IFL Project Report
03-05

April 2005

FishNose

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Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins
Icelandic Fisheries Laboratories Report Summary

Titill / Title	**FishNose** – Þróun á refnefi fyrir sjálfvirkt gæðaeftirlit á reyktum laxi / Development of an electronic nose system for the automated quality control of smoked fish		
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Skýrsla Rf /IFL report		*Útgáfudagur / Date:*	April 2005
Verknr. / project no.	1558		
Styrktaraðilar / funding:	*Evrópusambandið* QOL-K1-CT-2000-71304		

Ágrip á íslensku:

Skýrslan er samantekt á lokaskýrslum Evrópuverkefnisins FishNose. Markmið verkefnisins var að þróa tæki, byggt á rafnefstækni, til að framkvæma sjálfvirkar gæðamælingar á reyktum fiski og var stefnt að því að þróa hagkvæmt og einfalt tæki til notkunar í iðnaði. Í verkefninu voru skilgreindar þarfir iðnaðarins varðandi gæðamælingar á reyktum laxi. Þróun á rafnefstækninni fól í sér að útbúa sýnatökubúnað fyrir rafnef frá franska framleiðandanum AlphaMOS og vali á málmoxíðskynjurum með næmni og sérhæfni til að greina þau efni sem áhrif hafa á gæði.

Umfangsmiklar geymslutilraunir voru gerðar á reyktum laxi frá fjórum framleiðendum í Noregi, Þýskalandi og Íslandi þar sem svörun rafnefsins var borin saman við hefðbundnar mælingar á gæðum með skynmati, örverumælingum og efnamælingum. Jafnframt voru gerðar mælingar með gasgreini til að greina þau lyktarefni sem eru einkennandi fyrir reyktan lax og þær breytingar sem verða við geymslu á laxi. Þessar upplýsingar voru notaðar til að velja staðla til að meta næmni rafnefsins. Í ljós kom að þau efni sem eru einkennandi fyrir reyklykt eru til staðar í miklu magni, en rafnefið var ekki næmt fyrir þessum efnum. Hins vegar greindi rafnefið niðurbrotsefni örvera og gott samræmi var á svörun rafnefsins, örverutalningum og skynmatseiginleikum sem voru einkennandi fyrir lyktarbreytingar sem tengjast skemmd eins og sæt/súr skemmdarlyk.

Niðurstöður geymslutilrauna sýndu að flokkun á reyktum laxi eftir ferskleika var möguleg með rafnefinu. PLSR (partial least squares regression) módel sem byggðu á niðurstöðum frá einstökum framleiðendum voru mun betri en módel byggt á öllum gögnunum frá mismunandi framleiðendum. Niðurstöðurnar benda til þess að nauðsynlegt sé að þróa módel fyrir hvern framleiðenda þar sem skemmdarbreytingar í reyktum laxi eru mjög flóknar og eru háðar aðstæðum í hverju reykhúsi og meðhöndlun hráefnisins og geymsluaðstæðum. Mælingar með rafnefinu hjá framleiðenda í Frakklandi sýndu að tækið getur nýst til að fylgjast með framleiðslunni og hentar til að meta á fljótvirkan hátt breytingar sem verða á ferskleika og myndun skemmdareinkenna vegna örveruvaxtar.

Lykilorð á íslensku: *Rafnef , reyktur lax, gæði, skynmat, örverur, rokgjörn efni, GC*

Summary in English:

Fish is an important and popular food in the European Union. On average, each citizen of the EU consumes about 25 kg of fish per year, of which 10% is smoked fish. The production of smoked fish and the processing industry is dependent on the excellent freshness and high quality of their products. Due to a growing public awareness on a competitive food market, a high standard of quality control is essential. Currently, the industry employs conventional random sampling quality control methods like classical bacteriologic and chemical methods beside sensory evaluation. Normally, SME´s do not have good enough laboratories or sufficiently trained staff to carry out complex analytical tests. They have to outsource the time-intensive and expensive measurements.

Therefore, there is a great interest in having rapid, automated, in-situ and objective tools for process-monitoring and final quality assurance available. Odour is the first criterion for evaluation the fish freshness or spoilage. The project “FishNose” envisages the development of a new, efficient and easy to handle automated quality control system based on a gas-sensor array system - “Electronic Nose” - for detection of smoked fish product's freshness and 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.
- 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.

Today, there is no automated quality control system for the characterisation of smoked fish and related products, which can supply data ready for documentation to improve the production process reliability and reproducibility.

The FishNose prototype, generated during the current project, consists of a gas sampling unit, the sensor array system itself and a user-oriented software. Characteristic key components for spoilage of smoked fish have been identified and provide basis for sensor selection, calibration of the sensor system and optimisation of the sampling. The “FishNose” prototype has been tested and validated in laboratory trials and optimised on-site in the industrial smoked fish production process.

English keywords: *Electronic nose, cold smoked salmon, quality, volatile compounds, sensory analysis, microbial counts, gaschromatography*

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Project Summary

Introduction

Fish is an important and popular food in all countries of the European Union. On average, each citizen of the EU consumes about 25 kg of fish per year, from which 10% is smoked fish. The smoked fish producing and processing industry is crucially dependent on the excellent freshness and high quality of their products. Due to growing public awareness on a hard fought market, a high standard of quality control is essential. Currently, the industry employs conventional random sampling quality control methods like classical bacteriologic and chemical methods beside sensory evaluation. Especially small and medium-sized enterprises normally do not have sufficient laboratory capacity or correspondingly trained staff at their disposal to carry out complex analytical tests. They have to outsource the time-intensive and expensive measurements.

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Quality of smoked salmon

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 various factors related to the handling, smoking and hygienic conditions in the smokehouses and the storage conditions. Many papers have been published on spoilage and various quality indicators for smoked salmon. 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, color measurements, K value which is a measure of the breakdown of nucleotides and other techniques, have been reported for monitoring changes occuring during storage of fishery products. The use of an electronic nose to monitor spoilage changes in smoked products has not been reported before.

Development and optimisation of the FishNose

The FishNose prototype was developed by adapting the GEMINI electronic nose system (Alpha M.O.S, Toulouse, France) for the measurements of smoked salmon quality. A 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 diameter) that was placed on the fillets. Samples were covered with a 7 cm diameter pierced

aluminium paper to prevent cross contamination of samples. Aluminium was used because of its odourless property. Sampling was done at 5 °C and loading time of 7 s was used.

Manual injection optimisation of the FishNose system was performed by analysis of standard compound (2-butanone and ethanol). Validation of the performance of the FishNose system with repeated measurements of aqueous solutions of 2-butanone (20 ppm) in a 100 ml sample vial showed that mean RSD for the 6 sensors was 6.36 % (± 1.25%) and 5.86 % (± 1.41%) without purge between samples and with purge, respectively. Repeated measurements of grinded fish samples (5 g in 100 ml vial) showed RSD of 4.33 % (± 2.61 %) without purge and 5,34 % (± 4.31 %) with purge between samples indicating that purging was not necessary between samples.

A preliminary study was done see the influence of temperature during sampling on the responses of the sensors towards smoked salmon samples during sampling at 5 °C and 80 °C. This was done to study the influence of the compounds characteristic for the smoke flavor on the discrimination of the samples. GC analysis showed that some smoked salmon samples contained high levels of smoke related compounds. It was of concern that because of their high concentration, they would mask the lower molecular weight more 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 °C and 80 °C. Therefore, the sampling was done at 5 °C for the fish samples in this study. The manual injection volume was 10 ml (5g fish /100 ml vial) while the automatic injection volume was 2 ml (1g fish /10 ml vial).

Criteria for the selection of sensors was based on achieving diversity and fast recovery of the sensor. Six sensors out of 18 initial sensors were selected with three different metal oxide materials: SnO2 (P10/1, P40/1, P40/2, PA2), WO3 (LY2/LG) and Cr2-x-TiO3+y (LY2/G).

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. The main classes of compounds present in the headspace and examples of key compounds, are in agreement with earlier studies on volatile compounds produced by spoilage flora in cold smoked salmon. Ethanol and butanone were selected to represent spoilage compounds and furfural and guaicol 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.

The results of the standard compounds measurements 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 x 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. 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.

FishNose prototype testing – storage studies of smoked fish and correlation/classification modeling

Storage studies of smoked salmon samples from different producers in Europe were done. The samples were stored under different conditions (5 °C/10 °C) for up to 4 weeks of storage and 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 tesing.

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 odor attributes were found most relevant to compare to the electronic nose sensor´s responses.

Sensory analysis based on Quantitative Descriptive Analysis (QDA) was used to develop a detailed sensory scheme for smoked salmon. The assessors evaluated the samples each time by using 19 descriptors of odor flavor, appearance and texture. Chemical analyses of water, total fat, TVB-N, and salt content, were done according to AOCS official methods. The microbial analyses included total viable counts (TVC) (psychrotrophic counts) and lactic acid bacteria (LAB) counts.

Multivariate 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.

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. Sixty three % 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 would be of interest.

The best correlations were found between the gas sensors and the sensory odour / flavour attributes and the microbial counts (TVC and LAB).

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 (PLS) was validated by leave-one-out cross-validation.

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 4 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 the highest classification rates. The prediction of rancidity by the sensors is not good and indicates that the sensors are not able to detect the volatiles causing rancid off odour.

FishNose on-site testing

Smoked salmon fillets from 31different production batches were analysed, with 1-10 fillets of each production batch. In total, 87 salmon fillets were analysed during the on-site testing at the processors facilities, which lasted over 3 months. 44 of the samples were freshly processed samples, and the remaining 43 samples had been stored chilled for up to 30 days to generate samples of a poorer quality. The results showed that the background air was not stable during the on-site testing. However, after correction of the data and subtraction of the background, a good classification of the samples was achieved based on their age. Discriminant Partial Least Squares Regression (DPLSR) was used for the classification of respectively fresh and aged samples. The correct prediction of good samples in the different batches was overall 93-95% while the correct prediction of bad samples was slightly lower (81-93%).

Conclusions

It appears that the gas sensors in the FishNose are not sensitive to the compounds related to the smoke flavor characteristics, but are mainly detecting the changes in the very volatile compounds mainly representing microbial metabolism. Therefore, the FishNose appears to be ideal to monitor changes occuring during storage of smoked salmon. Local model based on samples from single producer shows better performance than a global model based on products from different producers to predict the quality related attributes like sweet/sour and off odor, and microbial counts based on the FishNose sensor array system.

FINAL REPORT

CONTRACT N°: QLK1-CT-2002-71304
PROJECT N°: CRAFT-1999-71304

ACRONYM: FISHNOSE

TITLE: Development of an electric nose system for automated quality control of smoked fish

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REFERENCE PERIOD: From the 1st January 2003 to the 31st of December 2004

STARTING DATE: 1st January 2003 **DURATION: 24 MONTHS**

Date of issue of this report: March 2005

Project funded by the European Community under the Programme „Quality of Life“ in FP5 (1998-2002)

CONTENTS

1 INTRODUCTION

Fish is an important and popular food in all countries of the European Union. On average, each citizen of the EU consumes about 25 kg of fish per year, from which 10% is smoked fish. The smoked fish producing and processing industry is crucially dependent on the excellent freshness and high quality of their products. Due to growing public awareness on a hard fought market, a high standard of quality control is essential. Currently, the industry employs conventional random sampling quality control methods like classical bacteriologic and chemical methods beside sensory evaluation. Especially small and medium-sized enterprises normally do not have sufficient laboratory capacity or correspondingly trained staff at their disposal to carry out complex analytical tests. They have to outsource the time-intensive and expensive measurements.
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The project “FishNose” envisages the development of a new, efficient and easy to handle automated quality control system based on a gas-sensor array system - “Electronic Nose” - for detection of smoked fish product's freshness and quality.
An automated quality control system for the characterisation of smoked fish and related products supplying data ready for documentation to improve the production process reliability and reproducibility is not available on the market today.

Objective and rapid quality evaluation techniques for cold smoked salmon are needed to help both producers and retailers to fulfill increasing demands of consumers for consistent food quality. In recent years attempts to use electronic nose technology to track the spoilage processes occurring in fish have been reported in numerous papers. Most of these are feasibility studies showing the ability of the electronic nose to discriminate between different spoilage levels or storage time of samples. Instruments based on different sensor technologies have been used such as metal-oxide chemoresistors sensors (Ólafsson *et al.,* 1992; Egashira *et al.,* 1990;1994; Ohashi *et al.,* 1991), MOSFET sensors (Haugen and Undeland, 2003), amperometric sensors (Schweizer-Berberich *et al.,* 1994; Olafsdottir *et al.,* 1997a; 1997b; 1998; 2000; 2002; 2003), conducting polymer sensors (Du *et al.,* 2001; 2002; Luzuriaga and Balaban 1999a; 1999b; Newman *et al.,* 1999) and quartz microbalance sensors (Di Natale *et al.,* 1996; 2001; 2003 Zhao *et al*., 2002).
Absolute estimation of quality and shelf life of smoked salmon products based on storage days is not relevant, because the various handling, smoking processes and the different storage conditions influence the freshness and the shelf life of the products (Hansen *et al*., 1995; Hansen et al, 1996; Cardinal *et al.,* 1997; Dondero *et al.,* 2004). Shelf life of smoked salmon products varies considerably from about 2 weeks to 2 months depending mostly on the temperature during storage. Bugeno *et al.* (2003) reported that shelf-life of salmon samples newly processed and stored for up to 30 days at 2°C under modified atmosphere and in vacuum packages was limited by microbial growth to 25 days and no relevant changes in chemical or physical parameters were observed. The shelf life for fillets evaluated by a sensory panel was longer (32-49 days) than for slices (21-36 days) of the same product (Hansen *et al.,* 1998). Different composition of the microflora in whole fillets compared to slices indicated the impact of the inhouse microflora in this study.
Characteristic spoilage off-odours and off-flavours are caused by microbial activity but autolytic enzymes have a major impact on the textural quality of cold smoked salmon during the early stage of deterioration (Hansen *et al*., 1996).
The microflora in cold smoked salmon appears to be related to the source of contamination i.e. the raw material and /or the smokehouses rather than being specific for the product (Hansen *et al.,* 1998). The spoilage potential of the microflora has been studied (Stohr *et al.,* 2001; Joffraud *et al.* 2001; Leroi *et al.,* 2001) and the predominance of lactic acid bacteria in vacuum-packed cold-smoked fish products at the end of shelf life of the products is generally acknowledged (Becker *et al*. 2002; Gonzalez-Rodriguez *et al*. 2002). *Enterobacteriaceae* has been identified in cold smoked salmon products as the main contributor to spoilage, related to the inhouse flora and hygienic conditions in the smokehouses.

Identification of spoilage indicators for cold smoked salmon has been the focus of numerous papers and various indicators have been suggested. Microbiological methods are commonly used to monitor the quality and safety of cold smoked salmon products, both total viable counts (TVC) and detection of Listeria and Salmonella (Sigurgisladottir, 1994). The validity of TVC measurements has been questioned for fresh fish and no obvious relationship has been found between sensory changes and TVC in smoked salmon (Hansen *et al.,* 1995; Leroi *et al.,* 1998; Cann *et al.,* 1984). Recently, Dondero et al (2004) conducted a study to find suitable objective quality indicators for vacuum packed cold smoked salmon stored at different temperatures (0 - 8°C). They reported that TVB (total volatile bases), K-value, total aerobic and anoerobic counts and Lactobacillus species were the most suitable indicators to determine the freshness of cold smoked salmon based on comparison with storage days and sensory analysis. Hypoxanthine, TVC, molds and yeasts and biogenic amines were not useful to detemine the deterioration of the products in the study.

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 colours and unpleasant odours and flavours. No standardized schemes are available for inspection of smoked fish, but smokehouses often use their own schemes or guidelines using only two categories such as the FDA scheme (Sado, 1993) and guidelines published by the Torry Research Station (Anon, 1963). 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, faecal, ammonia and cabbage were used by Hansen et al (1998) to describe the spoilage of vacuum packed smoked salmon fillets and slices. Cardianal *et al*, (2004) identified different quality classes based on sensory characteristics of cold smoked salmon from supermarkets in the European market. Colour, intensity of smoke related odours, amine odours and salty perception were the main sensory characteristics discriminating between quality classes.

2 MATERIAL & METHODS

Salmon samples were obtained from Partners in Norway, Iceland and Germany and storage studies were carried out in laboratories in the different countries. The raw material used for smoking in the different smokehouses was fresh and processed 2-3 days after slaughtering. All the smokehouses use traditional smoking and dry salting. Table 1 shows an overview of the smoking conditions in the different smokehouses.

Table 1: Smoking and storage conditions of samples from the different smokehouses and sample design for the prototype laboratory testing.

Company		FIEDLER	REMO	REYKO	TBB	Number of samples
Smoking conditions	Temperature (°C)	27	22	16-22	28	
	Time (hours)	0.5	5	14-18	6-12	
	Humidity (%)	40	50-60	50-60	50	
Storage conditions	Packaging	MAP/VAC	VAC	VAC	VAC	
	Temperature	5°C	5°C / 10°C	5°C / 10°C	5°C / 10°C	
Batch 1	Storage study	16	16	14	20	66
Batch 2	Process samples	4	4	4	4	16
Batch 3	Process samples	4	4	4	4	16
Total		24	24	22	28	98

The smoking procedures vary in the different smokehouses which can influence the smoke flavour intensity and the level of smoke related components like phenols in the final products. The cold-smoked salmon products were sliced and vacuum packed, but one producer (REYKO) vacuum packed the products as whole fillets.
The smoking temperatures at FIEDLER's and TBB's are higher than for the other smokehouses and the smoking times varies, being the shortest at FIEDLER's. A significant number of samples with different qualities and from different production batches were used for the prototype testing and optimization in order to obtain a reliable validation of the performance of the measurement system (Table 1). For the process samples each smoked salmon processor provided samples from the process. Two processors provided fresh samples, but the others provided both fresh and stored samples. One batch from TBB had been kept for 10 days in cooler before delivery to the laboratory and another batch from REYKO was selected from old stored products (15-22 months in freezer), to obtain samples of bad qualities reflecting frozen storage conditions. The storage experiments were performed with 16 freshly smoked samples delivered from each smokehouse to the laboratories. The samples were stored at two 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 Fiedler samples were all stored at 5 °C, one sample group in vacuum, and the other sample group in Modified Atmosphere Packaging (MAP).

2.1 Sampling

For each sampling 2 samples (fillets) were used and divided for the different analyses according to Figure 1.

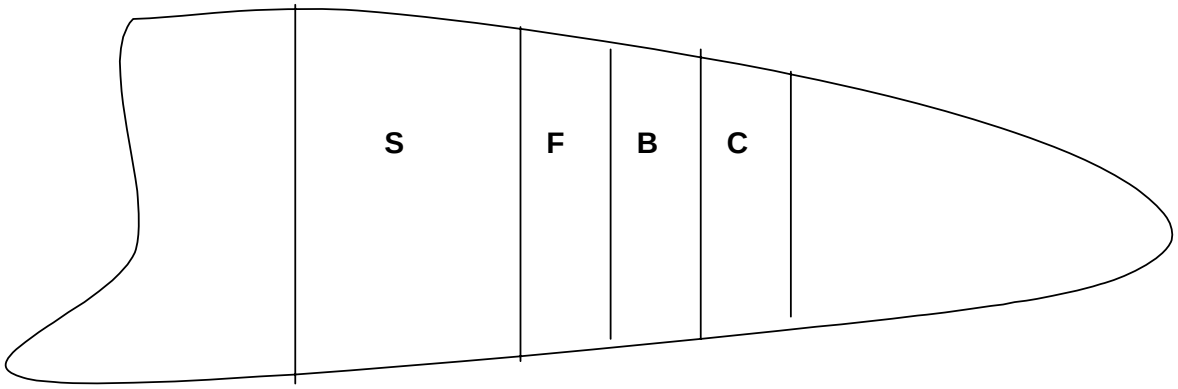


Figure 1: The division of the fillet for preparation of samples for sensory analysis (S), FishNose prototype measurement (F), microbial analysis (B) (TVC and LAB), and chemical analysis (C) (water, total fat, salt content).

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.

2.2 Methods

2.2.1 Reference methods:

Chemical analysis of fat, water, pH and TVB is recommended to characterize samples of smoked salmon

Chemical analysis	**Reference:**
Fat	AOCS Official Method BA 3-38 and application note Tecator no. AN 301. 1997
Salt	AOAC 16th ed. 1995 no. 976.18
Water	ISO 6496 (1999)
TVB	AOAC, 15th ed. 1990 920.03.

2.2.1.1 Selection of alternative reference chemical methods

Chemical analysis	**Reference:**
TMA	Malle, P. and Poumeyrol, M . (1989). A New Chemical criterion for the Quality Control of Fish: Trimetylamine/Total Volatile Basic Nitrogen (%). Journal of Food Protection, Vol 52, No 6, p 419-423
TBA	Pearson´s chemical analysis of foods.Vynke, W., Fette, Seifen Anstr. 77,6 (1975) Tarladgis, et. al., JAOCS, 37, 44(1960)
PV	AOAS Official Method Cd 8-53
Phenols	Singleton, V.L. and Rossi, J.A. 1965. Colorimetry and total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic*. **1965**, *16*, 144-158.

2.2.1.2 Selection of microbial methods

To monitor microbial spoilage relating to quality and general hygiene the following methods are needed: counts of TVC, lactic acid bacteria and Enterobacteriaceae.

Microbial analysis	**Method:**	**Reference :**
TVC: Total viable count Total cfu	Total psychrotrophic count (15°C): Modified Long & Hammer's medium (1% NaCl) use cooled Maximum Recovery Diluent (MRD, Oxoid) for serial dilution spread-plated incubated aerobically at 15°C for 5-7 days Detection limit: 20 cfu/g	Van Spreekens K.J.A. (1974) Archiv fur Lebensmittelhygiene 25 (10) 213-219
Lactic acid bacteria count	Nitrite-Actidione-Polymyxin medium (NAP, pH 6.1) pour-plated with overlay incubated anaerobically at 21-22°C for 5 days catalase test can be used for confirmation Detection limit: 10 cfu/g	Davidson, A.P.& Cronin, F. (1973). Medium for the selective enumeration of lactic acid bacteria from foods. Applied Microbiology, 26 (3) 439-440.
Entero-bacteria-ceae count	Violet Red Bile Glucose Agar (VRBGA) pour-plated with overlay incubated aerobically at 35°C for 24 h typical colonies counted oxidase test used for confirmation Detection limit: 10 cfu/g	British Standards Institution, BS 5763: Part 10. 1986. Enumeration of Enterobacteriaceae, (ISO 7402-1985).

2.2.1.3 Selection of alternative microbial methods

Microbial analysis	**Method:**
Presence of *Listeria*	Based on information from U.S. Department of Agriculture (USDA-FSIS,1989), the APHA (1992) and others. Enrichment broth is UVM modified Listeria broth (30°C, 24h). Then inoculated into Fraser broth (35°C for up to 40h). Growth from black tubes is streaked onto Modified Oxford Agar (MOX) (35°C,48h). Confirmation tests are done on 5 colonies and include Gram-staining, catalase and motility. Species identification includes haemolysis on Blood agar and testing on API Listeria (System for the identification of Listeria, bioMérieux SA/France).
Salmonella	First enrichment: Lactose broth (35°C, 24h). Second enrichment: Selinite broth and tetrathionate broth (35°C, 24h). From these broths we streak onto two solid media: BG agar and BS agar (35°C for 24/48 h). Typical colonies (2-4 or as needed) are inoculated into TSI-agar and LI-agar (35°C for 24h). Finally we test for urease-production. Species identification is carried out by serological methods.
E. coli / Coliforms	*Total and faecal coliforms.* Most probable number (MPN) method: Preenrichment in LST broth (35°C, 24/48 h) and confirmation tests done in BGLB broth for total coliforms (35°C, 48h) and in EC broth for faecal coliforms (44.5°C, 24h). Confirmation test for *Escherichia coli* is done by the MUG method (44.5°C, 24 h).
Yeasts / Moulds	The isolation medium used is Dichloran Rose-Bengal Chloramphenicol Agar (DRCB-Agar). Surface plating is used. Plates are incubated at 22°C for 120 hours.

For characterization of the different products chemical analysis of fat, water and salt were done and the proliferation of spoilage changes were monitored by sensory analysis, total aerobic and lactic acid bacteria counts.

2.2.2 Chemical analyses

Analysis of water content was done by heating the sample in an oven at 103°C +/-2°C for four hours. Water corresponds to the weight loss (ISO 6496, 1999). Total fat was determined by extraction with petroleum ether, boiling range 40-60°C. The extraction apparatus is 2050 Soxtec Avanti Automatic System (AOCS Official Method BA 3-38 and application note Tecator no. AN 301. 1997). Salt content was measured by extracting the soluble chloride from the sample with water containing nitric acid. The chloride content of the solution is titrated with silver nitrate and the end point is determined potentiometrically (AOAC 16th ed. 1995 no. 976.18)

2.2.3 Methods for the detection of valatile compunds

Different sampling methods have been tried for collection of volatiles prior to analysis by GC and detection by GC-MS and GC-O.

Air pump sampling – Pre concentration on TENAX - sample preparation for GC-MS
Headspace of samples (fillets) was collected by an air pump sampling (ALPIN-2, Air sampler, METEK). Approximately 300 g of sample was placed in the glass container (2.3 L, Ø 17 cm) and the headspace volatiles collected on 250 mg Tenax 60/80 (Alltech, IL, USA) in stainless steel tubes (Perkin-Elmer, Buchinghamshire, U.K.) for a combined ATD 400 and GC-MS measurement. Heptanoic acid ethyl ester was added as an external standard to samples by adding 1 mL of 10 ppb aqueous solution of the standard to a 25 ml beaker (Ø 3.5 cm) and placed with the sample in the glass container. Each sample was prepared in duplicate.

SPME sampling - sample preparation for GC-O
Frozen samples were thawed overnight at 4°C and prior to homogenisation with a Moulinex mixer. Approximately 15 g of sample was placed in a 25 ml vial and sealed with PTFE-faced silicone septum. The SPME device and fibers were purchased from Supelco (Bellefonte, PA, USA). The stationary phase used was polydimethylsiloxane (PDMS) of 100 µm thickness. The PDMS fibre was inserted through the septum of the sample vial and allowed to equilibrate with the headspace volatiles. The equilibration state needs to be optimised and should be comparable to conditions used for the electronic nose. The fibre was then retracted into the barrel of the syringe and immediately inserted into the injector of the GC for 2 min desorption of the entrapped volatile compounds. The fiber was left in the GC injector in position 3 on the manual holder. Volatile compounds are thermally desorbed in splitless mode (60s) in a split/splitless injection port, with helium as the carrier gas at linear velocity of 22.9 cm/s.

Purge and trap sampling on Tenax for GC-O and GC-MS measurements
Frozen samples were thawed overnight at 4°C and prior to homogenisation with a Moulinex mixer. Samples (100 ± 2 g) were homogenised in saturated NaCl solution (100 ± 5 g) and a purge and trap technique used for collection of volatiles on Tenax (Olafsdottir *et a*l., 1985). Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 1 mL of 10 ppb aqueous solution of the standard to the sample solution. The sample was purged at room temperature with nitrogen at about 100 mL/min for 2.5 hours. Volatiles were collected on 250 mg Tenax 60/80 (Alltech, IL) in stainless steel tubes (Perkin-Elmer, Buchinghamshire, UK) for the combined ATD 400 and GC-MS measurements or 150 mg Tenax in a Pasteur pipette for the GC-O measurements. Each sample was prepared in duplicate.

*Purge and trap sampling on Tenax for -MS measurement*s
Vacuumed frozen samples were thawed over 2 hours at room temperature. Just before weighing, samples were homogenised 30 sec. with a Moulinex mixer. The TBB samples were deskinned prior to homogenisation. 5 gram sample was transferred to a 250 mL Erlenmeyer glass for purge and trap sampling. 1 microliter (372 ng) ethyl-heptanoate in methanol was added as quantification standard. Volatile compounds were collected on 250 mg Tenax 60/80 GR (Alltech, IL) in stainless steel tubes (Perkin-Elmer, Buchinghamshire, UK) with 100 ml/min nitrogen and incubated for 30 min at 50 °C. Remaining water was removed by 10 min additional drying with 50 ml/min flow of nitrogen for the combined ATD 400 and GC-MS measurements.

Chromatography
GC-MS measurements:

Volatile compounds were thermally desorbed (ATD 400, Perkin Elmer) from the Tenax tubes and separated on a DB-5ms column (30 m ´ 0.25 mm i.d. ´ 0.25 µm, J&W Scientific, Folsom, CA) using helium as a carrier gas by GC-MS (HP G1800C GCD, Hewlett-Packard, Palo Alto, CA). The following temperature program was used: 50 °C for 7 min, 50 °C to 120 °C at 5 °C/min and from 120 °C to 220 °C at 10 °C/min. The injection temperature was 250 °C and the detector temperature was 280 °C. The mass detector ion range was 35-300 m/z.

Volatile compounds were thermally desorbed (ATD 400, Perkin Elmer) with 121 kPa pressure, 250 °C desorption temperature, 50 mL/min nitrogen flow, outlet split 10, inlet split 0. GC was performed on a separated on a DB-WAXetr column (30 m ´ 0.25 mm i.d. ´ 0.50 µm, J&W Scientific, Folsom, CA) using helium as a carrier gas on an Agilent 6890A GC interfaced to an Agilent 5973 Mass selective detector (MSD). The following temperature program was used: 30 °C for 10 min, 30 °C to 40 °C at 1 °C/min, from 40 °C to 70°C at 3 °C/min, from 70 to 230 °C at 4 °C/min. The MS ion source was set at 230°C with electron ionisation energy of 70 eV. The MS measured the total ion current (TIC) of positive ions over the mass area (m/z) 33-300. Tentative peak identifications were based on standard MS Wiley and NIST98 libraries.

GC-O measurements:

Volatiles were extracted from the Tenax traps with 1 mL diethyl ether. The sample was then concentrated by passing nitrogen over the solution leaving a small amount of sample, 20-30 µl. Headspace sample (1µl) samples were then injected splitless. Measurements were performed on a GC (HP 5890, Hewlett-Packard, Palo Alto, CA) with the same type of column and the same conditions as for the GC-MS measurements. The end of the column was split 1:1 between flame ionisation detector (FID) and an ODO-1 olfactory detector outlet (SGE, UK). Nitrogen, bubbled through water to add moisture, was used to drive the sample up to the sniffer. Two assessors describing the odour sniffed the effluent. Intensity (quality and duration/retention times) of each odour was determined using an intensity from 0-5, 0: not present; 5: very strong. The assessors were trained in recognising characteristic spoilage odours and smoke odours by injecting into the GC-O, mixtures of standard compounds dissolved in ether and sniffing the effluent. Two mixtures were prepared, i.e. rancid odours (hexanal, *cis*-4-heptenal, 2,4-heptadienal, 2,6-nonadienal, 2-nonenal and 2,4-decadienal) and smoke odours (2-methoxy-4-methyl phenol, 2-methoxy-4-[2-propenyl] phenol (eugenol), iso-eugenol, 2-methoxy-phenol (guaiacol), phenol and, 4-methyl-phenol (p-cresol)). All standards were purchased from Sigma-Aldrich.

Identification of the volatiles
Identification of the volatiles done by matching retention indices (RI) of ethyl esters and mass spectra of samples with authentic standards (Sigma-Aldrich and Merck). Tentative identifications were based on standard MS library data (Hewlett-Packard Co, 1997 and manually checked against literature sources and the database Flavornet (Acree and Arn, 1997).
Peak area ratio (PAR), i.e. the ratio between the total ion count of each peak and internal standard, was calculated for the GC-MS results.

2.2.4 Microbial analyses

The microbial analyses included total viable counts (TVC) (psychrotrophic counts) using modified Long & Hammer's medium (LH) (Van Spreekens K.J.A., 1974). Analysis of lactic acid bacteria (LAB) counts was done using NAP (Nitrite-Actidione-Polymyxin) medium slightly modified (Davidson, A.P. & Cronin, F., 1973).

2.2.5 Sensory analyses

A sensory scheme for smoked salmon was developed in the project based on Quantitative Descriptive Analysis (QDA) (Stone and Sidel, 1985). The scheme is a detailed description of the sensory profile for the changes occuring in smoked salmon during storage. With the QDA, all detectable aspects (odour, appearance, texture and flavour) of a product are described and listed by a trained panel. The list is used to evaluate the product and the panelists quantify the sensory aspects of the product using an unstructured scale. Despite of training of panelists in the different laboratories a significant inconsistency could be demonstrated for the data analysis of the sensory data between the three labs during pretrials in the project. Therefore, it was decided that one laboratory would do the sensory analysis of the samples.
Sensory assessments were carried out by six to nine assessors (age range 30 - 55). They were all trained according to international standards (ISO, 1993), including detection and recognition of tastes and odours, training in the use of scales, and in the development and use of descriptors. The assessors evaluated the samples each time by using 19 descriptors of odor / flavor, appearance, and texture. Odour and flavour attributes were: Smoked salmon odour/flavour, metallic odour/flavour, sweet/sour fruity odour/flavour, rancid odour/flavour, off- odour/flavour. Taste attributes included: salt and bitter taste. Appearance attributes evaluated were: fat secretion, translucent, hue, colour intensity and three texture attributes: elasticity, oilyness, juiciness.
A visual analog scale (0 to 100%) was used. The samples, approximately 30 g served as slices on plastic dishes, were allowed to equilibrate at room temperature for 30 min before evaluation. Each sample was evaluated in duplicate.

2.2.6 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 diameter) that was placed on the fillets. Samples were covered with a 7 cm diameter pierced aluminium paper to prevent cross contamination of samples. Aluminium was used because of its odourless property. Sample temperature (headspace generation temperature) was 5 °C in a refrigerator and loading time of 7 s was used.

Figure 2: On site testing at one of the smokehouses showing the FishNose with the sampling system developed in the project.

Validation of the performance of the system and the sensitivity of the sensors towards selected compounds that are known to be present in the headspace of smoked salmon is reported in Ólafsdóttir *et al.,*(2005).

2.2.7 Data handling

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). One-Way ANOVA was done to study if differences between sampling days of each storage group were significant (H_0= no difference between samples; significant difference $p < 0.05$). Multivariate analysis was performed by the Unscrambler Version 9.1 (CAMO Process, Norway). The main variance in the data set was studied using Principal Component Analysis (PCA) and 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. 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. PCA/SIMCA based on eucledian distance in the multidimensional space was also used for the classification of the samples. This method is already implemented in the Gemini software in a slightly modified version. The quality criteria established to discriminate good samples from bad samples were based on commercial critical limits for total viable counts (TVC) and sensory acceptance thresholds of selected attributes in the study.

2.2.8 Methods for pattern recognition

There exist a variety of methods for pattern recognition with the purpose of classification. The applications in the FishNose project will in most situations have two categories for the samples: Good (Pass), or Bad (reject). However, one might also foresee three quality classes. For that reason the methods chosen for comparison must be able to handle more than two classes. Below is a short description of the methods that were applied in this phase of the project. We refer to the literature for details about the mathematics and statistics. PCA/SIMCA This method is already implemented in the Gemini software in a slightly modified version. One attractive feature is that the model is based on samples for *one* class only. This might be an advantage for the FishNose applications because the "Good" samples are often quite well defined (no unwanted odour or high bacteria count) whereas a sample might be "Bad" due to many reasons. Therefore, any discrimination method that tries to find a decision line between "Good" and "Bad" might not be optimal. There exist established statistical criteria for allowing new samples to be inside the classes.

Support Vector Machines (SVM)
This method has gained a lot if attention the past years. The principle is to find a limited number of samples in each class ("support vectors") that define a discrimination line between the classes. Each class is "closed" by a polygon, thus this method also has a "one-class" option like the SIMCA method. It offers flexibility in terms of mapping the original variable space with a kernel function, such as a sigmoid function. However, with many options for a number of input parameters, it is challenging to find the "best" parameter settings.

Discriminant regression
Within the gas-sensor and chemometric community it is customary to apply the Partial Least Squares Regression (PLSR) as a tool for discrimination, especially in situations with two classes. A method based on cross-validation that performs best combination search to find e.g. a set of 6 sensors with the best classification potential was also evaluated.

Logistic regression
This type of regression works on the so-called logic function of the response variables in different categories. The outcome of the analysis gives the probability that the samples belong to the different classes. With only two classes, the method is named ordinary logistic regression.

Compensation software
A system drift correction method is implemented in the GEMINI software package. The principle is based on measuring chemical diagnostic products for weekly checking instrument stability and propanol as an internal reference for a new sequence. Alternatively, a stable reference product might be chosen. The user decides on correction approach during the training process.

3 RESULTS AND DISCUSSION

Different methods (sensory analysis, microbial counts of TVC, LAB and Enterobacteriacae (EB) and chemical analysis of TVB-N) to monitor smoked salmon quality were used in a pretrial in this study where reference methods were selected to use during the FishNose prototype testing. Three methods were selected as reference methods based on their continuous responses over the storage time which is useful for comparison with the electronic nose responses. The proliferation of TVC and LAB in the products showed a similar continuous trend and was in agreement with the sensory data and these were therefore selected as reference methods in this study.
Microbial counts of Enterobacteriacea were done in the pre-trials but they were not included in the study reported herein. EB counts were useful to explain high initial TVC counts in some of the products and reflected the hygienic conditions in the factories. The general trend was that EB counts decreased and LAB became predominant at the end of storage time.

3.1 Selection of reference methods for pre-trials

Selection of reference methods for pre-trials:

1. **Chemical analysis** of **fat, water, pH** and **TVB** were recommended to characterize samples of smoked salmon in the pre-trials at the beginning of the development**.**

2. Selection of **microbial methods.** To monitor microbial spoilage relating to quality and general hygiene the following methods were selected: **counts of TVC, lactic acid bacteria** and **Enterobacteriaceae.**

Sensory analysis is the most important method for quality evaluation of fish and fish products (Olafsdóttir *et al*., 1997). Quality monitoring of smoked salmon in the industry is based on using sensory quality schemes, evaluating appearance, texture, smell and taste. No standardized schemes are available for inspection of smoked fish as is the case for fresh fish, but less detailed schemes and guidelines are available using only two categories such as the FDA scheme (Sado, 1993) and guidelines published by the Torry Research Station (Anon, 1963).

