

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

F. Leroi, J.J. Joffraud, F. Chevalier and M. Cardinal

Laboratoire de Génie Alimentaire, IFREMER, Nantes, France

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Aims: 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 ($P < 0.05$), depending on hygienic conditions in the smokehouse. High correlations existed between the RSL and lactobacilli count ($P < 0.01$), yeast count ($P < 0.05$) and TVBN concentration ($P < 0.01$). A polynomial fitting the RSL as a function of those three factors was proposed ($R = 0.80$). Assuming that lactobacilli count could not exceed 10^9 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 10^4 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.

INTRODUCTION

Vacuum-packed, sliced, cold-smoked salmon is a highly perishable product, because of light preservative treatments (salt on product ranging between 2.5 and 3.5% w/w and phenol generally less than 0.5 mg 100 g⁻¹) and no other additives, such as nitrate or nitrite, being 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 microflora 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 microflora (Jorgensen *et al.* 2000; 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). Among these bacterial groups, *Lactobacillus sake*, *B. thermosphacta*, *Serratia liquefaciens* and *Photobacterium phosphoreum* have

Correspondence to: F. Leroi, Laboratoire de Génie Alimentaire, IFREMER, BP 21105, 44 311 Nantes Cedex 3, France (e-mail: fleroi@ifremer.fr).

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 Erol 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 amine production and pH. Biogenic amine 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 storage at 5°C. Parameters linked to raw material and process, i.e. lipid, water, salt and phenol contents, were measured and also the 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 five French smokehouses and transported to the laboratory in a frozen condition. The geographical origin of the raw material and smokehouses is 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 20–26°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. Microbial, chemical and sensory analyses were made each week from week 0 until 1 week after sensory spoilage was evident.

Sensory analysis

Two to three bags per lot were opened and divided into 20-g portions in aluminium foil to keep the odours intact. Fourteen trained panellists 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 sulphide,

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

Label	Smokehouse	Raw material	Origin	Shelf-life (weeks)	Water (% w/w)	Lipid (% w/w)	NaCl (% w/w)	Phenol (mg 100 g ⁻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

cabbage and faecal. Results of the profiling test were transformed in order 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, panellists classified each sample depending on the spoilage level as: 1, no off-odour, 2, weak off-odour and 3, strong off-odour. The sensory rejection time (SRT) was determined when at least seven judges estimated that the product was in class 3. The RSL of a sample was the difference between the SRT (in weeks) 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, stomached in 120 ml chilled diluent (0.85% NaCl and 0.1% peptone) for 2 min in a Stomacher 400 (Seward Laboratory Blender, London, UK). After resuscitation for 30 min at room temperature, 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 d 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). Nitrite actidione polymyxin agar and ROG plates were incubated at 20°C under 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, UK). Enterobacteriaceae counts were determined in pour plates of CASO agar (Merck) overlaid by Violet Red Bile Glucose agar (VRBG; Oxoid), incubated at 30°C for 2 d. Assuming that non-typical colonies could also belong to the Enterobacteriaceae family (unpublished data), two counts were prepared: typical colonies (VRBG typical), corresponding to red colonies with a diameter larger than 0.5×10^{-3} m, and total colonies (VRBG total), corresponding to all the colonies growing in the plate. *Brochothrix thermosphacta* was enumerated on spread plates of Streptomycin Thallium Acetate Actidion (STAA) (Gardner 1966) (2% peptone, 0.2% yeast extract, 0.1% KH_2PO_4 , 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 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.

Both 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 (Difco, Sparks, MD, USA) and partially identified by morphology

and motility examination, KOH Gram reaction and catalase and oxidase activity, as described by Leroi *et al.* (1998).

Chemical analysis

The remaining flesh in the three bags opened for microbiological analysis was homogenized in a Waring Blender (New Hartford, CO, USA). Each week, TVBN and TMA were measured in duplicate by the Conway microdiffusion method (Conway and Byrne 1933). The pH value was measured in the fivefold-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 13 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 each microbiological or chemical index successively. 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. The characteristics of cold-smoked salmon directly linked with the raw material composition and processing parameters (lipid, water, NaCl, phenol and initial pH) are summarized in Table 1. The 13 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 $3.1 \pm 0.3\%$ (w/w) (95% confidence limit calculated with Student's *t*-test = 2.179), corresponding to $5.2 \pm 0.5\%$ in the 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. The shelf-life observed by the panel ranged between 1 and more than 6 weeks (Table 1). The number of spoiled samples was identical for each sampling date (around two 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 13 cold-smoked salmon lots could be divided into three groups by Ward's hierarchical clustering method, each 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 hydrogen sulphide, cabbage and faecal off-odours.

