Significance of Volatile Compounds Produced by Spoilage Bacteria in Vacuum-Packed Cold-Smoked Salmon (*Salmo salar*) Analyzed by GC-MS and Multivariate Regression

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Changes were studied in the concentration of 38 volatile compounds during chilled storage at 5 °C of six lots of commercially produced vacuum-packed cold-smoked salmon and sterile cold-smoked salmon. The majority of volatile compounds produced during spoilage of cold-smoked salmon were alcohols, which were produced by microbial activity. Partial least-squares regression of volatile compounds and sensory results allowed for a multiple compound quality index to be developed. This index was based on volatile bacterial metabolites, 1-propanol and 2-butanone, and 2-furancarboxaldehyde produced by autolytic activity. Only a few of the volatile compounds produced during spoilage of cold-smoked salmon had an aroma value high enough to indicate contribution to the spoilage off-flavor of cold-smoked salmon. These were trimethylamine, 3-methylbutanal, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-penten-3-ol, and 1-propanol. The potency and importance of these compounds was confirmed by gas chromatography−olfactometry. The present study provides valuable information on the bacterial reactions responsible for spoilage off-flavors of cold-smoked salmon, which can be used to develop biosensors for on-pack shelf-life determinations.

Keywords: *3-Methylbutanal; 2-methyl-1-butanol; 3-methyl-1-butanol; 1-penten-3-ol; 1-propanol; 2-butanone; 2-furancarboxaldehyde; trimethylamine; sterile cold-smoked salmon*

INTRODUCTION

The world production of cold-smoked salmon has increased dramatically in the past two decades. In 1981 the world production of smoked salmon was ∼20000 metric tons; this increased to >70000 metric tons in 1996 with an approximate value of U.S. $950 million US$. The Danish production of smoked salmon has increased by a factor of 10 through the 1980s and 1990s. As a result, cold-smoked salmon is one of the most important products for the Danish fishery industry, which produces 10−20% of the world production and 50% of the smoked salmon sold on export markets (*1*).

Processors of cold-smoked salmon have to ensure that their products comply with a number of specifications, guidelines, and standards from customers and regulatory bodies. Most of these are microbiological criteria that do not comply with those set by Codex Alimentarius and the Scientific Committee for Food under the European Commission (*2*). The scientific basis of the microbiological criteria seems weak,and it has been shown that nonspecific "total viable counts" or "aerobic counts" are poorly correlated with the remaining shelf life and spoilage of cold-smoked salmon (*2*, *3*). Until recently, work on the identification of a quality index for cold-smoked salmon has been focused on changes in the concentration of a single compound such as acetic acid, ethanol, formic acid, hypoxanthine, or lactic acid, pH, and trimethylamine (*4*−*8*). Unfortunately, none of these single-compound quality indices (SCQI) has successfully been validated in cold-smoked salmon in repeated experiments. Recently, a multiple-compound quality index (MCQI) based on measurements of biogenic amines and pH was developed (*3*). Compared to SCQI, MCQI has the advantage that it may cover several spoilage domains with different microbial spoilage associations or specific spoilage organisms. Therefore, MCQI may be more robust and more applicable in practical seafood inspection. When measurements of cadaverine, putrescine, histamine, tyramine, and pH were performed, the spoilage level assessed by a sensory panel could be predicted by applying the suggested MCQI. Despite the usefulness of biogenic amines in MCQI for cold-smoked salmon, they are not the compounds responsible for the off-flavors produced during spoilage (*3*). It is more likely that volatile compounds such as alcohols, aldehydes, esters, and ketones individually or in combination are responsible for the spoilage off-flavors detected in chill-stored vacuum-packed cold-smoked salmon as their off-flavor and off-odor thresholds in foods are low compared to those of biogenic amines. The off-flavors normally perceived in commercially produced cold-smoked salmon during spoilage are not detected in sterile samples, suggesting that microbial activity is responsible for these off-flavors (*9*, *10*). Indeed, bacterial activity is responsible for biogenic amines produced in cold-smoked salmon. These were not produced in sterile cold-smoked salmon but by single bacterial cultures or cocultures when grown on cold-smoked salmon (*9*). In contrast to biogenic amine, little is known about the types of processes that are responsible for the formation of volatile compounds in cold-smoked salmon.

