Prediction of Microbial and Sensory Quality of Cold Smoked Atlantic Salmon (Salmo salar) by Electronic Nose

GUDRUN OLAFSDOTTIR, ERIC CHANIE, FRANK WESTAD, ROSA JONSDOTTIR, CLAUDIA R. THALMANN, SANDRINE BAZZO, SAID LABRECHE, PAULINE MARCQ, FRANK LUNDBY, AND JOHN ERIK HAUGEN

ABSTRACT: Quality changes of cold smoked salmon from 4 different smokehouses in Europe were monitored by a prototype gas-sensor array system, the FishNose. Samples were stored in different packaging (vacuum and Modified Atmosphere Packaging [MAP]) for up to 4 wk under controlled storage conditions at 5 °C and 10 °C. Quality criteria based on sensory attributes (sweet/sour, off, and rancid odor), and total viable counts and lactic acid bacteria counts were established and used for classification of samples based on the responses of the FishNose. The responses of the gas-sensors correlated well with sensory analysis of spoilage odor and microbial counts suggesting that they can detect volatile microbially produced compounds causing spoilage odors in cold-smoked salmon during storage. The system is therefore ideal for fast quality control related to freshness evaluation of smoked salmon products. Partial least squares (PLS) regression models based on samples from single producer showed better performance than a global model based on products from different producers to classify samples of different quality.

Keywords: electronic nose, cold smoked salmon, quality, descriptive sensory analysis, microbial counts, PLSR classification

Introduction

Objective and rapid quality control system for perishable food such as cold-smoked salmon are needed for the characterization of products and to supply data ready for documentation to improve the production process reliability and reproducibility. In recent years, attempts to use electronic nose technology to track the spoilage processes occurring in fish have been reported in numerous articles. Instruments based on different sensor technologies have been used such as metal-oxide chemoresistive sensors (Egashira and others 1990; Ohashi and others 1991; Olaflsson and others 1992), MOSFET sensors (Haugen and Undeland 2003), amperometric sensors (Schweizer-Bercher and others 1994; Olaflsdottir and others 1997, 1998, 2000, 2002), conducting polymer sensors (Luzuriaga and Balaban 1999a, 1999b; Newman and others 1999; Du and others 2001, 2002), and quartz microbalance sensors (Di Natale and others 1996, 2001, DiNatale 2003; Zhao and others 2002).

Microbiological methods are commonly applied for quality and safety monitoring of cold-smoked salmon products. The validity of total viable aerobic counts (TVC) has been questioned because contradictory results have been found between sensory changes and TVC in smoked salmon (Hansen and others 1995; Leroi and others 1998; Dondero and others 2004). The spoilage microflora in cold-smoked salmon is related to the source of contamination for example the raw material and/or the smokehouses rather than being specific for the product (Hansen and others 1998). The predominance of lactic acid bacteria in vacuum-packed cold-smoked fish products at the end of shelf life of the products is generally recognized (Becker and others 2002; Gonzalez-Rodriguez and others 2002). Enterobacteriacea has been identified in cold-smoked salmon products as the main contributor to spoilage, related to the in-house flora and hygienic conditions in the smokehouses.

Quality monitoring of smoked salmon in the industry is often based on sensory evaluation of appearance, texture, smell, and taste. Desirable attributes from smoking diminish during storage and the characteristic deterioration takes over, including softening of the fish flesh, fading colors, and unpleasant odors and flavors. Different schemes have been used in the various studies on cold-smoked salmon and sensory descriptors for spoilage have been suggested. Sensory descriptors like sweet/sour, bitter, fecal, ammonia, and cabbage were used by Hansen and others (1998) to describe the spoilage of vacuum-packed cold-smoked salmon stored at 5 °C. The main spoilage characteristics described by Leroi and others (1998) in cold-smoked vacuum-packed salmon stored for 5 wk at 8 °C were a pesty texture and pungent, acid, rancid, and sour flavors and odors. Cardinal and others (2004) identified different quality classes based on sensory characteristics of cold-smoked salmon from supermarkets in the European market. Color, intensity of smoke related odors, amine odors, and salty perception were the main sensory characteristics discriminating between quality classes.

Characteristic spoilage off-odors and off-flavors are caused by microbial activity. The spoilage potential of the microflora has been studied (Leroi and others 2001; Stohr and others 2001) and various volatile compounds produced by microbes have been suggested as spoilage indicators (Joffraud and others 2001, Jorgensen and others 2001). In some cases, spoilage of cold-smoked vacuum-packed fillets occurs with low microbial numbers indicating that shelf life of cold-
smoked salmon with very low microbial counts is caused by autolytic tissue degradation (Hansen and others 1998). Autolytic enzymes have a major impact on the textural quality of cold-smoked salmon during the early stage of deterioration (Hansen and others 1996). Other studies have shown that despite high microbial counts, no relevant changes in chemical or physical parameters were observed (Bugueno and others 2003).

For rapid monitoring of spoilage of cold-smoked salmon by an electronic nose, it is important to characterize the spoilage changes of the respective products and select quality indicators that correlate with the sensors responses. The use of an electronic nose to monitor spoilage changes in smoked products has not been reported before. This article presents results from a European project (QLK1-CT-2002-71304), where the aim was to study the possibility to use an electronic nose to monitor smoked salmon quality. A prototype instrument called FishNose was developed and adapted for the measurements of smoked salmon. Storage studies were done to compare the microbial and sensory changes with the FishNose responses. The main objective of the studies was to select quality indicators related to microbial counts and sensory odor attributes and to establish quality criteria to use in models based on the FishNose responses to classify cold smoked salmon of different quality.

