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Rapid control of smoked Atlantic salmon (*Salmo salar*) quality by electronic nose: Correlation with classical evaluation methods

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Abstract

A prototype solid-state-based gas-sensor array system including a gas sampling unit, the FishNose, for direct quality measurements of smoked salmon has been developed. Quality changes of smoked salmon during storage were monitored by the FishNose and compared with the results of traditional sensory, chemical, and microbial measurements. Gas-sensor selection was optimized for the detection of changes in the very volatile compounds mainly representing microbial metabolism during spoilage. Sensor readings of repeated measurements of calibration samples showed a repeatability for the six sensors of the array of 6.4% (±1.4%), and for repeated measurements of fish samples the repeatability was 4.3% (±2.6%) without purge of the system between the measurements.

The system was further tested on-site in a smoked salmon production plant. Due to varying ambient air conditions at the production plant during the measurements, the sensor readings had to be corrected for by subtracting the sensor readings for the background air. High classification rates were obtained of 95 and 93% for good and bad samples, respectively.

This work demonstrated that the FishNose, equipped with an application specific sampling unit, was suitable for monitoring quality changes occurring during storage of smoked salmon and that the system was able to predict the quality related attributes like sweet/sour and off odour, and microbial counts.
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1. Introduction

Quality evaluation of smoked salmon products is needed because of the wide range of quality of these products on the market. The shelf life varies depending on factors related to the handling, smoking and hygienic conditions in the smokehouses and the storage conditions. Quality indicators and microbial spoilage of smoked salmon products have been studied recently by many authors [1–4]. In general because of the complexity of the spoilage process related to the proliferation of the different spoilage flora, a single quality monitoring technique for these type of products is not existing.

Traditional microbial analysis of total viable counts (TVC), total volatile bases (TVB), sensory analysis, colour measurements, *K* value which is a measure of the breakdown of nucleotides and other techniques, have been reported for monitoring changes occurring during storage of fishery products. The paper presents results from an EU CRAFT project (QLK1-CT-2002-71304) where the aim was to study the possibility to use an electronic nose to monitor smoked salmon quality.

The main objectives of the project were:

- to develop an electronic nose system with specific sensors for detection of quality and freshness of smoked fish;
- to develop and optimise the gas sampling system which provides to the sensor system a reliable and reproducible sample for analysis;

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- to detect and determine specific volatile compounds for spoilage of smoked fish via GC–MS analysis, as a basis for the training of pattern recognition system for the electronic nose;
- to automate the electronic nose system for on-line application in the fish-smoking industry.

Herein selected data from the project will be presented to demonstrate the ability of the FishNose system to predict "good" or "bad" quality classes of smoked salmon products based on microbial and sensory critera.

2. Experimental

2.1. Samples

Smoked salmon from two different producers in Europe were collected. Freshly processed and vacuum packed smoked salmon fillets were received from the producers and stored under different conditions (5 °C/10 °C) for up to 4 weeks. Samples from the process of the smokehouses were also obtained to have the range of different qualities of smoked salmon products for the FishNose prototype testing. The overall results of the storage studies are described in Olafsdottir et al. [5], but herein selected data from one smoked salmon producer will be presented. Samples presented in this paper were stored in vacuum ($n = 18$) and modified atmosphere packaging ($n = 6$) at 5 °C up to 4 weeks. In addition, 87 samples were measured in a smoked salmon production plant in connection with the on-site performance test of the instrument prototype.

Studies in the project focused on selecting the appropriate reference methods, which were indicative of the proliferation of microflora contributing to the development of volatile compounds that the sensors could detect. Odour evaluation is one of the best measures of consumer's acceptance of a product. Therefore, sensory scores for odour attributes were found most relevant to compare to the electronic nose sensor's responses.

2.2. Reference methods

Sensory analysis with a trained panel was based on quantitative descriptive analysis (QDA) [6] to develop a detailed sensory scheme for smoked salmon. The assessors evaluated the samples each time by using 19 descriptors of odour flavour, appearance and texture. Chemical analyses of water, total fat and salt content were done according to The American Oil Chemist's Society (AOCS) official methods. Water was determined gravimetrically by heating the sample in an oven at 103 ± 2 °C for 4 h [7]. Total fat was determined by extraction with petroleum ether, boiling range 40–60 °C using an extraction apparatus 2050 Soxtec Avanti Automatic System [8]. 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 [9]. The microbial analyses included total viable counts (TVC) (psychrotrophic counts) [10] and lactic acid bacteria (LAB) counts [11].

