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Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon

J.J. Joffraud [a,*], F. Leroi [a], C. Roy [a], J.L. Berdagué [b]

[a] *IFREMER, Laboratoire de Génie Alimentaire, BP 21105, 44311 Nantes Cedex 03, France*
[b] *INRA de Theix, Station de Recherches sur la Viande, 63122 Saint Genès Champanelle, France*

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Abstract

This study investigated the volatile compounds produced by bacteria belonging to nine different bacterial groups: *Lactobacillus sake*, *L. farciminis*, *L. alimentarius*, *Carnobacterium piscicola*, *Aeromonas* sp., *Shewanella putrefaciens*, *Brochothrix thermosphacta*, *Photobacterium phosphoreum* and *Enterobacteriaceae* isolated from cold-smoked salmon. Each bacterial group was represented by several strains. In addition, combinations of the groups were examined as well. Sterile blocks of cold-smoked salmon were inoculated, vacuum-packed and stored at 6°C. After 40 days of storage at 6°C, aerobic viable count and pH were recorded, the volatile fraction of the samples was analysed by gas chromatography-mass spectrometry (GC-MS), and spoilage was assessed by sensory evaluation. Among the 81 volatile compounds identified by GC-MS, 30 appeared to be released as a result of bacterial metabolism. Some of the effects of inoculated bacterial strains on the composition of the volatile fraction seemed to be characteristic of certain bacterial species. Sensory analysis showed relationships between bacteria, the composition of the volatile fraction and the organoleptic quality of smoked salmon.

Keywords: Cold-smoked salmon; Spoilage; Bacteria; Volatile compounds

1. Introduction

Cold-smoked salmon is a lightly-preserved fish product which may involve health risks (Huss et al., 1995; Heinitz and Johnson, 1998), especially from the presence of *Listeria monocytogenes* (Ben Embarek, 1994; Jorgensen and Huss, 1998). Moreover, the sensory characteristics of lightly cured products are often affected with textural changes and the occurrence of off-odours after only 2 or 3 weeks of storage (von Rakow, 1977; Schulze and Zimmermann, 1983; Nieper, 1986; Hildebrandt and Erol, 1988; Truelstrup Hansen et al., 1995, 1996). Such spoilage effects are usually indicative of microbial activity. In recent years, some studies have considered the specific bacterial flora of cold-smoked salmon and determined the main taxonomic groups that occur frequently in this product (Leroi et al., 1998; Paludan-Müller et al., 1998; Truelstrup Hansen et al., 1998).

The identification of the bacteria responsible for the spoilage of smoked salmon is important to allow (i) a better understanding of the mechanisms in-

* Corresponding author. Tel.: +33-2-40-37-4088; fax: +33-2-40-37-4071.
E-mail address: joffraud@ifremer.fr (J.J. Joffraud).

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volved in spoilage and of any changes in the product during the salting and smoking process that limit the growth of spoilage flora; (ii) the development of convenient means of counting the spoilage flora; and (iii) the design of a model for predicting deterioration in the quality of smoked fish products.

The present study investigated the spoilage potential of bacteria isolated from smoked salmon (Leroi et al., 1998) and known to be present throughout the cold storage of this product. Spoilage potentials were assessed by analysing the compositions of the volatiles released from inoculated, spoiled, cold-smoked salmon.

2. Materials and methods

2.1. Isolates

Isolates were selected from the bacterial collection of the laboratory among strains belonging to the taxonomic groups: *Lactobacillus sake*, *L. farciminis*, *L. alimentarius*, *Carnobacterium piscicola*, *Aeromonas* sp., *Shewanella putrefaciens*, *Brochothrix thermosphacta*, *Photobacterium phosphoreum* and *Enterobacteriaceae*. Except the three isolates for *Enterobacteriaceae*, each group was represented by a mixture of five strains. In addition, five combinations of different species were also provided (Table 1).

Combination 1 included Gram-negative bacteria in an equal ratio; combination 2, all the bacteria except *Lactobacillus* spp. in an equal ratio; combination 3, all lactic acid bacteria together in an equal ratio; combination 4, all the species together, with Gram-negative bacteria comprising 75% of the total; and combination 5, all the species together, but in an inverse ratio to combination 4, with Gram-positive strains (LAB + *B. thermosphacta*) comprising 75% of the total.

Table 1
Compositions (Com) of the inocula of mixed cultures

Species	Combinations				
	Com. 1[a]	Com. 2[a]	Com. 3[a]	Com. 4[b]	Com. 5[c]
S. putrefaciens	X	X		X	X
B. thermosphacta		X		X	X
P. phosphoreum	X	X		X	X
Aeromonas sp.	X	X		X	X
L. alimentarius			X	X	X
L. farciminis			X	X	X
L. sake			X	X	X
C. piscicola		X	X	X	X

[a] Bacteria in equal ratio.
[b] Gram-negative bacteria comprising 75% of the inoculum.
[c] Gram-positive bacteria comprising 75% of the inoculum.