Shelf life and the sensory quality depend mostly on microbial growth, autolytic changes and oxidation of lipids that occur after smoking and cause changes in appearance, flavour and texture. Microbial activity has been found to cause characteristic spoilage off-odours and off-flavours but autolytic enzymes have a major impact on the textural quality of cold smoked salmon during the early stage of deterioration (Hansen *et al*., 1995a). Due to these changes the desirable attributes from smoking diminish and the characteristic of deterioration takes over involving softening of the fish flesh, fading colours and unpleasant odours and flavours.

Oxidative rancidity is often measured in smoked fish to monitor the quality. Peroxide values are of limited use in determining quality of fish (Connell, 1995) and that also applies to smoked fish. Measurements of TBA (thiobarbituric acid) are more useful and TBA value increased gradually during both cold storage (ca. 0 °C) and refrigerated storage (ca. 10 °C) of hot smoked Indian mackerel for up to 3 months and the free fatty acid content also increased during storage (Hanumanthappa and Chandrasekhar, 1987).
Biochemical or chemical methods are not commonly used in the smoking industry, according to Koteng (1992). In his research, only 5 % of the industry dealing with smoked salmon applied biochemical or chemical methods to estimate shelf life.

Fat content is a common quality parameter of salmon and producers of smoked salmon in Europe use a chemical method (Soxhlet) for fat determination (Sigurgisladottir, 1994). The importance of muscle lipid content (2.9 % to 10.7 %) on the eating quality of smoked Atlantic salmon was investigated by Robb et al. (2002) who found that lipid content had a highly significant effect on many of the texture and flavour attributes of the smoked fish. The hedonic ratings also showed an increased preference of the smoked salmon at the higher lipid levels over the range studied.

Physical methods: The most common physical method used for quality evaluation of smoked fish, is measurements of colour (Skrede, 1989; Sigurgisladottir, 1994), which is used to evaluate the quality of smoked fish in smoking plants in Europe. Texture is also an important quality attribute, but texture measurements of fish are primarily used for research, since these are complicated and require the use of expensive laboratory instruments and time-consuming procedures (Botta, 1995).

Microbiological methods: Lactic acid bacteria often dominate the microbial flora in smoked fish products during refrigerated storage (Magnusson and Traustadottir, 1982). As a result smoked fish products have a prolonged shelf life, since the Gram-negative spoilage flora is somewhat inhibited (Jeppesen and Huss, 1992). Lactic acid bacteria (LAB) have been used for centuries in the fermentation of a variety of dairy products. The preservative ability of LAB in foods is attributed to the production of anti-microbial metabolites including organic acids and bacteriocins that can improve the safety and quality of the final product
Microbiological methods are used to monitor the quality and safety of products (cold smoked salmon) in smoking plants in Europe, both total viable counts (TVC) and detection of Listeria and Salmonella (Sigurgisladottir, 1994). The validity of TVC measurements has been questioned for fresh fish and no obvious relationship has been found between sensory changes and TVC in smoked salmon (Hansen *et al.*, 1995; Leroi *et al.*, 1998; Cann *et al.*, 1984).

Stohr et al. (2001) evaluated the spoilage potential of nine bacterial groups (*Shewanella putrefaciens*, *Brochothrix thermosphacta*, *Aeromonas spp*., *Lactobacillus alimentarius*, *Lactobacillus sake*, *Lactobacillus farciminis*, *Carnobacterium piscicola*, *Photobacterium phosphoreum* and *Serratia liquefaciens*) isolated from cold-smoked salmon. Chemical and sensory changes were studied after five weeks of storage in vacuum packs at 6° C. The bacteria mainly responsible for spoilage were *L. sake*, *L. farciminis* and *B. thermosphacta*, which produced sulphurous, acidic and rancid off-odours respectively. Some strains of *S. liquefaciens*, produced rubbery, cheesy or acidic off-odours. Some *P. phosphoreum* isolates were characterized by an acidic effect.

Gonzalez-Rodriguez et al. (2002) studied the numbers and types of microorganisms in vacuum-packed cold-smoked freshwater fish from Spanish smokehouses at the retail level after 3 weeks storage at 2 +/-1 °C. According to their findings LAB predominated, with Carnobacterium and Lactobacillus being the genera most frequently found among 377 bacteria randomly isolated from aerobic 25 °C plate counts. The second and third major groups were Enterobacteriaceae and Micrococcaceae, respectively. *Salmonella spp*, *Escherichia coli* and *Listeria monocytogenes* were not detected in any samples.

Becker *et al*. (2002) studied microbiological quality and listeria-contamination of vacuum packaged smoked salmon at the retail level. The mesophilic, aerobic total count varied on the day of purchase between < 10(2) cfu/g and 1.1 x 10(8) cfu/g. At the end of the indicated "consume-by" date, 30 (75 %) samples exceeded the suggested guideline value of 10(6) cfu/g, whilst in 16 (40 %) of the samples the total detectable microbial numbers exceeded 10(8) cfu/g; the numbers of two samples actually exceeded 10(9) cfu/g. In 80 % of these highly contaminated samples, lactic acid bacteria were the dominating population, partly together with Enterobacteriaceae and pseudomonads. Listeria was detected in 26 samples (65 %) at the day of purchase. The contamination level is considered alarmingly high for the samples investigated.
Bugeno *et al*. (2003) reported that shelf-life of salmon samples newly processed and stored for up to 30 days under modified atmosphere and in vacuum packages was limited by microbial growth to 25 days and no relevant changes in chemical or physical parameters were observed. The parameters analyzed were pH, total volatile bases nitrogen, 2-thiobarbituric acid reactive substances, mechanical properties and colour.

3.2 Chemical analysis

3.2.1 Chemical analysis of smoked salmon

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. A significant variation in fat and water content of samples was found even within samples from the same batch. The Fiedler samples had the highest fat content and samples from REMO had the lowest fat content. This variation in fat content is expected and reflects both the age/size and feeding condition of the salmon.

Table 2: Range and mean of fat, water and salt content in smoked salmon samples from different smokehouses.

Smokehouse	% Fat	Mean	% Water	Mean	% Salt	Mean
FIEDLER	5.8 – 16.6	10.5	56.1 – 65.7	61.4	2.9 – 5.7	3.8
REMO	3.5 – 11.7	7.0	62.9 – 68.9	65.5	2.3 – 4.0	3.1
REYKO	5.8 – 12.9	9.0	60.2 – 66.2	63.3	2.1 – 6.6	4.7
TBB	4.7 – 11.4	8.5	57.3 – 63.8	61.2	4.3 – 7.0	5.3

Variation in salt content was also found within the samples, the highest values for TBB and lowest for REYKO. 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. Variation in salt content can influence the microbial growth and the proliferation of spoilage. Therefore, careful monitoring of the salting process is necessary to ensure consistent products.

3.2.1.1 TVB-N results

Figure shows the results of TVB-N analysis of samples from the different smokehouses. The range of the TVB-N values (mg N/100 g) varied and the range of the values was the following: Fiedler 13.0 - 34.8; REYKO 20.4-23.7, TBB 12.7-20.0; REMO 13.7-20.3. The highest values were detected in the FIEDLER samples after 21 days of storage. None of the samples exceeded the TVB-N of 35 mg/100g, the European standard for fresh salmon. Apparently the TVB-N values are not showing the same spoilage trend of the samples as the microbial analysis. Because of conflicting results, the TVB-N is not considered a good indicator for smoked salmon and is not commonly used in the industry.

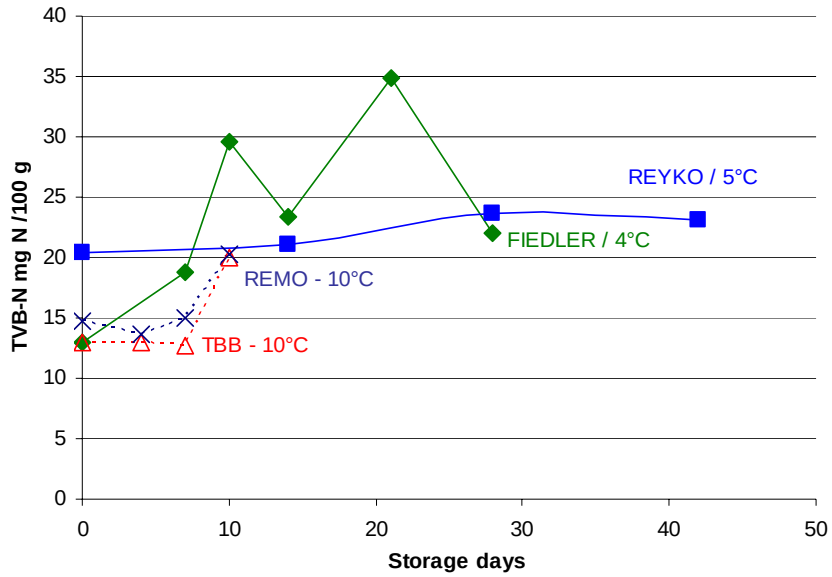


Figure 3: TVB-N values for the samples from REYKO, FIEDLER, TBB and REMO during storage at different temperatures. (dashed line for 10 °C)

3.2.1.2 Microbial results

The intial microbial counts varied considerably in the samples Fiedler 5.5; REYKO 2.0, TBB 6.1; REMO 2.7. At sensory rejection the TVC is typically 10^7-10^8 cfu/g in cold smoked products and the microflora differs depending on the processes involved in the different smokehouses. The quality of the raw material, hygienic conditions and smoking conditions (temperature and time of the smoking process) will influence the initial counts and the storage conditions in particular the temperature will influence the final counts. The limits for the end of shelflife are often set at 10^6 cfu/g in the industry. At the end of the storage study the TVC values were above 10^6 cfu/g for all the samples except in samples from REYKO.
The results show that LAB became predominant in the spoiled cold smoked salmon flora for all the samples. At lower storage temperature (4-5 °C), LAB development occurred slowly, but usually dominated towards the end of the storage periods (Figure 2). At 10 °C, LAB counts were similar to TVC throughout storage (Figure 3). This is in agreement with other studies showing that the LAB appear to be well adapted in vacuum packages and more resistant than Gram negative bacteria (i.e. *Pseudomonas* spp) to the high salt content found in smoked salmon products. The proliferation of LAB in smoked product is considered a positive trend since LAB can be effective in preventing/reducing the growth of *Listeria*.
High counts of Enterobacteriaceae in the initial product has been associated with conditions in the smokehouse and low hygienic quality of the products.

The slow spoilage rate and low microbial counts in the REYKO samples may be explained by the fact that the samples were pre-frozen (2 weeks at –24 °C) before storage at 5 °C which may have affected the proliferation of the microbial flora, hence influencing the development of volatile degradation compounds contributing to off odours. The high salt content may also have limited the growth of the microflora and the low temperature during smoking may have an effect on the slower growth of LAB.

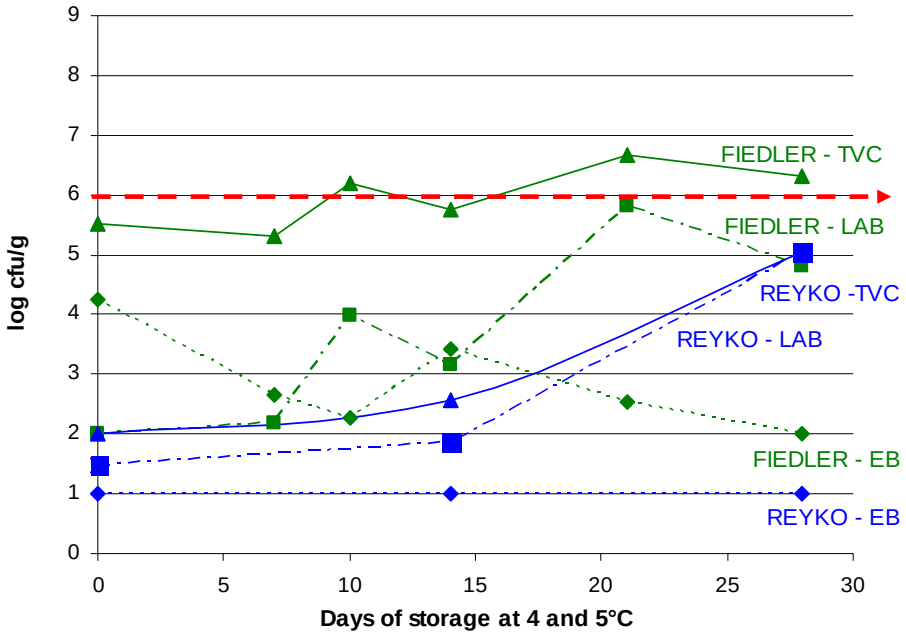


Figure 4: Microbiological analysis (log cfu/g) for samples from FIEDLER (TZZ) and REYKO (IFL) during storage at different temperatures (4 °C and 5 °C respectively).

The counts of EB were low in the beginning and their counts did not increase during storage, but the counts of LAB increased as expected for this type of products. The counts on the last day were very low and this could reflect the change in microbial flora often seen at the end of shelf life because of microbial competition and /or depletion of substrate for microbial growth (dying population). For the overall data analysis it is suggested not to include the microbial data for the last sampling day on week 6, which had probably passed sensory shelflife.

3.2.2 Chemical results of smoke

Smoke is a complex mixture of organic acids, alcohols, ammonia, CO2, CO, carbonyls, esters, furans, hydrocarbons, lactones, nitrogen oxides, particulates, phenols, sulphur dioxides and other miscellaneous compounds (benzene, indene, naphthalene, styrene, toluene, etc.). Over 400 volatile compounds have been identified in smoke. Volatile compounds are specifically related to each smoking technique which has a great influence on sensory characteristics of smoked salmon (Cardinal *et al.* 1997).

Results from the GC analysis of samples from the pre-trial at IFL showed that smoke related volatile compounds were in the highest concentration in the headspace.

Tables 3, 4, 5 and 6 show the results of the GC-MS analysis of samples from REYKO, REMO and TBB using purge and trap sampling technique. The main classes of compounds identified in all samples are the same, but some variation in the identity of individual compounds within each class. The quantification is based on an internal standard (PAR). As stated earlier the smoke related compounds are present in the highest amount in each sample and the compounds present because of microbial growth are in much lower concentrations.
Table 7 shows the results of the GC-O analysis of samples from REYKO and FIEDLER. Lower scores are in general noticed for the REYKO samples. This is not in agreement with the sensory analysis (QDA) of the IFL and TTZ panels. The reason for this may be that the GC-O panellists were trained in identifying smoke and rancid odours after the analysis of the REYKO samples and that may influence their scoring. It should be noted that the samples from TTZ were kept frozen for six months prior to GC measurements, which may have caused rancidity.

Spoilage related compounds
The characteristic odours of the samples were identified and quantified. Quantification of the main components detected in smoked salmon showed that compounds developed because of microbial growth are present in lower concentrations than compounds derived from the smoking process. Spoilage related compounds are very volatile and elute in the first half of the chromatogram. Among these compounds are short chain alcohols, aldehydes and ketones (e.g. ethanol, 3-methyl butanal, and 3-hydroxy-2 butanone). Odour thresholds for some of these short chain compounds are given in Table 6. In addition, odour thresholds for lipid-derived compounds (i.e. hexanal, 2,4-heptadienal, 2,6-nonadienal giving rancid, green like odours) are listed. Some of the components may have high odour impact like the compounds derived from lipid oxidation although they are not present in high concentrations.

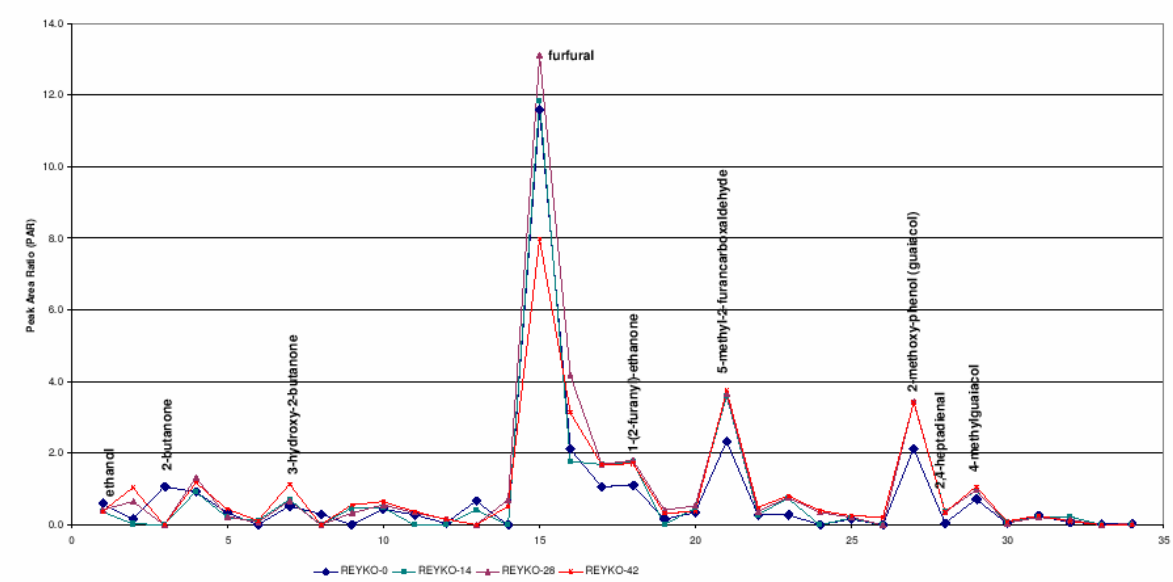


Figure 5: Quantities (PAR) of the key volatile compounds identified by GC-MS in REYKO samples vs. retention time.

Smoke related compounds
Less volatile compounds with characteristic smoke and burnt odours were most dominant in the samples. Examples of smoke related compounds are given in Table 9. These compounds are mainly detected in the second half of the chromatogram. Strong smokehouse-like odour together with smoke-like, wood and ash eluting close to each other, were detected in all samples. Guaiacol (2-methoxy phenol) was identified as the main compound contributing to this smokehouse odour. Phenolic compounds are important for preservation and flavour properties of smoked products. They are mainly produced by pyrolysis of lignin. The content of phenolic compounds in these products depends on the nature of wood (Rozum, 1992). Sérot *et a*l. (2004) studied the effect of smoke processes on the content of 10 major phenolic compounds in smoked fillets of herring *(Clupea harengu*s). Their results showed that guaiacol and 4-methylguaiacol were the main compounds in fish fillets, regardless of the process used. Furfural was in the highest concentration in REYKO samples, possibly explained by long smoking time as listed below. Furfural is a weak odorant (2 ppm) and does therefore not contribute much to characteristic smoked aroma. The REMO samples contained higher level of smoking compounds than the TBB samples, despite similar smoke processing conditions. This could probably be explained due to the fact that REMO use grinded beech wood, whereas TBB use grinded pine.

Company:	**REYKO**	**FIEDLER**	**REMO**	**TBB**
Temperature (°C):	16-22	27	22	28
Time (hours):	14-18	0.5	5	6-12
Humidity (%):	50-60	40	50-60	50

Quantities (PAR) of the key volatile compounds in REYKO samples during storage are shown in Figure 5. The figure illustrates clearly that the smoke related compounds are present in the highest concentration in the headspace.

Influence of sampling conditions on the composition of the headspace
The results from air pump sampling followed by GC-MS analysis shows that the characteristic compounds that are derived from microbial growth and lipid oxidation are detected but not the less volatile smoke compounds. The volatility of the compounds is influenced by temperature and it maybe possible to reduce the level of smoke related compounds in the headspace by lowering the sampling temperature (4-5 °C). This could be practical when sampling for the electronic nose if the aim is to detect the spoilage compounds. SPME sampling was not suitable for the analysis of volatile compounds but possibly another type of fibers will be more sensitive for this application.

Selection of key compounds
Based on these results recommendations for the selection of standard compounds to be used to test the performance of the electronic nose for monitoring smoked salmon quality can be given. The selection criteria are based on quantification of the compounds by using both GC-O and GC-MS analysis, but also keeping in mind the origin of the compounds and the processes involved in their formation. Following is a list of the main classes and the identity of key compounds present in the highest concentration in the headspace during storage of smoked salmon and contributing to the overall odour. Table 2 has a list of additional classes of compounds (aromatics, hydrocarbons, nitrogenated, terpenic and others) that were identified in smoked salmon samples during storage. These components may influence the sensors signals of the electronic nose but they were however not included in the list of key compounds for the standard cocktail.

Compounds developed in smoked salmon because of *microbial growth*

- → Alcohols (ethanol, 3-methyl-1-butanol, 1-penten-3-ol)
- → Carbonyls (3-methyl butanal, 2-butanone, 3-hydroxy-2 butanone)
- → Esters (ethyl acetate)
- → Amines (TMA)
- → Sulphur compounds (i.e. dimethyl disulfide - not detected)
- →

Compounds developed in smoked salmon because of oxidation

- → Aldehydes (hexanal, 2,4-heptadienal)

Compounds present in smoked salmon because of the smoking process

- → Furan and pyran derivatives (furfural)
- → Methoxyphenol derivatives (2-methoxy phenol, i.e. guaiacol)
- → Cyclic (cyclopentanone)
- → Acids (acetic acid)
- → Esters (ethyl acetate)
- → Hydrocarbons

Additional classes of compounds present in the headspace of smoked salmon

- → Aromatics
- → Hydrocarbons
- → Nitrogenated
- → Terpenic derivatives
- → Others

Table 3: Key volatile compounds (PAR) identified by GC-MS during storage study at 5°C in samples from REYKO, Iceland

Compound	RI	week 0	week 2	week 4	week 6
acid					
acetic acid	182	0.16	0.03	0.65	1.04
alcohol					
ethanol	<173	0.61	0.37	0.41	0.36
3-methyl-1-butanol	291	0.31	0.00	0.00	0.00
aldehyde					
3-methyl butanal	255	0.00	0.12	0.08	0.11
hexanal	371	0.28	0.00	0.34	0.36
3-hydroxy-butanal	382	0.04	0.00	0.16	0.15
2,4-heptadienal	674	0.00	0.00	0.00	0.21
nonanal	707	0.04	0.38	0.35	0.34
decanal	808	0.05	0.07	0.05	0.10
undecanal	907	0.07	0.23	0.13	0.08
ketone					
2-butanone	191	1.07	0.00	0.00	0.00
1-hydroxy-2-propanone	227	0.92	0.93	1.31	1.18
2-pentanone	245	0.35	0.22	0.24	0.43
3-hydroxy-2-butanone	264	0.52	0.72	0.68	1.13
3-pentanone	330	0.00	0.47	0.32	0.55
cyclo					
cyclopentanone	359	0.44	0.48	0.56	0.65
2-methyl-2-cyclopenten-1-one	498	1.05	1.69	1.67	1.69
2,3-dimethyl-2-cyclopenten-1-one	592	0.27	0.75	0.77	0.82
furan and pryran derivates					
furfural	396	0.68	0.41	0.00	0.00
3-furanaldehyde	400	0.00	0.00	0.70	0.51
furfural	417	11.59	11.83	13.10	7.98
2-furanmethanol	438	2.12	1.78	4.16	3.14
1-(2-furanyl)-ethanone	504	1.10	1.75	1.80	1.70
1-(2-furanyl)-ethanone	507	0.16	0.00	0.42	0.32
5-methyl-2-furancarboxaldehyde	515	0.35	0.45	0.53	0.37
5-methyl-2-furancarboxaldehyde	559	2.33	3.61	3.67	3.74
2,5-dihydro-3,5-dimethyl-2-furanone	595	0.00	0.00	0.35	0.39
methoxyphenol derivates					
phenol	578	0.28	0.28	0.37	0.49
2-methyl-phenol (cresol)	652	0.16	0.19	0.20	0.25
2-methoxy-phenol (guaiacol)	686	2.13	3.42	3.43	3.42
2-methoxy-4-me-phenol (4-me-guaiacol)	775	0.71	0.94	0.97	1.06
4-ethyl-2-methoxy-phenol	876	0.26	0.21	0.20	0.25
2-methoxy-4-vinylphenol (4-vinylguaiacol)	911	0.03	0.00	0.00	0.00
2,6-dimethoxy-phenol (syringol)	953	0.02	0.00	0.02	0.00
eugenol	958	0.04	0.00	0.03	0.00

Table 4: Volatile compounds (aromatics, hydrocarbons, nitrogenated compounds, terpenic derivatives and others) identified by GC-MS during storage study at 5°C in samples from REYKO, Iceland

Compound	RI	week 0	week 2	week 4	week 6
aromatic					
ethylbenzene	446	0.00	0.03	0.00	0.00
1,3-dimethyl-benzene	455	0.10	0.15	0.10	0.16
1,2-dimethyl-benzene	482	0.11	0.02	0.00	0.05
1,2-dimethoxy-benzene	746	0.03	0.02	0.05	0.00
1,4-dimethoxy-benzene	784	0.03	0.00	0.00	0.00
2-phenoxy-ethanol	823	0.03	0.00	0.00	0.00
hydrocarbon					
1-ethoxypropene	329	0.22	0.00	0.59	0.00
toluene	331	0.28	0.07	0.00	0.00
3-methylene-heptane	359	0.00	0.02	0.05	0.07
styrene	484	0.00	0.00	0.06	0.00
nonane	496	0.00	0.06	0.00	0.00
cis-3-decene og 2,6-dimethyl-4-octane	557	0.00	0.06	0.00	0.00
2,2,4,6,6-pentamethyl-heptane	588	1.29	1.42	1.55	1.89
(2-methyl-3-hexene)	596	0.00	0.15	0.00	0.00
decane	601	0.00	0.00	0.10	0.22
3,7-dimethyl-1,3,7-octatriene	606	0.00	0.00	0.02	0.00
2,2,4,4-tetramethyloctane	627	0.03	0.03	0.07	0.12
octadecane	628	0.00	0.04	0.00	0.00
2,5-dimethyl-undecane	649	0.02	0.00	0.08	0.09
(2,4-nonadiene)	697	0.05	0.00	0.00	0.20
naphthalene	787	0.00	0.09	0.06	0.11
2,3-dimethoxytoluene	846	0.01	0.00	0.00	0.00
hexadecane	899	0.00	0.03	0.00	0.00
tridecane	900	0.00	0.02	0.00	0.00
heptadecane eða hexadecane	902	0.00	0.00	0.00	0.05
eicosane	905	0.01	0.00	0.00	0.00
1-nonene	670	0.00	0.06	0.00	0.00
pentadecane	>1001	0.00	0.15	0.07	0.18
nitrogenated compounds					
pyrazine	300	0.00	0.16	0.35	0.32
pyridine	306	0.28	0.25	0.61	0.22
4-methyl-pyridine	400	0.03	0.00	0.00	0.00
methyl-pyrazine	412	0.01	0.04	0.08	0.08
pyridine	436	0.00	0.12	0.00	0.00
3-(4-)ethyl-pyridine	446	0.01	0.00	0.06	0.00
dihydro-2H-pyran-3(4H)-one	472	0.00	0.00	0.06	0.00
2H-pyran-2-one	541	0.03	0.02	0.05	0.00
2-hydroxypyridine	662	0.00	0.27	0.00	0.00
3-pyridinol	663	0.17	0.34	0.00	0.00
2-methoxy-5-methyl-pyrimidine	727	0.02	0.04	0.03	0.07
terpenic derivative					
limonene	628	0.03	0.06	0.07	0.14
other					
methyl-hydrazine	<173	0.10	0.03	0.34	0.35
chloroform	205	5.24	0.26	1.45	0.74
(2,4-octadiyne)	458	0.00	0.00	0.03	0.00
alpha pinene	529	0.11	0.22	0.18	0.18
1-ethoxy-but-1-ene-3-yne	565	0.00	0.00	0.00	0.05
benzonitrile	581	0.21	0.33	0.32	0.26
3-carene	608	0.00	0.04	0.06	0.00
(3,3-dimehtylbutyl)-oxirane	648	0.03	0.00	0.00	0.00

Table 5: Key volatile compounds (PAR) identified by GC-MS during storage study at 10°C in samples from REMO, Norway

Compound	RI	day 0	day 10
acid			
acetic acid	191	0.00	3.25
alcohol			
1-penten-3-ol	250	2.17	0.94
1-ethoxy-2-propanol	314	0.45	0.39
ethanol	<173	0.08	0.13
aldehyde			
3-methyl-butanal and 3-hexanone	264	0.00	0.19
hexanal	382	0.00	0.35
3-methyl-hexanal	588	0.06	0.03
2,4-heptadienal	597	0.02	0.00
2,4-heptadienal	611	0.02	0.00
nonanal	707	0.34	0.42
decanal	808	0.00	0.03
ester			
ethyl acetate	200	0.54	0.00
ketone			
2-heptanone	173	0.48	0.06
2-butanone	191	1.62	0.73
3-hydroxy-2-butanone	273	1.41	1.00
1-hydroxy-2-butanone	347	0.00	0.03
cyclo			
cyclopentanol or 2,3-pentanedinone	260	0.94	0.37
toluene or cyclopentanone	340	1.46	0.78
cyclopentanone	368	0.69	0.53
2-methyl-cyclopentanone	429	0.17	0.00
cyclohexanone	429	0.00	0.12
3-methyl-cyclopentanone	435	0.08	0.02
1,2,3-trimethyl-cyclopentane	484	0.00	0.06
2-methyl-2-cyclopenten-1-one	501	1.08	0.88
3,4-dimethyl-2-cyclopenten-1-one	536	0.06	0.19
furan and pyrant derivatives			
3-furanaldehyde	406	0.25	0.09
furfural	417	1.64	0.32
2-furanmethanol	438	1.15	1.12
1-(2-furanyl)-ethanone	507	0.76	0.63
5-methyl-2-furancarboxaldehyde	513	0.08	0.03
5-methyl-2-furancarboxaldehyde	516	0.10	0.07
5-methyl-2-furancarboxaldehyde	524	0.05	0.00
3-methyl-2(5H)-furanone	571	0.08	0.06
benzofuran	592	0.33	0.72
1-(2-furanyl)-1-propanone or 4-methyl-2-propyl-furan	606	0.11	0.04
methoxyphenol derivatives			
phenol	581	0.38	0.42
(guaiacol)	686	1.63	1.80
2-methoxy-6-me-phenol or 2-methoxy-3-me-phenol	778	0.09	0.11
2-methoxy-4-methyl-phenol (4-methylguaiacol)	790	0.61	0.72
4-ethyl-2-methoxy-phenol	879	0.11	0.13

Table 6: Key volatile compounds (PAR) identified by GC-MS during storage study at 10°C in samples from TBB, Norway

Compound	RI	day 4	day 7
acid			
acetic acid	191	0.20	0.17
alcohol			
ethanol	<173	0.75	0.74
1-penten-3-ol	250	0.79	0.39
2-methyl-1-propanol	218	0.17	0.09
3-methyl-1-butanol	305	0.18	0.09
2,2-dimethyl-1-hexanol	717	0.05	0.05
aldehyde			
3-methyl-butanal and -hexanone	264	0.00	0.24
hexanal	382	0.29	0.21
2,4-heptadienal	611	0.02	0.00
nonanal	707	0.18	0.19
decanal	808	0.06	0.06
ester			
ethyl acetate	200	0.11	0.09
ketone			
2-butanone	191	0.77	0.91
3-hydroxy-2-butanone	273	0.86	0.41
cyclo			
cyclopentanol or 2,3-pentanedinone	260	0.37	0.00
toluene or cyclopentanone	340	0.74	0.47
cyclopentanone	368	0.30	0.12
1-ethyl-cyclohexene	406	0.00	0.02
2-methyl-cyclopentanone	429	0.09	0.00
cyclohexanone	429	0.07	0.00
3-methyl-cyclopentanone	435	0.02	0.00
1,2,3-trimethyl-cyclopentane	484	0.04	0.00
2-methyl-2-cyclopenten-1-one	501	0.22	0.13
propyl-cyclohexane	529	0.05	0.04
3,4-dimethyl-2-cyclopenten-1-one	536	0.05	0.00
3,4-bis(methylene)-cyclopentanone)	555	0.02	0.00
furan and pyrant derivatives			
furfural	417	0.00	0.07
2-furanmethanol	438	0.25	0.10
1-(2-furanyl)-ethanone	507	0.16	0.08
methoxyphenol derivative			
2-methoxy-phenol	686	0.53	0.31
phenol	581	0.08	0.00
2-methoxy-4-methyl-phenol (4-methylguaiacol)	790	0.14	0.06
4-ethyl-2-methoxy-phenol	879	0.02	0.00

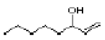
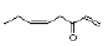
Table 7: Volatile compounds identified in smoked salmon by GC- O during storage at 4° C/ 5° C in samples from REYKO, Iceland and FIEDLER, Germany.

REYKO- 0 REYKO- 1 REYKO- 2 REYKO- 3 FO F2 F4

Compound no.	Possible compound	Odor description	rt (min)[a]	RI[b]	ID means[c]	REYKO-0 0 days	REYKO-1 14 days	REYKO-2 28 days	REYKO-3 42 days	FO 0 days	F2 14 days	F4 28 days
1		vanilla, karamel	35,-4,0	279-3003	2					4,5		4,0
2		sweet, caramel, flowery	4,4-5,2	315-340	2	1,0	2,3	2,5				
3		characteristic smoke odor solvent, geranium	5,3-5,5	344-350	2	3,0	4,0	3,5	3,0	4,0	3,0	3,0
4		bad, vomit	5,9-6,2	362-372	2	2,0	1,5	3,0	2,0			
5		sweet, strawberries, grass	5,9-6,2	362-372	2					3,8	4,0	4,0
6		undertone, salmon, flower, heavy, alcohol, solvent	6,6-7,3	384-406	2	2,0				3,0	3,0	3,0
7		flowery, sweet, alcohol	8,5-8,7	440-446	2		2,0	2,0		5,0	4,0	4,0
8		flowery, earthy, mushroom	8,9-9,2	451-460	2	2,0	0,8	1,8	2,3	2,5	3,5	3,5
9	hexanal	rancid	10,5-10,6	497-500	MS,1,2	2,5	2,0			5,0	5,0	5,0
10		characteristic salmon, boiled potato-like	10,8-11,0	505-509	2	4,5	4,3	4,5	4,3	4,5	5,0	5,0
11		smoked	11,0-11,4	509-519	2					3,0		
12		mushroom, boiled fish	13,1-13,4	558-565	2			1,5	2,0	2,0	2,5	2,5
13	1- octen-3-ol	mushroom, geranium	13,-14,1	577-581	1,2	2,5	3,5	3,3	2,3	3,5	4,0	4,0
14		smoke-like?	15,4-15,5	613-616	2	2,0	1,5					
15		sweet, fatty, lemmon	15,4-15,5	613-616	MS,1,2					5,0	5,0	5,0
16		caramel, sweet, mushroom	16,5-16,7	642-647	2			2,0		3,0	2,0	2,0
17		flowery, sweet, heavy	16,7-17,2	647-661	2	2,0	2,0			3,0	3,0	3,0
18		mushroom	17,17,5-17,7	668-674						2,5		
19		smoke-like	17,5	668	MS,1,2		2,0	2,5				
20	2-and 3-methly phenol	wood, burnt, ash, smoke	17,7-18,0	674-682	MS		3,5	2,5	3,5	4,0	5,0	5,0
21	guaiacol	smoke-house, ash, burnt, timber, sweet, phenol ?	18,1-18,6	684-697	Ms,1,2	3,8	3,8	4,5	5,0	5,0	5,0	5,0
22	2-mercarpto-phenol	sweet, esther?, fruity, ananas	20,0-21,1	739-742	2	2,0	2,8	3,5	2,5	3,0		
23		fresh, wood	20,2-20,5	745-754	2					2,5	2,8	2,8
24	2, 6-nonadienal	cucumber, rancid	20,7-20,9	760-766	1,2					4,3	5,0	5,0
25	4-methyl guaiacol	wood, smoke, sweet, heavy	21,5-21,6	785-788	MS,2	2,0	1,5	2,3	2,8	3,0		
26		smoked salmon, mushroom	22,0-22,4	800-816	2					4,3	3,0	3,0
27		mushroom, wood	226-22,9	824-835	2					4,0	2,5	2,5
28		burnt, smoke	22,8-22,9	820-930	2		1,5		2,0	4,0		
29		sweet, spice	24,9-25,1	<930	2					3,5	3,0	3,0
30		smoked salmoon	25,4-25,6	<930	2					2,8	2,0	2,0
31		sweet, cucumber	26,7-27,1	<930	2					3,0	2,5	2,5
32		sweet, smoke	27,3-27,5		2					2,5		

a Retention time (minutes)
b Caluclated ethyl ester retention index on DB- 5ms capillary column.
c Identification means: MS, mass spectra; 1, authentic standard; 2, odor identification
d Odor intensity from 0- 5, 0: not present; 5: very strong. Average scores of two assessors

Table 8: Example of compounds developed because of microbial growth or oxidation

Compound	Formula	Odour	Possible precursor	Odour threshold
3-methylbutanal		Malty	AA, leucine	0,06 ppm (a)
(Z)-4-heptenal		Rancid	FA	0,04ppb (b)
(E, Z)-2,6-nonadienal		Cucumber	PUFA (n-3)	0.001ppb (d)
(E, E)-2,4-decadienal		Fatty	PUFA (n-6)	ppb
1-octen-3-ol		Mushroom	PUFA (n-3)	10 ppb (e)
3-hydroxy-2-butanone		Buttery	FA	
1,5-octadien-3-one		Geranium	PUFA (n-3)	0,001ppb (f)

AA: amino acid; FA: fatty acid; PUFA: poly unsaturated fatty acid
(a) Sheldon *et al.* 1971; (b) McGill *et al.* 1974; (c) Guadagni *et al.* 1972); (d) Josephson 1991; (e) Pyysalo and Suihko, 1976; (f) Swoboda and Peers, 1977; (g) Buttery *et al.* 1976; (h) In: Baek og Cadwallader, 1997.