Materials and Methods

Preparation of samples

Salmon samples were obtained from smokehouses in Norway, Iceland, and Germany, and storage studies were carried out in laboratories in the different countries.

Table 1 shows an overview of the samples from the different smokehouses (labeled A1, A2, B, C, and D). smoking and storage conditions, and the number of samples used for the storage studies at 5 °C and 10 °C. In addition, samples from different production batches (process samples) of different quality were used for the FishNose testing. The raw material used for smoking in the different smokehouses was processed 2 to 3 days after slaughtering. All the smokehouses use traditional smoking and dry salting. The cold-smoked salmon products were sliced and vacuum-packed, but 1 producer (B) vacuum-packed the products as whole fillets. The storage experiments were performed with 16 freshly smoked samples delivered from each smokehouse to the laboratories. The samples were stored at 2 temperatures up to 4 weeks, and sampling was done on days 0, 7, 14, and 28 of storage for samples stored at 5 °C, but on days 0, 4, 7, and 10 of storage for samples stored at 10 °C. The A samples were all stored at 5 °C, 1 sample group in vacuum packages, and the other sample group in Modified Atmosphere Packaging (MAP). The microbial analysis included total psychrotrophic counts (TVC) and incubation at 15 °C. Analysis of LAB conditions A B C D

Storage study Batch 1 (n = 64) 16 14 16 16
Process Batch 2 (n = 16) 4 4 4 4
Process Batch 3 (n = 18) 4 4 4 6
Total n = 96 24 22 24 24

Microbial analysis

The microbial analysis included total psychrotrophic counts (TVC) (total viable counts) using modified Long and Hammer’s (LH) medium (Van Spreekens 1974) and incubation at 15 °C. Analysis of LAB counts was done using NAP (nitrite actidione-polymyxin) medium slightly modified (Davidson and Cronin 1973).

Sensory analysis

A sensory scheme for smoked salmon based on Quantitative Descriptive Analysis (QDA) (Stone and Sidel 1985) was developed before the shelf life studies. Nine trained panelists (age range, 30 to 55 years) from the Icelandic Fisheries Laboratories’ sensory panel participated in the sensory assessments. They were selected and trained according to international standards (ISO 1993), including detection and recognition of tastes and odors, training in the use of scales, and in the development and use of descriptors. The members of the panel were familiar with the QDA method and trained according to Intl. Standards (ISO 1994) for the QDA assessment. One 1.5-h session was used for training of the panel using freshly smoked salmon samples and samples that had been stored for 3 weeks at 5 °C. The panel adapted an already developed QDA sensory scheme used earlier at Matfor-sk in Norway and evaluated the attributes and developed the vocabulary to describe changes occurring in smoked salmon during storage. Thereafter the panel was trained in the use of an unstructured scale (15 cm) for the selected attributes. The scheme contained 19 descriptors of odor/flavor, appearance, and texture. Odor and flavor attributes included smoked salmon odor/flavor, metallic odor/flavor, sweet/sour fruity odor/flavor, rancid odor/flavor, off-odor/off-flavor. Taste attributes included salt and bitter taste. Appearance attributes

 Chemical analysis

Analysis of water content was done by heating the sample in an oven at 103 °C ± 2 °C for 4 hours. Water corresponds to the weight loss (ISO 6496 1999). Total fat was determined by extraction with petroleum ether, boiling range 40 °C to 60 °C using an extraction apparatus 2050 Soxtec Avanti Automatic System (AOCS 1998). Salt content was measured by extracting the soluble chloride from the sample with water containing nitric acid. The chloride content of the solution was titrated with silver nitrate and the end point determined potentiometrically (AOAC 1995).

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Sampling

Chemical analysis of fat, water, and salt were done to characterize the different products. The proliferation of spoilage changes were monitored by sensory analysis, total aerobic and lactic acid bacteria counts. For each sample, 2 fillets were used and the fillets were divided in the same way for the different analysis in the different laboratories to ensure that the same part of the fillet was always used for the respective analysis. Only the middle portion of the fillets was used, and they were divided from head to tail so that samples from sensory analysis were taken from the head end and consecutive to the tail end in the following order: samples for the FishNose prototype measurement, microbial analysis (TVC and lactic acid bacteria [LAB]), and chemical analysis (water, total fat, salt content). Duplicate samples were analyzed.

Microbial and chemical analyses were done in the participating laboratories in the different countries on each day of sampling. Samples for electronic nose measurements and sensory analysis were vacuum packed, frozen (−24 °C), and transported in a Styrofoam box by courier service at the end of the experiment to AlphaMOS and to Icelandic Fisheries Laboratories (IFL), respectively.

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<table>
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<th>B</th>
<th>C</th>
<th>D</th>
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<td>14</td>
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<td>Process</td>
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<td>Total n = 96</td>
<td>24</td>
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Table 1—Smoking and storage conditions of samples from the different smokehouses, number of samples from each smokehouse and treatments

4MAP = Modified Atmosphere Packaging; VAC = vacuum.
evaluated included fat secretion, translucent, hue, color intensity, and 3 texture attributes: elasticity, oiliness, and juiciness. Further training of the panel and testing of the scheme was done in a pre-trial, using samples that were stored for 14, 28, 35 and 42 d at 5 °C.

