relationships between RSL and different microbial and chemical parameters measured (TPC, LAB, lactobacilli, *B. thermosphacta*, Enterobacteriaceae and yeast counts, pH, TVBN and TMA) were established using the forward stepwise multiple regression method. Four batches (5, 9, 12 and 13) out of 13 were not rejected before the end of the experiment and RSL could not be determined. Stepwise regression was done with the other 9 batches corresponding to 47 samples (9 batches analysed weekly). Forty-four samples were used for calculation of the model and 3 samples have been left out for validation. Results showed that there was a statistically significant relationship at the 99% confidence level between the RSL and lactobacilli count and TVBN concentration, and at the 95% confidence level for yeast count. The equation of the fitted polynomial model was : $RSL_{(week)} = 5.65 - 0.31 \times \text{Log (OGA count)}_{\text{cfu g}^{-1}} - 0.25 \times \text{Log (ROG count)}_{\text{cfu g}^{-1}} - 0.06 \times (\text{TVBN})_{\text{mg-N 100 g}^{-1}}$. The model was successfully validated with the 3 left out samples F1 week 1, F2 week 0 and F8 week 4 (Figure 4). R^2 indicated that the model explained 80% of the variability in the RSL. Lactobacilli count had the major influence on RSL ($R^2 = 0.64$). Adding TVBN concentration in the model increased the R^2 up to 0.77 and finally yeasts count to 0.80. R^2 was not significantly increased by adding the other microbial and chemical descriptors

indicating they were either not good quality indices for smoked salmon, either highly correlated with the 3 selected factors. Different combinations of lactobacilli and yeasts counts and TVBN concentrations could lead to the rejection of a product (RSL = 0). Figure 5 represents the RSL as a function of lactobacilli count and TVBN concentration for a yeast count fixed to 10^4 cfu g^{-1}. Assuming that lactobacilli could not exceed 10^9 cfu g^{-1}, a minimum of 36 mg-N 100 g^{-1} were necessary for a product to be rejected. With lower values such as 10^7, 10^4 or 10^2 cfu g^{-1}, products were rejected for TVBN concentrations reaching 44, 57 and 65 mg-N 100 g^{-1} respectively. According to Dalgaard *et al*. (1993) and Leroi *et al*. (2000), the RSL of a product has been calculated with the decision that a product was rejected when 50% at least of the judges estimated the sample was in class 3. Another way to estimate the sensory quality of cold-smoked salmon was the percentage of judges who had noted the product in class 3 (% class3) (Jorgensen *et al*. 2000). Also a quality coefficient (QC) taking into account of the 3 classes with an arbitrary weighting factor attributed to each class has been calculated as follows : QC = [(1 x % class 1) + (2 x % class 2) + (3 x % class 3)] / 100. Those responses allowed to integrate in our model all the batches which had not been rejected before the end of our experiment. Results of the stepwise multiple regression showed a relationship with TVBN concentration but quality of the fitted models were lower than with RSL (R^2 = 0.62 and 0.53 for % class 3 and QC responses respectively).

Figure 4 : Correlation between observed remaining shelf-life and predicted with the model 4.78 - 0.34 Log (ROG count)$_{cfu\ g^{-1}}$ – 0.06 x (TVBN)$_{mg\text{-}N\ 100\ g^{-1}}$ (R^2=0.77). Cross indicate the 3 left out samples.

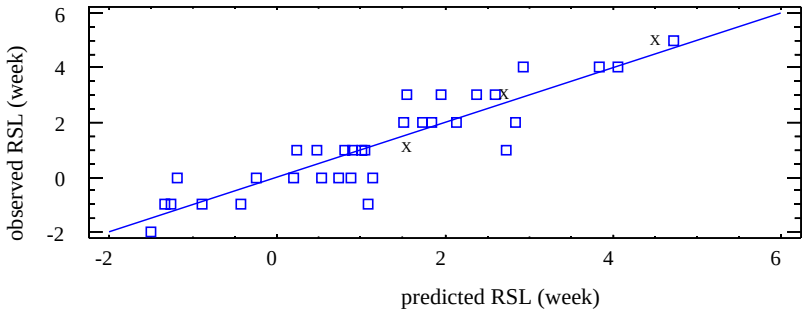
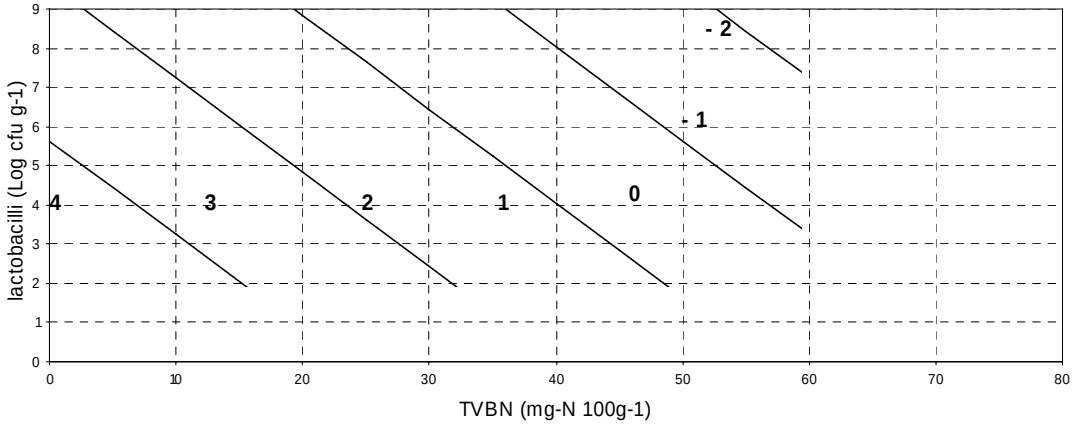


Figure 5 : Isoresponse curves for cold-smoked salmon remaining shelf-life (week) versus total volatile basic nitrogen concentration and lactobacilli count on Rogosa agar (yeast count fixed to 10^4 cfu g^{-1}).