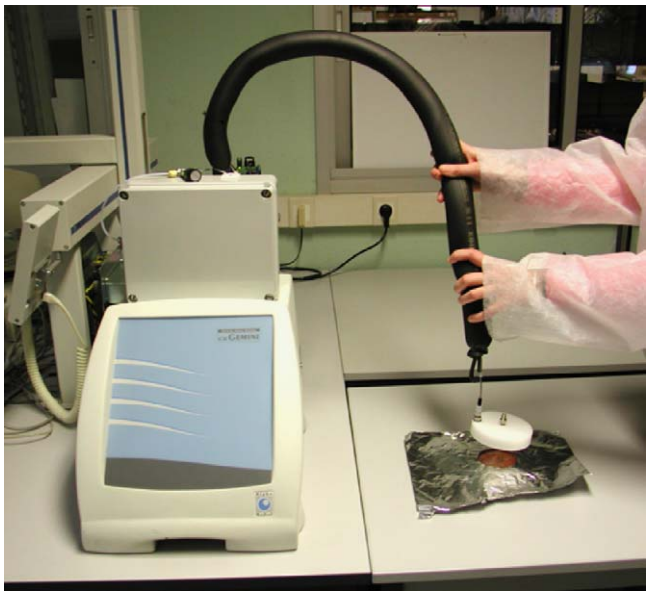

Fig. 1. FishNose—GEMINI system adapted for quality evaluation of smoked salmon.

2.3. Gas-sensor array system FishNose

The FishNose prototype was developed by adapting the GEMINI electronic nose system (Alpha MOS, Toulouse, France) for the measurements of smoked salmon quality. A prototype optimized gas sampling unit was developed by OPTOTEK engineering (Ljubljana, Slovenia) and interfaced with the sensor unit GEMINI (Fig. 1).

The sampling unit consists of the following main sub-units: VALCO 6 port valve with electronics box, transformer, driving electronics, control electronics, main power supply and heated inlet tube (55 °C) isolated with an Armaflex tube. The flow mechanical parts of the sampling system consist of the sample inlet, calibration inlet, pump inlet, column outlet, carrier inlet, 10 ml sample loop and pump with a flow rate of 200 ml/min. All tubes and fittings are made of seamless stainless steel type 316. All connectors are original from Valco. Two different sample loops were supplied with the unit: 10 ml (Fig. 1) and 20 ml. All the components are mounted in a closed box that is heated to 55 °C with an internal heater. Electronics at a main voltage of 230VAC drives the motor of the valve and gives the signal of the current valve position. The sampling unit was interfaced to the GEMINI gas-sensor unit with a heated tube at 60°.

The sampling was performed by inserting the inlet tube into a bell shaped unit 10 cm in diameter. The fillet was covered with a 7 cm diameter pierced aluminium foil to prevent cross contamination of samples. Aluminium was used because of its odourless property. The sampling bell was placed on the aluminium foil (see Fig. 1). Sampling was done at 5 °C and loading time of 7 s was used. Acquisition time was 120 s and no purging of sampling loop between sequential measurement was required. Total cycle time between subsequent measurements was 3 min including loading, acquisition and baseline recovery.

Manual injection optimisation of the FishNose system was performed by analysis of standard compounds. Validation of

Table 1
Key compounds identified in smoked salmon

Spoilage related compounds	
Alcohols	Ethanol[a], 3-methyl-1-butanol, 1-penten-3-ol
Aldehydes	3-Methyl butanal, hexanal, 2,4-heptadienal
Ketones	2-Butanone[a], 3-hydroxy-2 butanone
Esters	Ethyl acetate
Smoke related compounds	
Furan and pyran derivatives	Furfural[a]
Methoxyphenol derivatives	2-Methoxy phenol (guaiacol)[a]

[a]Selected for the standard compound measurements.

the performance of the FishNose system was done by repeated measurements of aqueous solutions of 2-butanone (20 ppm) in a 100 ml sample.

A preliminary study was done to investigate the influence of temperature (5 and 80 °C) during sampling on the responses of the sensors towards smoked salmon samples. This was done to study the influence of the compounds characteristic for the smoke flavour on the discrimination of the samples.

The sensitivity of the sensors towards selected compounds that are known to be present in the headspace of smoked salmon was studied. Identification and quantification of characteristic compounds in smoked salmon were determined by GC–MS analysis of samples from different producers [12]. The main classes of compounds present in the headspace and examples of key compounds shown in Table 1, are in agreement with earlier studies on volatile compounds produced by spoilage flora in cold smoked salmon [13].