Samples from each sampling day were kept frozen until analyzed for sensory analysis all at once at the end of the storage time. Approximately 30 g of smoked salmon served as slices on plastic dish were allowed to equilibrate at room temperature for 30 min before evaluation. The samples were coded with 3 random digit numbers. Each panelist evaluated duplicates of samples from 2 to 3 different storage days. The fish was served in a random order during 2 sessions for each day of the sensory evaluation.

Electronic nose

The GEMINI electronic nose (Alpha M.O.S, Toulouse, France) equipped with 6 metal oxide semiconductors (MOS) sensors (PA/2, P10/1, P40/2, P40/1, LY2/G, LY2/LG) was used in the project. A prototype sampling unit developed by OPTOTEK (Slovenia) was connected to the sensor unit GEMINI. The sampling unit has a 10-mL sample loop, a heated inlet tube (55 °C) and a pump (flow rate 200 mL/min). The sampling was performed by inserting the inlet tube into a bell-shaped unit (10-cm dia) that was placed on the fillets. Samples were covered with a 7-cm-dia pierced aluminum paper to prevent cross-contamination of samples. Aluminum was used because of its inert property. Sampling temperature (headspace generation temperature) was 5 °C and loading time of 7 s was used.

Validation of the performance of the system and the sensitivity of the sensors toward selected compounds that are known to be present in the headspace of smoked salmon was done in the project and will be published in a following article (Olafsdottir and others 2005).

Data analysis

Sensory analysis of smoked salmon was performed using the software Fizz (France). Statistical analysis was done on the sensory data using Number Cruncher Statistical Software (NCSS 2000 and Pass Trial, Kaysville, Utah, U.S.A.). One-way analysis of variance (ANOVA) was done to study whether differences between sampling days of each storage group were significant (H₀ = no difference between samples; significant difference P < 0.05). Multivariate analysis was performed by the Unscrambler Version 9.1 (CAMO Process, Oslo, Norway). The main variance in the data set was studied using Principal Component Analysis (PCA), and Partial Least Squares Regression (PLSR) models were used to describe the relationship of the data and to make predictions on quality of samples based on the sensor responses and the data from the reference methods. In this context prediction means cross-validated predictions, as there were no new independent sets of samples present for prediction. However, cross-validation is more conservative than just numerical fit of all samples. The quality criteria established to discriminate good samples from bad samples were based on overall results of the analysis in this study, taking into account commercial critical limits for total viable counts (TVC), results of earlier studies of cold-smoked salmon, and sensory scores of selected attributes in this study.

Results and Discussion

Quality indicators to use for calibration of the FishNose prototype were selected by studying the correlation of the gas sensor responses with the chemical, microbial, and sensory data. Correlations of gas sensor responses with results of chemical parameters (fat, water, salt) were low, but significant correlations were found for the sensory and microbial parameters. The correlations between gas sensors and selected sensory attributes were evaluated based on all the samples from different producers. Except for the rancid odor significant correlations were found for the odor attributes sweet sour (r = 0.3 to 0.5; P < 0.005), off-odor (r = 0.2 to 0.4; P < 0.005), and smoked odor, which was negatively correlated (r = -0.4 to -0.6; P < 0.005).

Univariate correlations were found between sensor responses and bacteria numbers. Highest correlation with the TVC and LAB numbers for the overall data set was found for the LY2/G sensor, r = 0.35 (P < 0.005) and r = 0.44 (P < 0.005), respectively. A high covariation between the single sensors of the array was also observed.

The data from the FishNose sensors for all the 96 samples were analyzed by Principal Component Analysis (PCA) (Figure 1). The 2 1st dimensions described 99% of the total variance in the data set. The...
Smoked salmon quality predicted by E-nose . . .

1st PC explaining 94% of the variation appears to be describing the spoilage level of the samples. Most of the samples located to the right were stored samples from producer A and a few from producer D with high bacterial numbers and high scores in sensory odor attributes (sweet/sour and off-odors). All the samples on the left had low microbial counts and low spoilage odor scores, indicating that the majority of the samples in the experiment were of good microbial and sensory quality. The 2nd PC explaining 5% of the variance in the data appears to be discriminating the different smokehouses: The samples were clustered together; however, samples from the same producers appear to be closer together, indicating differences in the headspace of the samples from the different smokehouses.

A “structural correlation” was defined when variables group induced a similar structure on the samples. Individual analyses showed that when the samples of a producer were structured, that is, showing changes in the measured variables, the sensor data revealed a similar structure. This was the case for producers A and D, that contained samples with obvious spoilage signs and these samples were located on the right side of the plot as mentioned before. Some of the sample groups did not show a clear indication of spoilage at the end of the storage time of the study. Therefore, no structural correlation was found and no obvious discrimination between the samples of the C and B sample groups was observed, as seen by the location of these samples on the left side of the plot.

To select quality indicators representative of microbial spoilage of the samples, it is of interest to investigate how the sensory attributes are related to the microbial counts (TVC). Therefore, all the sensory attributes, except the flavor attributes were subject to regression analysis with log TVC the response variable. The correlation loading plot from this analysis is shown in Figure 2. The attributes marked with small circles were found to be significant. The big circles indicate 50% and 100% explained variance, respectively. Smoked salmon and sweet/sour odor contribute in modeling TVC, although the correlation is not that high. Color intensity, hue, salt taste, and bitter taste contributed in modeling TVC, although the correlation is not that high. Color intensity, hue, salt taste, and bitter taste contributed significantly to the modeling in accordance with other studies, indicating the importance of color and salty taste for smoked salmon spoilage characteristics (Cardinal and others 2004). This is in agreement with earlier studies on cold-smoked salmon, indicating the importance of spoilage organism in product shelf life because characteristic spoilage off-odors were found only in samples of cold-smoked salmon with high bacterial loads (Hansen and others 1996).