Microbiological composition. The microbiological composition of cold-smoked salmon samples at week 0 and at the SRT is presented in Table 2. The initial flora was very different from one sample to another. The TPC in lots 2, 5, 9 and 13 were lower than 10² cfu g⁻¹, higher than 10⁵ cfu g⁻¹ in lots 3, 6, 10, 11 and 12 and between these two values for lots 1, 7 and 8. During the vacuum storage at 5°C, TPC increased to its maximum level (10⁷–10⁹ cfu g⁻¹) more or less quickly depending on the samples and remained at this level until spoilage, sometimes several weeks later (data not shown). Variations in the composition of the microflora between lots were very important and three scenarios could be distinguished. They are represented in Fig. 1a–c corresponding to the growth patterns of the different microorganisms in lots 3, 9 and 7, respectively. In scenario 1 (lots 3, 4, 6 and 10; Fig. 1a), TPC reached 10^{8–9} cfu g⁻¹ and the total LAB and count on Rogosa agar were equal to TPC, indicating that lactobacilli were the dominating flora. *Brochothrix thermosphacta*, yeasts and Enterobacteriaceae were in a minority, never exceeding 1% of TPC. In scenario 2, corresponding to lots 1, 2, 5 and 9 (Fig. 1b), the spoilage microflora was mainly represented by lactobacilli and Enterobacteriaceae and, to a lesser extent, yeasts. *Brochothrix 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; Fig. 1c) TPC was dominated by total LAB and *B. thermosphacta*. According to Leroi *et al.* (1998, 2000), LAB probably belonged to the *Carnobacterium* genus because the lactobacilli count on Rogosa agar was always 2 log lower than the count on NAP, except for lot 8, for which lactobacilli became dominant at the end of the storage (Table 2). Counts of yeasts and

Enterobacteriaceae were low, ranging between 10⁴ and 10⁵ cfu g⁻¹. The three samples 11, 12 and 13 could not be associated with any of the three scenarios because lactobacilli, *B. thermosphacta* and yeast counts were not determined. However, in the three samples, LAB were the dominating flora and the Enterobacteriaceae count always remained 1–2 log lower than TPC, indicating that these samples could follow scenario 1 or 3.

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, two groups of samples could be distinguished: Group 1 (lots 2, 5, 7, 8 and 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 and 13) in which TMA and TVBN always reached concentrations higher than 11 and 37 mg-N 100 g⁻¹, respectively. Figure 2 represents the kinetics of TMA and TVBN production during 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 (Fig. 2).

Relationship between shelf-life and initial composition

Despite the relatively homogeneous chemical composition of the 13 samples, the shelf-life ranged between 1 and 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 in Table 1 also indicate that shelf-life was not related to the geographical origin of the raw material. 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 the initial total count on VRBG agar, the shorter the shelf-life. However, the low R^2 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 a shorter shelf-life (1–3 weeks), had an initial Enterobacteriaceae load always higher than 10^{4.6} cfu g⁻¹, whatever the raw