Table 9: Examples of smoke related compounds

Compound	Formula	Odour threshold	Odour description
Furfural		2 ppm	
Phenol			Pungent (a) Sweet, smoky (c)
2-methoxy-phenol (guaiacol)		3 ppb	Smokehouse (c) Sweet, smoky, somewhat pungent (a)
2-methoxy-4-methyl-phenol (4-methylguaiacol)			Sweet, smoky (a)
4-(2-propenyl)-2-methoxyphenol (eugenol)		6 ppb	Mild smoky (c)
2-methoxy-4- (1-propenyl)- phenol (4-vinylguaiacol)			smoky aroma (b)

(a) Rozum, J. 1998; (b) Buttery, R. G. 1981; (c) GC- O sensory panel at IFL

The results of the GC analysis show that the compounds contributing to the smoke related characteristics dominate the headspace of smoked salmon samples. The compounds contributing to spoilage characteristics are present in lower concentration in smoked salmon samples during storage. This is in agreement with sensory analysis showing high intensity of smoked salmon odour/flavour and lower intensity of the spoilage attributes. It is of concern that the smoke related compounds may mask the spoilage compounds in the e-nose analysis and as a result the e-nose may not be able to detect spoilage changes. Sampling at lower temperature (i.e. 5°C) may decrease the level of smoke related component and increase the level of more volatile spoilage compounds. On the other hand the e-nose may be suitable to characterize the volatile profile of the smoked fish products as a quality check for the smoking process.

3.2.3 Standard cocktail of selected compounds for training of the pattern recognition system and calibration of the developed E-Nose system

Based on the results of GC analysis key compounds to be used to test the performance of the electronic nose for monitoring smoked salmon quality were selected.

Key volatile compounds
The main classes and the key compounds present in the highest concentration in the headspace during storage of smoked salmon and contributing to the overall odour have been identified. The key volatile compounds contribute to smoked fish characteristics and spoilage characteristics because of microbial growth and lipid oxidation.

Standard cocktail
Choices of potential compounds suitable as standard have been identified. According to stability and reliability it was decided, not to use them as a cocktail mixture but single-wise. Besides, commercial liquid smoke was suggested to be used for Sensor Testing.
The selection criteria for the standard cocktail were based on:

- compounds detected in the highest concentration by GC-MS
- compounds having a high odour impact by GC-O
-

but also keeping in mind the origin of the compounds and the processes involved in their formation. Thus the cocktail will contain smoke related compounds for monitoring the process and spoilage related compounds.

Table 10: Key compounds identified in smoked salmon

	Spoilage related compounds
Alcohols	ethanol *
	3-methyl-1-butanol, 1-penten-3-ol
Aldehydes	3-methyl butanal, hexanal, 2,4-heptadienal
Ketones	2-butanone *
	3-hydroxy-2 butanone
Esters	ethyl acetate
	Smoke related compounds
Furan and pyran derivatives	furfural *
Methoxyphenol derivatives	2-methoxy phenol (guaiacol) *

* selected for the standard compound measurements

From these selection criteria it is recommended that the following compounds will be used for the standard cocktail:

- Furfural
- 2-methoxy phenol (guaiacol)
- Ethanol
- 2-butanone
- 3-hydroxy-2-butanone
- Hexanal

3.3 Microbial analyses

An overview of microbial analyses of total viable counts (TVC) from samples from the different smokehouses is shown in Figure 3. The fresh samples on day 0 had TVC numbers in the range from 1.5 to almost 4 in log cfu. This wide range is reflecting the individual variation of the samples, batch to batch variation and the hygienic conditions in the different smokehouses. The increase in TVC numbers with storage time for samples stored at 5°C for 28 days is obvious for all the samples except the TBB samples that had very low counts (> 3cfu/g) at the end of the study which may be explained by the high salt content of those samples (see Table 2). Different handling procedures of smoked products such as dry salting and brine injection influence the microbial spoilage (Hansen *et al,* 1996). The Fiedler samples had the highest microbial counts which may be caused by the handling, salting and the smoking process. In particular it is of interest that the smoking time is very short for the Fiedler samples (Table 1) which may influence the level of smoke related compounds like phenols in the products and their impact on the quality changes.

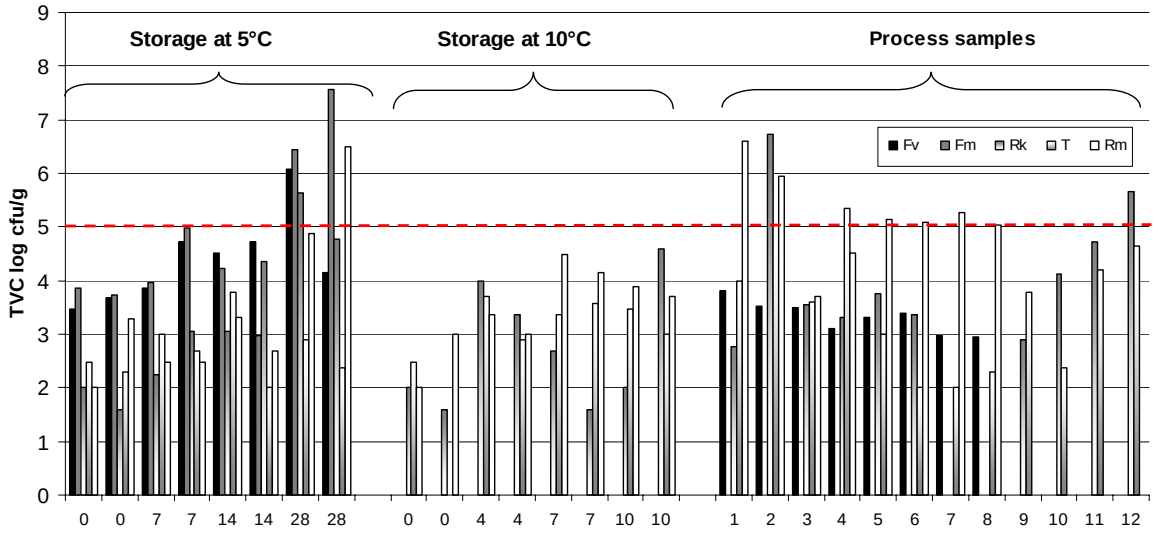


Figure 6: TVC (log cfu) for salmon samples stored at 5°C for 28 days, at 10°C for 10 days and selected samples from the process from all the smokehouses (labelled as Fv/Fm, Rk, Rm and T). Two replicate samples were analysed each day.

The microbial counts in samples stored at 10 °C for 10 days did not show as obvious increasing trend with storage time and only 3 samples exceeded 4 in log cfu. None of the samples exceeded log cfu = 6, the food safety limit for TVC, at the end of the study. This indicates that the end of the shelf life based on this criterion had not been reached at the end of the study and all the samples stored at 10 °C were still of acceptable microbiological quality despite the high storage temperature.
It had been decided that a quality criterion corresponding to a log cfu/g of 6 for total viable count should be applied to discriminate between accepted “good” and rejected “bad” samples, since this is the general microbiological safety guideline applied for food quality. However, in our study the majority of the samples were not spoiled based on the criteria of TVC of 10(6) cfu/g at the end of the study (Figure 3). Therefore, it was decided to use lower limits and discriminate between good samples and samples that are just starting to show spoilage signs but still of acceptable quality.
Only 6 samples, i.e. 6 % of the total sample set from different production batches from the respective smokehouses were of “bad” quality based on the log cfu = 6 criterion. Fifteen samples exceeded values above log cfu = 5 (16 %) and 33 samples (35 %) exceeded values above log cfu = 4. The end of shelf life according to microbial critera of log TVC = 6 was not reached unless for samples of FIEDLER stored for 28 days and two samples from the process. In fact according to the specification of the FIEDLER products this producer only indicates 2 weeks shelflife for the products.

The LAB counts appeared to increase with storage time at both storage temperatures (5 and 10 °C). The initial values in freshly smoked samples on day 0 were in the range < 1 to 2 log cfu and at the end of the study three of the samples exceeded log 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. In particular, for the MAP samples a dramatic rise in the LAB numbers were observed.

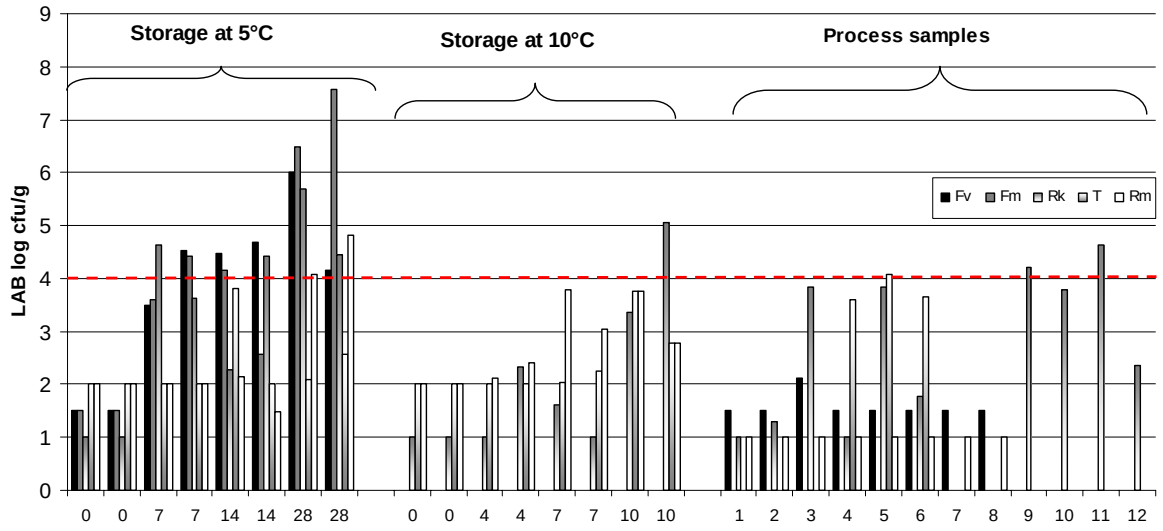


Figure 7: LAB results as log cfu/g for salmon samples stored at 5°C for 28 days, at 10°C for 10 days and selected samples from the process from all the smokehouses (labelled as Fv/Fm, Rk, Rm and T). Two replicate samples were analysed each day.

In some samples the counts of LAB exceeded the TVC value. It is expected that the LAB will grow on the modified Long and Hammer's medium (LH), but it may be speculated that an additional anaerobic flora may have grown on the LAB medium since 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.
LAB´s 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 odours. Therefore, a significantly high load of LAB will influence the headspace profile analysed with the FishNose sensor system and will be indicative of prolonged storage of products. A limit of log cfu/g = 4 was determined as the LAB criteria to distinguish between good and bad samples.

3.4 Sensory analysis

In total 96 samples were assessed by sensory analysis, thereof 70 for both odour and flavour 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.
The sensory analysis of the samples showed similar results as the microbial analysis indicating that quality changes of samples stored under these conditions were not obvious.
Statistical evaluation of the data using one way ANOVA showed that significant differences in the sensory attributes between storage days 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, oilyness, juiciness). Significant differences were found in odour and flavour attributes and colour intensity for some sample groups.

The descriptors used by the sensory panel for the odour and flavour attributes were the following: smoked salmon odour/flavour, metallic odour/flavour, sweet/sour fruity odour/flavour, rancid odour/flavour, off- odour/flavour. The scores of spoilage related attributes (sweet/sour and off-odour and flavour) showed a systematic increase with storage time for both the 5 and 10°C (Figures 5 and 6). However, significiant differences in sensory scores of samples between storage days for these attributes were only found for the Fiedler samples. The highest scores for spoilage related odour were observed for the Fiedler samples stored in MAP at 5 °C. This is in agreement with high microbial counts for these samples. Fresh unstored samples and samples stored at 5 °C for a week had sweet/sour scores less than 10, whereas the other stored samples showed scores from around 10 up to nearly 60. However, one fresh unstored sample from REMO obtained a high score, which was in agreement with a high TVC number.

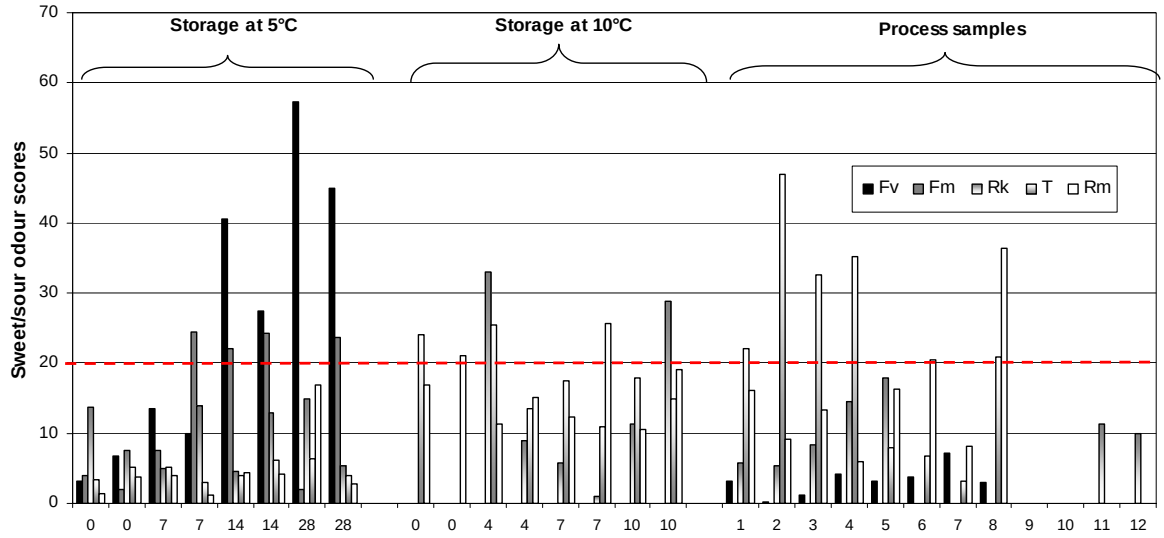


Figure 8: Sweet/sour odour scores for samples stored at 5°C and 10°C for 28 and 10 days, respectively, and samples from the process from the different smokehouses (labeled as Fv/Fm, Rk, Rm and T). Two replicate samples were analysed each day. Vertical line represents the quality criteria set at 20.

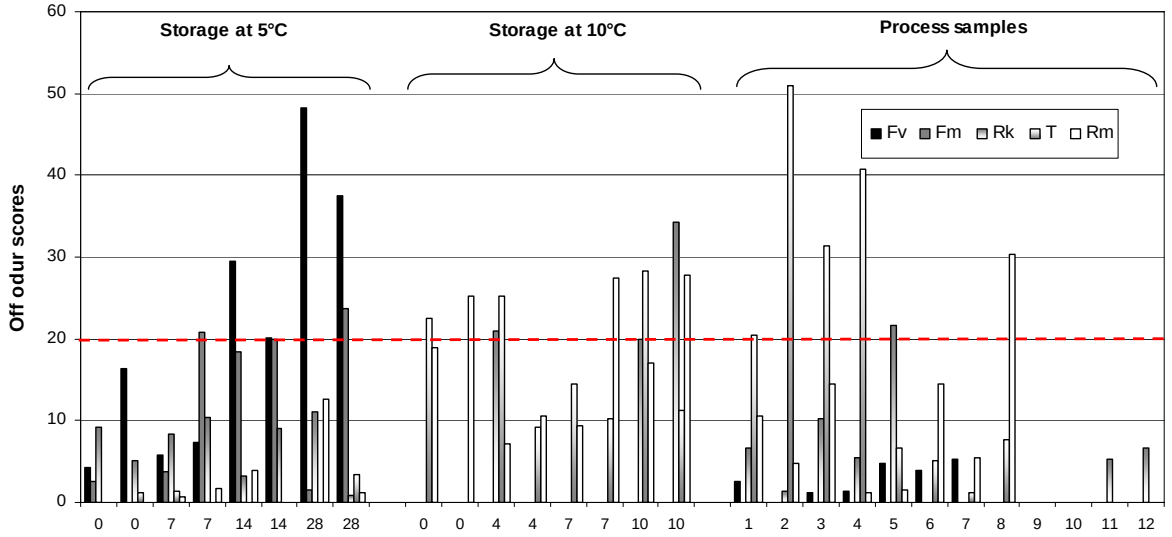


Figure 9: Off odour scores for samples stored at 5°C and 10°C for 28 and 10 days, respectively, and samples from the process from the different smokehouses (labeled as Fv/Fm, Rk, Rm and T). Two replicate samples were analysed each day. Vertical line represents the quality criteria set at 20.

A similar overall trend as for the sweet/sour scores could also be seen for the off-odour scores (Figure 6). 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 odours appears to be greater at the higher temperature. This is one of the reasons why the results of microbial counts may often be misleading (Gram and Huss, 1996; Hansen *et al.,* 1995; Leroi *et al.,* 1998), and no single quality criterion is adequate to explain the complex changes of spoilage and therefore multiple quality indicies have been suggested to assess the quality (Jörgensen, *et al.,* 2001).
The scores for the initial samples (0 days) from REMO and TBB and 4 days samples from REYKO for the sample groups stored at 10°C had already high scores for the spoilage attributes. Part of the samples from the process from TBB had high scores for the spoilage attributes that can be explained because the samples had been stored for 10 days before the delivery to MATFORSK.

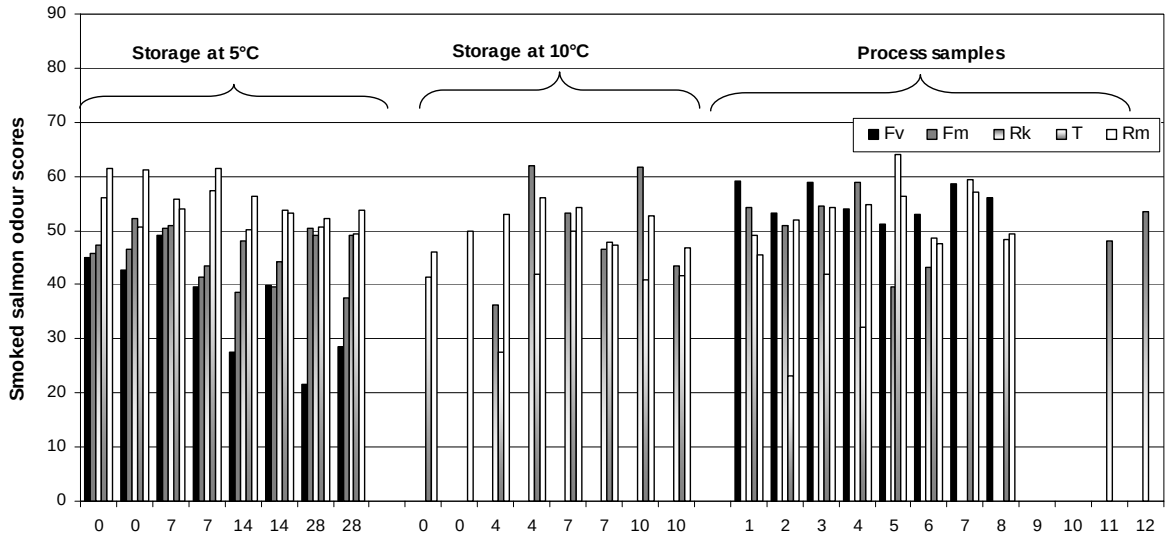


Figure 10: Smoked salmon odour scores for samples stored at 5°C and 10°C for 28 and 10 days, respectively, and samples from the process from the different smokehouses (labelled as Fv/Fm, Rk, Rm and T). Two replicate samples were analysed each day.

The smoked salmon odour showed a slight decreasing trend with storage time at 5°C in particular for the 5°C samples of REMO and the Fiedler samples (Figure 7). The trend is not as obvious for samples stored at 10°C. In addition it can be seen that there is a batch to batch variation of this attribute when comparing the fresh unstored process samples.
Different smoking techniques have great influence on the sensory characteristics of smoked salmon (Cardinal *et al.,* 1997). The short smoking time at FIEDLER´s may have influenced the lower smoking odor scores and higher spoilage rate of these samples.

3.5 Electronic FishNose development and construction

3.5.1 Development of the optimal gas sampler

OPTOTEK surveyed available gas sampling and concentration techniques. Several standards, articles, applications, methods and existing state-of-the-art of the gas sampling techniques have been surveyed. To ensure the compatibility at the hardware interfaces close the effort was coordinated with ALPHA and TTZ. Basic methods of gas sampling were evaluated: a gas syringe, a gas sampling valve, and a pneumatic device.
Gas sampling method with the 6 port sampling valve was chosen for the FishNose application.

Most important benefits of this method are:

- it requires small pressure differential between the process in and the process out,
- simple, safe and quick operation,
- low-cost.

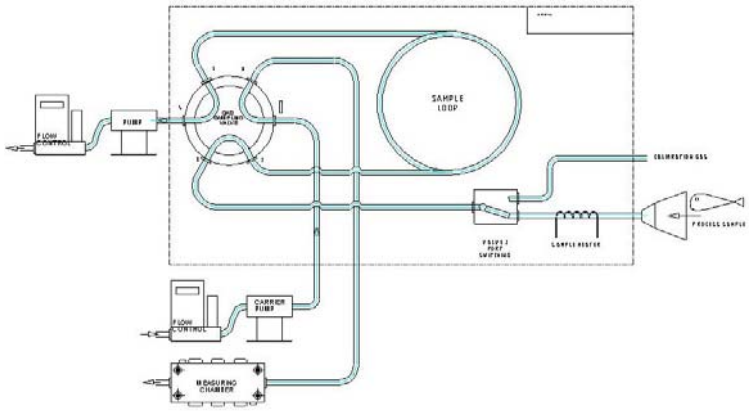


Figure 11: Design of the gas sampling unit and connections to the rest of FishNose

Having chosen the method, we designed the sampling unit. Drawings for measuring chamber, heater and other plumbing were made. Special components for the unit were purchased. After finishing the design, a model of the suggested sampling unit was assembled and basics of the method were tested on it.
A prototype of the optimized gas sampling unit was designed and produced. The design is characterized by the reliability of the components, their robustness, ease of procurement and adaptability when assembled into the sampling unit. The connection to the sensor unit were coordinated with ALPHA-MOS. A commercial Valco six-port valve was used for the injection of the sample into the sensor unit.

The prototype sampling unit consists of following main sub-units:

- VALCO 6 port valve with electronics box,
- transformer,
- Optotek driving electronics,
- Optotek control electronics ,
- main power supply ,
- heated inlet tube,
- accessories and documentation.

All components are mounted on a metal plate. Sample inlets and pump connectors are on the top side of the plate, inlet / output are fed through the plate down toward the ALHA-MOS sensors. Electrical connections are 230VAC and signal cable to the ALHA-MOS computer. All tubes and fittings are made of seamless stainless steel type 316. All connectors are original from Valco.

1. **VALCO 6 port valve with electronics box (Figures 4 and 5) –** original electronics has been modified. Electronics drives the motor of the valve and gives the signal of the current valve position. Electronics has two inputs: 115VAC/50Hz and +12VDC. For the final version (serial production) main voltage will be 230VAC only, without a need of a +12VDC. Flow inlets of the box are: sample inlet, calibration inlet, pump inlet, column outlet, carrier inlet.
   Two different sample loops were supplied with the unit: 10ml and 20ml.These two can be exchanged at will. Other loops could be used as well. Valco, the producer of the loops used, offers loops from 10 µl to 20 ml. As long as the fittings are standard one could use any loop volume, constrained only by the dimensions of the valve box.
   The valve itself is installed inside the heat insulated plastic enclosure, its electronics is located outside in the original metal enclosure.
2. **Transformer (Figure 5) –** transforms main voltage 230VAC to 115VAC needed for operation of the Valco box. For the final version (serial production) this transformer will not be needed.
3. **Optotek driving electronics (Figures 5 and 6)** is connected to Valco box via 6 pin. Electronics optical isolate control signals to / from the computer. It also drives the **2 port valve** used for switching the inlet from the sample to the calibration input. It also contains a **heater**, used to stabilize the temperature of the box, containing all valves and flow components to 55°C.
4. **Optotek control electronics –** is used for testing the system. It will be replaced by the ALHA-MOS computer.
5. **Main power supply (Figure 5) –** 230VAC to +12V switching power supply of 110W is used. It supplies all the electronics of the device.
6. **Heated inlet tube (Figures 8, 9 and 10) –** 230VAC/40W self regulating heater cable is twisted around the inlet tube. It heats the tube to 55°C. All is isolated with an Armaflex tube.
7. **Accessories and documentation –** extra stainless tubes, connectors and fittings are supplied, to allow ALHA-MOS to connect the sampling unit to their equipment. Technical documentation is supplied together with the prototype to provide technical information for installation and use.

The gas sampler was subjected to a number of tests. We tested the 6 port valve (switching between different positions, seals) and sampling with the device heated to 55 degrees C. We used two pumps to perform these tests. We also tested the 2 port valve for the switching between the sample and calibration gas. We tested the heating of the input tube and it also reached the 55 C, all in a lab at room temperature. With these tests the operation of electronics (valve position sensors, drivers) was tested as well.

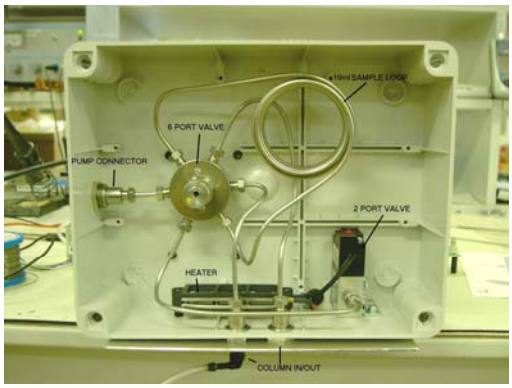


Fig. 12: Valve enclosure

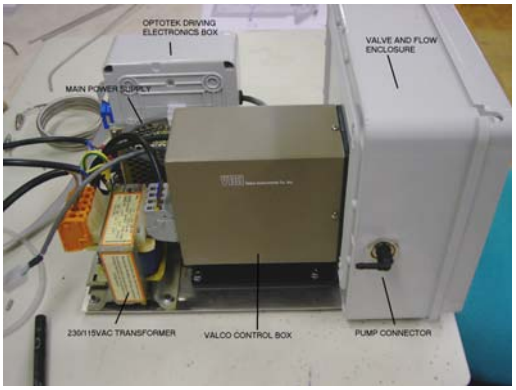


Fig. 13: Right side view

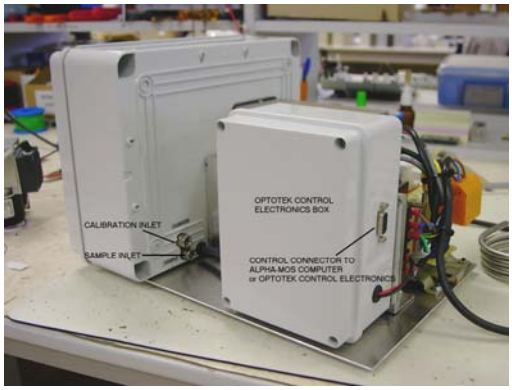


Fig. 14: Left side view

***Erratum**: "Optotek Control Electronics box" should be "Optotek Driving Electronics box"*

Fig. 15: 20ml sample loop installed

Fig. 16: Bottom view

Fig. 17: Heated inlet tube

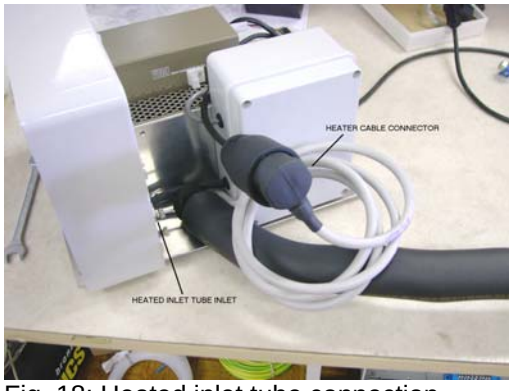


Fig. 18: Heated inlet tube connection

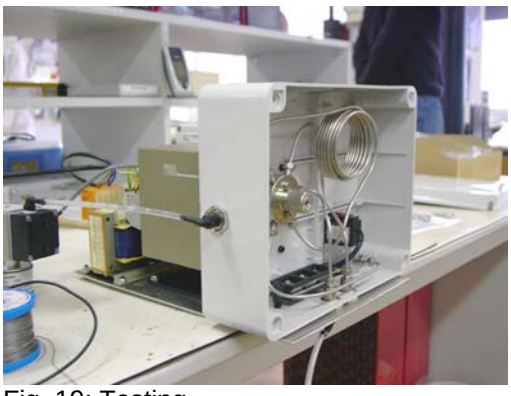


Fig. 19: Testing

3.5.2 Manufactured sensor array prototype

For large scale and industrial application a key criteria is sensor robustness and cost. Therefore Metal Oxide sensor technology was selected for the intended application as sensors have long lifetime (> 5 years) and sensitivity/selectivity can be tuned by changing sensing materials and dopants and by modifying operating conditions.
In order to test a high number of sensors, it was decided to use a laboratory instrument comprises of FOX system and headspace autosampler HS100. This system is the Research & Development platform and incorporates up to 18 different sensors. Sensor diversity is achieved through different materials (SnO2, WO3, Cr2-x-TiO3 + y…), different level of dopants (Pd, Pt) and different operating temperatures. Temperature modulation changes sensor selectivity. Depending upon target molecules, appropriate choice of operating conditions is achieved.

3.5.2.1 Phase 1: Sensor selection

38 samples provided by End-users partners have been qualified by reference analysis during storage trials by RTD Partners (GC/MS, physical-chemical parameters, microbiology, sensory panel). For sensor selection all samples have been analysed by the FOX instrument. The aim was to test several sensor materials and operating conditions so as to select the appropriate array.

Figure 20: FOX instrument – R&D platform for application validation and sensor selection

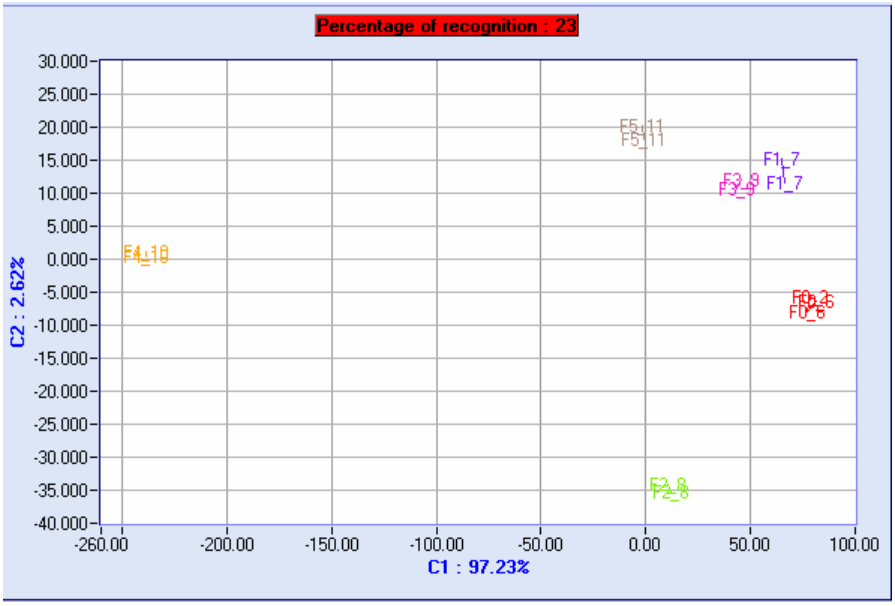
Used analysis parameters are listed in following table. The sensor responses are presented in the next figures.

Table 11: Parameters for sensor selection (FOX)

Carrier Gaz		**Injection**	
Carrier Gaz	Air TOC – Dry air	Injected volume	500 µl
Flow	150 ml/mn	Injection speed	500 µl/sec.
		Syringe	1 ml
Headspace generation		Syringe temperature	85 °C
Time	600 sec.	Flushing	120 sec
Temperature	80°C		
Agitation speed	500 rpm		
		Acquisition	
Sample preparation		Time	120 sec.
Sample volume	1 ml	Flushing	0.5 sec.
Vial	10 ml	Delay	1500 sec.

Sensor selection is based on optimal discrimination between samples without considering for the time being optimal correlation with quality criteria. Results shows sensor sensitivities to different samples and a Discriminant Function Analysis is also displayed for each samples group (Bad samples as regard to TVC criteria are labelled in Red).

Samples F0-F5 from TTZ:
Sensors selected : LY2/LG, LY2/AA, LY2/Gh and PA2



Results from the electronic nose (F0-F5)

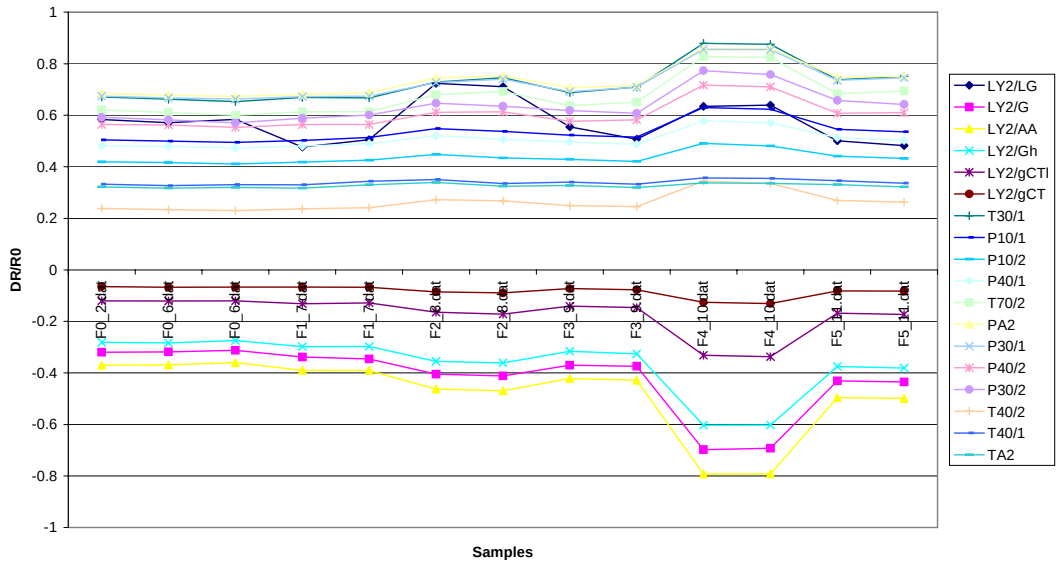
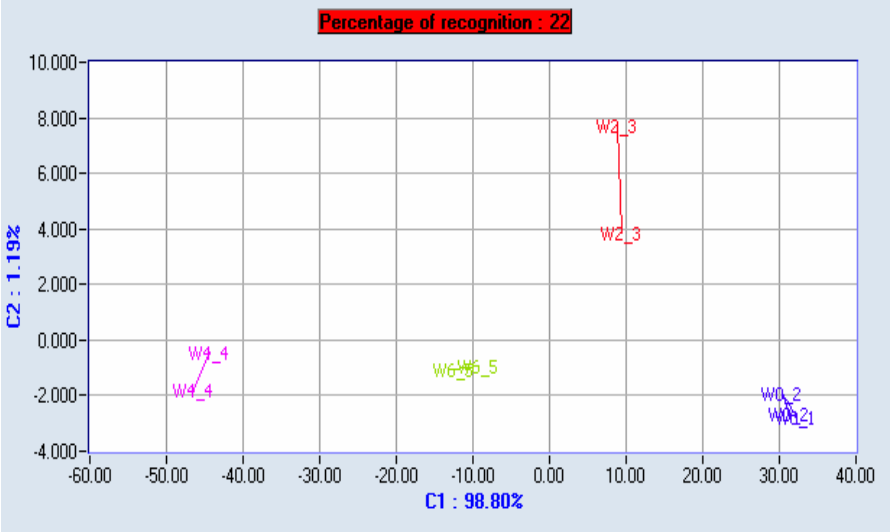


Figure 21 a/b: Sensor response according to FIEDLER / TTZ samples

Samples W0-W6 from IFL:
Sensors selected : LY2/LG, LY2/G and PA2



Results from the electronic nose (W0-W6)

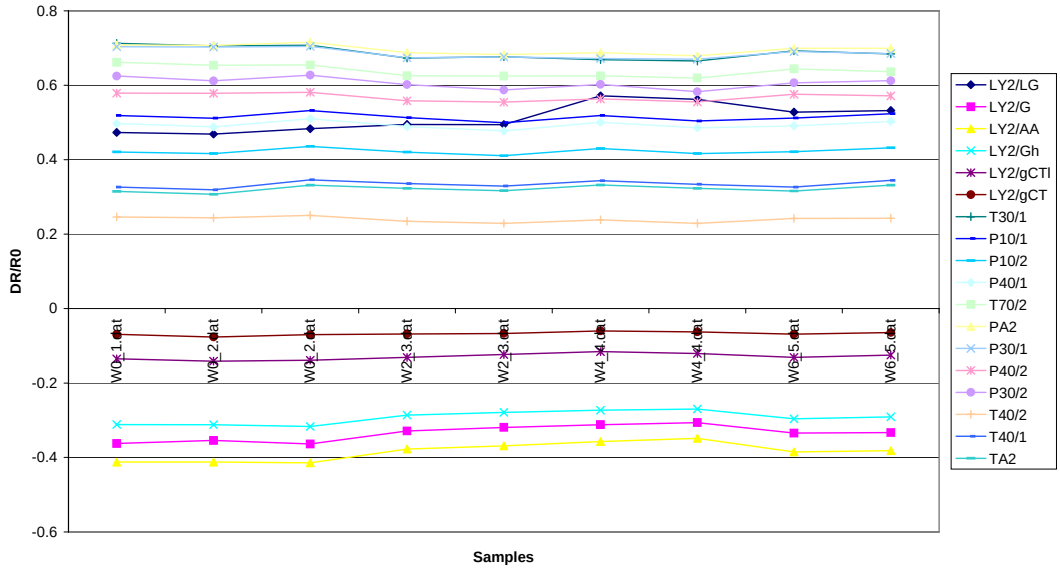
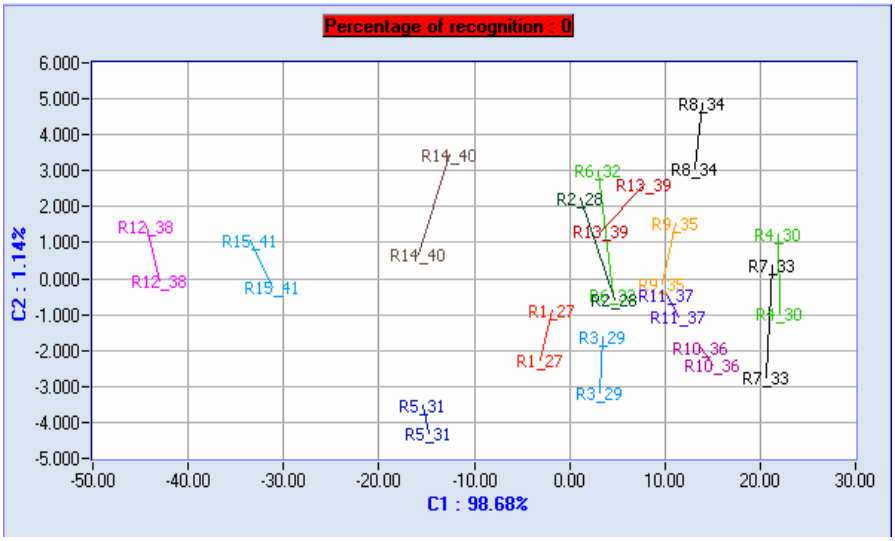