2.2. Sterile cold-smoked salmon model system

A sterile model system developed by an aseptic process and ionisation as described by Joffraud et al. (1998) was used as substrate. In this process, cold-smoked salmon were diced into approximately 5-mm-sided cubes before the ionisation treatment. The composition of this model system was 58% water, 18% lipids, 4.8% NaCl in water phase and 0.62 mg 100 g^{-1} phenols and the initial pH was 6.15.

2.3. Culture and inoculation procedure

Strains stored at −80°C were removed from cryovials and inoculated into appropriate culture media. *C. piscicola* strains were grown in Elliker broth (Elliker et al., 1956) (Biokar Diagnostics, Beauvais, France), *Lactobacillus* spp. isolates in Man, Rogosa and Sharpe (MRS) broth (de Man et al., 1960) (Merck, Darmstadt, Germany), and the other strains in brain–heart infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA).

Strains were grown at 20°C for 3 days and then in subculture at 20°C for 2 days. Five isolates belonging to the same genus or species were mixed and the mixture was twice diluted tenfold in sterile peptone water.

The second dilution of the mixture was used to inoculate cold-smoked salmon pieces of the model system. To achieve an inoculation rate of approx. 10^5 cells/g, 6.6 ml of culture at 10^7 cells/ml were added to 330 g of sterile cold-smoked salmon in a sterile flask and then stirred to homogenise the mixture. A control was created by replacing the inoculum with sterile peptone water.

The inoculated samples and the control were then vacuum-packed in polyamide/polyethylene bags (PA/PE 20/70, Euralpac, Alfo, Germany), the permeability for O_2 was 40–50 cm^3/m^2 and for CO_2, 146 cm^3/m^2 in 24 h, 1 atm at 23°C, 75% RH.

Before being vacuum-packed, salmon pieces were wrapped in an aluminium sheet to prevent volatile compounds from the bag material being absorbed by the product.

Each batch inoculated with a single bacterial group was distributed in four bags: two for volatile compound analysis, one for bacteriological and sensory analyses and an additional one if needed. Samples were stored at 6°C for 40 days before being withdrawn for GC-MS analysis, enumeration of bacteria and sensory evaluation.

2.4. Enumeration of bacteria and pH measurement

The aerobic viable count was determined on Long and Hammer's medium containing 1% NaCl (van Spreekens, 1974). Thirty grams of salmon samples were homogenised and diluted in 120 ml of chilled physiological saline containing 0.85% (w/v) NaCl and 0.1% (w/v) tryptone (Biokar) for 2 min in a stomacher (Lab. Blender, London, UK). After 30 min at room temperature, the homogenate was serially diluted 10-fold in physiological saline, and 0.1 ml of each appropriate dilution was spread-plated in duplicate on Long and Hammer's medium. Agar plates were incubated aerobically at 15°C for 5 days.

The pH value was measured in the five-fold dilution flesh with a pH meter (Mettler Delta 320, AES Laboratoire, Combourg, France).

2.5. Sensory evaluation

Sensory evaluation was carried out by seven trained panelists who sniffed the samples and chose free descriptors to define odours. According to this evaluation, they had to classify the samples into the following three groups defined by spoilage level: level 1, no spoilage noted; level 2, weak spoilage; level 3, strong spoilage.

The sample condition was regarded as spoiled when at least 50% of the panelists rated it in level 3.

2.6. Volatile compounds analysis

3.6 g diced portion of cold-smoked salmon was placed in a glass extraction cartridge of diameter 25 mm and length 135 mm (glass extraction cartridge M13, Etablissements Mailleres, Aubière 63170 France). The volatile components desorbing from the salmon were extracted at room temperature (20°C) by a stream of helium flowing at 60 ml min^{-1} for 45 min. They were adsorbed on a Tenax trap (Tenax Supelco, Alltech, Deerfield, IL. 60015, USA) of 60/80 mesh, 80 mm long, held at 25°C. The injection of volatile components into a gas chromatograph (Hewlett Packard 5890 series II, H.P., F 91947, Les Ulis, France) coupled to a mass spectrometer (Hewlett Packard 5791 A) was achieved by flash thermal desorption from the trap at 250°C with a desorption concentration injection system (DCI, DELSI Instruments, 92150 Suresnes, France). The components were then separated on a 60 m × 0.32 mm capillary column coated with a 1 μm film of DB5 (Supelco, C.H.-1196, Gland, Switzerland) using helium as carrier gas with a flow rate for injection through the column of 1 ml min^{-1}. The chromatograph oven was programmed for 5 min at 40°C, followed by a rise to 200°C at a rate of 3°C/min. Volatile components were detected by mass spectrometry with electron impact at 70 Ev and then identified by comparison of their experimental spectra with those in four data banks: NBS 75K, 1994; NIST/EPA/MSDC, 1996 (Royal Society of Chemistry, Milton road, Cambridge CB4 4WF, UK); NIST/EPA/NIH, 1996 (Mass spectral search program, National Institute of Standard and Technology, Gaithersburg, MD 20899, USA); Whiley 275 K, 1996. Their experimental Kovats indices (Tranchant, 1982) were compared with those in the data bank of Kondjoyan and Berdagué (1996). Peak areas of the volatile compounds expressed in arbitrary units of abundance (a.u.a) were integrated from the total ionic count.