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

Label	Date of analysis*	Remaining shelf-life*	pH	Total		Total psychrotrophic count‡	Lactic acid bacteria‡		Lactobacilli‡	<i>Brochothrix thermosphacta</i> ‡	Yeasts‡	Enterobacteriaceae (total colonies)‡	Enterobacteriaceae (typical colonies)‡
				volatile nitrogen†	basic amine†		nitrogen†	amine†					
F1	0	4	6.09	14.1	1.3	3.1	0.0	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	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	0.0
F2	5	0	6.14	21.9	5.9	7.4	7.3	7.2	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	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	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	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	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	0.0
F5	5	ND	6.19	27.5	3.9	8.5	7.8	7.9	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	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	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	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	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	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	6.4	4.6	4.3	0.0
F9	0	ND	6.17	13.8	1.3	2.2	0.0	1.7	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	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	5.6	5.0	4.7	5.0	2.3
F10	2	0	6.01	33.7	8.2	9.0	9.1	9.0	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	ND	2.1	1.0
F11	4	0	6.29	36.7	7.9	6.6	6.6	ND	ND	ND	ND	5.4	4.9
F12	0	ND	6.22	17.0	2.0	5.0	5.0	ND	ND	ND	ND	3.4	3.4
F12	6	ND	6.21	41.9	14.4	7.3	6.9	ND	ND	ND	ND	4.6	4.5
F13	0	ND	6.24	15.1	1.3	2.0	2.4	ND	ND	ND	ND	1.6	0.0
F13	6	ND	6.16	37.3	11.1	6.1	6.8	ND	ND	ND	ND	5.8	4.2

*Weeks.

†mg-N 100 g⁻¹.‡cfu g⁻¹.

ND, Not determined.

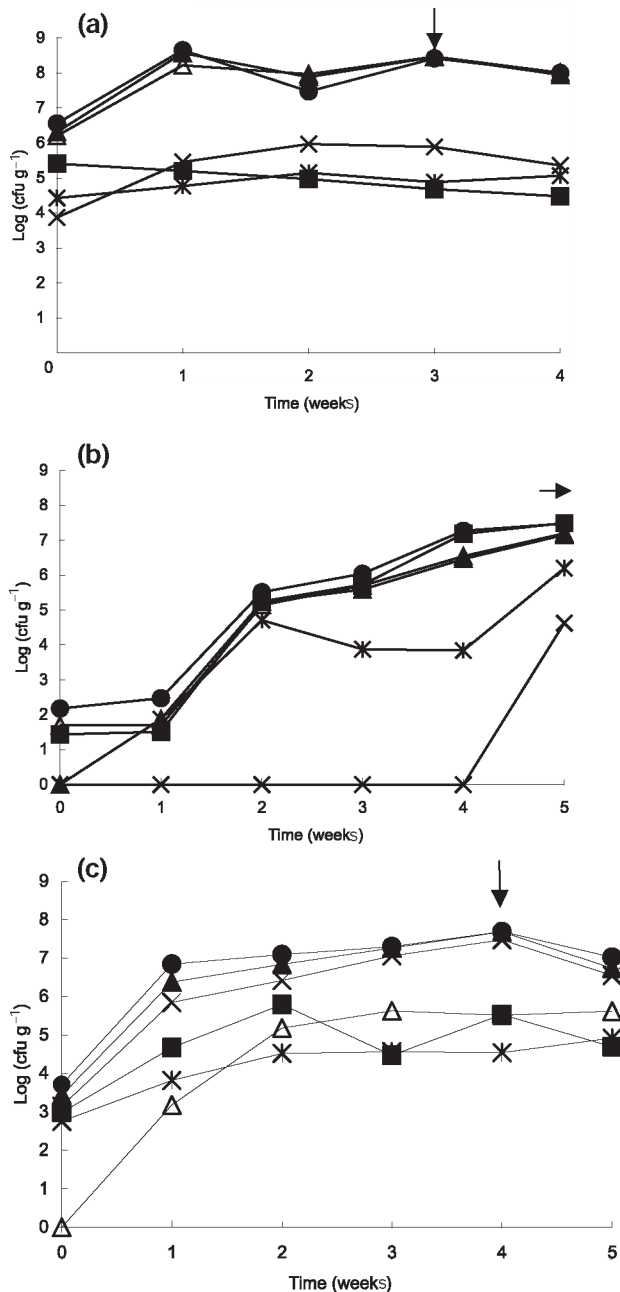


Fig. 1 Microflora evolution in samples (a) 3, (b) 9 and (c) 7 during the vacuum storage at 5 °C. ●, Total psychrotrophic count; ▲, total lactic acid bacteria; △, lactobacilli; ■, Enterobacteriaceae (violet red bile glucose agar total count); ×, *Brochothrix thermosphacta*; ✕, yeast. Arrow indicates the sensory rejection time

material processed in these plants, and samples from plants A, B and E, which had a longer shelf-life (4–> 6 weeks), had an initial load always lower than $10^{3.4}$ cfu g^{-1} .