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The objective of this study was to use dynamic headspace collection of volatile compounds followed by GC-MS analysis to study their production in a vacuum-packed cold-smoked salmon during chilled storage. The processes involved in production and reduction of volatile compounds in cold-smoked salmon were determined by applying the techniques to commercial and sterile cold-smoked salmon. Their association with spoilage off-flavors of cold-smoked salmon was studied by multivariate regression, calculation of aroma values, and GC−olfactometry detection.

MATERIALS AND METHODS

Commercial Samples of Cold-Smoked Salmon. Sliced vacuum-packed cold-smoked salmon (*Salmo salar*) from three smokehouses was studied. Two distinct lots from each smokehouse (98-1−t98-6) were frozen and transported to our institute. Packages were thawed overnight and stored at 5 ± 1 °C until 1−2 weeks in excess of sensory spoilage, as reported previously (*3*). Details on salting, drying, and smoking processes used in the three smokehouses were reported previously (*3*). At appropriate intervals during storage two samples were withdrawn from each lot for chemical analyses. In addition, two different packs were taken for sensory analyses.

Sterile Samples of Cold-Smoked Salmon. Samples of sterile cold-smoked salmon were produced as reported previously (*9*). In short, dry-salted, nonsliced, vacuum-packed, and previously frozen (−30 °C) cold-smoked salmon (*S. salar*) fillets were used for the production of sterile muscle blocks. Skin-on fillets were produced by a local smokehouse. The skin side of fillets was decontaminated with 70% ethanol in a sterile laminar airflow bench. A square of skin was removed aseptically, and a muscle block was cut free from the underlying dorsal muscle and placed into large sterile glass Petri dishes. Muscle blocks were cut into ~20 g blocks, vacuum-packed, and irradiated at 1.4 kGy. This procedure allowed for sterile samples to be produced without off-flavors (*9*).

Sample Preparation and Collection of Volatiles. On each sampling occasion 100 g of naturally contaminated cold-smoked salmon was taken from two individual packs and pooled into one sample. Muscle blocks of sterile cold-smoked salmon were analyzed individually. All samples were frozen with liquid nitrogen followed by grinding to homogenize the sample. The procedure took <2 min. The produced powder was immediately used for dynamic headspace analysis (*11, 12*). Briefly, 20 g of salmon powder was mixed with 25 mL of water in a 100 mL glass flask and purged at 45 °C with nitrogen at 340 mL/min for 20 min. Volatile compounds were collected on 225 mg of Tenax GR, mesh 40−60 (Chrompack Bergen op Zoom, The Netherlands) packed in 0.25 in. stainless steel tubes (Perkin-Elmer, Buckinghamshire, U.K.). After collection of the volatile compounds, Tenax tubes were dried by blowing nitrogen at 50 mL/min for 15 min. Collection of volatiles was done as triple analyses on each pooled sample.

GC-MS. Volatile compounds were thermally desorbed (ATD400, Perkin-Elmer) and separated on a DB-1701 column (30 m × 0.25 mm × 1 μm, J&W Scientific, Folsom, CA) using helium gas, which was split (5.0 mL min^{-1}/1.3 mL min^{-1}). The following temperature profile was used: 25 °C for 2 min, 25−45 °C at 2 °C min^{-1}, 45−165 °C at 4 °C min^{-1}, 165−240 at 30 °C min^{-1}, and hold at 240 for 5 min. Volatile compounds were identified and quantified by GC-MS (HP5890 IIA; HP5972 A, Hewlett-Packard, Palo Alto, CA) (*11, 12*). For quantification purposes hexanal (98%, Merck, Darmstadt, Germany) dissolved in fish oil was added to samples of 20 g of powdered fresh salmon with low concentration of volatiles. Hexanal was collected as described above, and the results were used to prepare a calibration curve.