2.7. Statistical analyses

Mean, median, minimum and maximum values were calculated for the volatile compounds. Principal component analysis (PCA) was performed on normalised data (Statistica, 1997) to study the effect of inoculated strains on these volatile compounds. This analysis was done from a 14-line (13 inoculated samples + the control) × 30-column (30 volatile compounds) matrix.

3. Results

3.1. Bacterial growth and pH

As the salmon inoculated with *P. phosphoreum* as pure culture was contaminated by other bacteria, this sample was not analysed, and no results were obtained for this bacterium. Bacterial counts and the pH of other samples at the end of the storage period are shown in Table 2.

No bacteria were recovered from the control sample. *Lactobacillus* spp. counts and those for *Lactobacillus*-containing combinations 3, 4, and 5 reached about 8.5 log cfu g^{-1}. This growth process led to a decrease in pH to about 5.6–5.8. Counts for other bacteria reached about 7.0–7.5 log cfu g^{-1} and the pH was in a range from 6.0 to 6.1.

3.2. Sensory evaluation

The results of the sensory evaluations are shown in Table 3. Most of the samples were regarded as spoiled, with the exception of samples inoculated with *C. piscicola*, *S. putrefaciens*, and combination 1.

Table 2
pH values and aerobic viable counts of sterile cold-smoked salmon inoculated with bacteria and stored vacuum packaged at 6°C for 40 days

Inoculum	pH	TVC (log cfu g^{-1})
Control	6.13	0
C. piscicola (Car)	6.10	7.5
L. alimentarius (Lba)	5.83	8.4
L. farciminis (Lbf)	5.63	8.5
L. sake (Lbs)	5.77	8.5
Aeromonas sp. (Aer)	6.01	7.5
S. putrefaciens (She)	6.12	7.1
B. thermosphacta (Bro)	6.03	6.8
Enterobacteriaceae (Ent)	6.11	7.2
Combination 1 (M1)	6.07	6.9
Combination 2 (M2)	6.12	7.3
Combination 3 (M3)	5.71	8.6
Combination 4 (M4)	5.69	8.4
Combination 5 (M5)	5.77	8.5

Table 3
Conditions and odours assigned by a seven members panel to samples of sterile cold-smoked salmon inoculated with bacteria and stored vacuum packaged at 6°C for 40 days

Inoculum	Condition	Sensory descriptors
L. alimentarius	Spoiled	Pungent, sour
L. farciminis	Spoiled	Sour, acid, milky
L. sake	Spoiled	H_2S, floorcloth
C. piscicola	Unspoiled	Butter, caramel, sour, fruity
Aeromonas spp.	Spoiled	Amine, socks, floorcloth
S. putrefaciens	Unspoiled	Sickly sweet
B. thermosphacta	Spoiled	Blue-cheese, sour, pungent
Enterobacteriaceae	Spoiled	Cheese, wine, butter, malty
Combination 1	Unspoiled	Smoke, wood fire
Combination 2	Spoiled	Socks, pungent, sour
Combination 3	Spoiled	H_2S, ham, acid
Combination 4	Spoiled	H_2S, acid, rancid
Combination 5	Spoiled	H_2S, floorcloth

Samples inoculated with *Lactobacillus* spp. were considered to be grossly spoiled, as they gave off strong unpleasant odours which were described as “sour”, “acid”, “pungent”, “floorcloth” and “hydrogen sulphide”. Samples inoculated with combinations containing *Lactobacillus* spp. were characterised by the same descriptors, particularly “hydrogen sulphide”.

Salmon inoculated with *S. putrefaciens* was not assessed as spoiled, even though the descriptor “sickly sweet” was used. The “amine” descriptor was cited for *Aeromonas* spp. The sample inoculated with *C. piscicola* was characterised by descriptors such as “butter” and “caramel”. As these odours were not unpleasant, the sample was not regarded as spoiled. The sample inoculated with *B. thermosphacta* was considered spoiled and was characterised by the descriptor “blue-cheese”.

3.3. Volatile compounds from GC-MS analysis

3.3.1. Volatile compounds of bacterial origin

One hundred and thirty-five compounds were isolated from samples and the control, most of which were identified although with various degrees of reliability. Only 30 of the compounds from bacterial origins were taken into consideration. These compounds, which were known to originate from the main microbial catabolic pathways of lipids, carbo-

hydrates and amino acids, are listed in Table 4 according to their main probable origins. Among these compounds, 2-butanone 3-hydroxy-, 2,3-butanedione, 1-butanol 2-methyl and 1-butanol 3-methyl were most abundant. About half of the compounds presented a "non-normal" content distribution, indicative of wide variability among the bacterial strains.