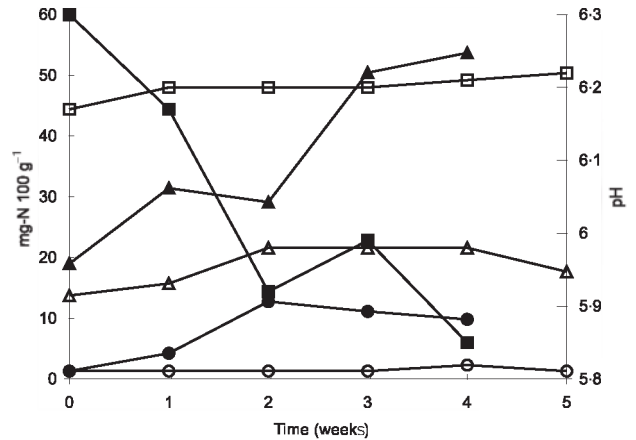


Fig. 2 Evolution of pH (■, □), trimethylamine (●, ○) and total volatile basic nitrogen (▲, △) in samples 3 (closed symbols) and 9 (open symbols) during the vacuum storage at 5 °C

Relationship between remaining shelf-life and microbiological and chemical data

Simple compound quality index. When observing the microbial growth curves of the 13 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 (Fig. 1a and c) and in other cases (samples 4, 6 and 10, data not shown) very early during the exponential growth phase of the micro-organisms. We have also seen that different micro-organisms dominated the spoilage microflora at the SRT. Lactobacilli, lactobacilli/Enterobacteriaceae and Carnobacteria/*B. thermosphacta* were present 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 had not yet been rejected by the panellists for any of the microbiological responses measured. For example, Fig. 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 3–5 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 in estimating the shelf-life. All samples in group 1 with low TMA and TVBN values had a shelf-life longer than 4 weeks, whereas six of eight samples in group 2 with high TMA and TVBN values had a shelf-life less than 4 weeks. One-way ANOVA confirmed that there was a significant difference ($P < 0.05$) in TVBN means between samples with an RSL of 1, 0, –1 and –2 weeks (Fig. 3b). The TMA was less discriminant as

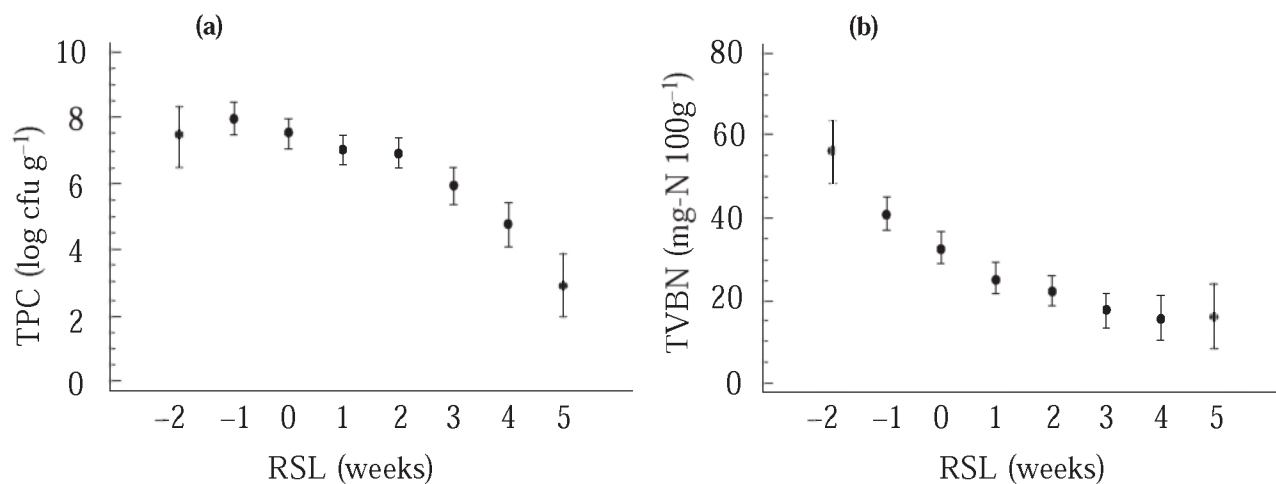


Fig. 3 Means plot and 95% intervals for (a) total psychrotrophic count (TPC) and (b) total volatile basic nitrogen (TVBN) vs remaining shelf-life (RSL)