Figure 22 a/b: Sensor response according to REYKO /IFL samples

Samples R1 to R15 from MATFORSK
Sensors selected : LY2/G and P40/1



Results from the electronic nose (R1- R15)

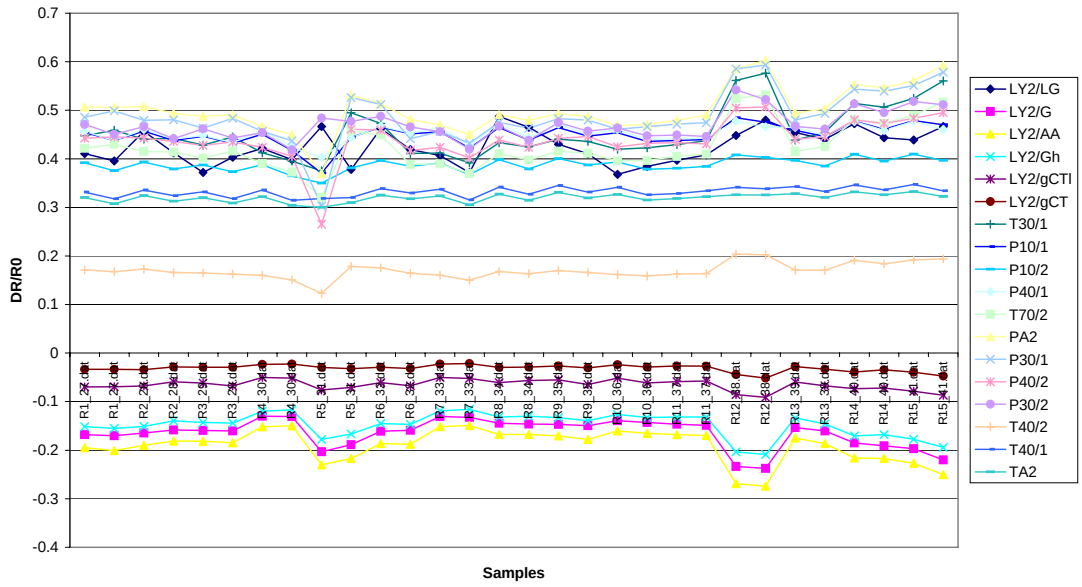
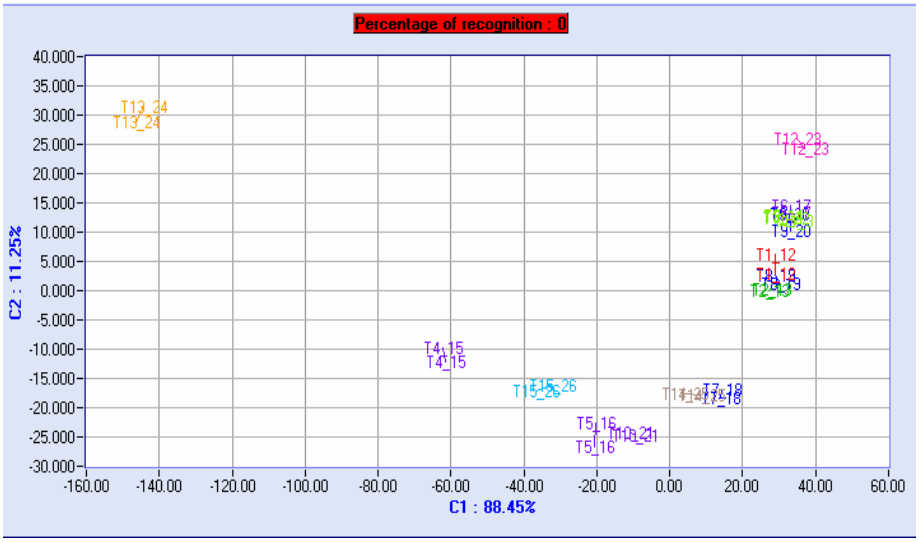


Figure 23 a/b: Sensor response according to REMO / MATFORSK samples

Samples : T1 to T15 from MATFORSK
Sensors selected : LY2/Gh, LY2/gCTI, LY2/gCT and PA2



Results from the electronic nose (T1-T15)

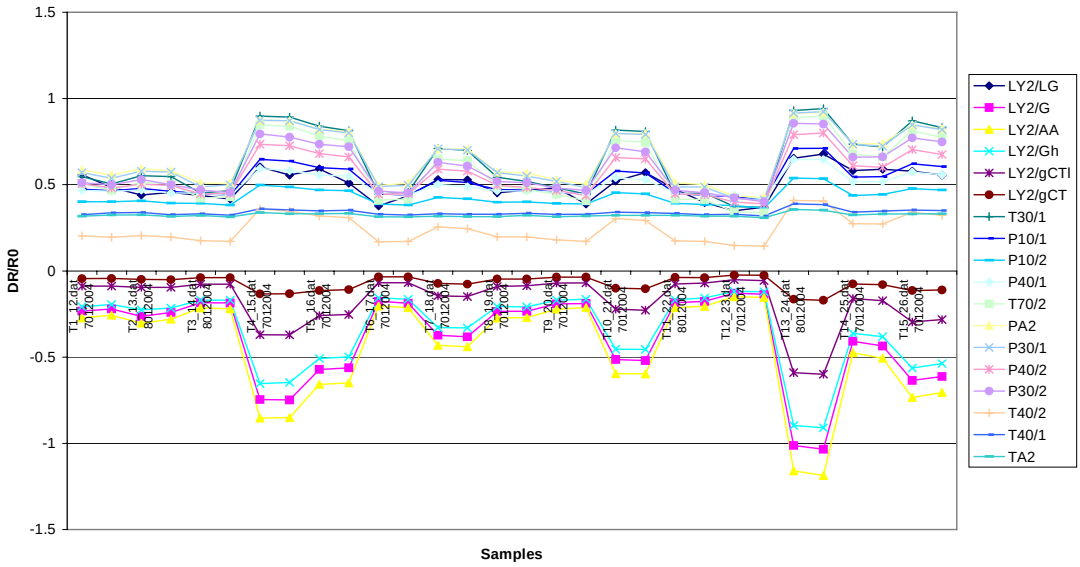


Figure 24 a/b: Sensor response according to TBB / MATFORSK samples

From those results and taking into account above described criteria, best sensor combination of 6 mixed metal oxide sensors was selected:

- Criterias: Sensor diversity, Discrimination, Return to base line.
- Selection of three Different Metal Oxide materials
  SnO2 (P/T sensors), WO3 (LY2/LG), Cr2-x-TiO3+y (LY2/G)
- Discrimination criteria : F samples (TVC=-1, TVC= +1)
- Selection: **P10/1, P40/1, P40/2, PA2, LY2/LG, LY2/G**

3.5.2.2 Phase 2: Design of specific sensor array module

A more compact design was then developed to host the new defined array. The electronic module is composed by the following PCB board:

- Conditioning board: Sensor polarisation, multiplex, Temperature controller
- Acquisition board: Programmable Gain Amplifier, ADC converter
- Signal processing board: Pre-processing, control/command, RS232 transmission
- Power board: Sensor heating, Power supply

A picture of the developed sensor module is shown in the figure below.

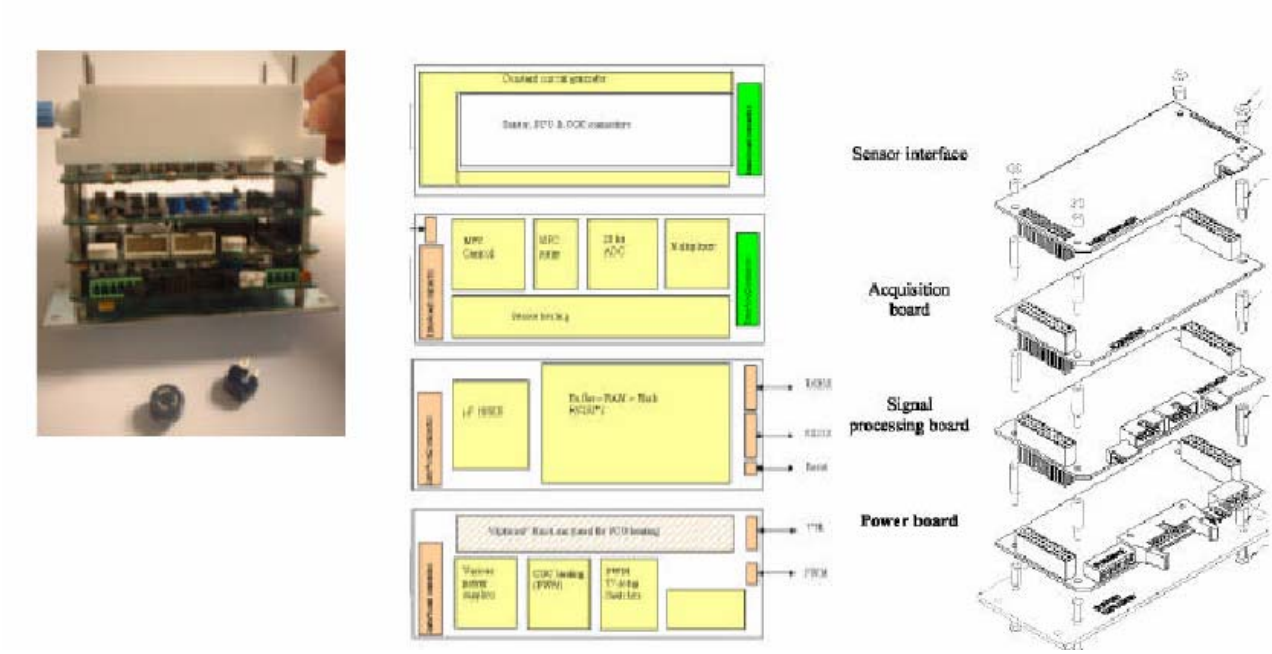


Figure 25: Synopsis of the electronic module and final sensor array sub-system

3.5.2.3 Phase 3: System integration and validation

The sub-system was integrated onto a specific hardware housing for further testing. In order to simplify data representation and results exploitation, it was decided to use one-class modelling. This model is suitable for Statistical Quality Control chart (SQC).
The aim was to monitor product variations. Supervised, this method defines a region/bandwidth of acceptable natural variations based on variability of reference good samples. All other samples are simply mapped.
Results from FOX system were compared with new results using the ultimate sensor array. F samples are taken as comparison.

Figure 26: Modified configuration for new sensor array validation

Results obtained with this industrial configuration were then directly comparable demonstrating proper sensor selection.

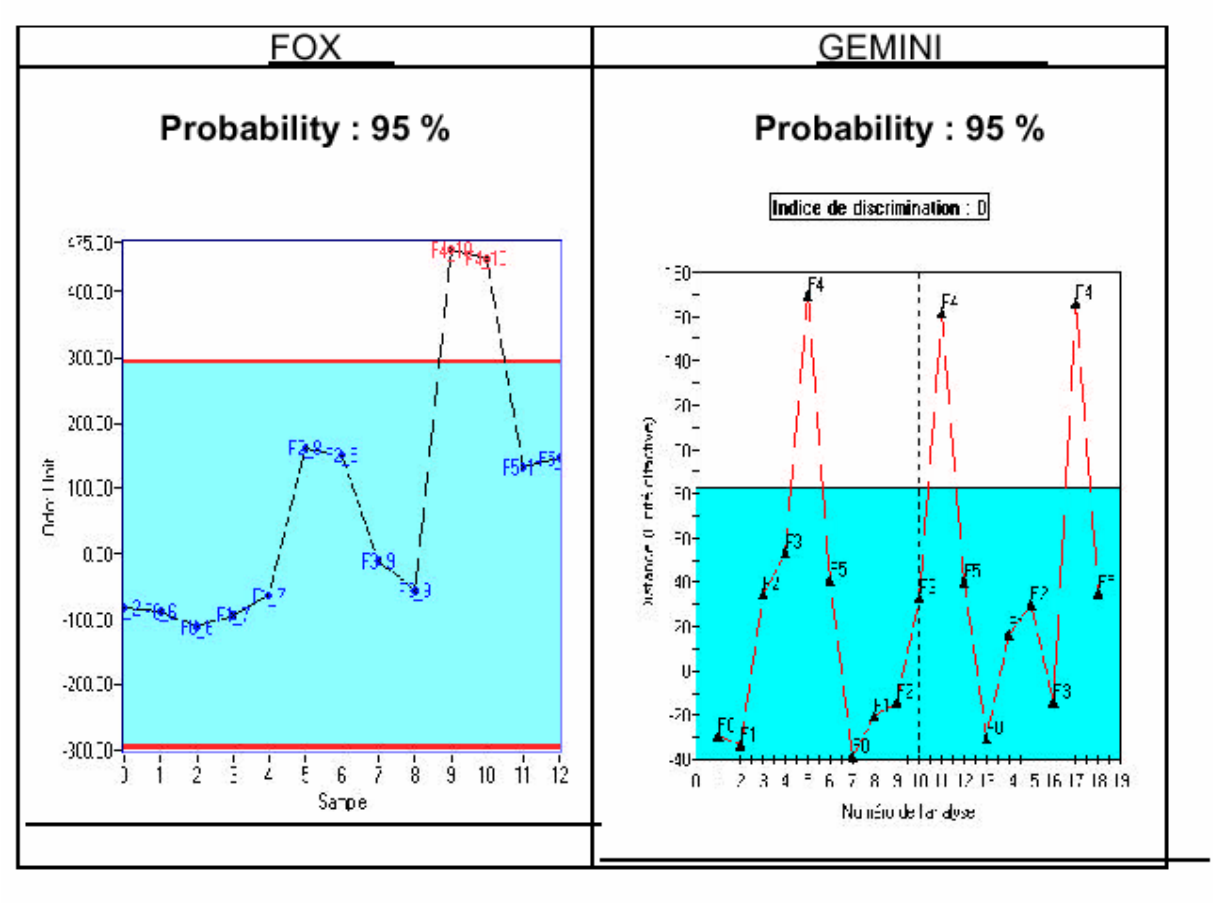


Figure 27 : SQC representation for both FOX and industrial system

3.5.2.4 Phase 4: Tests with real life incubation temperature

In order to get closer to process conditions (cold sample), it was decided to lower down sample temperature to 15 °C and 5 °C in order to assess the impact on discrimination. Decrease of incubation temperature was achieved using Peltier cooled tray. We notice that increase in sample volume injection was necessary but discrimination between good (F0,F1,F2,F3 and F5) and bad samples was maintained even do we notice a reduction in class distances.

The new analytical conditions are then described as follows :

Table 12: Parameters for sensor selection

	GEMINI
Carrier Gaz	
Carrier Gaz	Air TOC – Dry air
Flow	150 ml/mn
Headspace generation	
Time	0 sec
Temperature	The temperatures of the samples are set by cooling the tray at 5 or 15 °C.
Agitation speed	
Sample preparation	
Sample volume	1 ml
Vial	10 ml
Injection	2000 µl
Injected volume	500 µl/sec.
Injection speed	2.5 ml
Syringe	40 °C
Syringe temperature	
Acquisition	
Time	120 sec
Delay	600 sec

NB : In order to have a correct sensor response, the sample volume had been increased to 2 ml (optimisation of the volume with the F0 sample at temperature 15 °C).

Results for the three different incubation temperatures are shown below. Discrimination is maintained even do distances are decreased. From those results, it was decided to proceed with prototype validation and incubation temperature will be maintained at 5 °C so as to approach sample condition is the soking process. Samples are cold (5 °C) when entering slicing machine and packaging section.

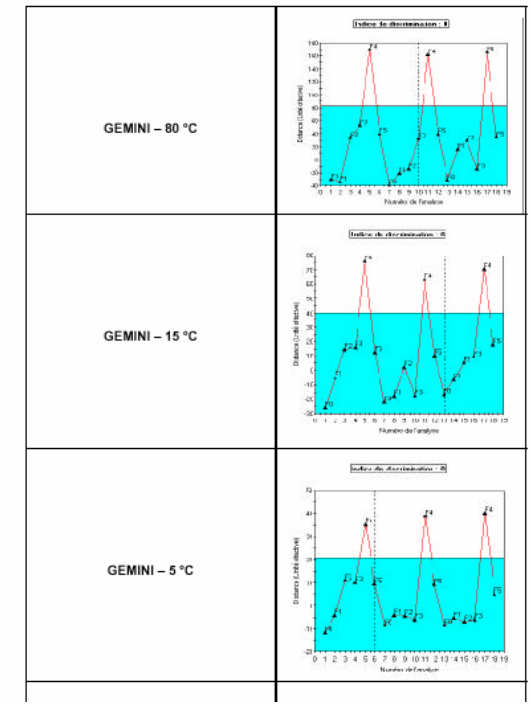


Figure 28: SQC representation for GEMINI system at different temperatures

First testing of the established Sensor Unit showed same discriminant response and identification of good/bad smoked fish quality as the previous used and more complex FOX system. Also reduction of sampling temperature until 5°C was successful: with little loss of sensitivity same bad samples could be identified.

3.5.3 Pattern recognition and compensation software

The objective with the data analysis was to establish correlation between the gas-sensors and the reference methods listed above. This main issues are;

1. Investigate correlation between gas-sensors and reference methods and find the significant correlations.
2. To establish criteria for classification of the fish samples.
3. Conduct data analysis with different methods to investigate to what extent the gas-sensors classify the samples correctly.

The most critical issue is the validation of the outcomes of the data analysis, with an objective of both high selectivity and specificity. Selectivity in this context means the percentage of cases where “Good” samples are classified as “Good”. Specificity is the percentage of “Bad” samples being classified as “Bad”.

Sensory data were acquired at different labs (i.e. with different trained panels) and numerical scale. For that reason, it was difficult to analyse all sensory data together and also to combine them in the correlation analysis to the gas-sensor signals.

Correlation analysis
Various models between microbial, chemical, sensory, GC and gas-sensors were computed. Table 12 shows the correlation between selected sensory variables and GC compounds, TVC and gas-sensor signals for the 24 MATFORSK samples. As expected, smoke odour has a significant correlation to most gas-sensors. Thus, this has a potential to monitor the quality of the smoking process. It is worth to noice that 3-hydroxy-2-butanone and ethanol have significant correlations to smoke odour as well as rancid and off-odour. These compounds are known to develop when there is microbial growth.
Table 13 shows correlations between gas-sensors, TVC and selected compounds identified in the GC analysis.

Classification with SIMCA
The procedure for this analysis was to first establish a model on the samples classified as “Good”. It was decided that 4 components from the PCA described the 18 gas-sensor signals.
Thereafter all samples were classified. The SIMCA method gave a total of 5 misclassified samples: 2 “Good” samples were erroneously classified as “Bad” (selectivity of 91%), and three “Bad” ones as “Good” (specificity of 77%). A closer examination of these three revealed that both the LAB count and off-odour values were lower than other “Bad” samples.
Based on the 6 sensors selected in the discriminant regression (see below), the same procedure was described above was followed for these sensors. This gave 6 wrongly classified samples; all of them were “Bad” samples classified as “Good”.

Support Vector Machines
SVM was run with various parameter settings for kernel type, and both 2-class and one-class appraches were investigated. However, the results gave a nigh number of misclassified samples compared to SIMCA and discriminant regression.

Discriminant regression
The results of this analysis showed a total of 6 misclassified samples; all of them being “Bad” samples classified as “Good”. A best combination search with 6 variables gave the same classification as using all 18 sensors. The 6 sensors selected were LY2/G, LY2/Gh, P10/2, PA2, P30/2, and TA2. However, there are many combinations of 6 sensors that give more or less the same result. The GEMINI system can handle 2 sensors of type LY and 4 of type P or T, and the sensors selected with the best combination search procedure fulfils this requirement.

Logistic regression

The cross-validated logistic regression gave a total of 7 misclassified samples, of which 6 were "Bad" samples classified as "Good". The regression coefficient for each of the 18 sensors is given in the figure below.

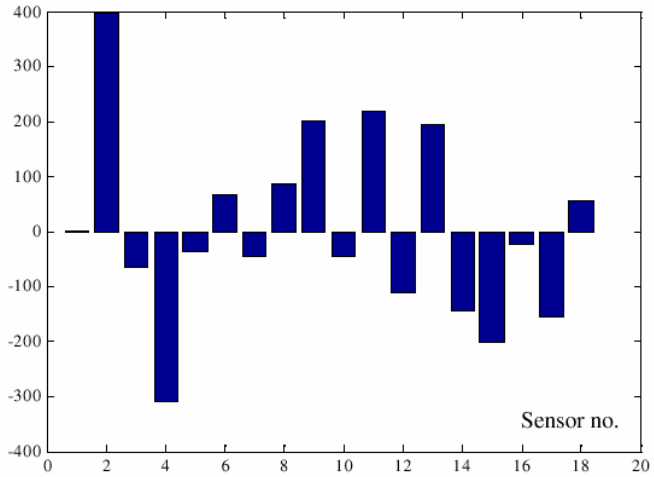


Figure 29: Coefficient in the logistic regression model.

The results show that there is correlation between sensory data and selected GC compounds, TVC and gas-sensors. Even though the individual correlations are not very high, a classification model using the gas-sensors give only 5 misclassifications out of 34 samples measured in this study. One of the major issues in the next phase of the project will be generation of large sample sets and to validated the existing findings. Also important is the choice of reference method(s) for classification. One might also foresee a system with three different quality classes.

Table 13: Correlations between selected sensory attributes, chemical compounds, TVC and gas-sensors for 24 samples. A correlation above 0.35 is significant at 95%.

	Smoke Odour	**Sweet/sour Odour**	**Rancid Odour**	**Off Odour**
Acetic acid ethyl ester	0.14	-0.30	0.16	0.11
2-Butanone	0.26	-0.22	0.03	0.07
Butanal, 3-methyl-	0.23	-0.03	-0.01	-0.10
Ethanol	-0.68	-0.30	0.44	0.48
Hexanal	0.57	-0.15	-0.08	-0.14
2-Butanone, 3-hydroxy	0.56	0.42	-0.37	-0.38
Nonanal/Tetradecane	0.37	-0.34	0.21	0.04
TVC (ex LAB) log	-0.65	-0.26	0.32	**0.37**
LY2/LG	-0.47	0.09	0.13	0.22
LY2/G	0.45	-0.03	-0.13	-0.18
LY2/AA	0.46	-0.03	-0.13	-0.18
LY2/Gh	0.45	-0.03	-0.13	-0.18
LY2/gCTl	0.40	-0.07	-0.09	-0.15
LY2/gCT	0.49	0.00	-0.16	-0.21
T30/1	-0.48	-0.02	0.17	0.20
P10/1	-0.41	0.04	0.10	0.15
P10/2	-0.41	0.05	0.10	0.15
P40/1	-0.37	0.05	0.08	0.13
T70/2	-0.46	-0.01	0.14	0.18
PA2	-0.49	-0.02	0.17	0.20
P30/1	-0.49	-0.03	0.17	0.20
P40/2	-0.47	0.00	0.15	0.19
P30/2	-0.46	0.00	0.14	0.18
T40/2	-0.47	0.01	0.14	0.19
T40/1	-0.16	0.13	-0.03	0.05
TA2	-0.11	0.16	-0.06	0.04

Table 14: Correlations between selected compounds from GC analysis, TVC and gas- sensors. A correlation above 0.35 is significant at 95%.

	TVC(e x LAB) log	Acetic acid, ethyl ester	2-Butanone	Butanal, 3-methyl	Ethanol	Hexanal	2-Butanone 3-hydroxy	Nonanal/ Tetradecane
TVC (ex LAB) log	1,0	-0,30	-0,08	-0,09	0,56	-0,51	-0,72	-0,38
LY2/ LG	0,32	-0,24	-0,41	-0,13	0,41	-0,37	-0,18	-0,32
LY2/G	-0,29	0,17	0,42	0,20	-0,39	0,37	0,33	0,26
LY2/AA	-0,29	0,17	0,42	0,20	-0,39	0,37	0,33	0,26
LY2/Gh	-0,29	0,17	0,42	0,20	-0,39	0,37	0,33	0,26
LY2/gCTI	-0,24	0,16	0,40	0,22	-0,3	0,34	0,31	0,24
LY2/gCT	-0,32	0,15	0,41	0,19	-0,42	0,40	0,35	0,27
T30/1	0,33	-0,16	-0,40	-0,10	0,40	-0,37	-0,32	-0,24
P10/1	0,30	-0,18	-0,41	-0,14	0,35	-0,32	-0,32	-0,21
P10/2	0,31	-0,19	-0,40	-0,16	0,38	-0,36	-0,32	-0,24
P40/1	0,29	-0,20	-0,40	-0,14	0,32	-0,30	-0,30	-0,19
T70/2	0,31	-0,16	-0,40	-0,10	0,37	-0,35	-0,31	-0,23
PA2	0,33	-0,16	-0,41	-0,12	0,41	-0,37	-0,31	-0,25
P30/1	0,33	-0,16	-0,40	-0,11	0,40	-0,37	-0,32	-0,24
P40/2	0,32	-0,17	-0,41	-0,13	0,40	-0,37	-0,32	-0,25
P30/2	0,33	-0,17	-0,41	-0,11	0,38	-0,35	-0,33	-0,22
T40/2	0,31	-0,17	-0,42	-0,15	0,39	-0,37	-0,32	-0,25
T40/1	0,31	-0,24	-0,32	-0,08	0,26	-0,29	-0,27	-0,19
TA2	0,29	-0,20	-0,25	-0,05	0,26	-0,29	-0,20	-0,22

3.5.4 Design of a user-friendly interface and control software

The developed software determines the degree of automation and simplicity versus variability of the system. It is expected that SME end-users who process only a moderate volume of fish or carry out quality monitoring for incoming inspection will prefer simplicity in handling and design, e.g. with three fixed measuring procedures installed. However, end-users with a wide variety of products (e.g. trout, salmon, eel etc.) and skilled personnel are expected to prefer a variable instrument with the option to adapt it individually according to their changing demands. The software developed for the FishNose sensor system is based on the existing Alpha MOS electronic nose software platform, GEMINI. The software uses VB and VC++ for programming and data are saved in ACCESS database.
Calibration/training data from the respective reference measurements defined in the standard analysis laboratory program represented the fish quality database, relating the quality calibration model to the gas-sensor output signal. A specific algorithm was developed which combines maximum information content with a user-friendly format. The developed FishNose is a self-training system, meaning that the software is not only processing the data of the current sample tested but also to compare it against data obtained by analytical reference methods through multivariate calibration models. The preferred format for the graphic user interface is Windows 9X/NT, the export of original data into a common Windows application, such as EXCEL, is supported.
One to four measuring procedures for the most common smoked fish products (e.g. salmon) are programmed as standard procedures. As an option, the software should be easily adaptable to individual measuring procedures by the end-users themselves.

Standard data format

- The raw data from the reference analyses is organised in a common Windows Excel Format.
- The calibration/training data matrix is organised in the following way:
  Each row in the data matrix represent respectively results from the reference data and gas-sensor measurements for one sample. Results from all the measured samples thereby make up a data matrix were each set of measurement results represent different blocks or category of measurement variable:
  Sample - Sample code – Measurement data (Reference methods, FishNose)

Application library

All applications are stored in a library with easy access to all necessary information about the instrument set-up, measurement conditions for the samples, and other relevant parameters.

Below are excerpts of the specifications for the user-interface:

The Gemini software has a built-in feature that specifies the ACCESS LEVEL for all users. There are three levels in the current implementation:

- Installer
  This is the level for certified personnel with access to both configure both hardware parameters and software parameters for different applications and analysis.
- Developer
  The developer has access to all software-related tasks such as training a new application, add or remove samples for training classes and tune parameters for optimal settings of the classification algorithms.
- Operator
  The operator can perform analysis of new sequence of samples. The operator can also start autotest and diagnostic test which are required at this level in order to ensure safe operation of the sensor system. It is assumed that the most users of the software in the fish industry will have access at this level.

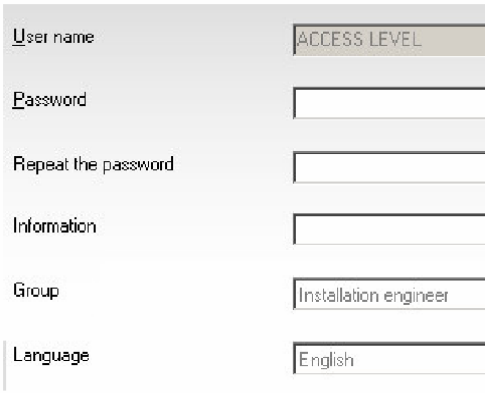


Figure 30: Dialog-window for set-up of users and their access level

Training of classification criteria

Although there will be a goal to make a global pattern recognition model for all kinds of samples, it is required of the software that the different SME's can build their own models for quality control. This is due to both known and unknown variations in the fish samples such as smoking procedure, origin and seasonal changes. The software has an interactive interface to assign measured samples to Good/Bad. This can be done based on the gas-sensor signals themselves and/or other quality criteria such as sensory data from the existing internal quality control in the companies. An example of the user-dialog is given in Figure below.

Figure 31: Samples can be removed and added to the training set

Sensor selection

Depending on the application, the sensors might have different ability to perform correct classification. There exist an ensemble of methods to select the best combination of a set of sensors to minimise the risk of false classification. The software has a feature that allows the user to either manually or automatically find the best set of sensors for the actual application (Figure below).

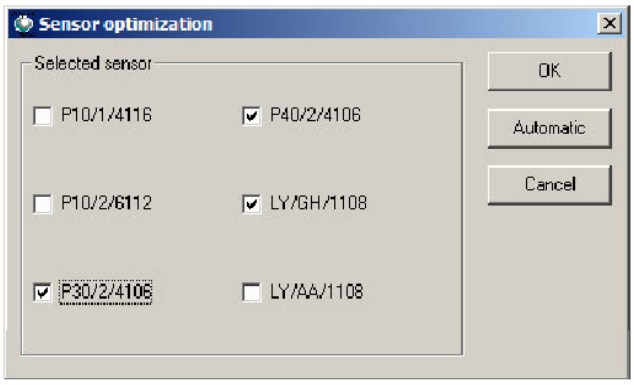


Figure 32: Example of user-dialog for sensor selection

Statistical quality control chart (SQC)

The results from analysis of samples are visualised in a SPC chart with critical limits shown as estimated in the training procedure for the current application. An example of a report-sheet is shown in Figure 33.

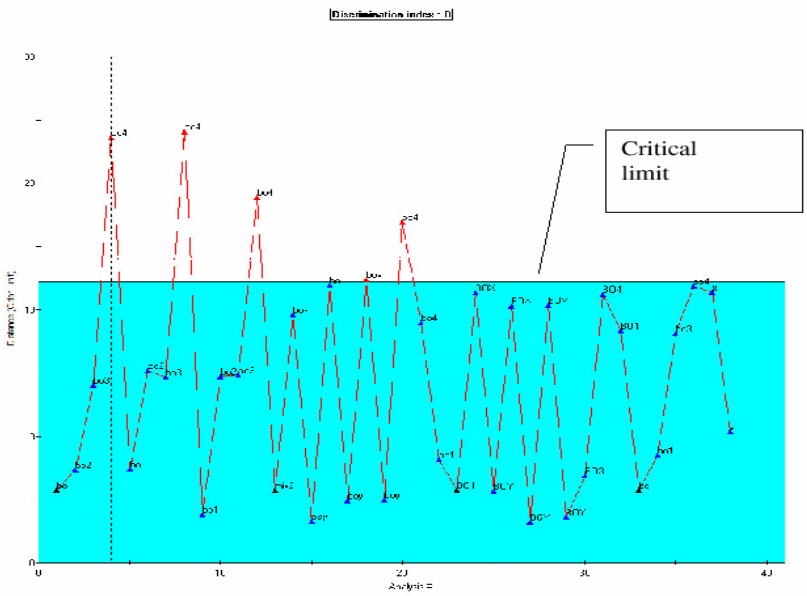


Figure 33: Example of classification results from the software. Accepted samples lie under the critical limit for distance to the model

3.5.4.1 Analysis reports

When a new set of samples are measured, an analysis report is generated automatically, as shown in Figure 34.

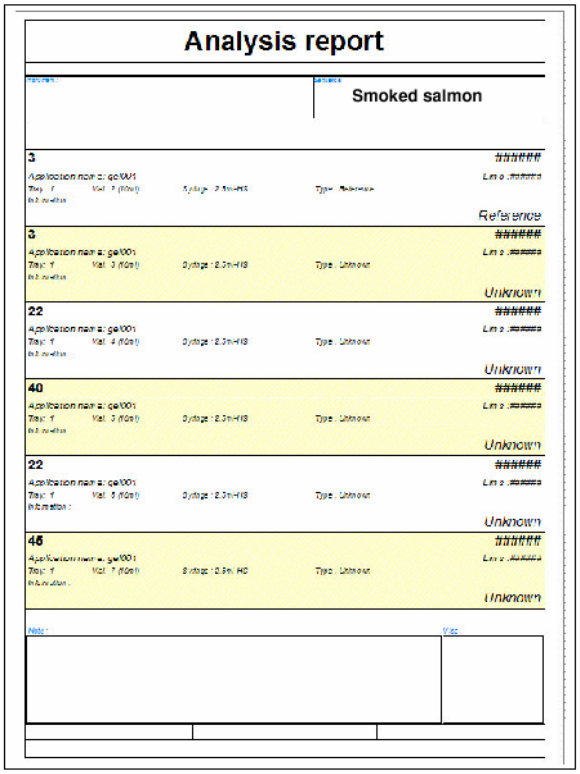


Figure 34: Analysis report from classification of new samples

Other relevant manu options and dialogs include autotest of the sensor system and diagnostic results.

3.6 Prototype FishNose measurements

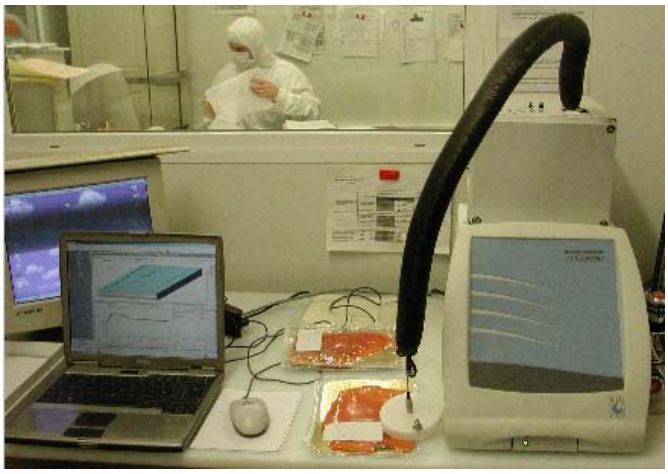


Figure 35: FishNose system with sampling with the bell sampling cup mounted on the sampling unit and PC installed at ARMORIC, QC Lab.

Vacuum packed and frozen stored samples from the different fish producers were analysed with the prototype sensor system. Total of 96 samples were analysed. The sensor responses for the different sensors showed a similar response pattern. The figure below gives an example of one the sensors PA/2 showing a pattern which is very similar to the microbial analysis of TVC and the spoilage related sensory attributes (sweet/sour odor and off odor).

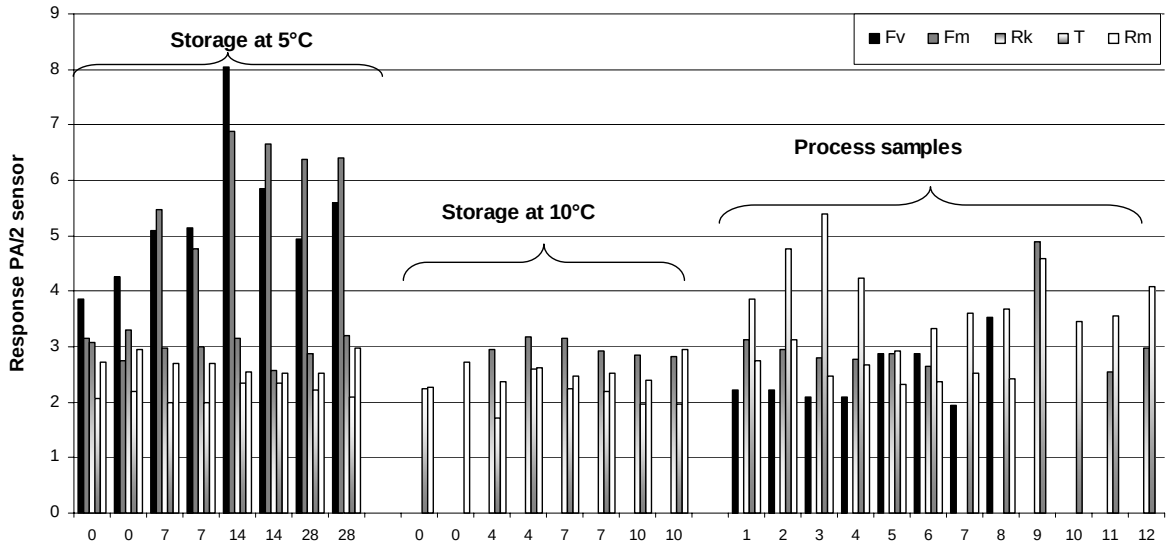


Figure 36: Sensor response distribution for samples measured with the FishNose sensor system for samples stored at 5 °C and 10 °C for 28 and 10 days, respectively, and samples from the process from the different smokehouses

When visually comparing the sensor responses with the TVC data a similar pattern is observed and high sensor readings correspond with high TVC numbers for two of the producers where samples showed spoilage signs according to the sensory analysis. Measurements with the FishNose of varying concentrations of standards compounds selected to represent spoilage related compounds (ethanol and butanone) and smoke related compounds (furfural and guaiacol), showed that the gas sensors were more sensitive towards the very volatile compounds e.g. ethanol and butanone, and were not sensitive enough to detect increasing concentrations of the smoke related compounds, furfural and guaiacol (Ólafsdóttir *et al.,* 2005). Therefore, it is concluded that the gas sensors are mainly detecting the changes in the very volatile compounds present in the headspace of the samples mainly representing microbial metabolism.