Table 4
Volatile compounds identified in cold-smoked salmon samples inoculated with bacteria and stored vacuum packaged at 6°C for 40 days

Compounds	Kovats indices[a]	Reliability of identification[b]	Extracted quantities (aua $\times 10^{-6}$)[c]
Amino acid catabolism			
1-Propanol, 2-methyl-	625	a	45; 0 (0–248)
Butanal, 3-methyl-	651	a	45; 5 (3–504)
Butanal, 2-methyl-	661	a	4; 1 (1–15.3)
1-Butanol, 3-methyl-[d]	731	a	218; 51 (2–1420)
1-Butanol, 2-methyl-[d]	735	a	102; 18 (0–748)
2-Butenal, 2-methyl-	741	c	3; 2 (0–13)
Disulphide, dimethyl-	746	a	18; 6 (2–76)
Residual glycogen catabolism			
2,3-Butanedione (diacetyl)[d]	588	a	130; 59 (4–632)
2,3-Pentanedione	690	a	9; 6 (4–24)
2-Butanone, 3-hydroxy-(acetoïne)[d]	707	a	219; 51 (0–1890)
2,3-Butanediol	779	a	5; 1 (0–21)
Fatty acid catabolism			
2-Butanone	600	a	75; 24 (18–225)
1-Penten-3-ol	679	a	31; 28 (18–68)
2-Pentanone	684	a	26; 20 (15–100)
3-Penten-2-one	721	b	2; 2 (1–9)
2-Pentanol	738	b	5; 5 (0–10)
3-Hexanone	783	a	2; 2 (2–6)
2-Hexanone	789	a	2; 1 (1–5)
Hexanal	798	a	3; 3 (1–11)
3-Hexanol	811		1; 1 (0–6)
2-Heptanone	888	a	1; 1 (1–2)
Nonanal	1104	a	4; 4 (2–8)
Decanal	1204	a	6; 6 (4–9)
TMAO reduction			
Trimethylamine	503	b	3; 0 (0–10)
Multiple origins			
2-Propanone	500	a	17; 14 (8–52)
1-Propanol	554	a	52; 14 (0–231)
Acetic acid	606	a	5; 4 (1–16)
Ethyl acetate	614	a	40; 31 (8–82)
n-Propyl acetate	712	b	14; 4 (0–69)
Ethyl butanoate	799		3; 2 (0–6)

They are classified according to their most probable origins.

[a] Kovats indices are calculated for the DB5 stationary phase of a capillary column.

[b] The reliability of the identification is indicated by the following letters: a, mass spectrum and retention time identical to those of an authentic sample; b, mass spectrum and Kovats indices in agreement with corresponding data in the literature; c, mass spectrum consistent with spectra found in the literature.

[c] The extracted quantities: mean; median (minimum − maximum) are expressed in arbitrary units of abundance $\times 10^{-6}$.

[d] Plentiful compound.

3.3.2. Effect of the bacteria

Results for principal component analysis are displayed in variable plots and sample plots (Figs. 1 and 2). The variable plot constitutes a graphic representation of the relationships between variables and the two axes. The first three axes accounted, respectively for 45%, 17% and 10% of the total variance of the data. Observations for the first two planes (1–2 and 2–3) indicated that there were five groups of samples.

The first group corresponded to samples inoculated with *Aeromonas* spp. (Aer), *Enterobacteriaceae* (Ent), *S. putrefaciens* (She), combination 1 (M1) and combination 2 (M2). This group released the greatest amounts of compounds, such as TMA, disulphide dimethyl, 2,3-butanediol and 2-pentanol.

The second group, in a central position, corresponded to samples inoculated, respectively with *L. alimentarius* (Lba), *L. farciminis* (Lbf) and *L. sake* (Lbs).