Ethanol and butanone were selected to represent spoilage compounds and furfural and guaiacol were selected as characteristic for the smoking process. The volume of 1 ml of different dilutions of the standards (0.01–2 ppm) in a 10 ml sample vial were measured at 5 °C using a 5 ml injection. Randomized injection sequence was used for repeatability assessment.

2.4. Data analysis

Multivariate data analysis was performed by the Unscrambler 9.1 software package (CAMO Process, Norway). The main variance in the data set was studied using principal component analysis (PCA) and partial least squares regression models (PLSR) were used to describe the relationship of the data and make predictions on quality of samples based on the sensor responses and the data from the reference methods. The quality criteria established to discriminate good from bad samples were based on commercial critical limits for total viable counts (TVC) and sensory acceptance thresholds of selected attributes determined in the storage studies of the project [5].

3. Results and discussion

3.1. Sampling and sensor selectivity

The gas chromatographic analysis showed that some smoked salmon samples contained high levels of smoke related compounds [12]. This was of concern due to the fact that they would mask the lower molecular weight volatile compounds characteristic for spoilage. The results showed that best discrimination of smoked salmon samples of different qualities was achieved by manual injection of samples at 5 °C compared with automatic injection at 5 and 80 °C (data not shown). The manual injection volume was 10 ml (5 g fish/100 ml vial) while the automatic injection volume was 2 ml (1 g fish/10 ml vial). A 5 °C is similar to the temperatures occurring under industrial conditions. Therefore, the sampling was performed at 5 °C for the fish samples in this study.

Sensor selection was based on following criteria; diversity with regard to different sensor materials (SnO_2, WO_3, CrO), high sensitivity to spoilage compounds and fast measurement time, i.e. within 3 min cycle time. Six sensors out of the 18 initial sensors, which fulfilled these criteria, were selected with three different metal oxide materials: SnO_2 (P10/1, P40/1, P40/2, PA2), WO_3 (LY2/LG) and Cr_{2-x}–TiO_{3+y} (LY2/G).

The results of the standard compounds sensitivity measurements (Table 2) showed that all the sensors were most sensitive towards butanone and the LY2/LG, LY2/G and PA/2 sensors had higher sensitivities than the others. Ethanol and furfural were best detected by the LY2/G and P40/2 sensors although their sensitivities towards butanone was more than 10× higher. The sensors P10/1 and P40/1 showed very low or negligent sensitivities towards the standard compounds selected. Only one of the sensors (LY2/LG) appeared to be sensitive enough to detect increasing concentrations of the smoke related compound guaiacol at the same concentration level as was found in the smoked salmon samples [12]. Based on this it appears that the gas sensors are mainly detecting the changes in the very volatile compounds like butanone. Earlier studies have shown that microbially produced ketones, aldehydes and alcohols are abundant in the headspace of cold smoked salmon products during storage [13,14].

During the measurement sequences of the fillets, the performance of the FishNose system was validated by repeated measurements of aqueous solutions of 2-butanone. The mean R.S.D. for the six sensors was 6.4% (±1.3%) and 5.9% (±1.4%) without purge between samples and with purge, respectively. Repeated measurements of grinded fish samples (5 g in 100 ml vial) showed R.S.D. of 4.3% (±2.6%) without purge and 5.3% (±4.3%) with purge between samples indicating that purging was not necessary between samples.

Table 2
Sensitivity (response/ppm) of the sensors towards spoilage and smoke related compounds

Sensor	Butanone	Ethanol	Furfural	Guiacol
LY2/LG	3.671	0.126	0.032	0.147
LY2/G	−3.904	−0.332	−0.283	−0.018
P10/1	1.003	0.080	0.034	−0.002
P40/1	1.263	0.087	0.043	−0.001
PA/2	3.242	0.215	0.191	0.018
P40/2	1.407	0.096	0.058	0.003

3.2. Classification and prediction modelling

A global model encompassing several producers is the final goal to validate if the principle of measurement can be generalised. A global model including data from all the producers ($n = 96$) based on PLS prediction of combined quality criteria for TVC, LAB, and the odour attributes (off odour, sweet/sour odour and rancid odours) by the gas sensors showed poor classification results of good and bad samples [5]. Sixty-three percent of expected bad samples were wrongly classified as good samples, while 17% of good samples were classified as bad. This is not satisfactory, but when studying the correlations of the variables for the individual producers it appears that local models could be more useful (Table 3). The best correlations were found between the gas sensors and the sensory odour/flavour attributes and the microbial counts (TVC and LAB) (Table 3). The poor outcome of the global classification model is most likely due to differences in the raw material and processing conditions between the producers. These factors may have a significant influence on the composition of characteristic volatile compounds, which makes it difficult to compare fish from different producers. Moreover, this may influence the spoilage pattern causing differences in the formation of volatile compounds between producers. Accordingly, validation of a set of samples from one producer based on a training set from another producer is not appropriate.