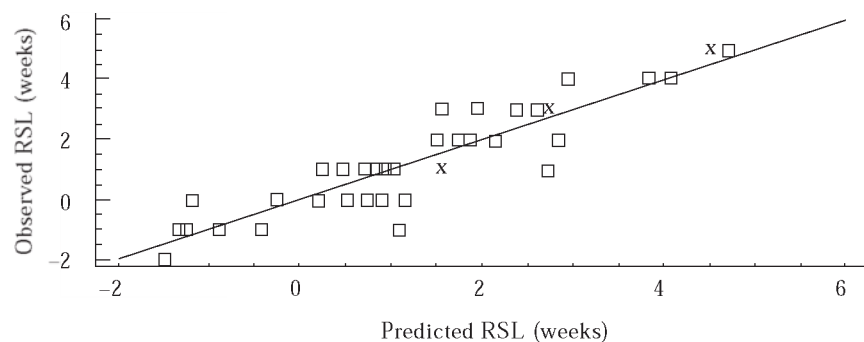


Fig. 4 Correlation between observed remaining shelf-life (RSL) and that predicted with the model $5.65 - 0.31 \log(\text{OGA count})_{\text{cfu g}^{-1}} - 0.25 \times \log(\text{ROG count})_{\text{cfu g}^{-1}} - 0.06 \times (\text{TVBN})_{\text{mg-N}100 \text{ g}^{-1}}$ ($R^2 = 0.80$). ×, Three omitted samples

there was a difference between samples with a -2, 0 and 2 week RSL but not between samples with a -1, 0 and 1 week RSL (data not shown). The average TVBN and TMA concentrations for samples at the SRT were 32.7 ± 3.6 and 7.4 ± 1.4 mg-N 100 g^{-1} , respectively. No statistical difference in the pH means was noticed. Although the 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.

Multiple compounds quality index. Relationships between RSL and different measured microbial and chemical parameters (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) of 13 were not rejected before the end of the experiment and RSL could not be determined. Stepwise regression was performed with the other nine batches corresponding to 47 samples (nine batches analysed weekly). Forty-four samples were used for calculation of the model and three 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

$$\text{RSL}_{(\text{week})} = 5.65 - 0.31 \times \log(\text{OGA count})_{\text{cfu g}^{-1}} - 0.25 \times \log(\text{ROG count})_{\text{cfu g}^{-1}} - 0.06 \times (\text{TVBN})_{\text{mg-N}100 \text{ g}^{-1}}$$

The model was successfully validated with the three omitted samples, F1 week 1, F2 week 0 and F8 week 4 (Fig. 4). R^2 indicated that the model explained 80% of the variability in the RSL. The lactobacilli count had the major influence on RSL ($R^2 = 0.64$). Adding the TVBN concentration to the model increased the R^2 to 0.77 and finally the yeast count to 0.80. R^2 was not significantly increased by adding the other microbial and chemical descriptors, indicating that they were either not good quality indices for smoked salmon, or not highly correlated with the three selected factors. Different combinations of lactobacilli and yeasts counts and TVBN