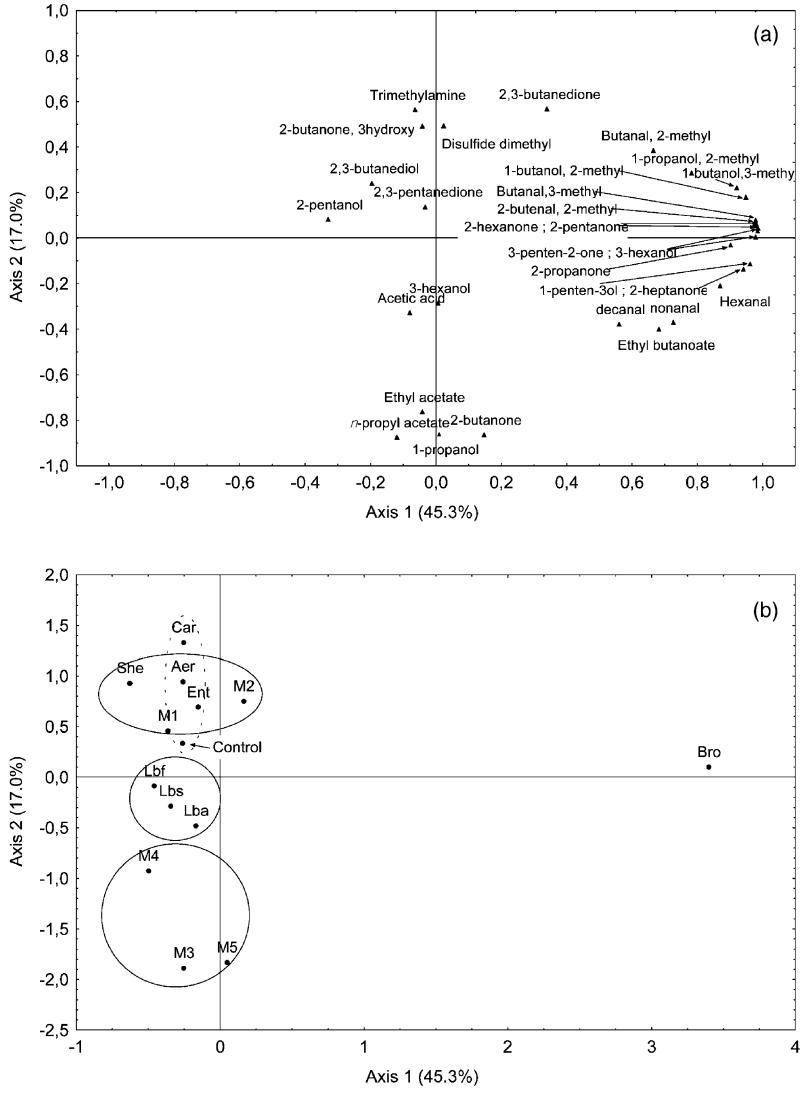

Fig. 1. Principal component analysis of volatile compounds, plane 1–2: (a) variable plot; (b) sample plot.

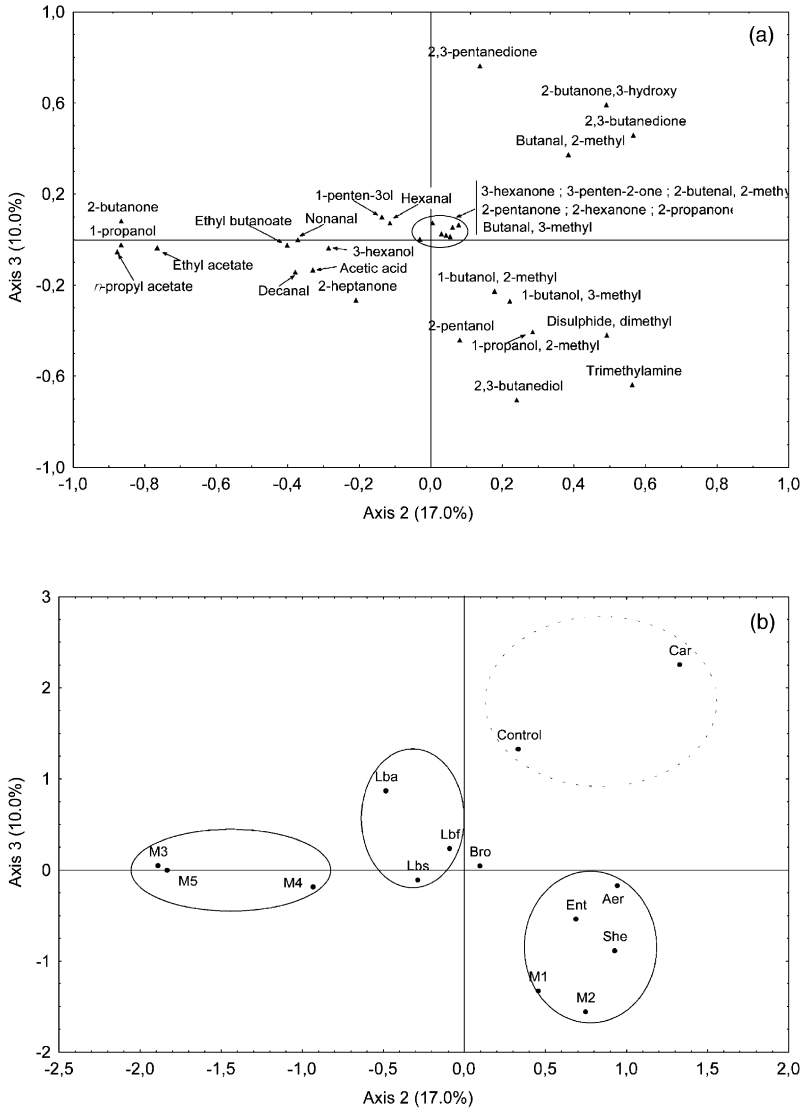

Fig. 2. Principal component analysis of volatile compounds, plane 2–3: (a) variable plot; (b) sample plot.