A local model based on data from one producer ($n = 24$) showed much better performance than the global model based on data from all the producers ($n = 96$). The predictive model based on partial least squares regression (PLSR) was validated by leave-one-out cross-validation. The PLSR models the covariance between gas-sensors (X) and quality (good/bad, Y). As there is noise present in the data, the PLSR might need to compensate for this by including some additional component(s). As an example: The model on the 24 samples with the combined criterion was of rank three; the first component explained 60% (cross-validated), increasing to 70% after three components, without changing the classification. This indicates that the PLSR extracts the relevant variance in the gas-sensor data related to quality.

Results from the classification based on the six FishNose sensors as the independent variables to predict the smoked salmon quality (good or bad) are shown in Table 4. The results are given

Table 3
Correlations of the sensory attributes for appearance, odour, flavour, taste and texture, the chemical and microbial reference methods and the gas sensors for all samples from one of the producers ($n = 24$)

	Gas sensors					
	PA/2	P10/1	P40/2	P40/1	LY2/G	LY2/LG
Appearance						
Fat secretion	0.09	0.00	0.11	0.00	0.11	0.14
Translucent	−0.44	−0.36	−0.41	−0.36	−0.43	−0.39
Hue	−0.34	−0.40	−0.33	−0.40	−0.32	−0.22
Colour intensity	−0.14	−0.12	−0.13	−0.12	−0.13	−0.04
Odour						
Smoked salmon odour	−0.80	−0.70	−0.79	−0.70	−0.81	−0.74
Metallic odour	−0.66	−0.68	−0.66	−0.67	−0.67	−0.51
Sweet/sour odour	0.77	0.62	0.77	0.63	0.78	0.74
Rancid odour	0.43	0.41	0.43	0.41	0.44	0.37
Off-odour	0.74	0.59	0.73	0.59	0.76	0.69
Flavour						
Smoked salmon flavour	−0.68	−0.59	−0.67	−0.59	−0.68	−0.61
Metal flavour	−0.64	−0.59	−0.63	−0.59	−0.64	−0.56
Sweet/sour flavour	0.74	0.60	0.75	0.60	0.76	0.71
Rancid flavour	0.11	0.13	0.08	0.13	0.09	0.01
Off-flavour	0.69	0.58	0.69	0.59	0.71	0.63
Taste						
Salt taste	−0.52	−0.49	−0.52	−0.49	−0.53	−0.45
Bitter taste	0.49	0.43	0.49	0.43	0.50	0.47
Texture						
Elasticity	−0.16	−0.13	−0.10	−0.12	−0.12	−0.16
Oilyness	−0.18	−0.20	−0.15	−0.20	−0.13	−0.23
Juiciness	−0.63	−0.61	−0.61	−0.61	−0.60	−0.58
Chemical						
Fat (%)	−0.06	−0.05	−0.08	−0.05	−0.07	−0.11
Water (/%)	0.16	0.18	0.19	0.19	0.17	0.19
Salt (NaCl) (%)	0.04	−0.17	0.04	−0.16	0.10	0.13
Microbial						
Log TVC	0.56	0.48	0.56	0.48	0.57	0.44
Log LAB	0.69	0.60	0.72	0.59	0.73	0.65

Table 4
A local PLS prediction model for one producer ($n = 24$) based on the six FishNose sensors as the independent variables to predict good and bad samples based on single (TVC, LAB, off; sweet/sour and rancid odours) and combined quality criteria

Criteria	Expected (number of samples)	Predicted	
		% Correct	% Wrong
TVC			
Good	15	73	27
Bad	9	100	0
LAB			
Good	15	80	20
Bad	9	89	11
Off odour			
Good	17	94	6
Bad	7	86	14
Sweet/sour			
Good	16	100	0
Bad	8	88	13
Rancid			
Good	21	100	0
Bad	3	0	100
Combined			
Good	14	79	21
Bad	10	90	10

as percentage of the number of good/bad samples predicted as good or bad using both single and combined criteria.

The main concern is that no "false positives" should occur, i.e. no bad samples should be predicted as good samples. The best result was obtained based on the TVC criterium, but on the expense of four good samples (27%) being classified as bad. The gas sensors gave similar prediction of off-flavour and the sweet/sour descriptors. The results show that the combined quality criteria gave overall the highest correct classification.