DISCUSSION

Initial chemical characteristics of cold-smoked salmon were consistent with the French Standard NF V45-065 (1997) specifications, *i.e.* lipid < 18 % (w/w), water content of the de-fatted product < 74 % and NaCl concentration ranging between 2.5 and 3.5 % (w/w). The wide variation in phenol content was concordant with results obtained in a large scientific investigation on French cold-smoked salmon (Leglise *et al*. 1996). The large variation in the

initial contamination of cold-smoked salmon coming from different smokehouses (10^2 to 10^6 cfu g^{-1}) and the differences in the quantitative and qualitative microbiological composition at the SRT had already been observed by Truelstrup Hansen and Huss (1998), Truelstrup Hansen *et al*. (1998) and Jorgensen *et al*. (2000). Two of the 3 scenarios proposed, *i.e.* domination of lactobacilli or a mixture of lactobacilli and Enterobacteriaceae have also been found by those authors whereas the last one , *i.e.* domination of Carnobacteria and *B. thermosphacta* was less current. Carnobacteria have been isolated from Danish (Paludan-Müller *et al*. 1998 ; Truelstrup Hansen and Huss 1998) and French products (Leroi *et al*. 1998, 2000) but *B. thermosphacta* have never been found at higher level than 10^4 cfu g^{-1} (Truelstrup Hansen *et al*. 1996). Marine vibrio/*Photobacterium* spp. have frequently been isolated in high number from cold-smoked salmon. Although not enumerated with selective culture medium in our study, characterization of colonies picked from LH indicated that they were present in samples 3, 4 and 7 and 9 (data not shown).

It was difficult to rely the sensory profiles of the samples to their microbiological composition, except for H_2S odour which could be due to the presence of lactobacilli in high number. Indeed, *Lact. sake* is able to produce H_2S in cold-smoked salmon (Truelstrup Hansen 1995 ; Leroi *et al.* 1999 ; Joffraud *et al*. in press). Moreover, samples which had developed H_2S odours corresponded to samples with high lactobacilli counts and in which pH had dropped to 5.8 – 5.9. Leroi *et al.* (1999) have shown that among nine bacterial group currently identified in cold-smoked salmon, *Lactobacillus* spp. was the only genus which was able to acidify the product to those values.

As shown by Truelstrup Hansen *et al*. (1998) in 3 different Danish processing plants, the shelf-life of cold-smoked salmon was highly related to hygienic conditions in the smokehouse rather than to the raw material quality or to the processing parameters. Some authors have established that cold-smoked salmon shelf-life was extended with increasing salt

and/or phenol concentration in the flesh (Shimasaki *et al.* 1994 ; Truelstrup Hansen *et al*. 1995 ; Leroi and Joffraud 2000), but those results were obtained with samples processed under otherwise identical conditions, and with higher differences in salt and phenol levels.

As demonstrated by many studies, no single chemical compound nor microbiological count could be used as an index of quality for vacuum-packed cold-smoked salmon. However, a combination of the three parameters TVBN and lactobacilli and yeast counts could be used to successfully predict the RSL. The quality of the fitted model ($R^2 = 0.80$) was identical to this of the model developed by Jorgensen *et al*. (2000) relying sensory data with biogenic amines and pH ($R^2 = 0.79$). According to Leroi *et al.* (1999), the higher producers of TVBN in cold-smoked salmon were Enterobacteriaceae, *Photobacterium* spp. and *Lactobacillus* spp. Thus, our model integrated most of the potential spoilage organisms identified by those authors. Although *B. thermosphacta* was found to be a strong spoiler organism (Leroi *et al.* 1999), it was not included in our model. In our set of experiments, *B. thermosphacta* never reached level higher than 10^{6-7} cfu g^{-1}, probably explaining that this organism was not considered to be of any importance in estimation of RSL. With the intention of lowering the number of routine analysis, for development of a standard for example, simplification of the model by eliminating the yeasts count could be proposed without losing too much precision ($R^2 = 0.77$). The simplified model was : $RSL_{(week)} = 4.78 - 0.34 \text{ Log (ROG count)}_{\text{cfu g}^{-1}} - 0.06 \text{ x (TVBN)}_{\text{mg-N 100 g}^{-1}}$. With this model, a product was rejected with counts on ROG agar of 10^9, 10^7, 10^4 or 10^2 cfu g^{-1} associated with TVBN concentrations of 30, 40, 57 and 68 mg-N 100 g^{-1} respectively. The multiple compounds quality index has been developed for samples having a shelf-life inferior to 6 weeks. The quality of the model was apparently lowered when adding samples which had a shelf-life higher than 6 weeks. This was mainly due to the use of a different way for assessing sensory quality (% class 3 and QC) rather than integration of samples with long shelf-life. Indeed, the R^2 of the fitted model calculated for % class 3 or QC

with the samples rejected before 6 weeks were lower (respectively 0.66 and 0.62) than the R^2 calculated for RSL (0.80). Nevertheless, more experiments would be necessary to validate the multiple compound quality index proposed in this study for sample with longer shelf-life.

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