3.7 Data analysis - correlation, principal component analysis and classification modelling

A total of 96 samples were measured by the FishNose gas-sensor array. Correlations of gas sensor responses with results of chemical parameters (fat, water, salt) showed low correlations, but significant correlations were found for the sensory and microbial parameters. The most appropriate reference methods that gave the best correlation with the sensor responses were indicative of the proliferation of microflora contributing to the development of volatile compounds that the sensors could detect. Odor evaluation is one of the best measures of consumers acceptance of a product. Therefore, sensory scores for odor attributes were found most relevant to compare to the electronic nose sensors´ 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 TVC, LAB counts, and sensory odour attributes: sweet/sour odour, off-odour and rancid odour. The correlations between gas sensors and selected sensory properties were evaluated based on 93 samples from different producers. Except for rancid odour, significant correlations were found for sweet sour (r=0.3-0.5, p<0.005), off-odor (r=0.2-0.4 p<0.005), and smoked odor which was negatively correlated (r=-0.4- -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.

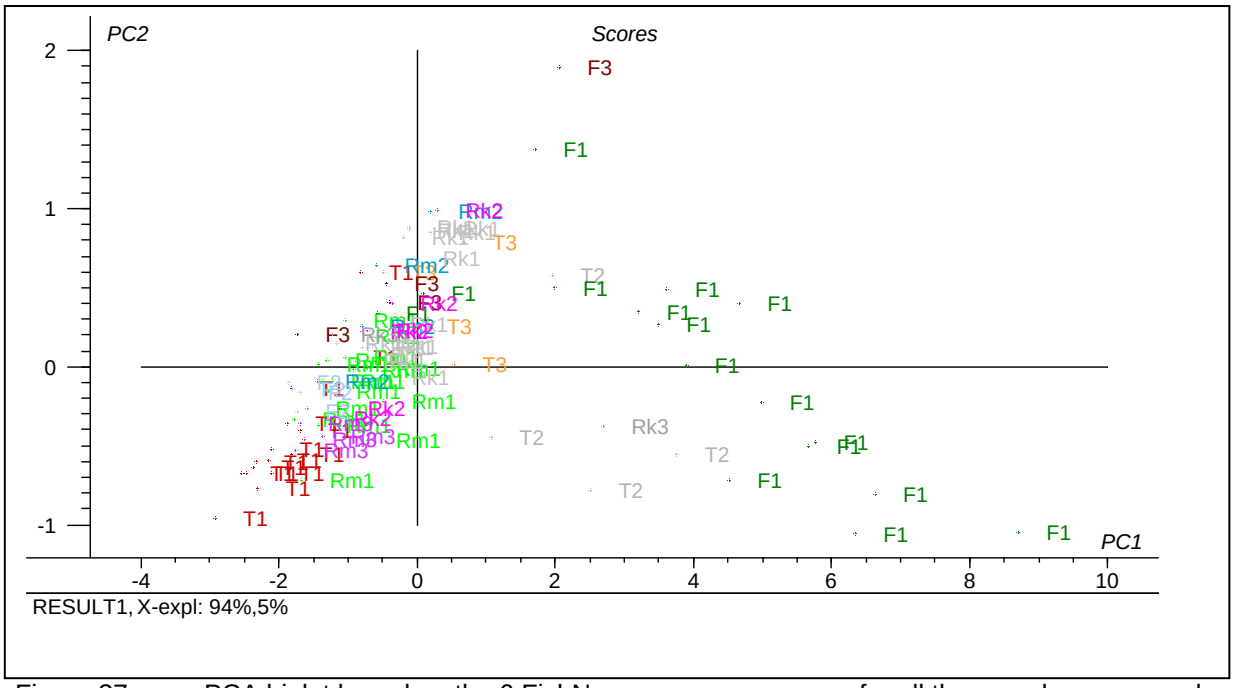


Figure 37: PCA biplot based on the 6 FishNose sensor responses for all the samples measured..

The sensor measurement data from the 6 FishNose sensors for all the 96 measured samples, that represent samples from all the suppliers, were analysed by Principal Component Analysis (PCA). The two first dimensions described 99 % of the total variance in the data set. Several of the samples located to the right, in particular most of the Fiedler samples represent samples with high bacterial numbers and high scores in sensory odour quality attributes (sweet/sour, off).

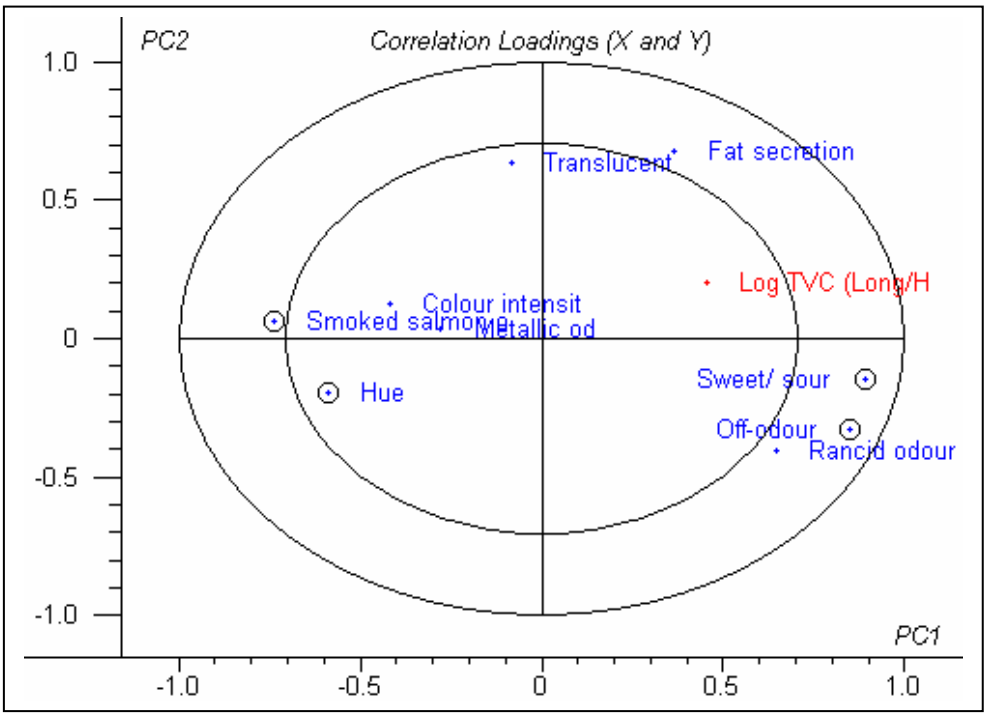


Figure 38: Correlation loadings for the first two components from PLS regression with sensory data as predictors and logTVC as reponse variable.

To justify the selection of the quality indicators representative of microbial spoilage of the samples, it is of interest to investigate how the sensory attributes are related to the TVC values. Therefore, selected sensory properties were subject to regression analysis with log TVC as the response variable. The attributes marked with small circles were found to be significant. The big circles indicate 50 and 100% explained variance respectively. Smoked salmon, sweet/sour and off-odour contribute in modelling TVC, although the correlation is not that high.

A PLSR regression model with gas-sensors as predictor variables and sensory attributes as response variables was also subject to investigation.
The plot shows that the gas-sensors are grouped on the same side as off-odour and sweet/sour odour, which concurs with their univariate correlations (data not shown). However, 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.

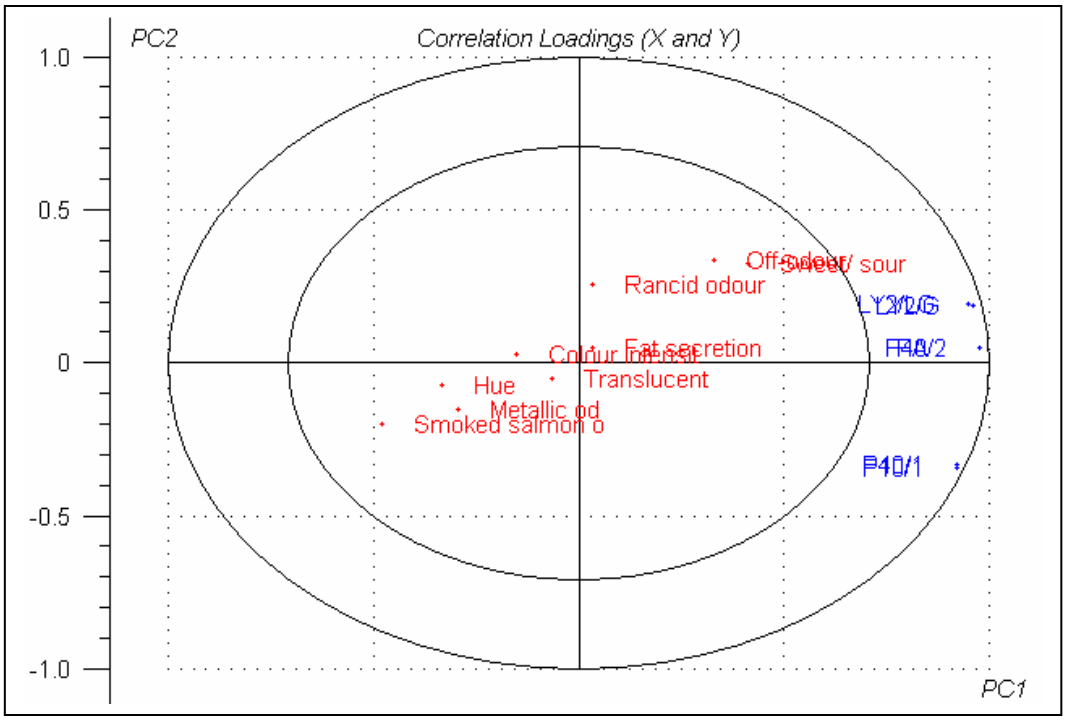


Figure 39: Correlation loading plot from the PLSR model based on gas-sensors as predictors and selected sensory attributes as response variables.

3.7.1 Global models

Different classification models have been investigated for prediction of samples of different qualities. Using single numeric criteria of separate reference parameters like TVC numbers or single sensory quality related parameters like sweet/sour odour, rancid odour or off-odour on all the combined measurement data gave in general low classification rates.
By using a combination of these parameters the classification rates were improved, compared to using single reference parameters alone. The following quality criteria established in the storage studies was applied for the Partial Least Squares Regression (PLSR) classification modelling for accepting and rejecting samples corresponding to respectively good and bad samples:

Good/accepted samples:

TVC < 5, LAB < 4, Off-odour < 20, Rancid odour < 10, Sweet/sour odour < 20

Bad/rejected samples:

TVC > 5, LAB > 4, Off-odour > 20, Rancid odour > 10, Sweet/sour odour > 20

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 15.

Table 15: PLSR classification results of a global model for samples from all producers

Total number of samples		Combined criteria	Expected number of samples	% correct prediction	% wrong prediction
96	Good / accepted samples	TVC < 5 Off-odour < 20 Rancid odour < 10 Sweet/sour odour < 20	65	92 (60 samples)	8 (5 samples)
	Bad / rejected samples	TVC > 5 Off-odour > 20 Rancid odour > 10 Sweet/sour odour >20	31	35 (11 samples)	65 (20 samples)
96	Good / accepted samples	LAB<4 TVC < 5 Off-odour < 20 Rancid odour < 10 Sweet/sour odour < 20	58	71 (41 samples)	29 (17 samples)
	Bad / rejected samples	LAB>4 TVC > 5 Off-odour > 20 Rancid odour > 10 Sweet/sour odour >20	38	37 (14 samples)	63 (24 samples)

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 three odour critera. However, the outcome of 65 % bad samples being classified as good samples is not satisfactory. 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 2-5 % good samples would be classified as bad, so 8 % is perhaps 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.

By including also the criterion for the LAB data, i.e. Log LAB = 4, the total number of expected bad sample increased, resulting in 58 and 38 samples being categorized as good and bad, respectively. However, the prediction was worse for the good samples but slightly better for the bad samples. The results showed that 41 of 58 good samples were classified as good, 71 % correct compared to 92 % correct without the LAB criteria, while 17 of 58 good samples were classified incorrectly as bad (29 %), which is much higher ratio than without the LAB criteria (8 %). Fourteen of the 38 expected bad samples were classified correctly as bad (37 %) while 24 of the bad samples were classified as good (63 %) which is not much better than the prediction without the LAB criteria.

These results suggest that it may be difficult to apply a global prediction model based on all the samples from the different smoked salmon producer. The results also showed that reference parameters as fat secretion and smoked salmon odour (data not shown) could be useful for local classification modelling, probably due to different fat content and smoking processing at the different suppliers of fish.

By inspection of the PCA plot, it appears that the samples tend to be grouped according to the different smokehouses, indicating that local prediction models for each supplier separately could be more suitable.

3.7.2 Local models

Correlation between sensor responses and the TVC, LAB counts, and sensory odour attributes for the individual producers are shown in Table 4. Two of the sample groups (Remo and Reyko) 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 two producers (Fiedler and TBB) where samples showed clear spoilage signs.
The samples that showed the highest correlation with TVC numbers were the Fiedler samples, except for the 28 days old samples, where an unexpected decrease was observed in the sensor response signal (Figure 9). By disregarding the 28 days 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 TBB samples from the process, but higher correlation of sensor responses with the TVC values, suggests that these samples had not been handled properly and indicate poor hygienic conditions in the factory (i.e. growth of Enterobacteriacea may contribute to the high TVC numbers). This assumption is based on the prestudy done in the project were counts of Enterobacteriacea were done.

Based on the findings above, local models based on Partial Least Squares Regression (PLSR) 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 17. The results are given as per cent of the number of good/bad samples predicted as good or bad.

Table 16: Correlation coefficients (r) between single sensor responses and selected quality properties for individual producers

	Parameter	PA/2	P10/1	P40/2	P40/1	LY2/G	LY2/LG
Fiedler	Sweet/ sour	**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**
	Log TVC	**0.56**	0.48	**0.56**	0.48	**0.57**	0.44
	Log LAB	**0.69**	**0.6**	**0.72**	**0.59**	**0.73**	**0.65**
TBB	Sweet/ sour	**0.87**	**0.72**	**0.88**	**0.72**	**0.91**	**0.89**
	Rancid odour	**0.74**	**0.73**	**0.74**	**0.72**	**0.7**	**0.68**
	Off-odour	**0.84**	**0.71**	**0.85**	**0.71**	**0.87**	**0.86**
	Log TVC	**0.56**	0.43	**0.57**	0.42	**0.62**	**0.58**
	Log LAB	-0.33	-0.16	-0.34	-0.16	-0.43	-0.44
Remo	Sweet/ sour	**-0.52**	-0.48	-0.23	-0.48	-0.22	-0.41
	Rancid odour	-0.47	-0.49	-0.27	-0.49	-0.25	-0.40
	Off-odour	-0.45	-0.45	-0.21	-0.45	-0.23	-0.40
	Log TVC	0.13	0.07	**0.51**	0.06	0.47	0.36
	Log LAB	0.31	0.01	0.25	0.02	**0.57**	**0.53**
Reyko	Sweet/ sour	-0.16	-0.29	-0.17	-0.26	-0.09	-0.16
	Rancid odour	0.37	0.22	0.35	0.22	0.41	0.42
	Off-odour	-0.09	-0.12	-0.11	-0.08	-0.06	-0.05
	Log TVC	-0.29	-0.34	-0.22	-0.34	-0.15	-0.21
	Log LAB	-0.04	-0.21	0.00	-0.21	0.06	0.02

Local models apparently show much better performance than the global model and the results show that both single criteria (TVC, LAB, sweet/sour odour, off odour and rancid odour) and combined quality criteria may be successful, but the outcome is dependent on the producer. The main concern is that no “false positives” should occur, i.e. no bad samples should be predicted as good samples.

TVC critera:
Correct prediction (100%) of bad samples using TVC criteria was obtained for REMO, FIEDLER and REYKO. No good sample was wrongly predicted as bad from REMO, but 8-38 % 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 TBB. Only 3 samples were expected bad and two of these were wrongly classified.

LAB criteria:
Wrong prediction of bad samples as good, based on the LAB critiera 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 sensors may be less sensitive to those volatiles (i.e. lactic acid).

Off odour criteria:
The gas sensors give the best prediction of off-flavour and sweet and sour descriptors as seen in Table 6. For instance a 100% correct classification was obtained for the TBB samples by using single sensory criteria, i.e. the off-odor or sweet/sour odor. The sensors are apparently detecting and predicting the spoilage odours caused by the improper handling of the TBB samples as seen by the 100% correct prediction of the bad samples from TBB .

Sweet sour criteria:
Correct prediction was obtained of bad samples for TBB and REMO and in fact no bad samples existed according to this criterium in the REYKO samples. One of the expected bad Fiedler samples was classified as good, but 100% correct prediction of the good samples was achieved.

Rancid criteria: The prediction of ranciditiy by the sensors is not good and indicates that the sensors are not able to detect the volatiles causing rancid off odour. 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 flavour, and the low values are thus not detected by the gas-sensors. In addition the odor thresholds of characteristic compounds causing rancid odour is very low so the sensory panel may be able to detect the odors even though these compounds are present in very low levels in the samples (much lower than the sensors can detect).

Combined criteria:
The combined criteria improved the overall predictions slightly for the Fiedler samples but not for the TBB samples where 8 samples were expected good and 8 bad but the combined criteria predicted all the samples as bad. The combined criteria was not used for the other sample groups (REMO and REYKO), because there were very few “bad” samples and therefore a lack of structural correlation of variables and sample groups. Moreover, a robust prediction was achieved with the single criteria for those samples.

Table 17: PLSR classification results of local models for samples from each producer

			Fiedler n=24			TBB n=16			Remo n=16			Reyko n=13		
	Criteria		Expected number of samples	% correct prediction	% wrong prediction	Expected number of samples	% correct prediction	% wrong prediction	Expected number of samples	% correct prediction	% wrong prediction	Expected number of samples	% correct prediction	% wrong prediction
TVC	< 5	Good	15	73	27	13	92	8	7	100	0	8	63	38
	> 5	Bad	9	100	0	3	33	67	9	100	0	5	100	0
LAB	< 4	Good	15	80	20	15	100	0	14	100	0	8	75	25
	> 4	Bad	9	89	11	1	0	100	2	0	100	5	100	0
Off odour	< 20	Good	17	94	6	12	100	0	15	100	0	13	100	0
	> 20	Bad	7	86	14	4	100	0	1	0	100	0		
Sweet/sour	< 20	Good	16	100	0	11	100	0	14	86	14	13	100	0
	> 20	Bad	8	88	13	5	100	0	2	100	0	0		
Rancid	< 10	Good	21	100	0	13	92	8	15	100	0	13	100	0
	> 10	Bad	3	0	100	3	67	33	1	0	100	0		
Combined		Good	14	79	21	8	0	100						
		Bad	10	90	10	8	100	0						

4 CONCLUSIONS

The evaluation of the quality of smoked salmon products is difficult because of the complexity of the spoilage of these products.

The overall analysis of the data generated with the FishNose of fresh and stored smoked salmon suggest that the gas-sensor system can be used for fast quality control of smoked salmon. The gas sensors appear to group the samples according to the fish processors indicating that there are differences in the composition of the headspace because of the different smoking and handling proccess. Additionally, the gas sensors show a similar trend regarding changes in the headspace that are representative of microbial growth (TVC and LAB) and sensory attributes (sweet/sour odour and off odour) related to microbially produced volatile compounds in the headspace. A “structural correlation” is thus defined when variables group induces a similar structure on the samples. Individual analyses have shown that when the samples of a producer are structured i.e. showing changes in the measured variables, the sensor data reveals a similar structure. This is the case for Fiedler and TBB producers, that contained samples with obvious spoilage signs and the results thus show a general agreement of the microbial and sensory parameters selected as reference parameters for the gas sensor responses. 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 significant discrimination of the REMO and REYKO samples was observed. However, local predictive models by quality attributes appeared to generate robust prediction of good samples and bad samples.

The analysis of the complete set of results, lead to the conclusion that the data generated with “FishNose prototype” shows a proper “structural correlation” between the sensory and microbial analyses with the FishNose prototype. The FishNose system is therefore ideal for fast quality control related to freshness evaluation of smoked salmon products and was able to predict good samples from bad ones based on microbial and sensory criteria. It can also be concluded that high classification rates can be obtained by using both single and combined quality criteria. The optimal classification with regard to lowest number of “false positives”, i.e. bad samples being predicted as good seems to rely on single criteria like log TVC or sensory off- odour or sweet/sour –odor when evaluating local models based on samples from individual processors, but taking into consideration that the samples were quite homogeneous and a few bad samples were expected. However, the combined criteria give the best overall classification, also taking into account the correct classification of good samples.

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24 Month PROGRESS REPORT

CONTRACT N°: QLK1-CT-2002-71304
PROJECT N°: CRAFT-1999-71304

ACRONYM: FISHNOSE

TITLE: Development of an electric nose system for automated quality control of smoked fish

PROJECT CO-ORDINATOR: ALPHA M.O.S. S.A.; TOULOUSE, FRANCE (A1)

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B3 Icelandic Fisheries Laboratories; Reykjavik, Iceland

REFERENCE PERIOD: From the 1st January 2003 to the 31th of December 2004

STARTING DATE: 1st January 2003 **DURATION: 24 MONTHS**

Date of issue of this report: February 2005

Project funded by the European Community under the Programme „Quality of Life“ in FP5 (1998-2002)

24 Month Progress Report

Title of the project: Development of an electric nose system for automated quality control of smoked fish		
Acronym of the project: FISHNOSE		
Type of contract: Co-operative Research (CRAFT)		**Total project cost** (in euro) 1.058.861,00 €
Contract number QLK1-CT-2002-71304	**Duration** (in months) 24 Months	**EU contribution (in euro)** 529.300,00 €
Commencement date 1st January 2003	**Period covered by this progress report** 1st January 2003 – 31st December 2004	

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Key words fish, sensor, fish nose, quality control, spoilage

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Rügen-Feinkost GmbH; Saßnitz, Germany	Contractor
Tonsberg Brygge AS; Tonsberg, Norway	Contractor
Optotek d.o.o.; Ljubljana, Slovenia	Contractor
ttz – Verein zur Förderung des Technologietransfers an der Hochschule Bremerhaven e.V.; Bremerhaven, Germany	RTD Performer
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1 OBJECTIVES AND EXPECTED ACHIEVEMENTS

Fish is an important and popular food in all countries of the European Union. On average, each citizen of the EU consumes about 25 kg of fish per year, from which 10% is smoked fish. The smoked fish producing and processing industry is crucially dependent on the excellent freshness and high quality of their products. Due to growing public awareness on a hard fought market, a high standard of quality control is essential. Currently, the industry employs conventional random sampling quality control methods like classical bacteriologic and chemical methods beside sensory evaluation. Especially small and medium-sized enterprises (SME's) normally do not have sufficient laboratory capacity or correspondingly trained staff at their disposal to carry out complex analytical tests. They have to outsource the time-intensive and expensive measurements.

Thus there is a great interest in having rapid, automated, in-situ and objective tools for process-monitoring and final quality assurance available.

Odour is the first criterion for evaluation the fish freshness or spoilage.

The project "FishNose" envisages the development of a new, efficient and easy to handle automated quality control system based on a gas-sensor array system - "Electronic Nose" - for detection of smoked fish product's freshness and quality.

An automated quality control system for the characterisation of smoked fish and related products supplying data ready for documentation to improve the production process reliability and reproducibility is not available on the market today. Some electronic noses for other products are being developed (e.g. computerized multi-sensor technologies for monitoring the quality of fresh fish, FAIR 979063 and FAIR 984076) - however due to the complexity of the smoked fish no system is available here. Because of the chemical structures and the physico-chemical properties of smoked fish products, approaches for their analysis will vary considerably from those used for fresh fish.

FishNose prototype, being generated during the current project, will consist out of a gas sampling unit, the sensor array system itself and a user-oriented software. Besides characteristic key components for spoilage of smoked fish will be identified and generated for training of the pattern recognition system. Finally the trained and lab-approved "FishNose" sensor will be established and optimised on-site in the industrial smoked fish production process.

2 PROJECT WORKPLAN

2.1 Introduction

The project envisages the development of a new automated quality control system based on a gas-sensor array system - “Electronic Nose” - for in-situ quality assessment of smoked fish. The system will be applied for raw material control and for product quality control during processing, storage, transport and delivery.
The project work will firstly involve specification of end-user requirements and the set of fish species and products to be tested. Sensor development will follow, investigating suitable sensor materials, development of the automated gas-sampling unit, device control and signal processing. The chosen approach will permit fabrication of sensors, which can be chosen a-priori for a measurement task, to produce an array, which is optimised for the fish quality assessment. An important area of development are the robustness of the sensor and the reproducibility of the gas-sampling technique. The development of a cost-efficient, simplified and automated gas-sampling unit is the key to success of large-scale applications of gas-sensor array systems in industrial processes which makes it’s use profitable for SME’s as well.
During sensor development, measurement results will be confirmed and compared by parallel reference measurements of fish quality and freshness by means of traditional methodologies such as sensory evaluation (human trained panel), microbial testing and GC-analysis of spoilage products (e.g. trimethylamine or other specific volatile compounds). Thereby the volatile key-compounds of smoked fish spoilage will be identified and a standard-mix will be developed to be used for training of the pattern recognition system of the electronic nose.

2.2 Project structure, planning and timetable

The work programme of the FishNose project is divided into work packages and tasks like the following:

Tab. 1: Work packages and Tasks of the FishNose project

Work package	Task	Description
WP A	1-3	Specification and characterisation
WP B	4-6	Design and manufacture of a prototype
WP C	7-8	Prototype testing and optimisation
WP D	9	Project management and dissemination

The individual work stages needed for the development of the FishNose Sensor are presented in the following figure 1:

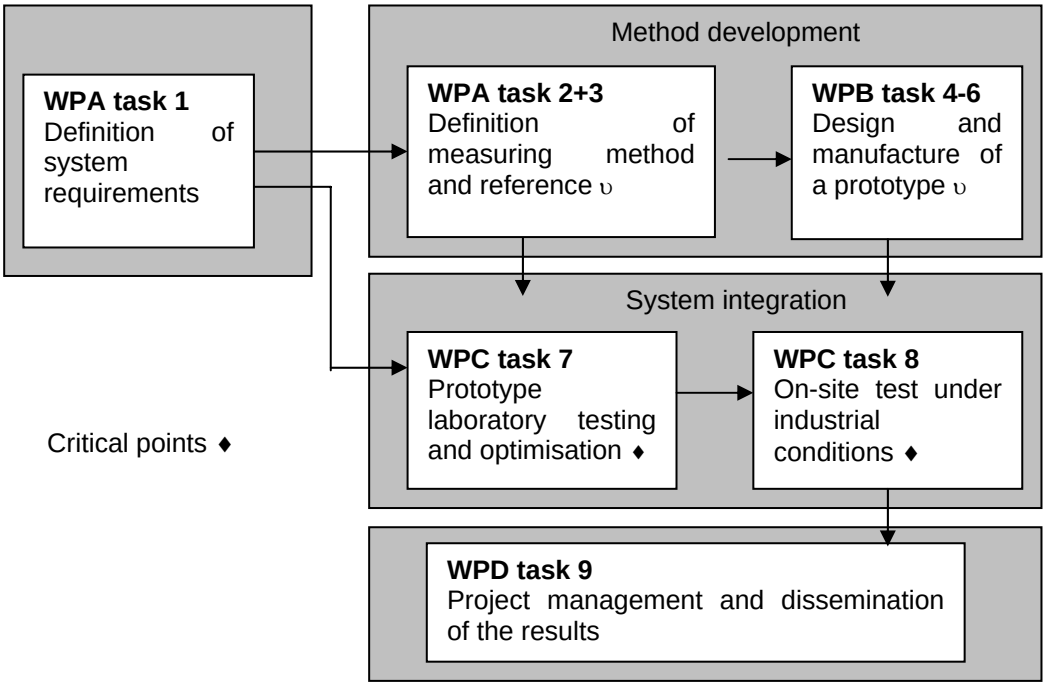


Fig. 1: Structure of the FishNose Project

The following table 2 shows the list and description of participating SME contractors and RTD performers with their assigned partner number and partner acronym.

Tab. 2: Participants of the FishNose project

Partner	Partner acronym	Contractual role	Business activities	Role in research project
A1	ALPHA	SME co-ordinator	Development and manufacture of analytical instrumentation and sensor technology	Development and optimisation of the gas sensor
A2	FIEDLER	SME proposer	Producer and trader of smoked fish	Testing of the developed electronic nose system under production conditions, end user
A3	ARMORIC	SME proposer	Producer and trader of smoked fish	Testing of the developed electronic nose system under production conditions, end user
A4	REYKO	SME proposer	Producer and trader of smoked fish	Testing of the electronic nose system and end user
A5	ANFACO	SME proposer	Commercial food laboratory	Performer of standard fish quality analysis for comparative measurements and end-user of developed E-Nose
A6	REMO	SME proposer	Producer and trader of smoked fish	Testing of the electronic nose and end- user
A7	Rügen-Feinkost	SME proposer	Processor of smoked fish – delicatessen producer	Testing of the electronic nose and end- user
A8	TBB	SME proposer	Producer and trader of smoked fish	Testing of the electronic nose and end user
A9	OPTOTEK	SME proposer	Producer of sensors and optoelectronic devices	Development of the air-sampling system, construction of the prototype
B1	TTZ	RTD performer	Contract R&D, technology transfer activities, food analysis	Standard fish quality analysis as reference, joining of gas sampling and sensor device, testing programme, sensory analysis and quality assurance,
B2	MATFORSK	RTD performer	Contract R&D, research in the sector of fish quality and electronic sensing	Development and optimisation of the gas sensor system, lab scale testing of E-Nose, standard analysis for fish quality as reference,
B3	IFL	RTD performer	Research in fish analysis and fish quality	Search for corresponding spoilage compounds via GC-MS, sensory evaluation with accredited panel, lab scale testing of E-Nose

Following table 3 shoes the project's overall time table and manpower recourses:

Tab. 3: Appropriateness of the resources

Work package descriptions	Partners													Duration																							
	Man Months													1st year												2nd year											
	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
WP A Specification and Characterization																																					
T1 Definition of requirements	1	0,75	0,75	0,75	1	0,75	1	0,75	0,5	1	1	1	10,25																								
T2 Definition of laboratory analysis	0,25	0,5	0,5	0,5	2	0,5	0,5	0,5		3	3	3	14,25																								
T3 Detection of key components				0,5	1	1		1		1	3	5	12,5																								
WP B Design & Manufacture Prototype																																					
T4 Design of gas sampler	1								5	3	2	1	12																								
T5 Sensor Design	3									3	2	1	9																								
T6 Software	2	0,25	0,25	0,25	0,5	0,25	0,5	0,25		2	3	1	10,25																								
WP C Prototype Testing																																					
T7 Prototype Tests Laboratory	1				2				0,5	5	3	3	14,5																								
T8 prototype Tests on site	1	3	4	2,5	1	2	2,5	2	0,5	2	2	2	24,5																								
WP C Project management and dissimination																																					
T9.2 Dissimination of results	0,75	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	2	1	1	8,75																								
T9.1 Project management	4												4																								
Total Man Month	**14**	**5**	**6**	**5**	**8**	**5**	**5**	**5**	**7**	**22**	**20**	**18**	**120**																								
in year 1	**9**	**1,5**	**1,5**	**2**	**5**	**2,5**	**2**	**2,5**	**6**	**16**	**15**	**14**	**77**																								
In year 2	**5**	**3,5**	**4,5**	**3**	**3**	**2,5**	**3**	**2,5**	**1**	**6**	**5**	**4**	**43**																								

The following table lists the deliverables scheduled fir the complete duration of the project and their status after 18 months:

Tab. 4: List of deliverables

Task	No.	Title	Planned Delivery	Responsible	Status of Delivery
1	**D01**	Detailed data on SME end-users processes	Month 3	ALPHA	Delivered
1	**D02**	Technical specification catalogue for the FishNose sensor	Month 3	ALPHA	Delivered
2	**D03**	Specified laboratory programme	Month 3	IFL	Delivered
2	**D04**	Reference Methods ready to use in the project partner labs (IFL, MATFORSK, TTZ, ANFACO)	Month 5	IFL	Delivered
3	**D05**	List of characteristic key compounds for spoilage of smoked fish	Month 5	IFL	Delivered
3	**D06**	Standard cocktail of selected compounds for training of the pattern recognition system and calibration of the developed E-Nose system	Month 5	IFL	Delivered
4	**D07**	Optimised gas sampler	Month 9	OPTOTEK	Delivered
5	**D08**	Manufactured sensor array prototype (hardware)	Month 9	ALPHA	Delivered
5	**D09**	FishNose prototype with specification regarding the standard mixture	Month 11	ALPHA	Updated/revised version delivered with this report
6	**D10**	Pattern recognition and compensation software	Month 12	MATFORSK	Delivered
6	**D11**	User-friendly interface and control software	Month 12	MATFORSK	Delivered
7	**D12**	Optimised prototype with specification of lab experiments	Month 14	MATFORSK	Updated/revised version delivered with this report
7	**D13**	Test formats and protocol schemes for on-site experiments	Month 12	MATFORSK	Updated/revised version delivered with this report
8	**D14**	Pre-competitive, optimised prototype with specification	Month 23	MATFORSK	Delivered with this report
9	**D15-1**	6-Month Management Report	Month 6	ALPHA	Completed
9	**D15-2**	12-Month Management Report	Month 13	ALPHA	Completed
9	**D15-3**	18-Month Management Report	Month 19	ALPHA	Completed
9	**D15-4**	24-Month Management Report	Month 24	ALPHA	Completed with this report
9	**D16**	Mid-Term Progress report	Month 13	ALPHA	Completed
9	**D17**	Training Report and programme	Month 24	ALPHA	Completed
9	**D18**	Leaflet	Month 21	ALPHA, TTZ	Completed
9	**D19**	TIP	Month 24	ALPHA	Completed
9	**D20**	Final progress report	Month 24	ALPHA	Completed

The following table lists the milestones scheduled for the complete duration of the project and their status after 18 months:

Tab. 5: List of milestones

WP	No.	Title	Planned Delivery	Responsible	Status of Delivery
A	**M01**	Detailed concept for E-Nose development including reference analysis	Month 5	ALPHA; OPTOTEK, ANFACO, TTZ, IFL, MATFORSK	Delivered
B	**M02**	FishNose prototype consisting of gas sampling, sensor	Month 12	ALPHA; OPTOTEK, MATFORSK	Delivered
C	**M03**	Approved and optimised FishNose prototype with specification of lab experiments	Month 16	ALPHA; MATFORSK	Delivered as combination of D12 and D13 together with this report
D	**M04**	Mid-term Progress report	Month 13	ALPHA	Delivered
D	**M05**	Final Review Report	Month 24	ALPHA	Delivered with final documents

2.2.1.1 Discussion-Conclusion

After the completion of project, all the partners are satisfied with the results achieved. The work programme was proceeding satisfactorily and in time. The key areas of technical progress from the hole project period of the FishNose project were like the following:

- Successful generation of the FishNose sensor prototype was performed and gained out of four preliminary procedures:
  - Definition of user's requirements and establishment of system specification for the FishNose sensor
  - Generation of simplified and "on-site suitable" direct gas-sampling unit for E-Nose technology and adaptation to the FishNose sensor prototype
  - Adaptation of ALPHA-MOS gas-sensor array to the FishNose sensor prototype including selection of 6 discriminating sensor-units for the characterisation of smoked salmon
  - Adaptation of ALPHA-MOS Software and Interface to the FishNose sensor prototype
- Performed storage trials and laboratory tests showed promising correlation and discrimination of FishNose prototype responses with fish quality and therefore with the selected and performed chemical, microbial and sensorial reference analysis results.
- Prototype on-site test by integration of the system into the SME's production chain were successfully fulfilled.
- First economic feasibility calculation lead to the follow expectations:
  - Mainly due to simplified gas-sampling procedure, FishNose price is estimated to be 10-15 kEuro, which is 25-35 % of existing common and commercial head-space based gas-sensor arrays.
  - Providing that the FishNose sensor will stand the test of further laboratory and on-site application and validation, it has got high potential to be of added value in quality insurance of smoked fish processing industry. Due to high graded quality and robustness of used materials the economic life-time is estimated to be > 5 years at a use of 24h/day.

Taking into account the results achieved, the consortium was encouraged of the of success after the completion of this project. The work performed during the project has been clearly focused on the fulfilment of the objectives fixed on the Technical Annex, and all important goals have been achieved.

The following pages summarize the work performed in each WP.

2.3 Description of the work packages

WPA: Specification and Characterisation

2.3.1 Task 1: Definition of user's requirements and system specification

Start date: Month 1
Completion date: Month 3
Current Status: Completed

Partners involved, including total and devoted person months:

Task 1													
Task leader	A1												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	1	0,75	0,75	0,75	1	0,75	1	0,75	0,5	1	1	1	10,25
MM devoted	1	0,75	0,75	0,75	1	0,75	1	0,75	0,5	1	1	0,6	9,85

Objectives:
The aim of this task is to define the end-users' demands so that they can be taken into account in the laboratory test programme and in the system specification for the E-Nose system to be developed.

Methodology and study material:
TTZ, IFL and MATFORSK will examine the end-user SME's production chains with regard to processes (smoking, processing, packaging, incoming and final inspection) and the products (type of fish etc.). An analysis will be carried out at each user company (A2-A8) to gain information about their requirements in terms of freshness and quality, system demands (handling, user's skills, data documentation, degree of automation, etc.) but also the frequency of measurements and the required sensitivity of the system have to be assessed. Furthermore on-site conditions under which the E-Nose will be applied later on (air temperature, humidity etc.) have to be measured.
The SME's will provide information according their special demands each: FIEDLER, ARMORIC, REYKO, REMO and TBB as fish-smokers concerning smoked-fish production and storage and RÜGEN-F as smoked-fish processor concerning incoming inspection of smoked fish. ANFACO as laboratory for fish quality and service provider will investigate needs of their potential clients.
Especially for RÜGEN-F and ANFACO, as sole representatives of their end-user-category each, a special and therefore more time-intensive definition is expected. Due to their experience in quality control (RÜGEN-F: sensory evaluation; ANFACO: phys.-chem. analysis) they also will contribute technical aspects.
The RTD performers TTZ, MATFORSK and IFL will act as interface to collect and filter the information and focus on the demands of the SME partners to prepare a specification of the sensor-system to be developed. OPTOTEK will be involved by providing it's experience in gas-sampling for specification preparation.
Thus all the partners in this task will have a supporting role in the planning of the design of the FishNose and identifying generically what functions it should have.