3.3. On-site validation

Totally 87 salmon fillets from 31 different production batches, with 1–10 fillets of each production batch, have been analysed at the production site during nine daily measurement sessions over a 3 months period. Forty-four of the samples were freshly processed samples, and the remaining 43 samples had been stored chilled for up to 38 days to generate samples of a poorer quality.

In general, the sensor readings from the on-site measurements showed low values around the background air levels, which basically, is in agreement with good samples, which is also expected. For several of the measurement sequences from different days however, the reference air readings showed a significant fluctuation and partly exceeded the fish sensor readings, even if the sensor readings of the aged samples seemed to be slightly higher than the fresh samples. These changes are not related to sensor drift, but are due to changes in the ambient air at the production plant, which influence the fish measurements. During gas sampling on top of the fish fillet, an amount of the surrounding air will also be sampled together with the fish volatiles that may

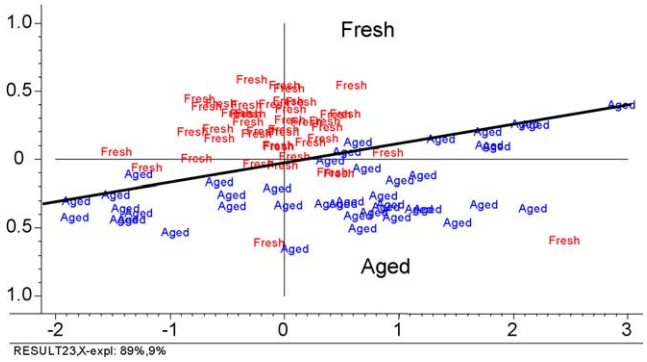

Fig. 2. PCA plot based on the six reference air corrected sensor readings from the on-site measurement of fresh (good) and aged (bad) samples.

contribute to the sensor signal. Analysis of the background air was therefore used as a zero calibration. The fluctuating pattern of the reference samples were also clearly reflected in the fresh and aged samples showing the effect of background ambient air at the production plant. Accordingly, the fluctuating reference air influences the fish sensor readings in a negative way by masking the expected real systematic variation in the fish measurement data with regard to, respectively, fresh and aged samples. To overcome this problem, all the sensor signal data of the measured fish samples have therefore been corrected for the fluctuating background air of the respective analysis date by simply subtracting them from the fish sample sensor readings. This has the positive effect on the data so that the structure in the data as expected is revealed as seen in the PCA plot based on the air reference corrected measurements data (Fig. 2). The PCA is not guided by the fresh/aged criterion, so the direction of the components is due to the variance in x (gas-sensor signals) only. This was not the situation for the PLSR model described earlier. Accordingly, the outcome of the on-site test results (Fig. 2) represent an unsupervised approach.

High classification rates were obtained and the outcome of the different sensor combinations were similar. Fresh (good) samples obtained a classification rate from 93 to 95%, whereas for the aged (bad) samples, a classification rate from 81 to 93% was obtained. The best classification in terms of lowest rate of "false positives", i.e. bad samples being classified as good, was obtained by combining all the six sensors. This corresponded to an overall classification rate of 94%, i.e. five samples classified wrongly, corresponding to three bad samples classified as good (false positives), and two good samples classified as bad, of a total of 87 samples.

4. Conclusions

The developed solid-state-based prototype gas-sensor system, FishNose, showed a good performance with regard to sampling, repeatability and sensitivity. This was partly due to the development of an application specific sampling unit interfaced with the sensor module, which had been optimised for direct analysis of smoked salmon. This allowed sequential sampling without purging the sampling system between the measure-

ments, thereby shortening the analysis time and maintaining a good repeatability of the system within 5% on real samples.

Measurement of standard compounds showed that the FishNose system was not sensitive to the compounds related to the smoke flavour characteristics like guaicaol, but was more sensitive to volatile compounds like butanone, originating from microbial metabolism causing spoilage of smoked salmon.

Local prediction modelling based on samples from a single producer showed better performance than a global model based on products from different producers to predict the quality related attributes like sweet/sour and off odour, and microbial counts based on the FishNose six sensor array system.

Due to the fluctuating ambient air quality at the production site during the on-site testing, correction of the sensor readings had to be made for the reference air readings to obtain a successful classification of good and bad samples. On the other hand, this was promising, since it showed that the system performance with regard to quality prediction of smoked salmon could be maintained, despite the harsh environmental conditions that may occur in fish production plants.

In conclusion, the FishNose appears to be promising for the quality control of smoked salmon, provided that the system has been calibrated for single producers, separately.

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