The third group, composed of samples inoculated with combinations M3, M4, and M5, produced large amounts of compounds, such as *n*-propyl acetate, 1-propanol, 2-butanone, and ethyl acetate.

The fourth group included the control and the sample inoculated with *C. piscicola* (Car). Increasing amounts of compounds, such as 2,3-butanedione, 2,3-pentanedione, were found in this sample (Fig. 2a).

Finally, the fifth group comprised the sample inoculated with *B. thermosphacta* (Bro), which was clearly distinct from the other samples with respect to axis 1 (Fig. 1b). This sample contained the highest amounts of 2-heptanone and 2-hexanone.

4. Discussion

The objective of the study was the investigation of spoilage potential of cold-smoked salmon flora. In this respect, only volatile compounds resulting from the bacterial metabolism were relevant and accord-

ingly only 30 of the 135 compounds identified were considered.

The compositions of some combinations were drawn from those of the natural flora observed on cold-smoked salmon. For instance, M2 did not contain *Lactobacillus* spp which sometimes occur late after the onset of spoilage, M5 was designed to provide a rough approximation of the actual proportion of the flora at the beginning of storage.

The results showed that *Lactobacillus* spp. developed well on cold-smoked salmon, reaching about 8.5 log cfu g^{-1}. In mixtures with other bacteria, *Lactobacillus* spp. were able to dominate the bacterial flora at the end of the storage period, as indicated by the low pH values. These findings are in agreement with reports in the literature in which the predominance of *Lactobacillus* spp. was observed at the end of the shelf life of naturally contaminated smoked salmon (Civera et al., 1995; Truelstrup Hansen, 1995; Truelstrup Hansen et al., 1995, 1996, 1998; Leroi et al., 1998). The *Lactobacillus* group occupied a central position on the first and second PCA planes showing that their growth did not result in a larger production of any particular compounds. On the other hand, *Lactobacillus* spp. containing combinations M3, M4, and M5 released large amounts of volatile compounds, such as acetic acid, ethyl acetate and *n*-propyl acetate. Any or all of these compounds could have been responsible for the odours perceived (sour, acid, pungent) when samples containing the three species of *Lactobacillus* were found to be spoiled after 40 days of storage. A dihydrogen sulphide smell was also described by the panelists who scored samples inoculated with *L. sake*. This finding is in agreement with data in the literature indicating that H_2S was produced by an *L. sake* strain during growth on cold-smoked salmon (Truelstrup Hansen, 1995) and by a homofermentative *Lactobacillus* sp. on a meat product (Borch and Agerhem, 1992). As this compound is very light, it was poorly or not at all detected by mass-spectrometry, possibly because it was lost during the preparation of the sample or after opening of the bags.

The other genus of lactic acid bacteria, *C. piscicola*, frequently occurs in cold-smoked salmon flora. *Carnobacterium* spp. were previously found to predominate in vacuum-packed lightly preserved fish products such as sugar-salted salmon (Leisner et al., 1994) and cold-smoked salmon (Leroi et al., 1998). In our experiment, this bacterium produced the greatest amount of 2,3-butanedione and 2,3-pentanedione, two substances which in pure condition give off a strong butter odour. Thus, these compounds could explain the butter odour noted by the panelists. A butter smell from *Carnobacterium* sp. has also been reported for meat products (Borch et al., 1996). Because these smells are not particularly unpleasant, the samples were not regarded as spoiled. Thus, a high level of *C. piscicola* growth did not lead to spoilage of the smoked salmon. These findings are in agreement with those of other studies in which *Carnobacterium* sp. were found in large number or were copiously inoculated into smoked salmon without affecting the sensory quality (Leroi et al., 1996; Paludan-Müller et al., 1998; Nilsson et al., 1999).

B. thermosphacta, which is associated with the spoilage of refrigerated meat products (Borch et al., 1996), has been detected in cold-smoked fish (Cann et al., 1984; Truelstrup Hansen, 1995; Truelstrup Hansen et al., 1996; Leroi et al., 1998), though usually in relatively low numbers. The present study showed that *B. thermosphacta* was clearly distinct from the other species, producing higher amounts of 2-heptanone and 2-hexanone, which were probably responsible for the blue-cheese odour detected by the sensory panel. The same strains of *B. thermosphacta* produced strong spoilage odours (sour, blue cheese, and butyric-acid-like) when cultured in sterile smoked salmon extract juice (Leroi et al., 1998).

Gram-negative bacteria and combinations M1 and M2 gave off relatively high levels of volatile compounds, such as TMA, disulphide dimethyl, 2,3-butanediol and 2-pentanol. These compounds were probably responsible for amine, sock and floorcloth odours in samples inoculated with *Aeromonas* sp., *Enterobacteriaceae* and M2, which were considered to be spoiled after 40 days of storage.