Task 1 comprises the following subtasks:

- Subtask 1.1 Analysis and assessment of the SME user requirements
- Subtask 1.2 Specification of hardware and software requirements

Progress and results during the project running time:
The aim of the task 1 was to collect data from all end-users partners in order to have a comprehensive survey about products and processes in the smoked salmon industry. RTD partners, ANFACO and ALPHA MOS have designed a specific questionnaire in order to define the end-users' demands so that:

- they can be taken into account in the laboratory test programme,
- a specification document for the e-nose system to be developed could be written.

A questionnaire was designed in close collaboration between partners to have a complete overview about:

- Intended application,
- Specification of the smoked fish production processes in each location, (Global European representativeness is achieved through location of SME's all over Europe –Spain, Norway, Germany, France, Iceland-),
- Specification of the processing of smoked fish,
- Current quality control activities for all product aspects (Incoming inspection, process monitoring, final control and general aspects, sensory analysis),
- Electronic nose comprehension in the fish industry,
- Exploitation strategy and market overview.

The questionnaire is presented in the deliverable D01 (Annex A01).
On the basis of the questionnaire, examination of the smoked fish production and processing have been carried out by RTD performers and the SME partners to define potential locations, test conditions and requirements for application of FishNose sensor.
Thereby TTZ, MATFORSK and IFL acted as interface to collect and filter the information: Meetings have been held between TTZ and FIEDLER, MATFORSK and TBB as well as between IFL and REYKO. Extensives telephone conferences have been performed between TTZ and RÜGEN-F, TTZ and ANFACO, ALPHA and ARMORIC as well as between MATFORSK and REMO.
Resulting conclusions are described in Deliverable D01 (Annex A01) and system specifications were issued. Detailed specifications were described in the deliverable D02 (Annex A02) and also constituted Milestone 01.
Smoked salmon process was described according to the following figure 2, in order to evaluate value chain and to select appropriate location of e-nose.

The decision was to promote the E-Nose use :

- for final QC of smoked products as first target applications is this market.

Intended Application :

- Quality Control on site – Correlation with sensory attributes and/or Spoilage detection

Process guidelines for cold Smoked Salmon

Raw material	slaughtered salmon with head; on ice (0 to –2°C)					
↓						
Thaw out	4°C					
↓						
Washing of		by				machine
the surface	➡	cell count reduction, desliming				
↓						
Cut off head	by hand or machine					
↓						
Filet cut,	➡	by	hand	or		machine
take off bone,	➡	by	hand	or		machine
take off skin	➡	by hand or machine				
↓						
Salting	a) dry salting at 4°C, then salting in brine					
↓	b) injection					
Curing	16h at 4°C					
↓						
Washing of surface	➡ remove salt and cell count reduction					
↓						
Cold smoke	6h at 25°C					
↓						
Freezing	to about -10°C					
↓						
Slicing	➡ by machine					
↓						
Packaging	total plate count 200 to 2000 cfu/g; storage at 4°C					
↓						
Distribution/Storage	below 7°C; for up to 21 days (4 weeks)					

E-NOSE IN HERE

Fig. 2: process of cold smoked salmon production

SPECIFICATIONS:

- Sort of fish : Application validation is performed on **salmon fillets**.
- Monitoring needs to be performed automatically with almost real-time capabilities.
- Frequency of measurement is not critical as end-users indicate a maximum of 5 analysis / hour. Few measurements per batch are going to be performed. Hypothesis here consists of an homogeneous batch composition (raw materials, storage conditions ..).
- A Good/bad output seems sufficient for QC purposes. No existing techniques are currently used for QC of final product and end-users are reported few quality problems.
- Qualified person are available to operate FISHNOSE sensor (Engineer, Production manager ..) as supervisor. Due to resources constraints and qualification, routine use should be simple.
- Representative samples are quite different between end-users and therefore at-line QC is more appropriate with the aim of analysing whole fillets without sample preparation (weighting, filling vials ..).
- Gas sampling interface would be required to automate the on-line smoking automate. Rugged design is expected to avoid contamination, poisoning … of gas sampling line. A gas volume is extracted from fillet gas surrounding in order to perform analysis. Controlling headspace generation conditions precisely (temperature, sample volume, incubation time) should be avoid if analytical results allow it.
- Market opportunity and features of the product were also detailed to promote system use by SME's end-users.

The overall description is described below:

Constraints about environment :

Sample extraction is performed with gas sampling accessory that allows extracting a given volume of sample into a sample loop before the measurement. Valves, loop etc need to be temperature-controlled to avoid condensation.
Pre-concentration techniques would also be discussed depending upon conclusion of Task3.
A source of clean-air is required for baseline of sensors (filtered compressed air). Generators are available as standalone unit.
Ambient temperature is in the range of 5-30 °C.
Housing allows protection to water. The module is installed close to **slicing or packaging machines**. Minor adaptations might be required depending upon smokehouse processes and organisation.

Data processing :

Results are presented in a simple manner (conformity or not). An acceptability range is defined during the set-up of the instrument by correlating sensor responses with reference methods (sensory ..). Software will be written when using Microsoft development tools (VB, VC++).

Hardware :

Module is linked to a process supervision PC that is performing computation and allows control/command of the sensor module.
Analysis time is below 5 min
Sensor module is composed by Metal Oxide sensors.

Sampling and Calibration :

An additional valve port should be available for calibration purposes. Inlet should allow calibration bottle connection. Gases used for calibration will be determined after quality factor determination.

Status of Deliverables:
Task 1 includes the following deliverables:

- D01 Detailed data on SME end-users' processes (ALPHA, due after month 3)
- D02 Technical specification catalogue for the FishNose (ALPHA, due after month 3)

Both deliverables, D01 and D02, have been completed until June 2003, which means 2 month delay compared to the estimated due date of the Technical Annex. They are included to this report as annexes A1 and A2. Time delay was due to date of kick-off meeting in middle of February compared to official project start on 1st January 2003.

Task has been completed

2.3.2 Task 2: Definition of standard analysis laboratory programme

Start date: Month 1
Completion date: Month 5
Current Status: completed

Partners involved, including total and devoted person months:

Task 2													
Task leader	B3												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	0,25	0,5	0,5	0,5	2	0,5	0,5	0,5	-	3	3	3	14,25
MM devoted	0,25	0,5	0,5	0,5	2	0,5	0,5	0,5	-	3	1,6	3,4	13,25

Objectives:
Based on the results of the assessment of the different production processes and products, IFL, TTZ, MATFORSK and ANFACO have designed the final laboratory programme supported by the SME's. Later on during prototype testing samples obtained from the end-user SME's will be analysed by means of standard methods for characterisation of the samples in addition to reference methods that have been established for correlating with the E-Nose.

Methodology and study material:
The FishNose prototype is based on gas sensors. For interpretation of their responses it is very important to compare and assess the obtained results with standard analytical methods. Reference methods have been established in every participating country by the RTD-performers and ANFACO to avoid long transport-time and non-standardised transport-conditions.
Storage trials were done to test the performance of the reference methods and to get an overview of the chemical composition of the products and their spoilage potential. Samples were provided by the SME's: FIEDLER, REYKO, REMO, and TBB. Each company provided freshly smoked samples that were stored at different temperatures to obtain a variety of quality categories RÜGEN-F supplied fresh salmon samples, but samples from ARMORIC will be supplied for the prototype testing.
At ANFACO, as service provider of fish analysis, most of the described methods are established and run as routine work. Therefore, they will contribute technical aspects, like technique of representative sampling etc., to the project work. Due to their variety of clients they will be able to provide and characterise different kind of samples.
Based on the results of the performance of the reference methods in the preliminary storage trials IFL, TTZ and MATFORSK, assisted by end-user ANFACO, will adjust procedures and test programmes which will be applied during prototype laboratory and in-site tests in task 7 and 8. Besides a first idea about regional variances will be given, covering the German, Norwegian, Icelandic and Spanish market.

Task 2 comprises the following sub-tasks:

- Subtask 2.1 Specification of laboratory test programme
- Subtask 2.2 Establishment of selected methods in participating laboratories (IFL, MATFORSK, TTZ, ANFACO)

Progress and results during the project running time:
A laboratory test programme has been developed by the RTD partners (IFL, TTZ, MATFORSK) and ANFACO. This includes descriptions of standardised procedures for sampling, storage and transport (Subtask 2.1.Annex A3 - D03), as well as selection of chemical, microbial and sensory reference methods for smoked salmon (Subtask 2.2. Annex A4 - D04).

Subtask 2.1 Specification of laboratory test programme
The harmonised laboratory test programme is necessary to characterise the different products from the participating smokehouses and identify common spoilage indicators for the typical smoked fish products in the different countries.

Preliminary storage studies were carried out at IFL, TTZ in May and June 2003 and at MATFORSK in September 2003. Samples were obtained from the SME's in the different countries and storage studies were done under controlled storage conditions, monitoring the microbial, chemical and sensory changes. Concerning comparability of the results the partners agreed on delivery of one sample batch from each fish processor instead of 5 different samples from batches of different quality. The Laboratory programme included a plan of different storage conditions to gain different qualities for characterisation. The different temperatures during storage (4°C, 5°C and 10°C for FIEDLER, REYKO and the Norwegian samples, respectively) were selected in the experiments to produce the range of qualities that possibly could occur during retail.

Subtask 2.2 Establishment of selected methods in participating laboratories (IFL, TTZ, MATFORSK, ANFACO)
The main criticism of the quality of the products refers to the appearance, the texture related to fat content, the level of salt and the taste. Methods to detect quality related changes influencing the odour of the products have been emphasised. Microbial growth is the main factor contributing to spoilage characteristic, especially odour development in refrigerated products.
The methods selected for the pre-trials were; Chemical analysis of fat, water, pH and TVB; microbial analysis of TVC, lactic acid bacteria and *Enterobacteriaceae* and; sensory analysis using QDA.

Chemical methods for characterisation of the samples:

- Fat content
- Water content
- Salt content

Methods to evaluate the freshness and onset of spoilage are microbial, chemical and sensory methods:

- TVC (total viable counts),
- LAB (Lactic acid bacteria)
- EB (*Enterobacteriaceae*) (viable counts on corresponding selective-media)
- TVB-N (total volatile basic nitrogen)
- Sensory analysis: QDA using a 6-8 member panel (Intensity scaling)

Results from the storage trials:
The variation in the handling and smoking conditions in the different smokehouses influences the resulting characteristics and spoilage patterns of the different smoked salmon products. Moreover the different storage temperature chosen in the different countries influences the spoilage rate. The samples stored at 10°C spoil most rapidly as expected.

Characterisation of samples:
Identification of common spoilage indicators has been achieved based on the results of the preliminary storage studies carried out at IFL, TTZ in May and June 2003 and at MATFORSK in September 2003.
The results show a variation in the fat and salt content in samples from the different countries. The fat and salt content were lowest in the smoked salmon from TBB in Norway (2.8 %). The salt content of the REYKO and FIEDLER samples was considerably higher, (4.0 % and 4.4 %, respectively) than the Norwegian products, which may influence the shelf-life of the products. The pH of the samples did not change during storage (pH = 6.0-6.2).

Spoilage profiles of smoked salmon samples - Identification of spoilage / quality indicators:
TVB-N analysis of samples from the different smokehouses. The range of the TVB-N values (mg N/100g) varied and the range of the values was the following: Fiedler 13.0 -34.8; IFL 20.4-23.7, TBB 12.7-20.0; REMO 13.7-20.3. The highest values were detected in the FIEDLER samples after 21 days of storage. None of the samples exceeded the TVB-N of 35 mg/100 g, the European standard for fresh salmon. The TVB-N values did not show the same spoilage trend of the samples as the microbial analysis and the sensory analysis. Because of conflicting results, the TVB-N is not considered a good indicator for smoked salmon.

TVC analysis:
The initial microbial counts (log cfu/g) varied considerably in the samples from the different producers (Fiedler 5.5; IFL 2.0, TBB 6.1; REMO 2.7). At the end of the storage study all the samples, except samples from REYKO, had TVC values above 10^6 cfu/g in,
LAB: The results show that LAB became predominant in the spoiled cold smoked salmon flora for all the samples. At lower storage temperature (4-5°C), the development of LAB occurred slowly, but usually dominated towards the end of the storage periods. At 10 °C, LAB counts were similar to TVC throughout storage.
A comparison of the smoking conditions (temperature and time) used at the 4 different plants based on the initial microbiological quality counts indicates that smoking at higher temperatures (30°C) appears to lead to higher initial TVC and LAB counts.
EB: High initial counts in the products of *Enterobacteriaceae,* has been associated with conditions in the smokehouse and low hygienic quality of the products.
TVC is recommended as a reference method for the prototype testing using a selective medium that allows the growth of LAB and is therefore suitable for monitoring smoked salmon.

Sensory analysis:
The results of the sensory analysis showed that the training of the sensory panels is critical. Even though all the panels carried out initial training before carrying out the pre-trials, the IFL panel appears to have been more sensitive to detect changes in the sensory attributes selected. The IFL panel was involved in the development of the sensory scheme and had therefore more training than the panels at MATFORSK and TTZ. Based on analysis of variance (ANOVA) of the data, significant differences in the changes of the sensory attributes with storage times were only found for the samples analysed by IFL.

Based on the QDA analysis a few quality criteria that best describe the changes of the samples have been selected. The sensory data from IFL shows significant decrease in smoked salmon odour/flavour during storage and the other samples analysed by TTZ and MATFORSK show similar trend although not significant. Rancid and off odour appeared to be increasing in the FIEDLER and TBB samples. The selection of attributes will be verified during the prototype testing and the sensory analysis scheme will be further evaluated. Suggestions will be made for a simplified sensory scheme that could be used by the end users as a reference method for the FishNose in the future.

Status of Deliverables:
Task 2 includes the following deliverables:

- D03 Specified laboratory programme (IFL, due after month 3, completed month 12)
- D04 Reference methods ready to use in the project partner labs (IFL, due after month 5, completed month 12)

The deliverables D03 and D04 were enclosed in the midterm report as Annex A3 and A4. The delay in completing D03 and D04 could be explained because it was considered necessary to carry out the pre-trials in all the laboratories and use the results to help defining the laboratory programme. The pre-trials were also necessary to make sure that the selected reference methods were comparable and efficient in monitoring spoilage changes of smoked salmon.

Milestones:
Work package A includes Milestone M01, which could be completed with 2 month delay in August 2004. Delay mainly was due to date of kick-off meeting in middle of February compared to official project start on 1st January 2003.

- M01 Detailed concept for E-Nose development including reference analysis (due after month 5)

This milestone is closely related to Deliverable D01 and D02 as the instrument functionalities were described to achieve prototype development. The concept includes:

- E-Nose hardware,
- Simplified QC software,
- Calibration procedure in order to get reliable results by verifying/calibrating the instrumentation when using a calibration cocktail.

During Task 2, the established laboratory program (Deliverable D03 and D04) allows to define and discriminate smoked fish quality criteria and to correlate E-nose results with reference method thus defining instrument performances.

Task has been completed

2.3.3 Task 3: Detection of key components and development of a standard

Start date: Month 1
Completion date: Month 5
Current Status: completed

Partners involved, including total and devoted person months:

Task 3													
Task leader	B3												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	-	-	-	0,5	1	1	-	1	-	1	3	5	12,5
MM devoted	-	-	-	0,5	1	1	-	1	-	1	2,8	5,2	12,5

Objectives:
This investigation will be carried out with Headspace-GC-MS whereby specific measurement methods have to be developed and calibrated for this purpose. Since several hundreds of substances are expected to occur (depending on the type of smoked fish and the smoking process) it will be necessary to identify the most predominant and characteristic volatile organic components (VOC's) in order to obtain a set of characteristic key components for quality and spoilage of smoked fish. IFL has the most experience in this area and will lead this task but will be supported by TTZ and MATFORSK.
For the training of the pattern recognition system and as standard for prototype testing at end-user sites, a standard cocktail of characteristic spoilage products is necessary.

Methodology and study material:
Gas chromatography-mass spectrometry will be used to identify the key volatiles that are most abundant in the headspace of the smoked product. This is important for selection of the right type of sensors for the smoked fish products and for the training of the pattern recognition system.
Possible spoilage indicators are for example microbial produced volatile compounds like ethanol, 3-methyl-1-butanol and 2-methyl-2-butenal. Characteristic products of the smoking process are described to be formaldehyde and acetaldehyde, phenol, guaiacol and 2,6-dimethoxyphenol among hundreds of other individual components of wood smoke (Baltes, Springer 1995).
IFL and MATFORSK have GC instruments and similar methodologies. Identification of the predominant 15 characteristic VOC's for fish quality and spoilage in smoked products will be done. Technologically, the focus will be on sampling methods based on static headspace sampling. Sampling is very critical and it is important to investigate the effect of different sampling conditions (i.e. temperature, static vs. dynamic system, pre-concentration techniques etc.) on the composition of the headspace sampling. Both gas-tight syringes and TENAX traps could be used to collect the headspace for GC analysis. IFL and MATFORSK use different GC-MS standard protocols. Therefore

they will co-operate in system-modulation, being suitable for determination and identification of the samples' key compounds.
IFL, assisted by TTZ, will generate the standard mixture of key-compounds, correlation with the quality of real sample-materials, according to the GC-MS results. IFL will perform stability tests of the standard-mix. The evaluation of the characteristics and stability tests of the standard cocktail will be carried out in collaboration with ALPHA and TTZ during prototype testing and the results will be reported in the final report.

Task 3 comprises the following subtasks:
Subtask 3.1 Determination of key compounds for quality and spoilage of smoked fish via GC-MS
Subtask 3.2 Preparation of a defined standard for pattern recognition training and calibration of the E-Nose prototype

Progress and results during the project running time:

Subtask 3.1 Determination of key compounds for quality and spoilage of smoked fish via GC-MS

Selection and optimisation of methods for GC analysis of the smoked salmon volatiles

a) Different sampling methods were tried for collection of volatiles prior to analysis by GC and detection by GC-MS and GC-O. These methods at include:
   - Air pump sampling – Pre-concentration on TENAX
     - Based on sweeping the volatiles from the surface of the fillet in a closed sampling vial (2,3L).
   - Purge and trap sampling on TENAX
     - Salmon fillets mixed with NaCl aqueous solution and volatiles collected by purging through the mixture.
   - SPME sampling (Supelco, Bellefonte, PA, USA)
     - was also tried but was not sensitive enough

   The purge and trap sampling on Tenax was most sensitive and Matforsk used the same procedure.

b) Analysis of volatile compounds by GC-O and GC-MS using a purge and trap TENAX method was done for all samples during the storage study at IFL and selected samples from TTZ and MATFORSK. GC-MS analysis was also done by MATFORSK for samples from the Norwegian SME's. Samples from the storage studies at MATFORSK and TTZ were transported in a similar way to IFL for the GC-MS measurements carried out at IFL in November and December 2003

Analysis of key components in smoked salmon - results from the storage trials
About 35 characteristic volatiles belonging to different classes of compounds (acids, alcohols, aldehydes, ketones, esters, cyclic compounds, furan and pyran derivatives and methoxyphenol derivatives) were identified and selected as potential key compounds for the standard cocktail.
Quantification of the most abundant components detected in smoked salmon showed that compounds developed because of microbial growth are present in lower concentrations than compounds derived from the smoking process. Among these microbial derived compounds short chain alcohols, aldehydes and ketones were present in detectable amounts (e.g. ethanol, 3-methyl butanal, and 3-hydroxy-2 butanone). Some of the compounds derived from lipid oxidation were present in low concentrations (i.e. hexanal, 2,4-heptadienal, 2,6-nonadienal giving rancid, green like odors). They may however have high odour impact because of their low odor thresholds.
Less volatile compounds with characteristic smoke and burnt odours were most dominant in the headspace of the samples. Strong smokehouse-like odour together with smoke-like, wood and ash eluting close each other from the GC column, were detected in all samples. Guaiacol (2-methoxy phenol) was identified as the main compound contributing to this smokehouse odour.
Based on these results recommendations for the selection of standard compounds to be used to test the performance of the electronic nose for monitoring smoked salmon quality can be given.

The selection criteria for the standard cocktail were based on:

- compounds detected in the highest concentration by GC-MS
- compounds having a high odour impact by GC-O

but also keeping in mind the origin of the compounds and the processes involved in their formation.

The main classes of compounds identified in all samples are the same, but some variation in the identity of indvidual compounds within each class. Smoke related compounds were present in the highest amount in each sample and the compounds present because of microbial growth were in much lower concentrations.

Subtask 3.2 Preparation of a defined standard for pattern recognition training and calibration of the E-Nose prototype

Development of the standard cocktail:
Choices of potential compounds suitable as standard have been identified and were presented at the midterm meeting in Bremerhaven. According to stability and reliability it was suggested by IFL, TTZ and MATFORSK, not to use them as a cocktail mixture but single-wise. This will be confirmed in stability tests during laboratory testing in the next months Besides, commercial liquid smoke was suggested to be used for the prototype testing. From these selection criteria it is recommended that the following compounds will be used for the standard cocktail:

- Furfural
- 2-methoxy phenol (guaiacol)
- Ethanol
- 2-butanone
- 3-hydroxy-2-butanone
- Hexanal

Status of Deliverables:
Task 3 includes the following deliverables:

- D05 List of characteristic key compounds for spoilage of smoked fish (complete and delivered with 12 months report)
- D06 Standard cocktail of selected compounds for training of the pattern recognition system and calibration of the developed E-Nose system (delivered with 12 months report – will be updated during prototype testing)

The deliverables D05 and D06 were enclosed to the midterm report as Annex A5 and A6. The delay in completing D06 can be explained because it was considered necessary to carry out the pre-trials in all the laboratories and use the results to identify the key compounds to characterise smoked salmon.

Task has been completed

2.3.4 Task 4: Design of the gas sampler

Start date: Month 3
Completion date: Month 10
Current Status: completed

Partners involved, including total and devoted person months:

Task 4													
Task leader	A9												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	1	-	-	-	-	-	-	-	5	3	2	1	12
MM devoted	1	-	-	-	-	-	-	-	5	3	0,3	1	10,3

Objectives:
The design of a cheap but precise gas-sampling unit is one of the most challenging parts of the development. The gas sampler to be developed has to assure a reproducible quality of the gas sample independent of the surrounding conditions at affordable costs. Therefore different designs will be developed and tested with the standard cocktail (D06).

Methodology and study material:
OPTOTEK, with the support of ALPHA and TTZ, will be responsible for the design of the gas-sampling system. IFL and MATFORSK will assist according to their experience of sampling conditions of GC-MS measurements in task 3.
A applicable collection of commercially available gas-controlling-systems (probably 5-8 different systems) will be selected and integrated into a prototype gas-sampling unit. The design depends on different variables such, for example, as desired flow rate, sample volume (depending e.g. on the results of task 3), temperature, humidity etc. It comprises the design of the opening where the gaseous phase is sucked into the sensor head and the sensor head in the housing itself. The diameter of the opening must be optimised with regard to the intake behaviour of the sensor housing and the wetting behaviour of the gaseous substances selected.
The designs obtained will be characterised according to their price, their robustness, their reliability and their potential to be adapted to the sensor unit, developed in task 5.
By using calibrated flow-measuring devices at inlet and outlet of the sampling units, TTZ will be involved in validation. Test-Series and long running assays at different conditions (temperature, humidity, simulated industrial conditions...) will be performed. The most promising sampling design will be chosen for further testing. OPTOTEK will be responsible for the housing of the gas sampler and, with support of ALPHA, for the connection to the sensor unit.
Since a commercially available gas-controlling device is intended to be integrated into the gas-sampler prototype, “other specific costs” are assessed for OPTOTEK in the project’s budget.

Progress and results during the project running time:
OPTOTEK surveyed available gas sampling and concentration techniques. Several standards, articles, applications, methods and existing state-of-the-art of the gas sampling techniques have been surveyed. To ensure the compatibility at the hardware interfaces close the effort was coordinated with ALPHA and TTZ. Basic methods of gas sampling were evaluated: a gas syringe, a gas sampling valve, and a pneumatic device.
Gas sampling method with the 6 port sampling valve was chosen for the FishNose application. Flow scheme is presented in figure 3.
Most important benefits of this method are:

- it requires small pressure differential between the process in and the process out,
- simple, safe and quick operation,
- low-cost.

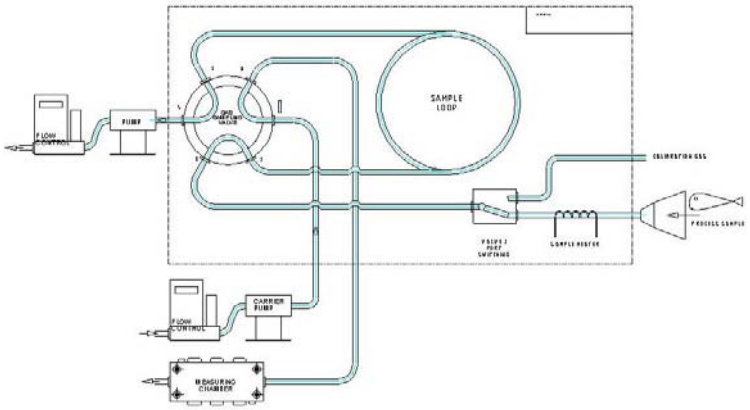


Fig. 3: Design of the gas sampling unit and connections to the rest of FishNose

Having chosen the method, we designed the sampling unit. Drawings for measuring chamber, heater and other plumbing were made. Special components for the unit were purchased. After finishing the design, a model of the suggested sampling unit was assembled and basics of the method were tested on it. The design was confirmed on the 6 month meeting in Alesund, Norway.
A prototype of the optimized gas sampling unit was designed and produced. The design is characterized by the reliability of the components, their robustness, ease of procurement and adaptability when assembled into the sampling unit. The connection to the sensor unit were coordinated with ALPHA-MOS. A commercial Valco six-port valve was used for the injection of the sample into the sensor unit. As planned and according to the Technical Annex, the prototype of the unit was finished and supplied as a deliverable D07 with all appropriate documentation at end of October 2003 to ALPHA-MOS for connection to the sensor unit.

The prototype sampling unit consists of following main sub-units:

- VALCO 6 port valve with electronics box,
- transformer,
- Optotek driving electronics,
- Optotek control electronics ,
- main power supply ,
- heated inlet tube,
- accessories and documentation.

All components are mounted on a metal plate. Sample inlets and pump connectors are on the top side of the plate, inlet / output are fed through the plate down toward the ALHA-MOS sensors. Electrical connections are 230VAC and signal cable to the ALHA-MOS computer. All tubes and fittings are made of seamless stainless steel type 316. All connectors are original from Valco.

1. **VALCO 6 port valve with electronics box (Figures 4 and 5) –** original electronics has been modified. Electronics drives the motor of the valve and gives the signal of the current valve position. Electronics has two inputs: 115VAC/50Hz and +12VDC. For the final version (serial production) main voltage will be 230VAC only, without a need of a +12VDC. Flow inlets of the box are: sample inlet, calibration inlet, pump inlet, column outlet, carrier inlet.
   Two different sample loops were supplied with the unit: 10ml (Figure 1) and 20ml (Figure 4). These two can be exchanged at will. Other loops could be used as well. Valco, the producer of the loops used, offers loops from 10 µl to 20 ml. As long as the fittings are standard one could use any loop volume, constrained only by the dimensions of the valve box.
   The valve itself is installed inside the heat insulated plastic enclosure, its electronics is located outside in the original metal enclosure.
2. **Transformer (Figure 5) –** transforms main voltage 230VAC to 115VAC needed for operation of the Valco box. For the final version (serial production) this transformer will not be needed.
3. **Optotek driving electronics (Figures 5 and 6)** is connected to Valco box via 6 pin. Electronics optical isolate control signals to / from the computer. It also drives the **2 port valve** used for switching the inlet from the sample to the calibration input. It also contains a **heater**, used to stabilize the temperature of the box, containing all valves and flow components to 55°C.
4. **Optotek control electronics –** is used for testing the system. It will be replaced by the ALHA-MOS computer.
5. **Main power supply (Figure 5) –** 230VAC to +12V switching power supply of 110W is used. It supplies all the electronics of the device.
6. **Heated inlet tube (Figures 8, 9 and 10) –** 230VAC/40W self regulating heater cable is twisted around the inlet tube. It heats the tube to 55°C. All is isolated with an Armaflex tube.
7. **Accessories and documentation –** extra stainless tubes, connectors and fittings are supplied, to allow ALHA-MOS to connect the sampling unit to their equipment. Technical documentation is supplied together with the prototype to provide technical information for installation and use.

The gas sampler was subjected to a number of tests. We tested the 6 port valve (switching between different positions, seals) and sampling with the device heated to 55 degrees C. We used two pumps to perform these tests. We also tested the 2 port valve for the switching between the sample and calibration gas. We tested the heating of the input tube and it also reached the 55 C, all in a lab at room temperature. With these tests the operation of electronics (valve position sensors, drivers) was tested as well.

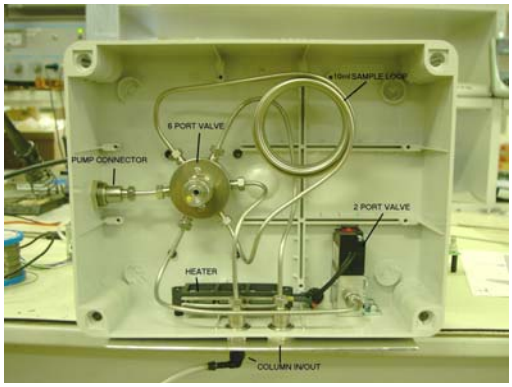


Fig. 4: Valve enclosure

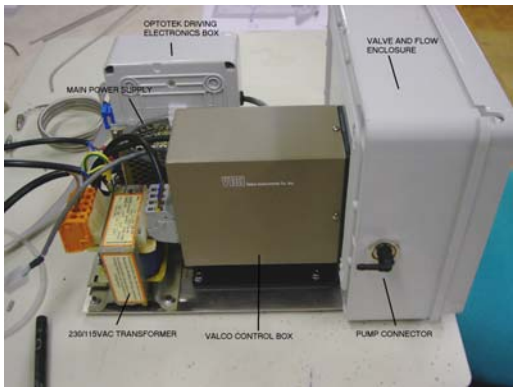


Fig. 5: Right side view

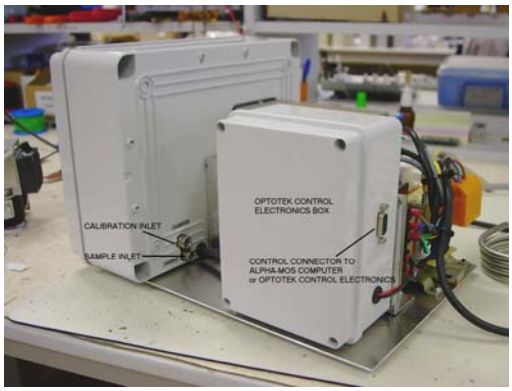


Fig. 6: Left side view

***Erratum**: "Optotek Control Electronics box" should be "Optotek Driving Electronics box"*

Fig. 7: 20ml sample loop installed

Fig. 8: Bottom view

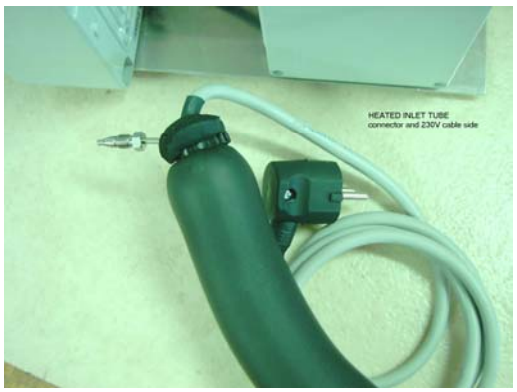


Fig. 9: Heated inlet tube

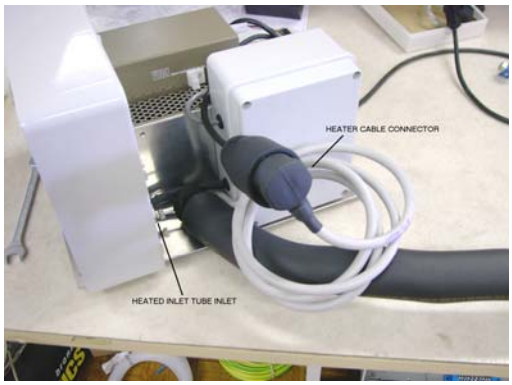


Fig. 10: Heated inlet tube connection

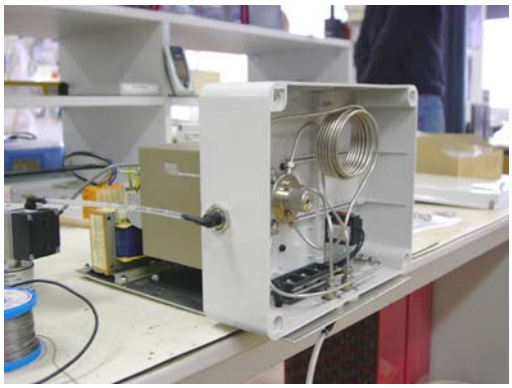


Fig. 11: Testing

Status of Deliverables:
Task 4 includes the following deliverable:

- D07 Optimised gas sampler (OPTOTEK, due after month 9)

Deliverables D07 has been completed in time in September 2003. The developed and established gas-sampling-unit prototype has been supplied to ALPHA-MOS. Corresponding specification is included in this report as Annex A7.

Task has been completed.

2.3.5 Task 5: Sensor design

Start date: Month 2
Completion date: Month 11
Current Status: completed

Partners involved, including total and devoted person months:

Task 5													
Task leader	A1												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	3	-	-	-	-	-	-	-	-	3	2	1	9
MM devoted	3,25	-	-	-	-	-	-	-	-	3	3,4	0,4	10,05

Objectives:
For large-scale applications and industrial projects, a key criterion will be sensor robustness and cost. Different gas-sensor technologies to be considered for the application are quarts microbalance (QMB) sensors, conducting polymers (CP), metal oxide semiconductor sensors (MOS). Alpha would assess mainly a metal oxide sensing platform. The aim is to develop gas-sensitive resistors which, when used in an array to address a complex vapour measurement problem, maximise the variance across the array, and this in a stable, reliable and repeatable fashion.
This development work will require considerable effort from ALPHA, TTZ, MATFORSK and IFL concerning in particular the signal processing.

Methodology and study material:
Based on the characterisation of the volatile key quality compounds identified by headspace GC-MS (Task 3) and end-user specifications (Task 1), a selection of the suitable sensor technology regarding selectivity, sensitivity, stability and robustness will be chosen. Since metal oxide sensors are robust and have a broad selectivity, they are especially qualified for detecting single compounds in the complex mixture and varying background of smoked fish gas samples under industrial conditions.
ALPHA and MATFORSK, with support of TTZ, will select and test the appropriate sensors. The approach is based not on the development of completely new sensor materials for such a task, with all the consequent uncertainties, but aims for a novel combination of known materials, sensor fabrication, device control and signal processing. The chosen approach will permit fabrication of sensors, which can be chosen *a-priori* for a measurement task, to produce an array, which is optimised for the fish quality assessment. Furthermore sensor housing and materials, sensor size and geometry will be selected for manufacture.
ALPHA is well experienced in this area and will lead the task. Together with MATFORSK the sensor materials as well as the electronic module will be selected with regard to long service life, durability and cost. Thereby IFL will deliver support concerning their experience and the results of key compound identification and characterisation in Task 3.
The final prototype will be linked by ALPA and MATFORSK to the gas sampler of Task 4 in order to allow validation and assessment of the system as total by the use of the standard-mixture (D06). This will be performed by TTZ.
Since commercially available sensor devices are intended to be integrated into the prototype, “other specific costs” are assessed for ALPHA in the project’s budget.

Task 5 comprises the following subtasks:

- Subtask 5.1 Sensor selection
- Subtask 5.2 Design of sensor array
- Subtask 5.3 System integration and validation regarding the standard-mixture

Progress and results during the project running time:

For large scale and industrial application a key criteria is sensor robustness and cost. Therefore Metal Oxide sensor technology was selected for the intended application as sensors have long lifetime (>5 years) and sensitivity/selectivity can be tuned by changing sensing materials and dopants and by modifying operating conditions.

In order to test a high number of sensors, it was decided to use a laboratory instrument comprises of FOX system and headspace autosampler HS100. This system is the Research & Development platform and incorporates up to 18 different sensors. Sensor diversity is achieved through different materials (SnO2, WO3, Cr2-x-TiO3+y…), different level of dopants (Pd, Pt) and different operating temperatures. Temperature modulation changes sensor selectivity. Depending upon target molecules, appropriate choice of operating conditions is achieved. Several key compounds relative to spoilage have been identified during task 3.