A PLSR regression model with gas-sensors as predictor variables and sensory attributes as response variables was also subject to investigation (Figure 3). The results showed a general agreement of the microbial and sensory odor parameters selected as reference parameters for the gas sensor responses. The correlation loading plot shows that the gas-sensors are located on the same side as off-odor and sweet/sour odor, which concurs with their univariate correlations (data not shown). Although these correlations are significant, the numerical ranges for the attributes are not that high, and the distributions are quite skewed as seen in the histograms (Figure 6 and 7). However, based on their location on the plot, it is suggested that the sensors are detecting the volatile compounds contributing to the sweet/sour and off-odors. The position of the rancid attribute in the middle of the plot indicates that this attribute was not important in explaining the spoilage level in the samples.

Sensory evaluation of odor changes caused by the development of microbially produced volatile degradation compounds is often the most reliable method to determine the freshness or spoilage level of chilled fish (Olafsdottir and Fleurence 1998). The composition of volatile compounds in the headspace can be related to the odor and the gas sensors are responding to volatile compounds present in the highest concentration in the headspace. Therefore, sensory scores for odor attributes were found most relevant to compare to the electronic nose responses. Moreover, selection of quality indicators to use for calibration of the FishNose prototype was based on attributes that showed increasing responses to samples in the storage study and significant responses for the aged process samples. The parameters giving the best correlation to the sensor responses were related to the proliferation of the microflora like TVC and LAB counts, and the sensory odor attributes indicative for the development of microbial metabolites like sweet/sour odor and off-odor.

Chemical analysis

The correlation of the chemical parameters and the gas sensors were low. Therefore, they were not selected to calibrate the FishNose
Smoked salmon quality predicted by E-nose . . .

responses. However, variation in salt content can influence the microbial growth and may explain the proliferation of spoilage. An overview of the chemical composition (fat, water, and salt) of the cold-smoked salmon samples from the different producers is shown in Table 2. Considerable variation in fat and water content of samples was found even within samples from the same batch. The A samples had the highest fat content, and samples from C had the lowest fat content. This variation in fat content is expected and reflects both the age/size and feeding condition of the salmon.

Variation in salt content was also found within the samples, the highest values for D and lowest for B. Analysis of a subset of the data showed that the size of the fish can influence the salt content. Salt uptake appeared to be slower in the large fillets resulting in overall lower salt content. Therefore, careful monitoring of the salting process is necessary to ensure consistent products. Different handling procedures of smoked products such as dry salting and brine injection influence the microbial spoilage (Hansen and others 1996).

Microbial analysis—TVC

The limits for the end of shelf life in the industry are often set at \(10^6\) colony-forming units (CFU)/g. However, this limit has been questioned and not in agreement with the results of sensory panelists who estimated that samples with counts of \(3 \times 10^6\) CFU/g had not exceeded the limit of shelf life (Leroi and others 1998). At sensory rejection, the TVC is typically \(10^3\) to \(10^6\) CFU/g in cold-smoked products, and the microflora differs depending on the processes involved in the different smokehouses (Hansen and others 1995, 1998). Estimation of shelf life of smoked salmon products based on storage day is not practical because the various handling and smoking processes and the different storage conditions influence the spoilage processes and the shelf life of the products (Hansen and others 1995, 1996; Dondero and others 2004). Accordingly, the shelf life of smoked salmon products varies considerably from about 2 wk to 2 mo depending primarily on the temperature during storage.

Storage at 5 °C. The initial TVC numbers for the day 0 samples in the storage studies varied from 1.5 to almost 4 in log CFU/g (Figure 4). The increase in TVC numbers with storage time for samples stored at 5 °C for 28 d is obvious for all the samples except from smokehouse D. The D samples had very low counts (<\(10^3\) CFU/g) throughout the study, which may be explained by the high salt content (mean = 5.3%) of those samples (Table 2). Slow spoilage rate was also observed for samples from smokehouse B, and at the end of the study, the TVC values did not exceed \(10^6\) CFU/g. Samples from smokehouse B were packaged as whole fillets, but not as slices. This may have influenced the spoilage rate for the B samples. Other researchers have reported longer shelf life for fillets (32 to 49 d) than for slices (21 to 36 d) of the same product evaluated by a sensory panel (Hansen and others 1998). The initial high counts of the A samples probably reflected the hygienic conditions in the smokehouse. Additionally, the A samples had the highest microbial counts throughout the study, which may also be explained by the handling, salting, the smoking process, and the packaging. In particular, it is of interest that the smoking time was very short for the A samples (Table 1).

**Storage at 10 °C.** The microbial counts in samples stored at 10 °C for 10 d did not show a clear trend with storage time, and only 3 samples exceeded counts of \(10^4\) CFU/g. None of the samples (B, C, and D) stored at 10 °C exceeded the food safety limit for TVC (\(10^6\) CFU/g) at the end of the study. This indicated that the end of the shelf life based on this criterion was not reached when the study finished and all the samples were still of acceptable microbiological quality, despite the high storage temperature (10 °C).