S. putrefaciens, a spoilage bacterium for fresh fish and meat products, is known to produce volatile compounds such as TMA from the TMAO present in fish muscle and sulphurous compounds, especially disulphide dimethyl (Miller et al., 1973; Dainty and Mackey, 1992; Gram and Huss, 1996). In our study, both of these compounds produced off-odours of the type usually found at spoilage. The five strains used in our study were previously grown in sterile smoked

salmon extract juice (Leroi et al., 1998) where they produced strong off-odours (H_2S, putrid). On the other hand, sterile smoked salmon inoculated with a mixture of the five strains was not clearly spoiled in the present study after 40 days of storage. Moreover, in naturally contaminated cold-smoked salmon, *S. putrefaciens* is dominated by Gram-positive bacteria and lactic acid bacteria in particular (Cann et al., 1984; Leroi et al., 1998). Thus, this bacterium represents only a small part of the total flora after one or 2 weeks of storage and does not seem capable of spoiling the product.

Strains of *Enterobacteriaceae* inoculated into different cold-smoked salmon models (juice or muscle blocks) displayed a spoilage potential by producing off-odours (the present study; Truelstrup Hansen, 1995). These bacteria have been frequently encountered in cold-smoked salmon or trout (Declerck, 1976; Civera et al., 1995; Truelstrup Hansen, 1995; Lyhs et al., 1998), but usually at low levels. However, these bacteria, together with lactic acid bacteria or psychrotrophic marine vibrio, have sometimes been found to dominate the flora (From and Huss, 1991, as cited by Truelstrup Hansen, 1995; Truelstrup Hansen et al., 1998). In this case, they express their spoilage potential through the production of compounds such as TMA and disulphide dimethyl detected in the present study.

The volatile compounds of biological origin found in smoked salmon inoculated with the different species were also detected in naturally contaminated commercial smoked salmon samples (data not shown), whereas no other volatile compounds of microbial origin appeared in these samples. This indicates that these compounds are relevant to microbial activity and the spoilage of cold-smoked salmon.

In conclusion, this study shows that some bacterial strains isolated from cold-smoked salmon seem to be well-characterised by certain volatile compounds, e.g. *C. piscicola* by 2,3-butanedione (diacetyl) and 2,3-pentanedione, *B. thermosphacta* by 2-heptanone and 2-propanone. Moreover, the relationships between some bacteria, volatile compounds and certain smells were rather precisely defined, e.g. *C. piscicola*, 2,3-butanedione and butter smell, or *B. thermosphacta*, 2-heptanone and blue-cheese odour. Such results for cold-smoked salmon should improve our understanding of spoilage mechanisms since they indicate the bacteria mainly responsible for the sensory deterioration of products.

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References

Ben Embarek, P.K., 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. International Journal of Food Microbiology 23, 17–34.

Borch, E., Agerhem, H., 1992. Chemical, microbial and sensory changes during the anaerobic cold storage of beef inoculated with a homofermentative *Lactobacillus* sp. or a *Leuconostoc* sp. International Journal of Food Microbiology 15, 99–108.

Borch, E., Kant-Muermans, M.L., Blixt, Y., 1996. Bacterial spoilage of meat and cured meat products. International Journal of Food Microbiology 33, 103–120.

Cann, D.C., Houston, N.C., Taylor, L.Y., Smith, G.L., Smith, A.B., Craig, A., 1984. Studies of salmonids packed and stored under a modified atmosphere. Report of the Torry Research Station, Aberdeen, Scotland.

Civera, T., Parisi, E., Amerio, G.P., Giaccone, V., 1995. Shelf life of vacuum-packed smoked salmon: microbiological and chemical changes during storage. Archiv Für Lebensmittelhygiene 46, 1–24.

Dainty, R.H., Mackey, B., 1992. The relationship between the phenotypic properties of bacteria from chilled-stored meat and spoilage process. Journal of Applied Bacteriology (Symposium Supplement) 73, 103S–114S.

Declerck, D., 1976. Organoleptic, chemical and microbiological aspects of vacuum packed and unpacked smoked pink salmon. Medelingen Van Het Rijksstation Voor Zeevisserij (C.L.O. Gent) Publikatie nr. 113-VB/VV(I.W.O.N.L.). 18, 1–9.

de Man, J.C., Rogosa, M., Sharpe, M.E., 1960. A medium for the cultivation of *Lactobacilli*. Journal of Applied Bacteriology 23, 130–135.

Elliker, P.R., Anderson, A.W., Hannesson, G., 1956. An agar culture medium for lactic acid Streptococci and Lactobacilli. Journal of Dairy Science 39, 1611–1612.

Gram, L., Huss, H.H., 1996. Microbiological spoilage of fish and fish products. International Journal of Food Microbiology 33, 121–137.

Heinitz, M.L., Johnson, J., 1998. The incidence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* in smoked fish and shellfish. Journal of Food Protection 61 (3), 318–323.