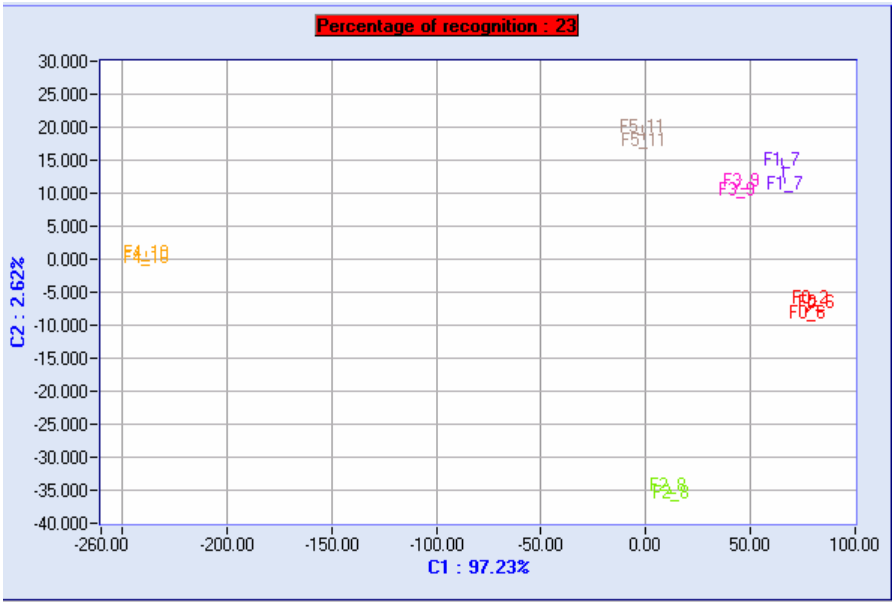
38 samples provided by End-users partners have been qualified by reference analysis during storage trials by RTD Partners (GC/MS, physical-chemical parameters, microbiology, sensory panel). For sensor selection all samples have been analysed by the FOX instrument. The aim was to test several sensor materials and operating conditions so as to select the appropriate array.

Used analysis parameters are listed in following table 6. The sensor responses are presented in figures 12-15.

Tab. 6: Parameters for sensor selection (FOX)

Carrier Gaz		**Injection**	
Carrier Gaz	Air TOC – Dry air	Injected volume	500 µl
Flow	150 ml/mn	Injection speed	500 µl/sec.
		Syringe	1 ml
Headspace generation		Syringe temperature	85 °C
Time	600 sec.	Flushing	120 sec
Temperature	80°C		
Agitation speed	500 rpm		
Sample preparation		**Acquisition**	
Sample volume	1 ml	Time	120 sec.
Vial	10 ml	Flushing	0.5 sec.

Samples F0-F5 from TTZ:
Sensors selected : LY2/LG, LY2/AA, LY2/Gh and PA2



Results from the electronic nose (F0-F5)

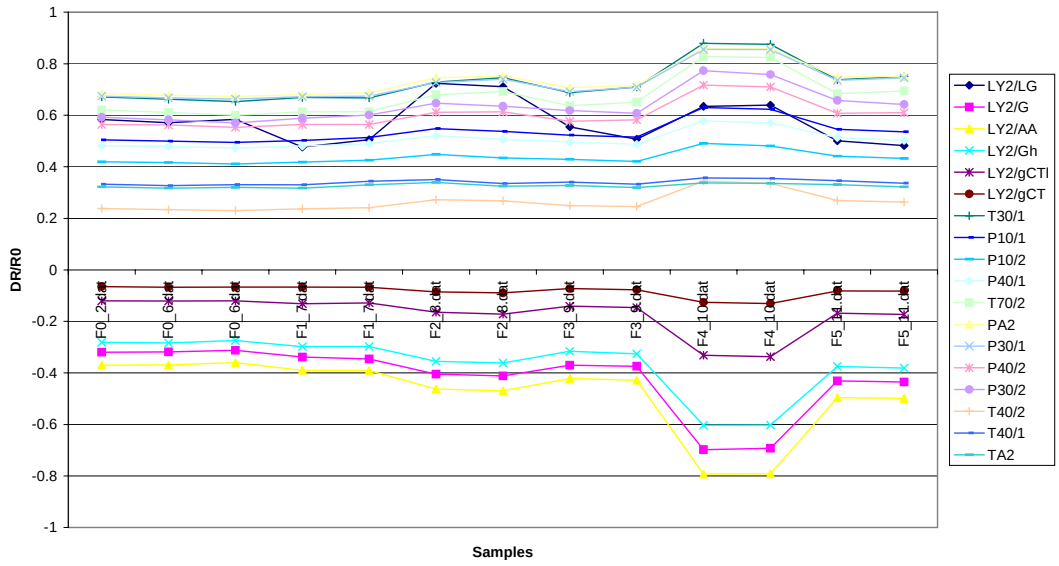
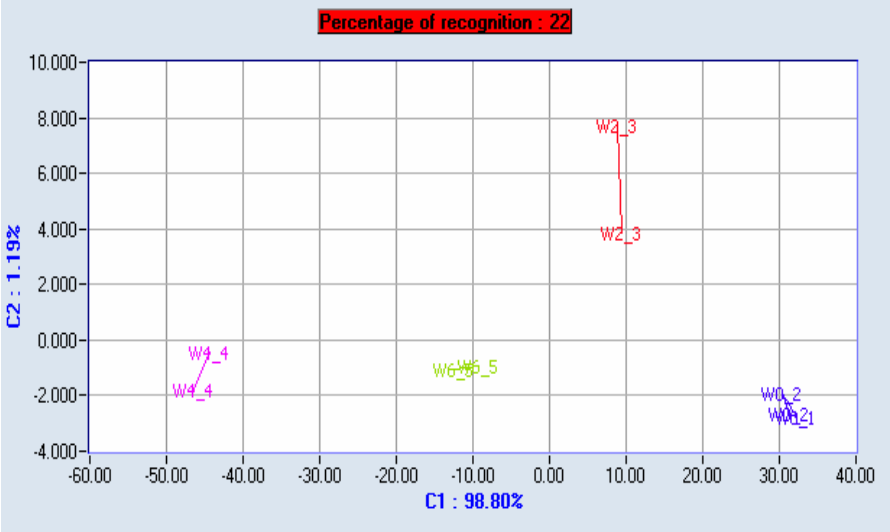


Fig. 12 a/b: Sensor response according to Fiedler / TTZ samples

Samples W0-W6 from IFL:
Sensors selected : LY2/LG, LY2/G and PA2



Results from the electronic nose (W0-W6)

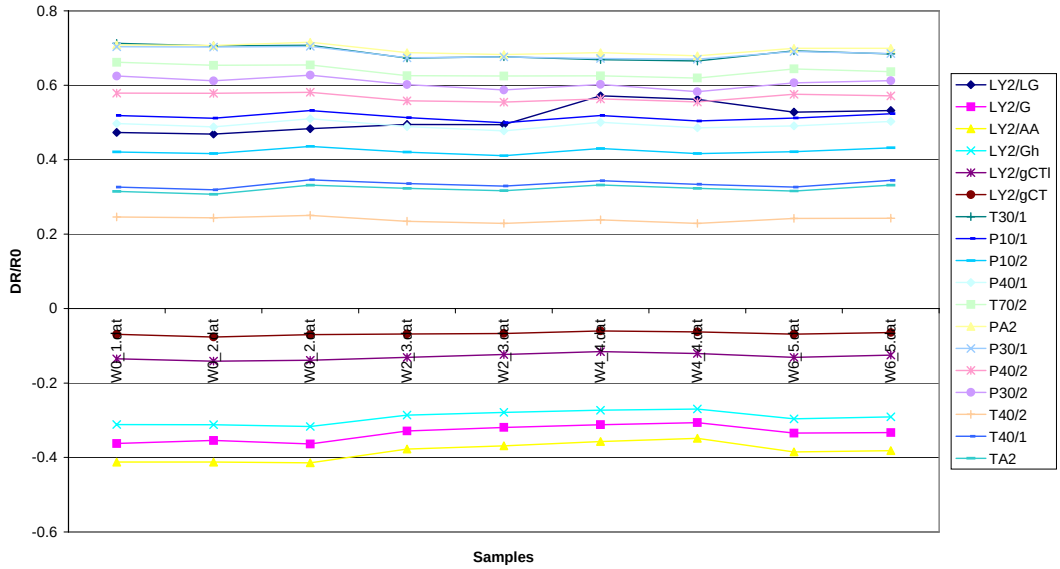
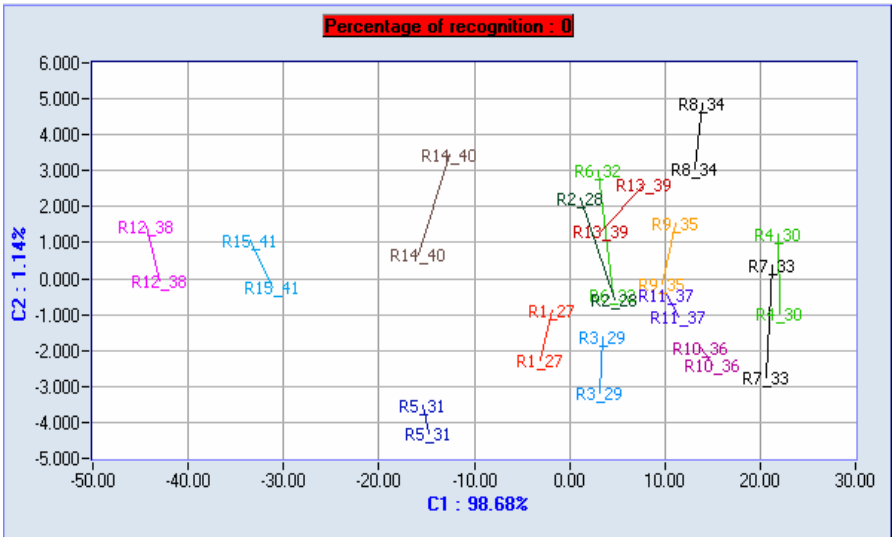


Fig. 13 a/b: Sensor response according to REYKO /IFL samples

Samples R1 to R15 from MATFORSK
Sensors selected : LY2/G and P40/1



Results from the electronic nose (R1- R15)

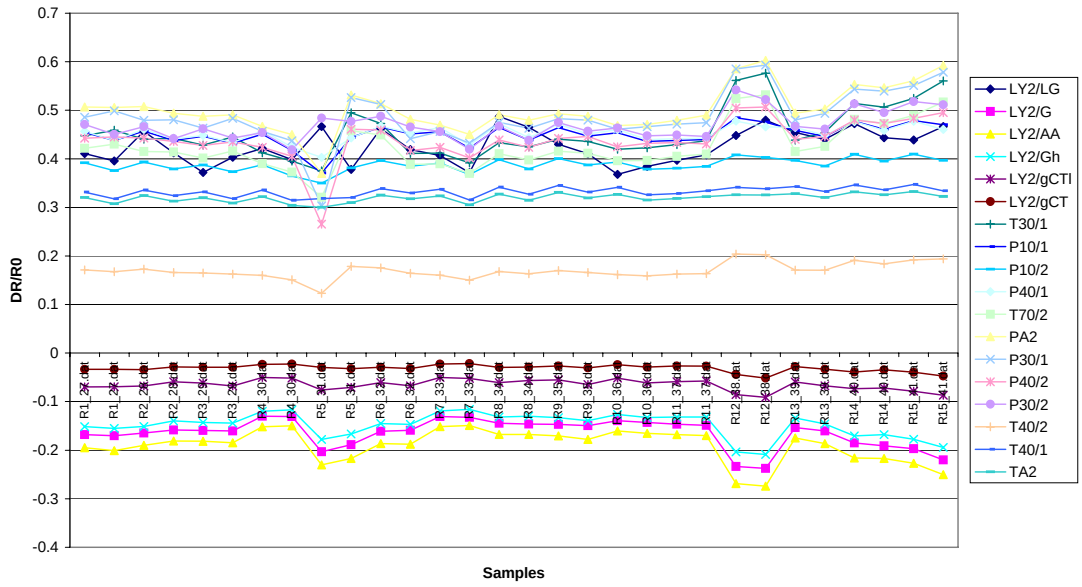
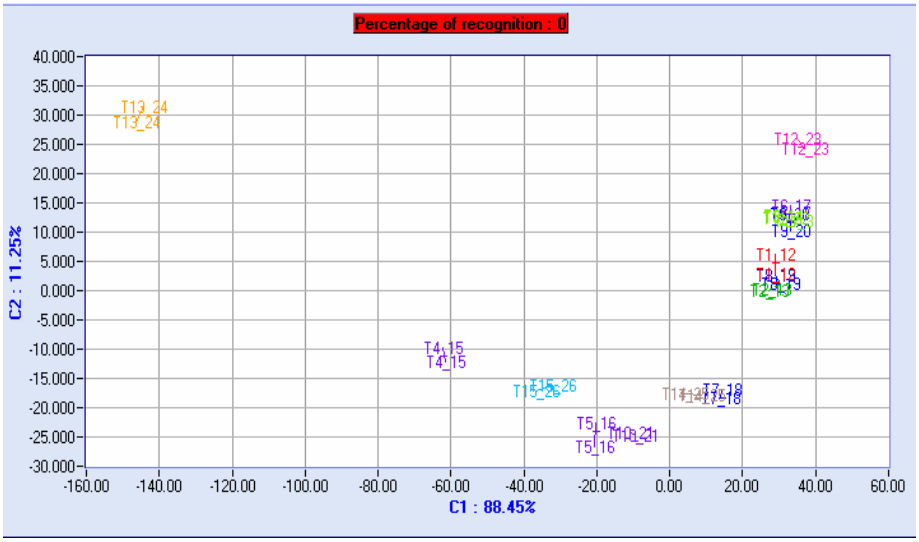


Fig. 14 a/b: Sensor response according to REMO / MATFORSK samples

Samples : T1 to T15 from MATFORSK
Sensors selected : LY2/Gh, LY2/gCTI, LY2/gCT and PA2



Results from the electronic nose (T1-T15)

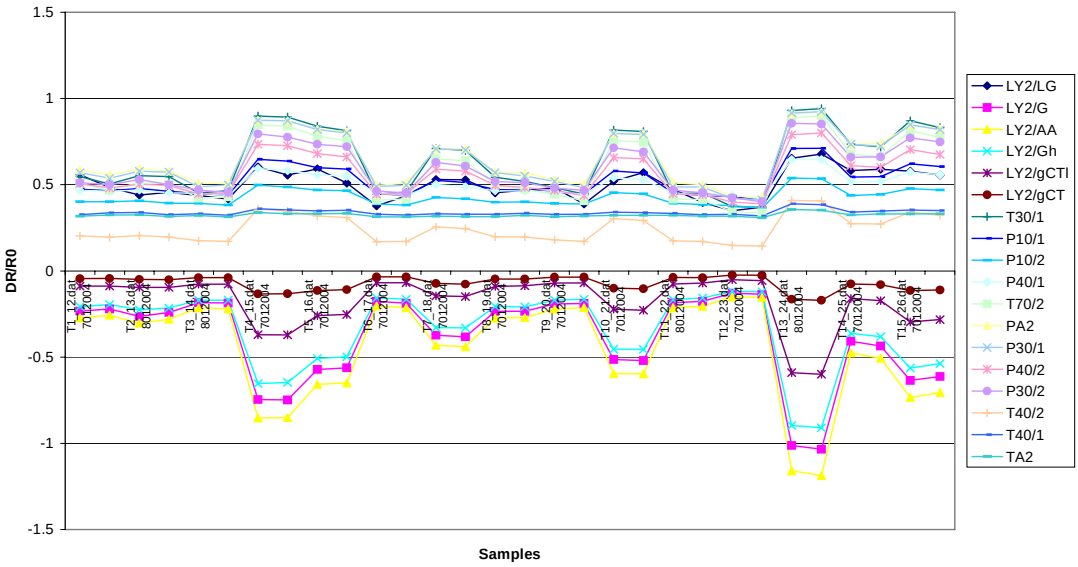


Fig. 15 a/b: Sensor response according to TBB / MATFORSK samples

Correlation of all sensor-responses and reference analysis results have been performed with assistance of the RTD partners. Generated bad samples successfully were discriminated by the FOX-Sensors with Headspace sampling technique at 80°C. Results show sensor sentivities to different samples and a discriminant function analysis is displayed for each samples group.
The 6 most discriminating metal oxide sensors were selected and integration into a sensor housing (ALPHA GEMINI-System). A picture of the developed sensor module, representing the Deliverable D08 (see Annex A8), is shown in figure 16.

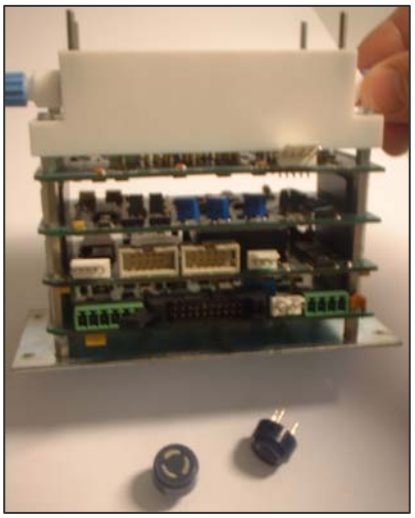


Fig. 16: Sensor Module of FishNose

First testing of the established Sensor Unit showed same discriminant response and identification of good/bad smoked fish quality as the previous used and more complex FOX system. Also reduction of sampling temperature until 5°C was successful: with little loss of sensitivity same bad samples could be identified (see Annex A8).
Overall, the generation and first evaluation of the sensor unit led to promising results. The OPTOTEK gas sampling device has been connected to sensor module onto a single equipment to generate the Deliverable D09 (Annex A9). The established Sensor Prototype will be tested, optimised and validated in laboratory and on-site in Tasks 7 and 8 with new set of salmon samples during the second year.

Status of Deliverables:
Task 5 includes the following deliverables:

- D08 Manufactured sensor array prototype (hardware) (ALPHA, due after month 9)
- D09 FishNose prototype with specification regarding the standard-mixture (ALPHA, due after month 11)

Sensor Array Prototype and FishNose prototype were **completed in January 2003** and available for laboratory testing. D08 and D09 are delivered as Annex A8 and A9 to this report.

Task has been completed.

2.3.6 Task 6: Software design

Start date: Month 2
Completion date: Month 12
Current Status: completed
Partners involved, including total and devoted person months:

Task 6													
Task leader	B2												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	2	0,25	0,25	0,25	0,5	0,25	0,5	0,25	-	2	3	1	10,25
MM devoted	2,15	0,25	0,25	0,25	0,5	0,25	0,5	0,25	-	2	3	0	9,4

Objectives:
The software to be developed determines the degree of automation and simplicity versus variability of the system. It is expected that SME end-users who process only a moderate volume of fish or carry out quality monitoring for incoming inspection will prefer simplicity in handling and design, e.g. with three fixed measuring procedures installed. However, end-users with a wide variety of products (e.g. trout, salmon, eel etc.) and skilled personnel are expected to prefer a variable instrument with the option to adapt it individually according to their changing demands.

Methodology and study material:
The software will be developed by ALPHA and MATFORSK and execute the pattern recognition, classification algorithms and user interface. As basis input to this task, existing ALPHA-MOS software package will be used. This software platform will be modified to incorporate sensor modelling and compensation algorithm developed as modules by MATFORSK. The user interface comprises data acquisition and result presentation according market needs and will be defined with additional assistance of TTZ.
A specific algorithm has to be developed which combines maximum information content with a user-friendly format. The FishNose will be a self-training system, meaning that the software has not only to process the data of the current sample tested but also to compare it against data obtained by analytical reference methods. The preferred format for the graphic user interface will be Windows 9X/NT, the export of original data into a common Windows application, such as EXCEL, is supported.
One to four measuring procedures for the most common smoked fish products (e.g. salmon) are programmed as standard procedures. As an option, the software should be easily adaptable to individual measuring procedures by the end-users themselves.
All end-user SME's in the consortium are actively involved in the development of the software with regards to the definition of the performance criteria, stability and reliability as well as the user-friendliness since the FishNose is not intended to be usable only by academics. The RTD performers TTZ, MATFORSK and IFL thereby will act as interface to collect and filter the information and focus on the demands of FIEDLER, ARMORIC, REYKO, ANFACO, REMO, RÜGEN-F and TBB. Besides the group of fish smokers where 1-4 fixed applications will be defined according to their product range, ANFACO and RÜGEN-F will have special demands that have to be investigated and defined in detail: ANFACO according to flexibility for service application and RÜGEN-F according to different applications in the field of incoming inspection. Thereby TTZ will be involved in describing demands of an suitable user-interface structure.

Task 6 comprise the following subtasks:

- Subtask 6.1 Development of the pattern recognition and compensation software
- Subtask 6.2 Development of the user interface

Progress and results during the project running time:

Task 6.1 Development of the pattern recognition and compensation software:

MATFORSK and ALPHA with support of TTZ developed the software system for the FishNose sensor. Working meetings took place in Toulouse for analysis of the actual ALPHA-MOS software and strategy development.

Results of correlation analysis of sensor responses and reference analysis for pattern recognition is given in detail in Annex A10. It was difficult to combine the sensory data from different panels in the different labs due to different use of scales. For future trials this will be avoided by the use of only one sensory panel at IFL (see Task 2).

Accepted total microbial count in terms of log (cfu) according to guidelines for smoked salmon was chosen as the criterion in these analyses. However, the sensory attributes off-odour and rancid odour were also taken into account in interpretation of the classification.

GOOD/BAD classification is anticipated. Thereby good quality will be defined during training phase. Bad samples are defined to be outside this class. Thereby it is essential that bad samples definitely not will be classified as good.

Different methods for pattern recognition have been tested. The SIMCA model based on local class for the “good” samples seemed to be the best choice for the FishNose applications. 85% correct classification was achieved for a combined model on samples from the four producers REMO, TBB, FIEDLER and REYKO.

Task 6.2 Development of the user interface

Data will be stored in an ACCESS data base. As main interface (D11) 3 access levels are available:
certified personnel (configuration of soft- and hardware)
training personnel (training, calibration of the Sensor)
normal operator for routine analysis

Sensor stability is handled by periodically measuring internal reference compounds. Other main features include selection of sensors, training of models for classification for different producers/product groups, statistical quality control and analysis reports for new samples to be classified.

Status of Deliverables:

Task 6 includes the following deliverables:

- D10 Pattern recognition and compensation software (MATFORSK, due after month 12)
- D11 User-friendly interface and control software (MATFORSK, due after month 12)

Deliverables D10 and D11 are completed in time and reported in Annexes A10 and A11.

Milestones:

Work package B includes one milestone which could be completed in time in January 2004:

- M02 FishNose prototype consisting of gas sampling and sensor array system and developed Software (due after month 12)

This milestone is closely related to Deliverables D07-D11 (Annex A07-A11), representing the single needed components. The FishNose prototype was generated by connecting developed Gas-Sampling Unit, Sensor Array and Software as presented in figure 17. It will be tested and optimised in WPC during the second project year.

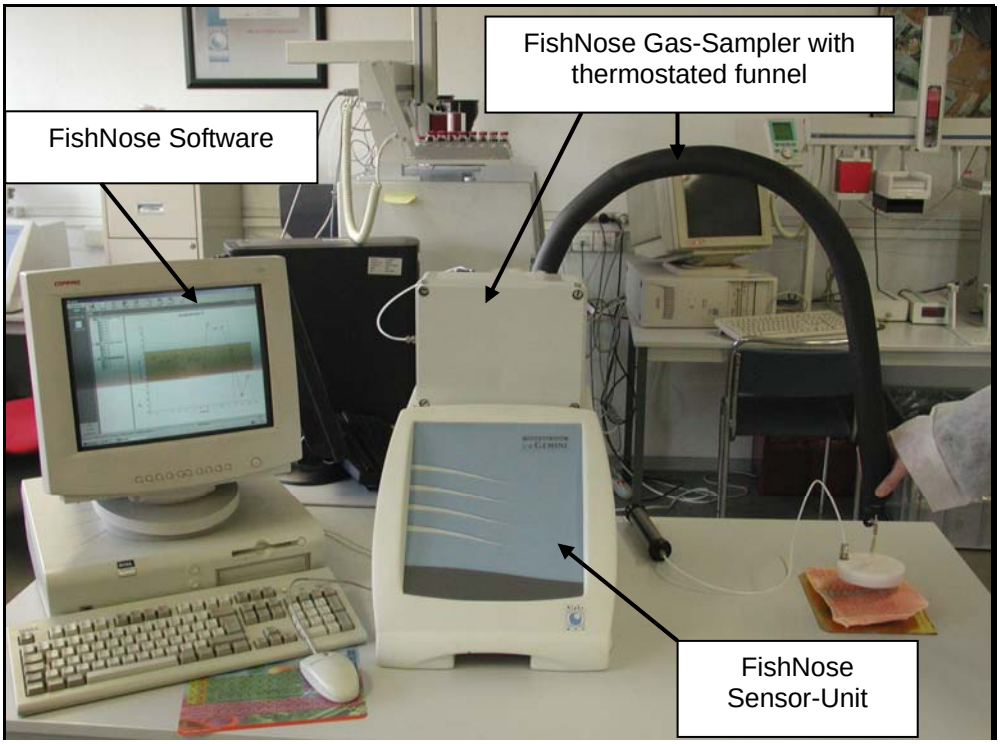


Fig. 17: FishNose prototype

Task has been completed.

WPC: Prototype Testing and Optimisation

2.3.7 Task 7: Prototype laboratory tests

Start date: Month 5
Completion date: Month 16
Current Status: in progress

Partners involved, including total and devoted person months:

Task 7													
Task leader	B2												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	1	-	-	-	2	-	-	-	0,5	5	3	3	14,5
MM devoted	3	-	-	-	2,2	-	-	-	0,5	5	4	6,4	21,1

Objectives:
After assembling in WP B, the FishNose prototype will be tested by means of a comprehensive laboratory test programme. This includes a basic functional testing of the FishNose, comparative measurements with analytical reference methods (see Task 2), and the optimisation of the software developed. It is the objective to have an optimised prototype at the end of this task ready to be tested under real production conditions

Methodology and study material:
ALPHA will deliver the FishNose prototype to the RTD laboratories and ANFACO for calibration and fine-tuning. Thereby the prototype will be forwarded form one partner to the next, starting at MATFORSK followed by IFL and TTZ after 2 month stay at each RTD. Finally laboratory tests will be performed at ANFACO concentrating on the prototype optimisation before transition to on-site tests described in Task 8.
Overall, MATFORSK will be the partner responsible for the testing and training of the FishNose with support of TTZ and IFL. The complete instrument with sensor arrays and gas sampling module will be tested with the standard cocktail (Task 3) and real smoked fish samples for the following parameters:

- Long-term stability
- Accuracy and sensitivity relative to alternative testing methods
- Consistency and reliability of the result

Thereby support of OPTOEK and ALPHA will be performed concerning optimisation of the gas-sampling unit. TTZ and MATFORSK will optimise the software.
FIEDLER, ARMORIC, REYKO, REMO, RÜGEN-F and TBB each will provide 20 samples of 5 different quality categories.
Besides IFL, TTZ, MATFORSK and ANFACO provide established analytical reference methods according to the analytical test programme (task 2). Correlation of sensor results to reference method results will be performed by TTZ.
The results of optimisation experiments under lab conditions will be summarised by the RTD partners MATFORSK, TTZ and IFL by generation of specification. Concluding from this, TTZ will design evaluation test formats and protocol-schemes for the final on-site tests in Task 8.

Progress and results during the project running time:
The prototype laboratory tests have been planned in detail regarding the harmonisation of the reference analysis program (Task 2, D03) and the experimental design and timing of second round with storage experiment for the laboratory testing on new smoked fish samples. Planning discussion have been performed by RTD partners at the 6 months meeting (Alesund, Norway), technical meeting (Toulouse, France) and midterm meeting (Bremerhaven, Germany) and via e-mail. The SME's have also been involved in the discussion of selection of samples and sample delivery.

The prototype optimisation has been performed by ALPHA in collaboration with the RTD's and will be finalised within time. In addition, specifications of lab experiments have been discussed at the midterm meeting and will also be finalised within time. Accordingly, the deliverable D12, Optimised prototype with specification of lab experiments, is in good progress and will be finalised in due time (14 months).
Test formats and protocol schemes for laboratory testing of prototype sensor system (D13) are partly finalised and will be delivered together with D12. In the context of discussion about the prototype testing at the midterm meeting, it was agreed by the partners and in presence of the project's PTA to postpone the deadline for D13 until the 18 Month Meeting / Report. With regard to the on-site testing, test formats and protocol will be finalised on the basis of the experience and optimisation gained during the prototype laboratory testing.
According to the gained results and conclusion of the first project year it was agreed, not to follow the original work-program of the Technical Annex due to lack of efficiency and time. Instead new proceedings were established by the technical partners on the technical meeting in Bremerhaven, on 12th February 2004. On the basis of the results and conclusion from Task 2 and 7 it is required that the original working plan, as stated in Task 7, with regard to the practical performance of the prototype laboratory testing needs to be revised. The motivation for this are the following:

- Despite the use of standardised and harmonised reference methodology with regard to the sensory analysis labs, a significant inconsistency could be demonstrated for the data analysis of the sensory data between the three labs and thereby also for the correlation computations between the overall sensory data and references analyses. It has therefore, during the discussions during the 6 months and midterm meeting finally been agreed that only one of the sensory laboratories, i.e. the one at the IFL will be used in connection with the prototype laboratory testing. This laboratory also was demonstrated to obtain most consistent results with regard to the sensory attributes related to quality of smoked salmon.

- According to the original working plan it was foreseen to carry out the laboratory testing of the prototype at the RTD labs. Due to the slight delay in the proceeding deliverables and the limited time left for the project, it has been discussed and agreed at the midterm meeting that the prototype will be kept at the manufacturer, ALPHA, and that the laboratory testing will be carried out at their laboratory with their skilled and dedicated staff. The reference analysis will be performed as planned at the RTD's on new samples delivered from their respective SME's. All the samples will be shipped to ALPHA for measurement with the prototype sensor system.

The justification and benefit from the revised plan will imply an enhanced efficiency of resource use, i.e. with regard to training of personal at the RTD's, which will be unnecessary, including also instrument shipment and travel costs, gain of time, and quality assurance of reference methodology, in particularly the sensory assessment.

Sample material:
A significant number, about 120, of new samples will be used for the prototype laboratory testing in order to obtain a reliable validation of the performance of the measurement system. Samples will be selected for analysis at the RTD's according to the sample distribution shown in Table 8. A few samples of fresh, unprocessed salmon samples will also be analysed to evaluate the background volatiles from the smoking process which may be distinguished from the typical spoilage volatiles.

Two sample qualities will be defined (good and bad) on the basis of the reference analysis results. The sample set will represent about 60-70 % good quality samples from three different production batches from the respective SME's. The poor quality samples will be generated during a new storage trial at the RTD-laboratories and ANFACO. Storage conditions used will be similar to the conditions used during the first round of storage experiment reported from Task 2, D03. All end-user SME's will provide sample material and are involved in the planning and performance.

Tab. 7: Sample design for the prototype laboratory testing

SME/Batch	**1***	**2**	**3**	**Total**
FIEDLER	14+2	4	4	**24**
REMO	14+2	4	4	**24**
TBB	14+2	4	4	**24**
REYKO	14+2	4	4	**24**
ARMORIC	-	4	4	**8**
RÜGEN-F	-	10**	10**	**20**
Total number of samples				**124**
*Storage trials		** Fresh samples		

Reference analyses and sampling
Results from the reference analyses (Task 2, D03 and D04) and data analysis (Task 6, D10) concluded the following reference analysis program to be used according to Task 2 (D04) during the prototype laboratory testing:

- Chemistry (Fat, Water, Salt)
- Total viable count (TVC)
- Sensory analysis with only spoilage and smoke related attributes

In addition there will be carried out GC analyses on a selected number of good and bad samples.
The revised reference analyses program on the samples as well as the FishNose validation will be performed according to Table 9.

Tab. 8: Analyses program of the RTD's and ALPHA for the prototype laboratory testing

	Sensor test	Chemical analysis	Microbial TVC/LAB	Sensory	GC
Alpha	X				
IFL		X	X	X	X
TTZ		X	X		
Matforsk		X	X		
ANFACO		X	X		

The sampling for the respective analyses will be performed according to the following figure 18:

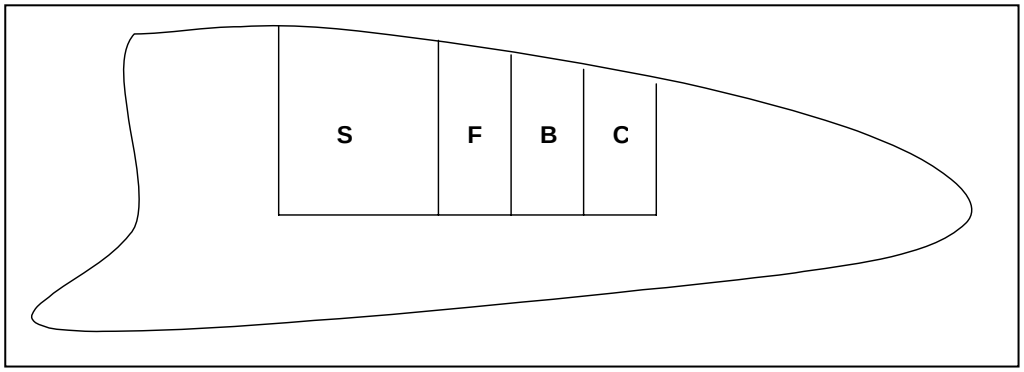


Fig. 18: Sampling location on salmon fillet for the different analyses.
S=Sensory, F=FishNose, B=Bacteria (TVC), C=chemistry.

Storage experiment:
The storage experiment will be performed with 16 new samples delivered from REMO, TBB, REYKO and FIEDLER to the RTD's. For each storage condition (time and temperature) there will be used 2 samples per SME. The samples will be stored at two temperatures up to 4 weeks according to the following scheme:

Tab. 9: Storage conditions

T in °C					
5	0	7	14	28	Days
10	0	4	7	10	Days

Alternatively, two different types of packaging - vacuum and modified atmosphere - are used.

Data analysis:
The new measurement data of both reference (RTD's) and FishNose sensor data (ALPHA) will be merged into an overall reference/calibration data base according to the previously defined standard data format (Task 6) and new predictive models generated.

Performance evaluation of the FishNose sensor system:
Accuracy, reproducibility, repeatability, sensitivity and reliability to alternative testing methods will be evaluated. Critical QA routines of instrument performance will be performed. This will be done on the basis of frequent measurement of control samples (standard cocktail of key substances and liquid smoke).

Timing:
It was decided during the midterm meeting that the start of the storage experiment at each RTD lab will be in the middle of March (18th). Sample generation and analyses with the reference methods will be performed during March/April 2004 in the respective RTD laboratories. After sample transfer, FishNose prototype tests will be performed at ALPHA during May 2004 - as well as sensory evaluation and GC on selected samples at IFL. The timetable on the actions for laboratory tests is given in Table 10.

Tab. 10: Time schedule for the prototype laboratory testing (Task 7).

Action	Responsible	Feb. 04	Mar. 04	Apr. 04	May 04	June 04
Detailed lab test planning	RTD's/ALPHA	X	X			
Delivery of samples to RTD's	SME's/RTD		X			
Storage experiment	RTD's		X	X		
Reference analysis	RTD's/ANFACO		X	X	X	
Delivery samples to ALPHA	RTD's				X	X
Delivery of samples to IFL	MATFORSK/TTZ				X	X
Sensor testing at ALPHA	ALPHA/RTD's			X	X	X
Sensory analysis	IFL				X	
Data analysis	MATFORSK/ALPHA				X	X
M03 Approved an optimised FishNose prototype	MATFORSK/RTD's				X	
Reporting 18 month meeting	RTD's/ALPHA				X	X

Data evaluation of the overall measurements from reference analyses and sensor system were performed during May/June 2004 (D12). Accordingly, the laboratory tests were finalised until 18 Month Meeting in June 2004. Based on this, detailed planning of on-site tests including test protocols were performed until June 2004 (D13).

Initial prototype testing at Alpha M.O.S

Two sampling methods have been evaluated: one based on an aluminium recipient and one with a bell. Analysis have been carried out on both grinded and full fillet samples. With these 2 methods it was observed a good discrimination oft he samples analysed and a good reproducibility on 3 repetitions.

The choice should then depend on several parameters taking into account the advantages and disadvantages specific for each sampling method.

Sampling method with aluminium recipient

- Advantages:
  - Impossible of contaminating the system between 2 analysis because there is no direct contact with the sample
  - Sensor response is more intense because the V.O.C amount is more important in the generated headspace
  - Sensors respond to V.O.C of the full filet
- Disadvantages:
  - Fillets can have different weights and then different V.O.C amount that can influence on the discrimination of the system

Sampling method with the bell

- Advantages:
  - The use of pierced aluminium paper of known diameters for analysis prevents the sensor responses from variation due to the different sample weighs. The exchange surface is constant.
- Disadvantages:
  - The pierced aluminium paper can be placed on a bad part of the sample
  - The contamination of the bell is possible if the sample is bumped- this problem can be eliminated by adapting the high of the bell to the shape of the fillets

Second round laboratory experiment

The objective of the second round with laboratory experiments was to monitor quality changes related to spoilage of smoked salmon from different producers and provide samples for the laboratory testing of the prototype FishNose sensor system that was kept at the manufacturer Alpha M.O.S.

A significant number of samples (128 samples) with different qualities and from different production batches were selected for prototype testing and optimisation in order to obtain a reliable validation of the performance of the measurement system. Moreover, samples of different quality were generated during new storage trial at the RTD-laboratories and ANFACO. Storage conditions were similar to the conditions used during the first round of storage trials performed in 2003.

All the samples were analysed using the reference measurement methods established during the first year of the project: chemical, microbiological and sensory methods. In addition, there have been carried out gas chromatographic (UC) analyses on a selected number of good and bad samples.

The results obtained are detailed in Deliverable D12 included as Annex 4 to this 18 months management report.

FishNose sensor system

Vacuum packed and frozen stored samples from the different fish producers were shipped to ALPHA by TTZ, IFL, ANFACO and MATFORSK, and analysed with the prototype sensor system. Totally 96 samples were analyses and the sampling was performed with the bell method. The results are included in D12 included as Annex 4 to this 18 months management report.

All the data obtained with the reference methods were analysed together with the obtained with the FishNose sensor. Different models have been investigated for prediction of samples of different qualities. Using single numeric criteria of separate reference parameters like TVC numbers or single sensory quality related parameters like sweet/sour dour, rancid odour or off-odour gave in general low classification rates. By using a combination of these parameters the classification rates were significantly improved, than using single reference parameters alone. The following quality criteria have been applied for the Partial Least Squares Regression (PLSR) classification modeling for accepting and rejecting samples corresponding to respectively good and bad samples:

Good/accepted samples:

TVC < 5, Off-odour < 20, rancid odour < 10, Sweet/sour odour < 20

Bad/rejected samples:

TVC > 5, Off-odour > 20, rancid odour > 10, Sweet/sour odour > 20

The global PLSR discrimination model using the sensor data from all 96 samples and the combined criteria gave the following classification results:

- 60/65 good samples classified as good (92 % correct)
- 5/65 good samples classified as bad (8 %)
- 11/31 bad samples classified as bad (35 %)
- 20/31 bad samples classified as good (64 %)

In total, 71 samples i.e. is 74 % of the samples were classified correctly into their respective quality class and 26 % were classified wrongly. However, the outcome of 64 % bad samples being classified as good samples is not very satisfactory. In principle. 0 % bad samples should be classified as good ones, so the observed rate is far too high. For the fish producers also 2-5 % good samples classified as bad is acceptable, so 8 %, which was found is still 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.