**Process samples.** The TVC in samples from the process (labeled: a, b, c, ...h) varied from \(10^5\) to almost \(10^7\) CFU/g. This variation is explained by the different age of the samples. Two processors (A and C) provided fresh samples from the process, but the others (B and D) provided both fresh and stored samples. One batch from D had been kept for 10 d at 2 °C before delivery to the laboratory and another batch from B was selected from old stored products (15 to 22 mo in

![Correlation loading plot from a Partial Least Squares Regression (PLSR) model based on the 6 gas sensors as predictors and selected quality attributes (sweet/sour, off-odor, rancid, smoked odor, and microbial counts of total viable counts [TVC] and lactic acid bacteria [LAB]) as response variables](image)
Initially, it was decided that a quality criterion corresponding to a 10^6 CFU/g for total viable count should be applied to discriminate between accepted “good” and rejected “bad” samples in this study because this is the general microbiological safety guideline applied for food quality. Only 6 samples (6%) of the total sample set from different production batches were of “bad” quality based on the TVC criterion 10^6 CFU/g. These were samples A1 and A2 stored for 28 d and 2 samples from the process. It should be mentioned that according to the specification of the A products the indicated shelf life was only 2 wk. Because the majority of the samples were not spoiled based on the TVC criteria of 10^6 CFU/g at the end of the study (Figure 4), it was decided to use lower limits (10^5 CFU/g) and aim at discriminating between good samples and samples that are just starting to show spoilage signs but still of acceptable quality. Fifteen samples exceeded values above 10^5 CFU/g (16%), and 33 samples (35%) exceeded values above 10^4 CFU/g.

**Microbial analysis—LAB**

The overall results of the lactic acid bacteria counts in Figure 5 shows that the pattern is very similar to the TVC numbers. The LAB...
Counts appeared to increase with storage time at both storage temperatures (5 °C and 10 °C). The initial values in freshly smoked samples on day 0 were in the range <10^1 to 10^2 CFU/g, and at the end of the study, 3 of the samples reached counts of 10^6 CFU/g. Comparison of the TVC and LAB numbers during storage showed that the TVC dominated the LAB in the fresh samples but their numbers seemed to converge with increased storage time. The highest LAB counts were in the MAP samples (A1).

In some samples, the counts of LAB exceeded the TVC value. It is expected that the LAB will grow on the modified LH medium, but it may be speculated that an additional anaerobic flora may have grown on the LAB medium because higher LAB than TVC counts were found in some samples. It should be specified that the LAB medium is incubated anaerobically as opposed to aerobically for LH.

Lactic acid bacteria do not represent typical spoilage bacteria, but a high load of these bacteria will affect the sensory quality of the product because they can produce volatiles that contribute to the spoilage odors. Therefore, a significantly high load of LAB will influence the headspace profile analyzed with the FishNose sensor system and will be indicative of prolonged storage of products. A limit of 10^4 CFU/g was determined as the LAB criteria to distinguish between good and bad samples.

Figure 6—Sweet/sour odor scores for samples stored at 5 °C and 10 °C for 28 d and 10 d, respectively, and 8 samples from the process from the different smokehouses (A1, A2, B, C, and D). Two replicate samples were analyzed each day. Line represents the quality criteria set at 20.

Figure 7—Off odor scores for samples stored at 5 °C and 10 °C for 28 d and 10 d, respectively, and 8 samples from the process from the different smokehouses (A1, A2, B, C, and D). Two replicate samples were analyzed each day. Line represents the quality criteria set at 20.
Sensory analysis

In total, 96 samples were assessed by sensory analysis, 70 thereof for both odor and flavor attributes. Samples that had been stored at 10 °C were not tasted to avoid the health risk for panelists associated with the growth of pathogenic bacteria.

Sensory analysis of the samples showed similar results as microbial analysis indicating that quality changes of samples stored under these conditions were not obvious.

Statistical evaluation of the data using 1-way ANOVA showed that significant differences in the sensory attributes between the storage day of samples from the same producer within the same sample treatment were not found in the sensory attributes for taste (salt and bitter taste), appearance (fat secretion, translucent, hue), and the texture attributes (elasticity, oiliness, juiciness). Significant differences (P < 0.05) were found in odor and flavor attributes and color intensity for some sample groups (data not shown). Because flavor was not evaluated in all the samples, the odor scores were used for the quality criteria and data from the odor evaluation are shown herein (Figure 6 through 9).

The descriptors used by the sensory panel for the odor and flavor attributes were as follows: smoked salmon odor/flavor, metallic odor/flavor, sweet/sour fruity odor/flavor, rancid odor/flavor, off-odor/off-flavor. The scores of spoilage related attributes (sweet/sour, rancid, and off-odor) increased slightly with storage time for both 5 °C and 10 °C (Figure 6 and 7). However, significant differences in sensory scores of samples between storage days for these attributes were found only for the A samples. The highest scores for spoilage related odor were observed for the A1 samples stored in MAP at 5 °C. This is in agreement with high microbial counts for these samples. On day 0, the fresh samples from producer A had sweet/sour scores of approximately 10, but after 2 wk of storage, the sweet/sour scores exceeded 20 and increased further up to nearly 60 after 28 d of storage. Other sample groups had much lower scores.

A similar overall trend as for the sweet/sour scores were observed for the off-odor (Figure 7) and the rancid odor (Figure 8). The spoilage related attributes had generally higher scores in samples stored at 10 °C than 5 °C, even though the microbial counts were not higher at the end of the study at 10 °C. This indicates that even though the microbial counts were lower at 10 °C, the spoilage potential of the microflora and production of off-odors appeared to be greater at the higher temperature. This is 1 of the reasons why the results of microbial counts may often be misleading (Hansen and others 1995; Gram and Huss 1996; Leroi and others 1998). Oxidation may also have been causing the off-odor and rancid odor development. It has been emphasized that no single quality criterion is adequate to explain the complex changes of spoilage of smoked salmon products and therefore multiple quality indices have been suggested to assess the quality (Jörgensen and others 2001).