GC—Olfactometry. Volatile compounds were thermally desorbed (ATD400, Perkin-Elmer) from Tenax traps and separated on a DB-1701 column (30 m × 0.32 mm × 1 μm, J&W Scientific) using the temperature program described above. Odors were perceived at a sniffing port (olfactory detector outlet, OD-1, SGE, Ringwood, Australia).

Sensory Evaluation. Panels of five to eight persons experienced and trained in seafood evaluation were used for sensory evaluations. At every evaluation, a sample from each production lot stored at 30 °C was thawed and used as a reference sample (fresh). A three-class evaluation scheme was used: class 1, no off-flavors, equal to reference sample; class 2, slight off-flavors, but not spoiled; and class 3, clearly recognizable off-flavors. A lot was classified as spoiled when 50% or more of the panel members determined the samples to be in class 3.

Data Analysis. Single ion peaks were integrated by HP-ChemStation and imported to spreadsheets (MS Excel 97) for further calculations. Single ion areas of the peaks were taken as relative measures of the concentrations of compounds (peak area/gram). The peaks were tentatively identified on the basis of their mass spectrum and retention index as trimethylamine, ethanol, 2-propanone, 2-propanol, butanal, 1-propanol, acetic acid ethyl ester, 2-butanone, 2-butanol, 3-methylbutanal, 2-methyl-1-propanol, 2-pentanone, 1-butanol, 3-pentanone, 1-penten-3-ol, methylbenzene, 3-methyl-1-butanol, 2-methyl-1-butanol, 2-pentanal, 3-hexanone, butyl acetate, hexanal, (*E*)-2-penten-1-ol, cyclopentanone, 1,2-dimethylbenzene, 3-methylcyclopentanone, 2-cyclopenten-1-one, 2-furancarboxaldehyde, pentamethylheptane, 2-furanmethanol, 2-methyl-2-cyclopenten-1-one, 1-(2-furanyl)ethanone, benzaldehyde, 2-ethyl-1-hexanol, phenol, 2-methoxyphenol, 2-methylphenol, and 2-methoxy-4-methylphenol. Chemical data (*X* variables) were used to predict results obtained from sensory analyses (*Y* variable), that is, the percentage of panelists rejecting the sample (%-class 3). This was done by partial least-squares regression (PLSR) on relative concentration of volatile compounds (peak area/gram) and sensory data. Data were centered by subtraction of the mean over all samples. Unscrambler (version 6.1, CAMO A/S, Trondheim, Norway) was used for multivariate data analysis. Full cross-validation of the obtained models was used. To test if concentration of the individual volatile compounds changed during storage, the GLM procedure in Statgraphics *Plus* (version 7, Manugistics, Inc., Rockville, MD) was applied. Storage time was used as the quantitative factor and log(peak area/gram) of the individual volatiles as dependent variables.

To evaluate the sensory importance of volatile compounds produced during spoilage of cold-smoked salmon, semiquantitative determination was done for samples of cold-smoked salmon at the time of spoilage. These were calculated as hexanal equivalents based on the hexanal standard curve. From this, aroma values of volatiles were calculated by dividing the concentration with the odor threshold of the respective volatiles in water (*13*).

RESULTS AND DISCUSSION

Development of a Multiple-Compound Quality Index. A PLSR model was calculated from the relative peak area/gram of the 38 volatiles studied (*X* variables) that predicted the results obtained by the sensory panel (%-class 3) in commercial samples. The first latent variable described 65% of the variation in the sensory data, *Y* variable. Including two additional latent variables increased the degree of described variation of the sensory data to 75 and 83%, respectively (Figure 1). This PLSR model with three latent variables was able to predict the sensory results obtained by the panel from measurements of volatile compounds determined by GC-MS ($r^2 = 0.82$). The volatile compounds included in this model were a combination of trimethylamine, alcohols, aldehydes, and ketones, some of which increased in concentration during storage while others decreased (Table 1). From a seafood inspection point of view a quality index with 38 volatile compounds is not applicable in practice. In an attempt to produce a less