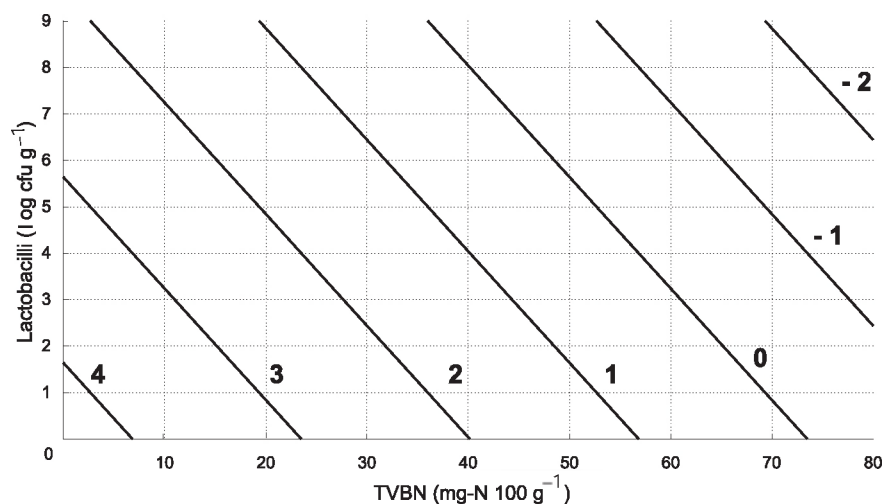


Fig. 5 Isoresponse curves for cold-smoked salmon remaining shelf-life (weeks) *vs* total volatile basic nitrogen (TVBN) concentration and lactobacilli count on Rogosa agar (yeast count fixed to 10^4 cfu g^{-1})

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 at least 50% of the judges estimated that the sample was in class 3. An alternative way of estimating the sensory quality of cold-smoked salmon was the percentage of judges who had noted the product to be in class 3 (% class 3) (Jorgensen *et al.* 2000). A quality coefficient (QC), taking into account the three classes with an arbitrary weighting factor attributed to each class, has also been calculated as follows

$$QC = [(1 \times \%class1) + (2 \times \%class2) + (3 \times \%class3)] / 100$$

These responses allowed the integration in our model of 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 the quality of the fitted models was lower than with RSL ($R^2 = 0.62$ and 0.53 for percentage class 3 and QC responses, respectively).

DISCUSSION

The 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 defatted 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 – 10^6 cfu g^{-1}) and the differences in the quantitative and qualitative microbiological composition at the SRT had previously been observed by Truelstrup Hansen and Huss (1998), Truelstrup Hansen *et al.* (1998) and Jorgensen *et al.* (2000). Two of the three scenarios proposed, i.e. domination of lactobacilli or a mixture of lactobacilli and Enterobacteriaceae, have also been found by these authors whereas the last scenario, 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 levels higher than 10^4 cfu g^{-1} (Truelstrup Hansen *et al.* 1996). Marine vibrio/*Photobacterium* spp. have frequently been isolated in high numbers 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, 7 and 9 (data not shown).

It was difficult to relate 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 numbers. 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 the pH had dropped to 5.8–5.9. Leroi *et al.* (1999) have shown that, among nine bacterial groups currently identified in cold-smoked salmon, *Lactobacillus* was the only genus which was able to acidify the product to these values.

As shown by Truelstrup Hansen *et al.* (1998) in three 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 the shelf-life of cold-smoked salmon was extended with increasing salt and/or phenol concentration in the flesh (Shimasaki *et al.* 1994; Truelstrup Hansen *et al.* 1995; Leroi & Joffraud 2000), but these results were obtained with samples processed under otherwise identical conditions and with greater 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 three parameters, TVBN, 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 that of the model developed by Jorgensen *et al.* (2000) fitting sensory data to 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 these authors. Although *B. thermosphacta* was found to be a strong spoilage organism (Leroi *et al.* 1999), it was not included in our model. In our set of experiments, *B. thermosphacta* never reached levels greater than 10^{6-7} cfu g⁻¹, probably indicating that this organism was not of any importance in the estimation of RSL. Simplification of the model, by eliminating the yeast count, could be proposed, without losing too much precision ($R^2 = 0.77$), with the intention of lowering the number of routine analyses (e.g. for development of a standard). The simplified model was

$$\text{RSL}_{(\text{week})} = 4.78 - 0.34 \log(\text{ROG count})_{\text{cfu g}^{-1}} - 0.06 \times (\text{TVBN})_{\text{mg-N}100 \text{ g}^{-1}}$$

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 of less than 6 weeks. The quality of the model was apparently lowered when adding samples which had a shelf-life of greater than 6 weeks. This was mainly due to the use of a different method of assessing sensory quality (% class 3 and QC) rather than integration of samples with a long shelf-life. Indeed, the R^2 of the fitted model calculated for percentage class 3 or QC with the samples rejected before 6 weeks was lower (0.66 and 0.62, respectively) 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 samples with a longer shelf-life.

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