The scores for the initial samples (0 d) from C and D and samples from day 4 from B for the sample groups stored at 10 °C had already high scores for the sweet/sour, rancid, and off-odor spoilage attributes in agreement with higher microbial counts for these samples. Part of the samples from the process from D had high scores for the spoilage attributes that can be explained because they had been stored for 10 d before the delivery to the laboratory.

The smoked salmon odor decreased slightly with storage time at 5 °C in particular for the A and C samples (Figure 9). The trend was not clear for samples stored at 10 °C. When comparing the process samples, a batch-to-batch variation was observed for the smoked odor, which may have been influenced by the different smoking conditions at the smokehouses (Table 1). The smoking temperatures at producers A and D were higher than for the other smokehouses and the smoking time was the shortest at producer A. The short smoking time at smokehouse A may have resulted in lower smoking odor scores and the high smoking temperature may have influenced the proliferation of the microflora in these samples and the higher spoilage rate. Earlier studies on cold-smoked products have shown that the smoke flavor intensity and the level of smoke related components like phenols in the final products were influenced by the different smoking procedures in different smokehouses (Cardinal and others 2001).

**FishNose measurements**

The sensor responses of the FishNose prototype sensor system showed a similar pattern for the 96 samples analyzed, suggesting co-
Smoked salmon quality predicted by E-nose . . .

text continues...

Figure 9—Smoked salmon odor scores for samples stored at 5 °C and 10 °C for 28 d and 10 d, respectively, and 8 samples from the process from the different smokehouses (A1, A2, B, C, and D). Two replicate samples were analyzed each day.

Figure 10—Sensor response distribution for samples measured with the FishNose PA/2 sensor for samples stored at 5 °C and 10 °C for 28 d and 10 d, respectively, and 8 samples from the process from the different smokehouses (A1, A2, B, C, and D). Two replicate samples were analyzed each day.
or off-odor on all the combined measurement data gave in general low classification rates. It should be emphasized that the establishment of quality criteria in this study is aimed at detecting samples that are showing initial spoilage signs, but not necessarily of unacceptable quality, because the sample set did not contain many samples that were spoiled. Therefore, the sensors sensitivity is challenged because the concentration of spoilage volatiles may be low and as a result the classification rates may be poorer than otherwise expected if the samples were indeed spoiled.

By using a combination of the quality parameters the classification rates were improved, compared with using single reference parameters alone. The quality criteria established in the storage studies (Table 3) was applied for the Partial Least Squares Regression (PLSR) classification modeling for accepting and rejecting samples corresponding to respectively “good” and “bad” samples.

The global PLSR discrimination model using the sensor data from all 96 samples and the combined criteria of 4 or 5 parameters gave the classification results shown in Table 4.

In total, 71 samples or 74% of the samples were classified correctly into their respective quality class and 26% were classified wrongly (25 samples) when using the criteria for TVC, and the 3 odor criteria. By also including the criterion for the LAB and using 5 criteria, the overall classification was not improved as seen by fewer samples classified correctly (57%, 55 samples) and more samples were classified incorrectly (43%, 41 samples) (Table 4). In principle, 0% bad samples should be classified as good ones, so the observed rate is far too high. For the fish producers, it is acceptable that 1% to 5% of good samples would be classified as bad, so 8% is far too high. Increasing or decreasing the bacterial criterion, in combination with the sensory criteria, did not show much improvement of the number of correctly classified samples. However, considering that the criteria are very strict and aimed at detecting samples of marginal quality, the overall classification can be justified.

The results suggested that it was difficult to apply a global prediction model based on all the samples from the different smoked salmon producers. Moreover, the results showed that other reference parameters like fat secretion and smoked salmon odor (data not shown) could be useful for local classification modeling, probably due to different fat content and smoking conditions at the different suppliers.

By inspection of the PCA plot (Figure 1), it appeared that the samples were grouped according to the different smokehouses, indicating that local prediction models for each supplier separately could be more suitable.

Local models. Correlation between sensor responses and the TVC, LAB counts, and sensory odor attributes for the individual producers for samples from the storage studies are shown in Table 5. Two of the sample groups (C and B) did not have any structural correlations. No obvious trends or correlations for the responses of the spoilage indicators and gas sensors were observed in those samples. On the other hand, significant correlations were found for the 2 producers (A and D).

The tables that showed the highest correlation with TVC numbers were the A samples, except for the 28-d old samples, where an unexpected decrease was observed in the sensor response signal (Figure 10). By disregarding the 28-d samples, a correlation of $r = 0.92$ ($P < 0.005$) was obtained for the PA/2 and P40/2 sensors and $r = 0.94$ ($P < 0.005$) for both the LY2/G and LY2/LG sensors with storage time. The low correlation of sensor responses with LAB numbers for the D samples from the process, but higher correlation of sensor responses with the TVC values, suggests that other bacteria than LAB may have contributed to the TVC counts like Enterobacteriaceae. This indicates that these samples were not handled properly and perhaps exposed to poor hygienic conditions in the factory. In a prestudy done in the project, Enterobacteriaceae counts were indeed high in samples from producer D (unpublished data).

Based on these findings, local Partial Least Squares Regression (PLSR) models for each producer were evaluated and validated by leave-one-out cross-validation. The values determined for the sensory and microbial variables to establish the quality criteria of good and bad samples were the same as for the global model. Results from the classification based on the 6 FishNose sensors as the independent variables to predict the smoked salmon quality (“good” or “bad”) are shown in Table 6. The results are given as the percentage of the number of good/bad samples predicted as good or bad.