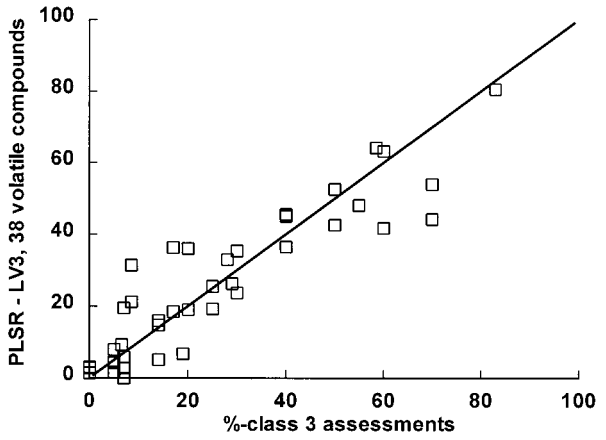

Figure 1. Correlation between the PLSR model of 38 volatile compounds and 3 latent variables and percentage of class 3 sensory assessments of cold-smoked salmon, $r^2 = 0.82$.

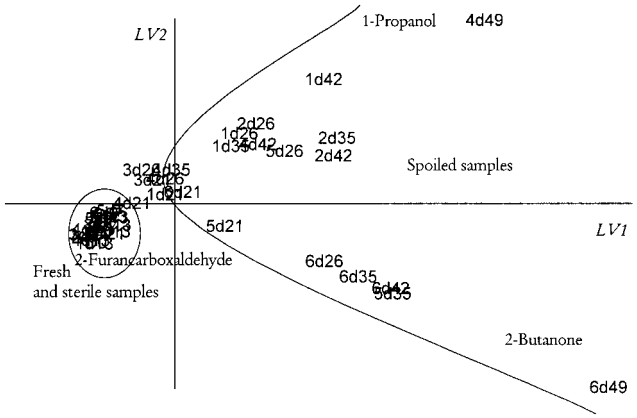

Figure 2. Biplot of the *X* scores and loading weights of the PLSR model of 2-butanone, 2-furancarboxaldehyde, 1-propanol, and sensory assessments, *Y* variable. The explained variations of the sensory results (*Y* data) were 67% for latent variable 1 (LV1) and 1% for latent variable 2 (LV2), whereas the explained variations of *X* data were 52 and 42%, respectively. Data point notation is (lot no.) d (storage time at 5 °C), for example, 6d26.

complicated model loading weights and regression coefficients of the model were examined. This enabled the model to be reduced to only three original variables. Reduced in this way the model explained 67% of the variation in the sensory data. The second latent variable added only an additional 1% point to the degree of *Y* data explanation. This simple PLSR model included 1-propanol, 2-butanone, and 2-furancarboxaldehyde (Figure 2). When sterile samples were included, they formed a tight group together with the fresh samples, which was characterized by high concentration of 2-furancarboxaldehyde and very low concentrations of 1-propanol and 2-butanone (Figure 2). As storage time increased and spoilage in the commercial samples initiated (≥21 days), the concentration of 2-furancarboxaldehyde decreased and the concentrations of 1-propanol and 2-butanone increased. This moved the samples toward the spoilage area to the right in Figure 2.

The mathematical equation of the MCQI for cold-smoked salmon is probably specific to the analytical unit used (headspace sampling equipment and GC-MS). As a result, the equation is not stated, but knowledge of the compounds produced might allow for specific rapid methods to be targeted at these. Developments in sensor technology and electronic noses have many potential applications in the food industry. For seafood, work has been published on the correlation of electronic nose sensor outputs with sensory results (*14*−*16*), but these correlations have been studied with little knowledge of the specific volatiles measured. Knowledge of relationships between specific volatile compounds in cold-smoked salmon and sensory data might allow for the development of an electronic nose with a high specificity toward 1-propanol, 2-butanone, and 2-furancarboxaldehyde. This could be obtained either with more specific sensors or mass spectrometric detection.