Hildebrandt, G.V., Erol, I., 1988. Sensorische und mikrobiologis-

che Untersuchung an vakuumverpackten Räucherlachs in Scheiben. [Sensory and microbiological analysis of vacuum packed sliced smoked salmon] Archiv Für Lebensmittelhygiene 39, 120–123.

Huss, H.H., Ben Embarek, P.K., From Jeppensen, V., 1995. Control of biological hazards in cold smoked salmon production. Food Control 6, 335–340.

Joffraud, J.J., Leroi, F., Chevalier, F., 1998. Development of a sterile cold-smoked fish model. Journal of Applied Microbiology 85, 991–998.

Jorgensen, L.V., Huss, H.H., 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. International Journal of Food Microbiology 42, 127–131.

Kondjoyan, N., Berdagué, J.L., 1996. A compilation of relative retention indices for the analysis of aromatic compounds, Edition du Laboratoire Flaveur. Station de Recherche sur la viande, INRA de Theix, St Genes Champanelle, France, 234 pp.

Leisner, J.J., Millan, J.C., Huss, H.H., Larsen, L.M., 1994. Production of histamine and tyramine by lactic acid bacteria isolated from vacuum-packed sugar-salted fish. Journal of Applied Bacteriology 76, 417–423.

Leroi, F., Arbey, N., Joffraud, J.J., Chevalier, F., 1996. Effect of inoculation with lactic acid bacteria on extending the shelf-life of vacuum-packed cold-smoked salmon. International Journal of Food Science and Technology 31, 497–504.

Leroi, F., Joffraud, J.J., Chevalier, F., Cardinal, M., 1998. Study of the microbial ecology of cold smoked salmon during storage at 8°C. International Journal of Food Microbiology 39, 111–121.

Lyhs, U., Björkroth, J., Hyytiä, E., Korkeala, H., 1998. The spoilage flora of vacuum-packaged, sodium nitrite or potassium nitrate treated cold smoked rainbow trout stored at 4°C or 8°C. International Journal of Food Microbiology 45, 135–142.

Miller, A., Scanlan, R.A., Lee, J.S., Libbey, L.M., 1973. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas putrefaciens*, *Pseudomonas fluorescens*, and an *Achromobacter* species. Applied Microbiology 6, 18–21.

Nieper, L., 1986. Untersuchung und Beurteilung von vakuumverpacktem geräuchertem Lachs im Hinblick auf die deklarierte Mindesthaltbarkeit. [Analysis and assessment of vacuum packed smoked salmon with regard to the declared minimal shelf life] Rundschau für Fleischuntersuchung und Lebensmittelüberwachung 38, 135–136.

Nilsson, L., Huss, H.H., Gram, L., 1999. Growth control of *Listeria monocytogenes* on cold-smoked salmon using a competitive lactic acid bacteria flora. Journal of Food Protection 62, 336–342.

Paludan-Müller, C., Dalgaard, P., Huss, H.H., Gram, L., 1998. Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum and modified atmosphere-packed-smoked salmon stored at 5°C. International Journal of Food Microbiology 39, 155–166.

Schulze, Kv., Zimmermann, T., 1983. Untersuchungen an Räucherfischen unter besonderes Berücksichtigung von Makrelen. [Analysis of smoked fish with special emphasis on mackerel] Archiv für Lebensmittelhygiene 34, 67–70.

Statistica, 1997. Comprehensive system for statistics, graphics and application development. Version 5.1 for Windows; Statsoft, Charenton-le-pont, France.

Tranchant, J., 1982. Manuel Pratique de Chromatographie en Phase Gazeuse. Editions Masson, 120 Bd St Germain, Paris, pp. 301–337.

Truelstrup Hansen, L., 1995. Quality of chilled vacuum-packed cold-smoked salmon. PhD Thesis. Danish Institute for Fisheries Research and The Royal Veterinary and Agricultural University of Copenhagen, Denmark.

Truelstrup Hansen, L., Gill, T., Huss, H.H., 1995. Effects of salt and storage temperature on chemical, microbiological and sensory changes in cold-smoked salmon. Food Research International 28 (2), 123–130.

Truelstrup Hansen, L., Gill, T., Drewes Rontved, S., Huss, H.H., 1996. Importance of autolysis and microbiological activity on quality of cold-smoked salmon. Food Research International 29 (2), 181–188.

Truelstrup Hansen, L., Drewes Rontved, S., Huss, H.H., 1998. Microbiological quality and shelf life of cold-smoked salmon from three different processing plants. Food Microbiology 15, 137–150.

van Spreekens, K.J.A., 1974. The suitability of a modification of Long and Hammer's medium for the enumeration of more fastidious bacteria from fresh fisheries products. Archiv für Lebensmittelhygiene 25, 213–219.

von Rakow, D., 1977. Bemerkungen zum Keimgehaltvon im Handel befindlichen Räucherfischen. Archiv für Lebensmittelhygiene 28, 192–195.