Local models apparently show much better performance than the global model, and the results show that both single criteria (TVC, LAB, sweet/sour odor, off-odor, and rancid odor) and combined

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### Table 3—Quality criteria for good and bad samples based on microbial counts and sensory odor attributes

<table>
<thead>
<tr>
<th>Good/accepted samples</th>
<th>Bad/rejected samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVC &lt; 5</td>
<td>TVC &gt; 5</td>
</tr>
<tr>
<td>LAB &lt; 4</td>
<td>LAB &gt; 4</td>
</tr>
<tr>
<td>Off odor &lt; 20</td>
<td>Off odor &gt; 20</td>
</tr>
<tr>
<td>Rancid odor &lt; 10</td>
<td>Rancid odor &gt; 10</td>
</tr>
<tr>
<td>Sweet/sour odor &lt; 20</td>
<td>Sweet/sour odor &gt; 20</td>
</tr>
</tbody>
</table>

*LAB = lactic acid bacteria; TVC = total viable counts.

### Table 4—PLSR classification results of a global model for samples from all producers based on microbial and sensory quality criteria (TVC, sweet/sour, rancid and off-odor, and LAB)

<table>
<thead>
<tr>
<th></th>
<th>Expected nr of samples</th>
<th>Correct prediction (%)</th>
<th>Wrong prediction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 criteria</td>
<td>Good/accepted 65</td>
<td>92 (n = 60)</td>
<td>8 (n = 5)</td>
</tr>
<tr>
<td></td>
<td>Bad/rejected 31</td>
<td>35 (n = 11)</td>
<td>65 (n = 20)</td>
</tr>
<tr>
<td>5 criteria</td>
<td>Good/accepted 58</td>
<td>71 (n = 41)</td>
<td>29 (n = 17)</td>
</tr>
<tr>
<td></td>
<td>Bad/rejected 38</td>
<td>37 (n = 14)</td>
<td>63 (n = 24)</td>
</tr>
</tbody>
</table>

*LAB = lactic acid bacteria; PLSR = Partial Least Squares Regression; TVC = total viable counts.

### Table 5—Correlation coefficients ($r$) between single sensor responses and selected quality properties for individual producers

<table>
<thead>
<tr>
<th>Sensors</th>
<th>Attributes</th>
<th>PA/2</th>
<th>P10/1</th>
<th>P40/2</th>
<th>P40/1</th>
<th>LY2/G</th>
<th>LY2/LG</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sweet/sour</td>
<td>0.77</td>
<td>0.62</td>
<td>0.77</td>
<td>0.63</td>
<td>0.78</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Rancid odor</td>
<td>0.43</td>
<td>0.41</td>
<td>0.43</td>
<td>0.41</td>
<td>0.44</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Off-odor</td>
<td>0.74</td>
<td>0.59</td>
<td>0.73</td>
<td>0.59</td>
<td>0.76</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Log TVC</td>
<td>0.56</td>
<td>0.48</td>
<td>0.56</td>
<td>0.48</td>
<td>0.57</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Log LAB</td>
<td>0.69</td>
<td>0.6</td>
<td>0.72</td>
<td>0.59</td>
<td>0.73</td>
<td>0.65</td>
</tr>
<tr>
<td>B</td>
<td>Sweet/sour</td>
<td>0.87</td>
<td>0.72</td>
<td>0.88</td>
<td>0.72</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Rancid odor</td>
<td>0.74</td>
<td>0.73</td>
<td>0.74</td>
<td>0.72</td>
<td>0.7</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Off-odor</td>
<td>0.84</td>
<td>0.71</td>
<td>0.85</td>
<td>0.71</td>
<td>0.87</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Log TVC</td>
<td>0.56</td>
<td>0.43</td>
<td>0.57</td>
<td>0.42</td>
<td>0.62</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Log LAB</td>
<td>0.33</td>
<td>0.16</td>
<td>0.34</td>
<td>0.16</td>
<td>0.43</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*LAB = lactic acid bacteria; TVC = total viable counts.
quality criteria may be successful, but the outcome is dependent on the producer. The main concern is that no “false positives” should occur, that is, no bad samples should be predicted as good samples.

**TVC criteria.** Correct prediction (100%) of bad samples using TVC criteria was obtained for C, A, and B. No good sample was wrongly predicted as bad from C, but 8% to 38% of good samples were classified as bad from the other producers. The only producer that had wrong prediction of bad samples as good using TVC criteria was D. Only 3 samples were expected bad and 2 of these were wrongly classified.

**LAB criteria.** Improved correct prediction of good samples was observed using the LAB criteria compared with the TVC criteria, but wrong prediction of bad samples as good was higher for producers A, D, and C. This may possibly be explained because the growth of the LAB may lead to the production of different volatiles than produced by the psychrophilic spoilage flora and the gas sensors may be less sensitive to those volatiles.

**Off-odor criteria.** The gas sensors gave the best prediction of off-odor and sweet and sour odor as seen in Table 6. For example, a 100% correct classification was obtained for the D samples by using single sensory criteria, that is, the off-odor or sweet/sour odor. The sensors are apparently detecting and predicting the spoilage odors caused by the improper handling of the D samples as seen by the 100% correct prediction of the bad samples from D.