Microbiological Growth and Spoilage Microflora. The microbiological data of the present study have previously been reported (*3*). Briefly, microbial counts of commercial cold-smoked salmon showed a variable initial contamination [10^2−10^5 colony-forming units (CFU)/g]. During the first 2 weeks of storage, bacteria in the product grew and reached counts of 10^7−10^8 CFU/g and remained at this level until spoilage several weeks later (*3*).

Production of Volatiles during Storage. Alcohols dominated the volatile compounds produced in commercial samples during storage both in numbers and in produced amounts (Table 1). Among the 12 alcohols studied, the concentrations of 1-propanol, 2-methyl-1-propanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and (*E*)-2-penten-1-ol increased in all lots of cold-smoked salmon by 0.5−3 log(peak area/g) (Table 1). In contrast, only 3-methylbutanal of the aldehydes increased in concentration, whereas other aldehydes neither increased nor decreased in concentration (Table 1). 2-Butanone was the only ketone that increased during storage in all lots of commercial cold-smoked salmon. None of the alcohols, aldehydes, or ketones that increased during storage in commercial samples of cold-smoked salmon were produced in sterile samples of cold-smoked salmon. In fact, several of the alcohols decreased during storage of sterile samples, that is, 2-methyl-1-propanol, 1-butanol, and 2-ethyl-1-hexanol (Table 1). Production of 1-propanol was a result of bacterial activity in all commercial samples (Figure 3), whereas bacterial production of 2-butanone was detected in only four of six lots of commercial cold-smoked salmon (Figure 4). Clearly, alcohols, aldehydes, and ketones produced during storage of commercial samples of cold-smoked salmon resulted from microbial activity.

It has been shown that a number of volatiles were not produced in sterile fish (*Sebastes melanops*), but when sterile muscle blocks were inoculated with *Pseudomonas perolens*, 3-methyl-1-butanol and 1-penten-3-ol were produced among others (*17*). Similar results have been obtained for volatile sulfur compounds and trimethylamine in cod (*Gadus morhua*) (*18*, *19*). Future studies are needed to show what bacterial species are producing the volatile compounds in cold-smoked salmon. *P. phosphoreum* and *Lact. curvatus* were recently identified as specific spoilage organism of cold-smoked salmon (*9*). This identification was based on production of biogenic amines in pure cultures of the organisms that was comparable to production in commercial samples. Preliminary results have shown that mixed bacterial cultures of spoilage organism isolated from cold-smoked salmon produce volatile compounds when grown on cold-smoked salmon similar to those detected in this study (Leroi and Joffraud, personal communication). Cold-smoked salmon samples showing enhanced production of 2-butanone (lots 98-5 and 98-6, Table 1)

Table 1. Changes in Volatile Compounds during Storage of Vacuum-Packed Commercial and Sterile Cold-Smoked Salmon (5 °C)