**Sweet/sour criteria.** Correct prediction was obtained of bad samples for D and C and in fact no bad samples existed according to this criterion in the B samples. One of the expected bad A samples was classified as good, but 100% correct prediction of the good samples was achieved.

**Rancid criteria.** The prediction of rancidity by the sensors is not good and indicates that the sensors may not be able to detect the volatiles causing rancid off-odor. It should also be stated that the odor scores were very low as detected by the sensory panel, and a few of the samples had values >10 for rancid flavor. The low values indicate that the volatiles causing rancid odor were present in low concentration and may therefore not be detected by the gas-sensors. In addition, the odor thresholds of characteristic compounds causing rancid odor are very low so the sensory panel may be able to detect the odors even though those compounds are present in very low levels in the samples.

**Combined criteria.** The combined criteria improved the overall predictions slightly for the A samples but not for the D samples where 8 samples were expected to be good and 8 to be bad, but the combined criteria predicted all the samples as bad. The combined criteria was not used for the other sample groups (C and B) because of a lack of structural correlation of variables and sample groups. Moreover, a robust prediction was achieved with the single criterion for those samples.

The developed FishNose system with the application-specific sampling unit interfaced with the sensor module was tested onsite at 1 of the producers location and showed a good repeatability of the system within 5% on real samples. Due to the fluctuating ambient air quality at the production site during the onsite testing, correction of the sensor readings had to be made for the reference air readings. However, the system showed good performance with regard to quality prediction of smoked salmon and successful classification of good and bad samples (95% to 93%, respectively) under the harsh environmental conditions occurring in fish production plant was achieved (Haugen and others Forthcoming 2005). This is encouraging for the future use of the system as a quality classification tool in the smokehouses.

### Conclusions

The overall analysis of the data of fresh and stored cold-smoked salmon from different smokehouses showed a “structural correlation” between the sensory and microbial analyses with the FishNose prototype responses. More specifically, the FishNose sensors showed a similar pattern in their responses as microbial counts (TVC and LAB) and sensory scores for spoilage attributes (sweet/sour odor and off-odor). The FishNose system is therefore ideal for fast quality control and freshness evaluation of smoked salmon products related to microbially produced volatile compounds and was able to predict “good” samples from “bad” ones based on the established microbial and sensory criteria.

The majority of the samples in this study were of “good” quality, and most of the samples defined as “bad” were of marginal quality because the criteria for the individual quality attributes were set lower than the commercial rejection criteria. Only a few of the samples would have been judged unacceptable based on commercial quality criteria. The practical implication of this study for the smoked salmon producer is that the FishNose system was able to discriminate samples of marginal quality from the good samples. This allows more effective quality control of samples in the production and could also be useful in retail and distribution. Rapid quality grading of samples is possible before the samples are actually spoiled. Additionally, the FishNose gas sensors appeared to group the samples according to the fish processors, indicating that there were differences in the composition of the headspace because of the different smoking and handling processes.

When classifying samples using all the data from different producers.

<table>
<thead>
<tr>
<th>Quality criteria</th>
<th>Expected nr of samples</th>
<th>Correct prediction (%)</th>
<th>Wrong prediction (%)</th>
<th>Expected nr of samples</th>
<th>Correct prediction (%)</th>
<th>Wrong prediction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TVC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>15</td>
<td>73</td>
<td>27</td>
<td>13</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Bad</td>
<td>9</td>
<td>100</td>
<td>0</td>
<td>3</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td><strong>LAB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>15</td>
<td>80</td>
<td>20</td>
<td>15</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Bad</td>
<td>9</td>
<td>89</td>
<td>11</td>
<td>9</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td><strong>Off-odor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>17</td>
<td>94</td>
<td>6</td>
<td>12</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Bad</td>
<td>7</td>
<td>86</td>
<td>14</td>
<td>4</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td><strong>Sweet/sour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>16</td>
<td>100</td>
<td>0</td>
<td>11</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Bad</td>
<td>8</td>
<td>88</td>
<td>13</td>
<td>5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td><strong>Rancid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>21</td>
<td>100</td>
<td>0</td>
<td>13</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Bad</td>
<td>3</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td><strong>Combined</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Good</td>
<td>14</td>
<td>79</td>
<td>21</td>
<td>8</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Bad</td>
<td>10</td>
<td>90</td>
<td>10</td>
<td>8</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

*LAB = lactic acid bacteria; PLSR = Partial Least Squares Regression; TVC = total viable counts.*
Smoked salmon quality predicted by E-nose...

in a global model, 74% of the samples were classified correctly into their respective quality class and 26% were classified incorrectly when using the criteria for TVC, and the sweet/sour, off-odor, and rancid odor criteria. Local predictive models based on samples from individual processes using the same quality criteria appeared to generate more robust prediction of good and bad samples than the global model. High classification rates (100%) were obtained for the FishNose prediction using both single and combined quality criteria. When evaluating local models, the optimal classification with regard to lowest number of "false positives ("bad samples predicted as "good") appeared to rely on single criteria like log TVC or sensory off-odor or sweet/sour odor. Combined criteria gave the best overall classification for the sample group A with the highest number of expected bad samples. This suggests that multiple quality indices are favorable to predict the complex spoilage changes occurring in smoked salmon products and a model based on the FishNose responses adapted for individual product may be useful for quality classification in the smokehouses.

Further studies should include characterization of the volatile compounds contributing to the spoilage changes including different processes used for smoked salmon products and storage conditions. This would allow selection of suitable sensors in the FishNose for the detection of quality related volatile compounds and help establishing useful limits for quality criteria based on different products.

Acknowledgments

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