		increase during storage [log(peak area/$g_{spoiled}$) − log(peak area/g_{fresh})]						
		A		B		C		
compound	RI[a]	smokehouse lot 98-1	smokehouse lot 98-2	smokehouse lot 98-3	smokehouse lot 98-4	smokehouse lot 98-5	smokehouse lot 98-6	sterile[b]
alcohols								
ethanol	645	−[c]	−	0.60***	0.41***	−	−	−
1-propanol	688	1.85***	2.33***	1.00***	2.02***	2.45***	1.50***	−
2-propanol	657	−0.24*	−	−	−	−	−	−
2-methyl-1-propanol	737	1.14***	1.71***	1.35***	1.65***	0.92**	0.93***	−1.13**
1-butanol	774	1.28**	0.68**	1.24***	1.26***	−	0.57**	−0.91**
2-butanol	709	1.31***	1.68***	1.64***	2.45***	2.41***	3.52***	−
2-methyl-1-butanol	850	1.21***	1.47**	1.21***	2.01***	1.40***	1.51***	−
3-methyl-1-butanol	848	1.76***	2.27***	0.95***	2.43***	2.01***	2.26***	−
1-penten-3-ol	795	−	−	0.56***	0.73***	0.76**	0.63**	−
(*E*)-2-penten-1-ol	893	0.49**	0.46**	0.64***	0.83***	0.74***	0.71***	−
2-ethyl-1-hexanol	1136	1.22***	1.22***	−	0.18**	−	0.49*	−0.29*
2-furanmethanol	1020	−	0.23*	0.43***	0.34***	0.48*	0.01*	−
aldehydes								
butanal	669	−	−	−	−	−	−	−
3-methylbutanal	731	0.70**	1.21**	−0.26**	−	0.73***	0.54**	−
(*E*)-2-pentenal	860	−	−	−0.28***	−	−	−	−
hexanal	885	−0.23**	−0.42**	−0.37***	−	−	−0.65*	−1.12***
benzaldehyde	1091	−0.66***	−0.39**	−0.86***	−	−0.52***	−0.87***	−
2-furancarboxaldehyde	973	−1.61***	−1.24***	−1.17***	−2.02***	−1.24***	−1.84***	−2.21**
esters								
ethyl acetate	689	−	−	0.22*	0.42***	0.43*	0.64***	−0.99*
butyl acetate	879	−	−	−	−	0.94*	0.62***	−
ketones								
2-propanone	650	−0.55***	−0.80***	−	−	−0.30**	−	−
2-butanone	697	0.03*	0.83***	−0.10*	0.91***	1.16***	1.78***	−
2-pentanone	769	−0.40**	−	−0.48*	−0.27*	0.05**	−0.36*	−
3-pentanone	777	−	−	−	0.63***	−	−	−
cyclopentanone	901	−	−	0.18*	0.11**	−	−	−
3-methylcyclopentanone	961	−	−	−	0.10*	−	−	−
2-cyclopenten-1-one	969	−	−	−	−	−	−	−
2-methyl-2-cyclopenten-1-one	1028	−0.16*	−	0.11*	−	−	−	−
3-hexanone	868	−	−	−	−	−	−	−
1-(2-furanyl)ethanone	1044	−	−	−	−	−	−	−
phenols								
phenol	1221	−0.19*	−	0.19**	0.16**	−	−	−
2-methylphenol	1269	−	0.38*	0.10*	0.09*	−	−	−
2-methoxyphenol	1236	−	−	0.10*	0.08**	−	−	−
2-methoxy-4-methylphenol	1339	−	0.44*	−	0.08*	−	−0.29**	−
miscellaneous								
trimethylamine	630	2.92***	0.86**	−0.60***	−0.84***	1.06*	1.43**	−
methylbenzene	811	−0.31*	−	−	−	−	−	−
1,2-dimethylbenzene	920	−	−	−	−	−	0.14**	−
pentamethylheptane	992	−0.25***	−0.69***	−	−	−	−	−

[a] Kovats indices calculated from capillary column DB-1701 (J&W Scientific). [b] Increase from 0 to 63 days of storage, as sterile samples did not spoil. [c] − indicates no significant changes in log(peak area/g) during storage. *, **, and *** indicate significant changes in log(peak area/g) at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

have also been shown to have enhanced production of putrescine (*3*). Enhanced putrescine production results from metabiosis between lactic acid bacteria (*Lactobacillus sakei* or *Carnobacillus divergens*) and Enterobacteriaceae (*Hafnia alvei* or *Serratia liquefaciens*) during growth in cold-smoked salmon. Thus, enhanced 2-butanone production as found for lots 98-5 and 98-6 (Figure 2) might also be linked to metabiosis between lactic acid bacteria and Enterobacteriaceae.

Hexanal and other aldehydes and ketones are generally regarded as secondary products of lipid oxidation when produced in foods (*20*). As none of these aldehydes and ketones increased in commercial or sterile samples of cold-smoked salmon it can be concluded that lipid oxidation did not take place to a measurable extent and therefore is unlikely to be important in spoilage of chill-stored vacuum-packed cold-smoked salmon. This confirms results obtained by measuring thiobarbituric acid reacting substances (TBARS) during chilled storage of vacuum-packed cold-smoked salmon (*4*−*6*, *21*). The volatile trimethylamine has been suggested as an SCQI in cold-smoked salmon (*6*). Nevertheless, this study showed trimethylamine to increase in some samples of cold-smoked salmon, whereas it decreased in other samples (98-3 and 98-4) and as such cannot be used as an SCQI in cold-smoked salmon, in agreement with other studies (*5*, *7*, *8*, *22*). In addition, trimethylamine concentration as determined by GC-MS remained constant in sterile samples while it decreased in some commercial samples, which suggests that trimethylamine was metabolized by the spoilage microflora in samples from lots 98-3 and 98-4 during storage (Table 1).

2-Furancarboxyaldehyde was the only volatile compound that decreased in concentration in all commercial and sterile samples during storage (Table 1). The decrease in concentration was 1.17−2.21 log(peak area/g) depending on the storage time of the samples. The

Table 2. Aroma Values of Volatile Compounds in Samples of Spoiling Cold-Smoked Salmon (5 °C)

		aroma value of volatiles in spoiled cold-smoked salmon[a]					
		A		B		C	
compound	odor threshold[b] (ppb)	smokehouse lot 98-1	smokehouse lot 98-2	smokehouse lot 98-3	smokehouse lot 98-4	smokehouse lot 98-5	smokehouse lot 98-6
ethanol	25000	0.03	0.01	0.08	0.2	0.05	0.02
1-propanol	6600	2	2	0.5	2	2	0.1
2-methyl-1-propanol	1000	0.03	0.1	0.2	0.2	0.02	0.02
1-butanol	500	1	0.7	0.6	0.6	0.4	0.4
2-butanol	500	0.2	0.3	0.4	2	1	16
2-methyl-1-butanol	320	1	1	8	6	1	1
3-methyl-1-butanol	250	4	10	30	20	4	4
1-penten-3-ol	400	10	8	20	20	7	4
2-butanone	23000	0.06	0.3	0.02	0.1	0.2	0.8
3-methylbutanal	0.2	650	1100	1400	400	200	140
ethyl acetate	3000	0.001	0.005	0.02	0.02	0.004	0.006
trimethylamine	0.5	1200	900	400	40	700	1000

[a] Aroma value calculated from hexanal equivalences of volatile compounds in cold-smoked salmon at time of spoilage divided by odor threshold of the odorant in water (*13*). [b] Odor threshold in water (*25*, *26*).

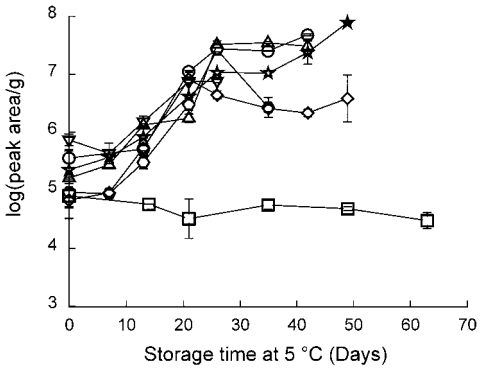

Figure 3. Changes in 1-propanol concentration, log(peak area) of six lots of commercial (98-1−98-6) and sterile vacuum-packed cold-smoked salmon during chilled storage. Samples were from storage trial of lot 98-1 (○), 98-2 (△), 98-3 (▽), 98-4 (☆), 98-5 (⬡), 98-6 (◇), and sterile samples (□). Error bars indicate standard deviation of triplicate determinations.

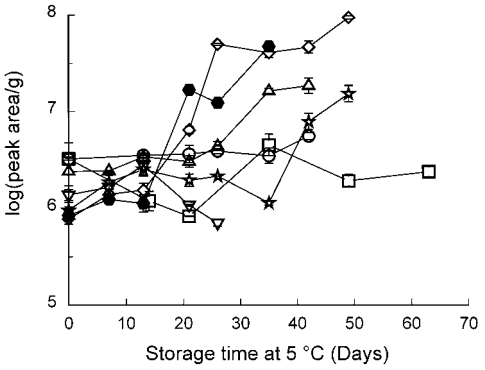

Figure 4. Changes in 2-butanone concentration, log(peak area), of six lots of commercial (98-1−98-6) and sterile vacuum-packed cold-smoked salmon during chilled storage. Samples were from storage trial of lot 98-1 (○), 98-2 (△), 98-3 (▽), 98-4 (☆), 98-5 (⬢), 98-6 (◇), and sterile samples (□). Error bars indicate standard deviation of triplicate determinations.

fact that 2-furancarboxaldehyde decreased in commercial and sterile samples suggests that microbial activity is not crucial for the conversion of this compound. It is more likely that an unknown autolytic process is involved in the degradation of 2-furancarboxaldehyde in vacuum-packed cold-smoked salmon. 2-Furancarboxaldehyde originates from the smoke (*23*) and has not been detected in fresh salmon (*11*, *12*).

Sensory Importance of Volatile Compounds. A few of the volatile compounds produced during storage were detected (in hexanal equivalents) above the odor threshold of the compounds in water (Table 2). When judged by the aroma value, trimethylamine and 3-methylbutanal were the most potent volatile compounds detected during spoilage of cold-smoked salmon (Table 2). 3-Methylbutanal had an aroma value of 140−1400 in these samples; that is, the detected concentrations were 140−1400 times the odor threshold in water. For samples in which the concentrations of trimethylamine increased during storage (98-1, 98-2, 98-5, and 98-6), the aroma value was between 700 and 1200 (Table 2). Trimethylamine and 3-methylbutanal are most likely to contribute to the spoilage off-flavors of cold-smoked salmon in samples in which their concentrations increased during storage. Compounds such as 2-methyl-1-butanol, 3-methyl-1-butanol, 1-penten-3-ol, and 1-propanol may also contribute alone or in combination to the spoilage off-flavor of cold-smoked salmon as the aroma values for these compounds were >1 (Table 2). The GC−sniffing technique confirmed a number of offensive odors in the first part of the GC chromatogram of spoiled samples. A strong fishy odor was detected together with trimethylamine, and sweet buttery and etheric odors were detected with 1-propanol and 2-butanone. 2-Butanol, 2-methyl-1-butanol, and 3-methyl-1-butanol coeluted with strong alcoholic and glue-like odors, whereas a pungent "old-fish" odor was detected together with 1-penten-3-ol. The potent odorant 3-methylbutanal was detected with a sweet "malty" and "sour" odor. The odors detected in the second half of the chromatogram resembled smoked and burned odors and not that of cold-smoked salmon spoilage.

Conclusion. Development of quality indices for specific foods is laborious as large numbers of samples need to be analyzed and the relationship between these results and sensory assessment of the samples has to been established. However, the benefits lie in establishing a relationship between a chemical parameter and sensory assessments. Chemical measurements can easily be standardized and automated, whereas sensory assessments are expensive and hard to standardize. Recently, an MCQI based on biogenic amines was proposed, but this was only possible after years of studies on microflora of cold-smoked salmon and microbial activity (*3*). If a quality index for newly formulated or unstudied foods needs to be developed, a more stringent approach is needed. The combined use of dynamic headspace sampling of large numbers of vola-

tile compounds, analyses on GC-MS for detection and identification, and determination of important volatile compounds by PLSR is a powerful methodology. These analyses allow for the development of new and rapid methods targeted at important compounds. In combination with the use of sterile muscle blocks the methodology gives detailed information on the process and compounds that are involved in product deterioration. This knowledge would not have been obtained if gas sensor array systems or other biosensors had been applied directly. Knowledge of the compounds produced during spoilage of a particular food allows for the bacterial origin to be identified.

It is likely that production of volatile compounds during spoilage of cold-smoked salmon can be used in gas-phase biosensors for on-pack shelf-life determination, as an alternative to time−temperature indicators (*24*). The success of any biosensors for on-pack shelf-life determinations is limited by lack of knowledge of the spoilage reactions responsible for the off-flavors produced. Therefore, these bacterial reactions have to be studied in depth before such biosensors are developed and applied by the industry.

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