These results suggest that it may be difficult to apply a global prediction model based on all the samples from the different suppliers to every separate fish producer. The results also showed that reference parameters as fat secretion and smoked salmon odour could he useful rather for local classification modelling than global. probably due to different fat content and smoking processing at the different suppliers of fish.

By inspection of the data, it seems that the samples tend to be grouped according to the fish producer, indicating that local prediction models for each supplier separately could he more suitable.

ALPHA also performed a treatment of the data by means of Principal Component Analysis (PCA). They make a global analysis, taking into account all the variables examinated in the sensorial, chemical, microbial and analytical (FishNose prototype) measurements with all the samples and also individual analyses executed on each producer for each variables group of measurements. During the interpretation of the data, ALPHA observed that when the samples of one producer have a significant structure, this structure is revealed by each variables group. This result is a consequence of the good correlation, by producer, between the variable groups.

Additionally ALPHA makes a classification via partial least squares (PLS). For each producer, PLS models were built to predict of the 5 following descriptors: TVC, I.~AB, Sour/sweet odour, off odour and rancid odour. The results are included in Dl 2 attached as Annex 4 to this 18 months management report.

After the exploration of the data, ALPHA can conclude that a proper “structural correlation“ between the different analyses: sensorial, chemical, microbial and analytical (FishNose prototype) is observed. One defines ~‘structural correlation“ the fact that each variable group (sensorial group, chemical group, microbial group and analytical group). induce a similar structure on the samples.

individual analyses have shown that when the samples of a producer are structured, the most part of the variable groups induce a similar structure. This is the case for FIEDLER and TBB producers. ALPHA has not observed significant discrimination of the REMO and REYKO samples. But local predictive models by quality attributes seem also to generate robust prediction.

In general can be concluded that local modelling by attributes and producers looks more promising than global quality classification.

Further prediction modelling and calculations on the measurement data from the second storage trial have been investigated and have been included in an updated final revised version of D12 (Annex No. 2) delivered with the 24 month reporting. The final conclusions could be drawn: Individual analyses have shown that when the samples of a producer are structured, the most part of the variables groups induce a similar structure. This is the case for Fiedler and TBB producers. No significant discrimination of the REMO and REYKO samples was observed, but local predictive models by quality attributes seem also to generate robust prediction. High correct classification rates can be obtained by using both single or combined quality criteria, and that this is dependent also on the single smoked salmon producer. However, the optimal classification with regard to lowest number of “false positives”, i.e. bad samples being predicted as good seems to be to rely on the single criteria like Log TVC or sensory off-odour or sweet/sour odour. In the case of the TBB samples, a 100 % correct classification could be obtained based on the single criteria off-odour or sweet/sour odour.

Status of Deliverables:
Task 7 includes the following deliverables:

- D12 Optimised prototype with specification of lab experiments (MATFORSK, due after month 14)
- D13 test formats and protocol-schemes for on-site sensor evaluation (MATFORSK, due after month 12)

Milestones:
This work package includes one milestone which is was completed in the second year of the project:

- M03 Approved and optimised FishNose prototype with specification of lab experiments (due after month 16)

Task has been completed.

2.3.8 Task 8: Prototype on-site tests

Start date: Month 13
Completion date: Month 23
Current Status: Completed

Partners involved, including total and devoted person months:

Task 8													
Task leader	B2												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	1	3	4	2,5	1	2	2,5	2	0,5	2	2	2	24,5
MM devoted	2,5	3	3,5	2,5	1,3	2,1	2,7	2,1	0,5	2	3,3	0	25,5

Objectives:
The prototype optimised under laboratory conditions has to prove its applicability and reliability under everyday use and production conditions. The measuring data obtained must be easy to use and to interpret, so that it helps the end user SME's to improve their quality assurance as well as the entire production process. Therefore the objective is to verify that the FishNose shows sufficient concentration sensitivity, reliability and robustness.

Methodology and study material:
The FishNose prototype will be delivered to the end user SME's and integrated into their production (FIEDLER, ARMORIC, REYKO, REMO, TBB), their monitoring system of incoming inspection (RÜGEN-F) and their spectrum of analysis they offer (ANFACO). Each SME user is supervised by one of the research subcontractors TTZ, MATFORSK or IFL so that a modification based on the user's needs and requirements can be carried out efficiently. Thereby the prototype will stay at each end-user about 1 month and will be forwarded from one partner to the next, starting at ANFACO. During this month in each company one to four persons will be trained on the sensor equipment and run the prototype. Additionally providing sample material has to be guaranteed and possible integration into the existing quality control system has to be discussed and substantiated.
The sensor systems is evaluated and assessed regarding concentration sensitivity, reliability, stability and reproducibility in an extended time test according to the test format evaluated in Task 7. Modifications and corrections of the prototype can be performed on-site by the supervising organisations.
In addition to the monitoring of end-users everyday processed samples 5 samples each of 5 different qualities, the SME's provide, will be included in random order. The corresponding responds of the FishNose Sensor will be investigated.
In parallel correlation with conventional analytical methods and sensory panels (see Task 2) will be executed on 10 samples taken from end-users, which are transported under controlled conditions to the food labs. IFL, TTZ, MATFORSK and ANFACO provide established analytical reference methods according to the analytical test programme (Task 2). Correlation of sensor results to reference method results will be performed by TTZ.
The results of on-site tests of the developed sensor array system will be summarised by the RTD partners MATFORSK, TTZ and IFL by specification generation.

Progress and results during the project running time:
Discussions on the planning of the on-site testing of the prototype sensor system including also technical/practical issues have been discussed during the 6 and 12-month meetings. In agreement with the end-user specifications (Task 1) it is has been suggested that the sensor system should be applied on the processing line after the smoking process and before storing of finished product, i.e., during the slicing and packaging process. It has therefore been decided at the midterm meeting that type on-site testing will be performed at this processing stage.

The on-site instrument testing was the final objective of FishNose European project. The optimised industrial prototype FISHNOSE system was installed at the ARMORIC (Quimper France) factory on the 2nd November 2004 by staff from FishNose manufacturer company ALPHA MOS. Installation, performance testing and on-site training on the instrument with the ARMORIC personal was carried out according to the Training report and Programme as reported in **D17**. The setup of the FishNose measurement system for at-line analysis on-site of processed smoked salmon fillet is shown in Figure 18.

In total, 70 Salmon fillets have been analysed at the ARMORIC company during 7 daily measurement sessions over the period from 19th October 2004 to 4th January 2005 (Table 11). The first three days were spent on performance check and training of dedicated personnel by ARMORIC company. Smoked salmon fillets from 31different production batches were analysed, with 1-10 fillets of each production batch. This has been less than the originally planned number of samples, due to the fact that the tests were performed during the high production season with a high working load on the company personnel.

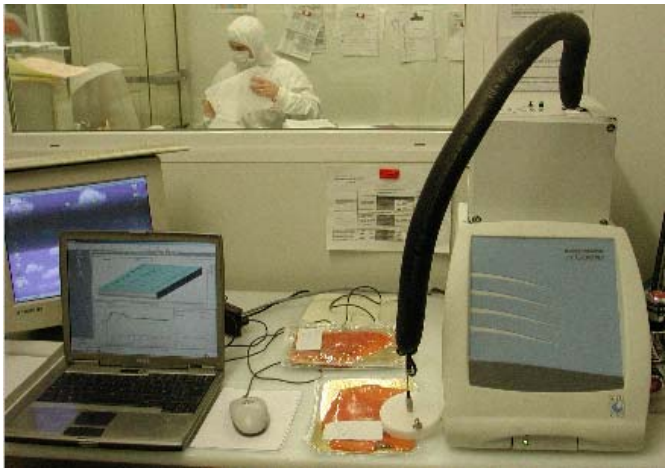


Fig. 19: FISHNOSE system with sampling with the bell sampling cup mounted on the sampling unit and PC installed at ARMORIC, QC Lab.

Tab. 11: Overview of FishNose on-site testing activities at ARMORIC company.

DATE	Tasks done
02.11.2004	Installation (Alpha-MOS)
03.11.2004	Training and samples analysis (Alpha-MOS)
04.11.2004	Training and samples analysis (Alpha-MOS)
29.11.2004	Samples analysis (ARMORIC)
30.11.2004	Samples analysis (ARMORIC)
01.12.2004	Samples analysis (ARMORIC)
09.12.2004	Samples analysis (ARMORIC)
16.12.2004	Samples analysis (ARMORIC)
22.12.2004	Samples analysis (ARMORIC)
January	Analysis of 30 days stored ARMORIC samples

In addition to the analysis of freshly processed samples, it was also planned to analyse samples that were aged at 3°C up to one month in the refrigerator to create bad samples in order to validate the SQC prediction model. Unfortunately, the analysis of the aged samples could not been performed on-site as it was planned.

Sampling:
The bell (10 cm in diameter) sampling was used for the sampling. Analysis have been carried out on full fillet smoked fish. Samples have been covered by a 7 cm diameter pierced aluminum paper (disposable) in order to avoid contamination between samples. Headspace is aspirated by centering the bell on this piece of paper. The bell has a 10 cm diameter.

Sampling conditions:
- Direct manual injection sampling
- Measurement at 5°C,
- Pump flow rate of 200ml/min,
- 10 ml sample loop volume,
- loop loading time 7 sec,
- acquisition time 120 sec,
- no purging of sampling loop between sequential measurement is required.

Before each daily salmon fillet measurement sequence, background ambient reference air was also measured as a control.

Results:
Sensor readings
In general the sensor readings of the ARMORIC samples low values around the background air levels, which is in agreement with good samples, which also is being expected. For several of the measurement sequences from different days, the reference air readings showed a significant fluctuation and partly exceed the fish sensor readings. 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. The effect is obvious, by inspecting the raw data of single sensor readings. Even if the aged samples seem to have a tendency to be slightly higher than the fresh samples, the reference air reading exceed by far both categories of samples. Since the different categories of samples are plotted in time order, the fluctuating pattern of the reference samples are clearly also reflected in the fresh and aged samples showing the effect of background air at the production plant. The same effect can also be seen in the combined sensor data in the PCA plot. To overcome this problem, all the sensor signal data of the measured fish samples have therefore been corrected for the fluctating background air of the respective analysis date by simply subtracting them from the fish sample 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.

Classification
Discriminant Partial Least Squares Regression (DPLSR) was used for the classification of respectively fresh and aged samples. There outcome of different combination of sensor for the predictions are summarised in Table .

Table 12. Classification rates based on different combinations of sensor readings.
The number of samples are respectively 44 fresh and 43 aged.

Sensor combination		% correct prediction	% wrong prediction
No. 1-6	Fresh	93	7
	Aged	93	7
No. 1-3, 5-6	Fresh	95	5
	Aged	91	9
No. 2-3,5-6	Fresh	95	5
	Aged	88	12
No.2,5-6	Fresh	95	5
	Aged	88	12
No. 5-6	Fresh	95	5
	Aged	81	19

High classification rates were obtained and the outcome of the different sensor combination were similar. Fresh samples obtained a classification rate from 93-95 %, whereas for the aged samples a classification fate from 81 to 93% was obtained. The best classification in terms of lowest rate of “false positives”, i.e. aged samples being classified as fresh, was obtained by combining all the 6 sensors corresponds to an overall classification rate of 94 %, i.e. 5 samples classified wrongly, corresponding to 3 aged samples classified as fresh (false positives), and 2 fresh samples classified as aged, of a total of 87 samples.

Comparison with previously analysed samples
For comparison the sensor readings of the ARMORIC samples have been combined with the sensor readings from the FIEDLER samples (reported in D12). A direct comparison should basically be comparable since identical since identical FishNose measurement conditions have been used during the two trials. A line plot showing the single sensor readings for selected sensors are shown in Figures 20 and 21.

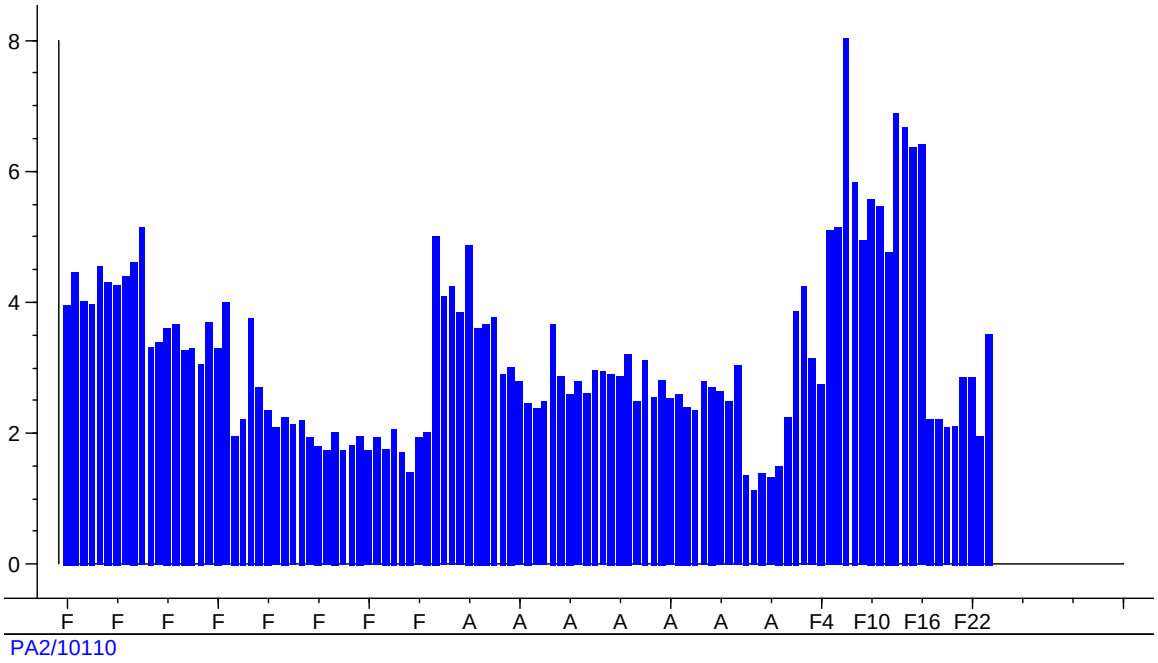


Fig. 20: Sensor readings of the first sensor (PA2/10110) of the array for combined ARMORIC and FIEDLER samples. ARMORIC samples are labelled F (Fresh) and A (Aged) and FIEDLER samples as numbered F.

The sensor readings basically fall within the same measurement range as the ARMORIC samples, despite the influence of fluctuating air reference background for the ARMORIC samples as pointed out earlier. Interestingly, however, is to notice that the samples of highest readings also correspond to the bad Fiedler samples with regard to the defined quality criteria (microbiology, sensory) as discussed in D12. On the other hand, by looking at the distribution of the last two sensors, exemplified by the fifth sensor in Figure 8, the Fiedler readings show lower values than the ARMORIC samples. Bearing in mind that it was shown earlier (D5) that these sensor are detecting smoking compounds, suggests that the ARMORIC samples are more heavily smoked than the FIEDLER samples., again also suggesting local modelling.

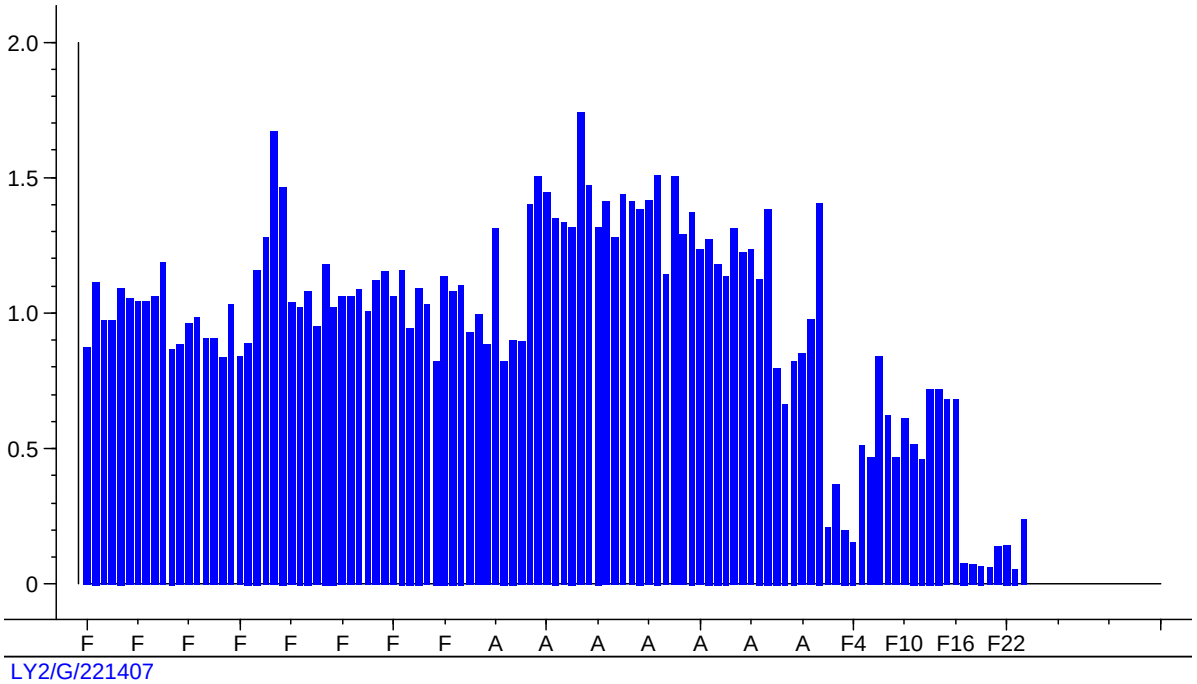


Fig. 21: Sensor readings of the fifth sensor (LY2/G/221407) of the array for combined ARMORIC and Fiedler samples. ARMORIC samples are labelled F (Fresh) and A (Aged) and FIEDLER samples as numbered F.

By combining all the 6 sensor readings for the ARMORIC and FIEDLER samples together, the results shown in Figure 22 was obtained. Also here a time shift seems to be reflected between the ARMORIC and FIEDLER data.

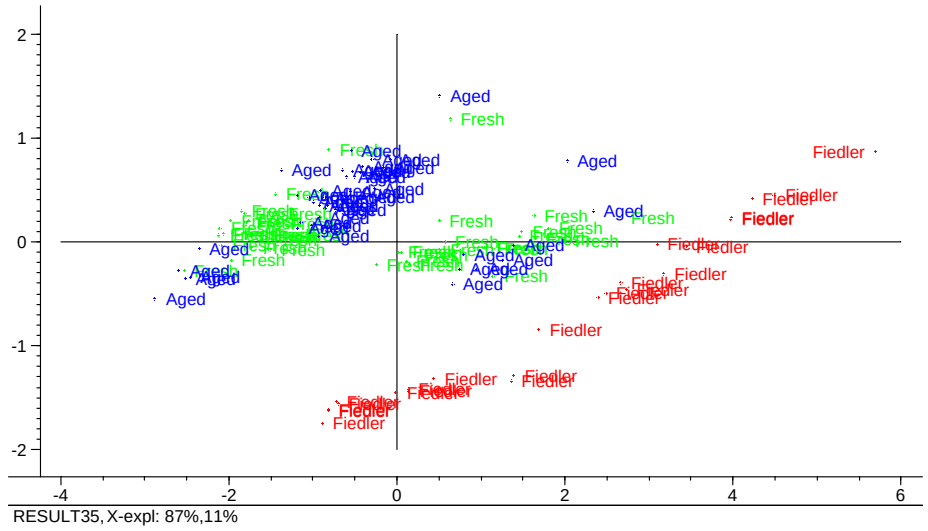


Fig. 22: PCA plot, PC1 against PC2, based on the 6 sensor readings of ARMORIC, fresh (in green) and aged (in blue) and FIEDLER samples (in red).

The major shift/drift along axis 2 probably reflects day to day sensor drift due to fluctuating background reference air between the measurement sequences.

To some extent this drift effect, since it seemed to give the major contribution along the first principal component, the same result from the PCA is plotted in dimension 1 and 3 as seen in Figure 22. The bad quality Fiedler samples are located below to the right. The correspond to the 12 of the bad Fiedler samples fullfilling the following quality criteria:

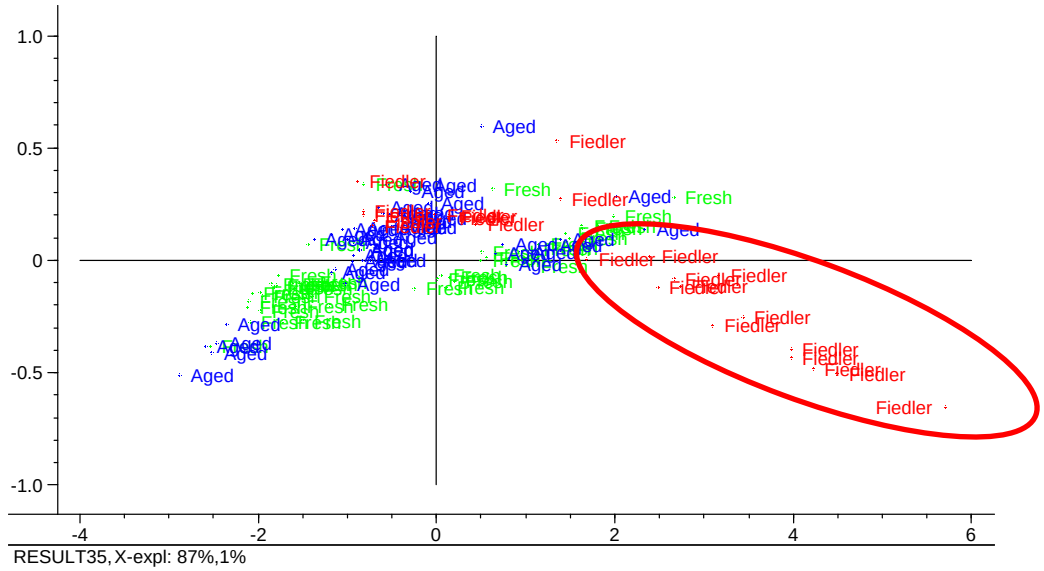


Fig. 23: PCA plot, PC1 against PC3, based on the 6 sennsor readings) and aged (in blue) and FIEDLER samples (in red).

A: Log TVC > 4 (except for 2 samples)
B: Off-odour > 20 (except for 4 samples, 3 different from than A)
C: Rancid odour >10 (3 samples, among A and B)
D: Sweet/sour odour > 20: 8 samples (except for 4)

Overall there are 3 samples of the 12 that fail on these criteria.

It is expected that the aged ARMORIC samples should still be better in quality, suggesting. It was previously described that Fiedler samples are aging much more quickly and strongly due to the smoking process used.

Summary
The analysis of the complete set of results, generated by "FishNose prototype" in laboratory trials lead to the conclusion that the data generated with this instrument shows a proper "structural correlation" between the: sensory, and microbial analyses and the FishNose prototype sensor readings. Thus the FishNose system appeared to be promising for the rapid quality control related to freshness evaluation of smoked salmon products. The FishNose sensor system was therefore used for the on-site testing at ARMORIC without further optimization.
I was analysed and explored the data generated at ARMORIC site. The results obtained with the different statistical methods on the corrected data show that more than 90% of the samples are correctly identified.
It can be conclude that the approach adopted in the FishNose project, particularly the selection of reference methods, the instrument and the mathematical models used are useful for the purpose of Quality Control of smoked salmon fillet.
However additional validation is required by integrating the sensing module directly onto a fish equipment in order to validate classification rate on thousands of samples measured on-line.

Status of Deliverables:
Task 8 includes the following deliverable:

- D14 Pre-competitive, optimised industrial prototype with specification of in-site experiments (due after month 23)

In the context of discussion about the prototype testing at the midterm meeting, it was also agreed by the partners to postpone the deadline for D13 (Test formats and protocol-schemes for on-site sensor evaluation) until the 18 Month Meeting / Report. With regard to the on-site testing, test formats and protocol will be finalised on the basis of the experience and optimisation gained during the prototype laboratory testing, but in due time.
Due to the tight time schedule and limited budget for travel costs in the project, it has been discussed during the midterm meeting whether the on-site testing should be performed at a selected number of end-users instead of by all end-users.

Task has been completed.

WPD: Project Management and Dissemination

2.3.9 Task 9: Project Management and Dissemination

Start date: Month 1
Completion date: Month 24
Current Status: in progress

Partners involved, including total and devoted person months:

Task 9													
Task leader	A1												
Partners	A1	A2	A3	A4	A5	A6	A7	A8	A9	B1	B2	B3	Total
MM total	4,75	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	2	1	1	12,75
MM devoted	4,75	0,5	0,5	0,4	0,5	0,5	0,5	0,5	0,5	2	1	1	12,65

Objectives:
The objective of this task is to ensure effective project management and co-ordination over the entire project duration. Since FishNose will be a totally new measurement system, diverse dissemination activities are intended as far as possible patenting activities are not tangented to. Besides, preparation of a work-shop for staff training will be implemented in the order to facilitate the absorption of the results by the SME's and future clients.

Methodology and study material:
The project management method is detailed in section 4 and will include the following elements:

- Project meetings with all partners every 6 months
- Special partner group meetings
- Preparation of project reports

Secondments of SME staff to the research providers will assist in their post-project exploitation of the technology by transferring expertise. Training will involve familiarisation with the handling of the FishNose system, software utilisation and calibration of the FishNose, as well as safety procedures. A training report will be produced in order to document in detail the knowledge and skills transferred and the progress of each staff member.

The dissemination strategy, additionally described in section 5, will include - as far as patenting activities are not tangented to -:

- Presentation at relevant conferences, symposia and exhibitions: FISH international (Germany), ANALYTICA (Germany), SIAL (France) or IFT Fair (USA) are anticipated.
- Direct contact with end-users and relevant food, engineering and trade associations
- Documentation through papers in scientific journals by TTZ, MATFORSK and IFL, e.g. in J. of Food Science, European Food Research and Technology, J. Agricultural and Food Chemistry, Trends in Food Science, Sensor Technology and Sensors and Actuators etc.
- Generation of a project web page by ALPHA and the RTD performers TTZ, MATFORSK and IFL. The web page is intended for a) data exchange of all involved project-partners and b) project presentation and result dissemination to the industry.
- Generation of a leaflet by ALPHA and TTZ to provide compact project and product information to potential clients. Beside 1500 hardcopies, the leaflet will be available as PDF-file for E-Mail propagation and will be integrated on the generated web page.

Besides it is anticipated by the SME's that patents will be required to protect the knowledge generated on electronic nose development for industrial application in the fish industry, comprising the measuring procedure, sensor development and the evaluation and documentation of the data and information.
With experience of RTD partners during establishing the prototypes at the SME companies (Task 8), a programme and script will be developed by TTZ, MATFORSK and IFL, supported by ALPHA, preparing future work-shops and staff training.
In general all partners are intending to disseminate their involvement in the development and use of the new FishNose-Sensor technology to demonstrate improved state-of-the-art resulting in competitive advantages
Finally expected market potential of the developed Sensor-System will be updated by ALPHA and TTZ according to the project results and calculated sensor costs.

Task 9 comprise the following subtasks:

- Subtask 9.1 Project management
- Subtask 9.2 Dissemination and knowledge protection
- Subtask 9.3 preparation of Work-Shop / Staff-Training
- Subtask 9.4 updating of sensor's market potential

Progress and results during the project running time:
The project management was performed by the co-ordinator ALPHA since the beginning of the project. For details see "4. Project-Management and Coordination".
Dissemination and Exploitation already was started in the first year of the project. Details are listed in "5. Exploitation and Dissemination Activities"

Status of Deliverables:
Task 9 includes the following deliverables:

- D15 Four progress reports (after month 7, 13, 19 and 24)
- D16 Mid-term review report (after month 12)
- D17 Training report and programme (after month 24)
- D18 leaflet (after month 21)
- D19 Technology implementation plan (after month 24)
- D20 Final reports (after month 24)

Deliverables D15a, the 6-month report, has been completed in time in June 2003. Deliverable D 15 b and D16 have been combined after affirmation by the PTA and are represented by the current report. After agreement between the partners and affirmation by the PTA, deliverable D18, the project leaflet, has been generated earlier than estimated in the Technical Annex. It has been delivered in month 12 and is included in this report as Annex A13. A first draft of Technological Implementation Plan (TIP) as contribution to deliverable D19 has been established according to the commission's demands. It is enclosed to this report as Annex A16. All other deliverables were completed after month 24.

Status of Milestones:
The work package includes the following milestones:

- M 04 Mid-term assessment report (month 13)
- M 05 Final review (after month 24)

Milestone M04 was reached successfully. It is represented by the current report and delivered in month 14. The 1 month delay was conceded by EC due to the date of Midterm Meeting in month 14. Milstone M05 will be reached after the second year of the FishNose project.

Task has been completed.

3 ROLE OF PARTICIPANTS

3.1 Alpha-MOS S.A., France (ALPHA) Co-ordinator

Participant number: A1

Address: ALPHA-MOS S.A.
20, Avenue Didier Daurat
F-31400 Toulouse
France

Scientific Team: Mr. Eric Chanie, Mrs. Sandrine Bazzo, Mr. Hicham Amine, Mr. Pierre-Olivier Michel, Mr. Francois Loubet, Mr. Pascal Boilot

Contractual link to other participants: SME contractor / co-ordinator

Objectives:
ALPHA is a public analytical instrumentation company set up in 1992 to develop, manufacture and sell Smart Sensing Systems. ALPHA employs 35 persons including 8 Ph.D., based in France, Germany, Great Britain and the United States. They are fully dedicated to this technology and to its applications from R&D/QC laboratory to on-line production as well as development of gas sensor applications.
With over 250 systems installed world-wide, ALPHA is a leader in sensor technology for odours, aroma measurement and control, covering several fields of application (food industry, perfumes & cosmetics, chemistry & petrochemistry, packaging, environment ...).
Besides the project management as project co-ordinator, ALPHA mainly will be involved in specification, generation and optimisation of the FishNose Sensor to be developed.

Workplan:

Partner	A1									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	1	0,25	-	1	3	2	1	1	4,75	14
MM devoted Y 1	1	0,25	-	1	2,5	0,15	-	-	2,5	7,4
MM devoted Y 2	-	-	-	-	0,75	2	3	2,5	2,25	10,5

Deliverables:
ALPHA is responsible for the following deliverables:

- D01 Detailed data on SME end-users' processes (due after: month 3)
- D02 Technical specification catalogue for the FishNose (due after month 3)
- D08 Manufactured sensor array prototype (hardware) (due after month 9)
- D09 FishNose prototype with specification regarding the standard-mixture (due after month 11)
- D15 Four progress reports (due after month 7, 13, 19 and 24)
- D16 Mid-term review report (due after month 12)
- D17 Training report and programme (due after month 24)
- D18 leaflet (due after month 21)
- D19 Technology implementation plan (due after month 24 – first draft after month 12)
- D20 Final reports (due after month 24)

The Deliverables D01 and D02 have been completed until June 2003, which means 2 month delay compared to the estimated due date of the Technical Annex. They were included to the midterm report as Annexes A1 and A2. Time delay was mainly due to date of kick-off meeting in middle of February compared to official project start on 1st January 2003.
The Deliverables D08 and D09 were completed and presented in Annex A8 and A9 of the midterm report and were available for laboratory testing during the second year. They were delivered end of February 2004 to the RTD partners. The project leaflet (D17) was generate earlier than planed in the Technical Annex, to promote the FishNose Sensor already during the operating time of the project.

Regarding the project management, deliverable D15a, the 6-month report, has been completed in time in June 2003. Deliverable D 15 b and D16 have been combined after affirmation by the PTA and were presented in time. Deliverables 15c, the 18 month report has been generated and submitted in time. Deliverable 15d (24 month progress report) are represented by the current report.
A first draft of Technological Implementation Plan (TIP) as contribution to deliverable D19 has been established according to the commission's demands. It was enclosed to the midterm report as Annex A16. The final Technological Implementation Plan (TIP) will be submitted with this report and the further final documents (Final report, project summary and final cost statements).

Research activities during project running time:
The main activities were:

- Contribution to system specifications and end-user requirements collection;
- Sensor array module design and sensor selection for appropriate measurement of smoked fish quality;
- Sensor selection and correlation with reference methods;
- System integration with software, sensor module and gas sampling device.
- Project management
- presentation of FishNose on the SISQA fair, Toulouse, France (traceability in food industry - December 2003)

The second year of the project was devoted to the validation of developed apparatus for the intended application. Initial tests were confirmed on the prototype using new gas sampling device. After those tests, a technology transfer was operated with the RTD partners in order to perform laboratory test program as well as field-test at SME's location.
Full software including correlation capabilities were developed in collaboration with MATFORSK.

3.2 Hans Fiedler Söhne Lachs- und Aalräucherei GmbH, Germany (FIEDLER)

Participant number: A2

Address: Hans Fiedler Söhne Lachs- und Aalräucherei
Am Lunedeich 149
D- 27572 Bremerhaven
Germany

Scientific Team: Mr. Andre Fiedler, Mr. R. Röper

Contractual link to other participants: SME contractor

Objectives:
Fiedler is located in Bremerhaven, North Germany, and was established in 1949. Since then it has operated a smoked fish plant (cold and hot) where they mainly process trout, salmon and eel. In 1994 the company built a new plant for the smoking of fish. It is one of the most modern fish-smoking plants in Germany.
The company sets great store by its quality standards in production as well by the quality of its products. It orientates itself to the state of the art. This includes a strong interest in preventive measures especially concerning hygiene in production and processing. Approximately 90 % of annual turnover comes from business with wholesalers. H.F. Fiedler & Söhne is an accredited fish-processing company in accordance with EU Directive No.: 91/493/EU (Permit No. D-HB-EFB-013)
As end-user, FIEDLER mainly will be involved in specification and testing of the FishNose Sensor to be developed.

Workplan:

Partner	A2									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	0,75	0,5	-	-	-	0,25	-	3	0,5	5
MM devoted Y 1	0,75	0,5	-	-	-	0,25	-	0,3	0,1	1,9
MM devoted Y 2	-	-	-	-	-	-	-	2,7	0,4	3,1

Deliverables:
FIEDLER is not the main responsible of any deliverable, although according to the Technical Annex collaborates in those where its contribution has been required.

Research activities during project running time:
FIEDLER contributed to the specification phase of tasks 1 and 6 by providing detailed information about their applied process technology and user's requirements. They supplied sufficient amount of fresh smoked salmon fillets for performance of first storage trials at TTZ related to task 2, 7 and 8. Thereby they actively have been involved in the planning of storage conditions. The samples additionally have been provided for detection of key compounds (task 3) as well as for sensor-design (task 5). Additionally first dissemination have been performed by promoting the intended FishNose sensor at "Bundesverband der deutschen Fischindustrie" in Hamburg, Germany.
In the second year FIEDLER provide the RTD partners with further smoked fish sample material and was involved in additional storage trials for the on-site tests to be performed in task 8. Thereby, among others, especially different kind of packaging methods and their influence on resulting freshness parameters and FishNose sensor signals were of highest interest.

3.3 Armoric S.A., France (ARMORIC)

Participant number: A3

Address: ARMORIC S.A.
55, Avenue de Keradennec
F-29556 Quimper Cedex 9
France
Scientific Team: Mr. Jean-François Feillet, Mrs. Christell van Bambost

Contractual link to other participants: SME contractor

Objectives:
Armoric S.A. is a well-established company in Brittany (France) with more than 50 employees. Their main products are smoked salmon and trout on an industrial scale but they approach their trade as craftsmen. Their quality system guarantees compliance with the most stringent specifications. Two new systems have been developed to guarantee material yield and full traceability: NEGOPTI, a database listing over 70 000 slices used for the automation of the slicing/assembly production lines, and the VISIO process for slice dimensional control.
ARMORIC has a 25% market share in the stores where it is present (source: Nielsen).
As end-user, ARMORIC mainly will be involved in specification and testing of the FishNose Sensor to be developed.

Workplan:

Partner	A3									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	0,75	0,5	-	-	-	0,25	-	4	0,5	6
MM devoted Y 1	0,75	0,5	-	-	-	0,25	-	-	-	1,5
MM devoted Y 2	-	-	-	-	-	-	-	3,5	0,5	4

Deliverables:
ARMORIC is not the main responsible of any deliverable, although according to the Technical Annex collaborates in those where its contribution has been required.

Research activities during project running time:
ARMORIC contributed to the specification phase of tasks 1 and 6 by providing detailed information about their process technology and their idea of user's requirements. They supplied samples for first storage trials and reference analysis at ANFACO related to task 2.
In the second year of the FishNose project, ARMORIC provided sample material and was involved in the on-site tests to be performed within task 8. Additionally dissemination activities were done contacting and informing further clients about the new sensor and its potential application in the fish industry.

3.4 Reykofninn ehf, Iceland (REYKO)

Participant number: A4

Address: Reykofninn ehf
Skemmuvegur 14
IS-200 Kopavogur
Island

Scientific Team: Mr. Kari P. Olafsson, Mr. Olafur Georgsson, Mr. Ole Pedersen, Mr. Tomas Kristinsson

Contractual link to other participants: SME contractor

Objectives:
REYKO was established in 1975 but changed to a limited liability company in 1979. It is now totally family-owned. REYKO has been located in Kopavogur, Iceland since 1980. Their main products are smoked salmon, trout and arctic char besides smoked meat products.
As end-user, REYKO mainly will be involved in specification and testing of the FishNose Sensor to be developed.

Workplan:

Partner	A4									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	0,75	0,5	0,5	-	-	0,25	-	2,5	0,5	5
MM devoted Y 1	0,75	0,5	0,5	-	-	0,25	-	-	-	2
MM devoted Y 2	-	-	-	-	-	-	-	2,5	0,4	2,9

Deliverables:
REYKO is not the main responsible of any deliverable, although according to the Technical Annex collaborates in those where its contribution has been required.

Research activities during project running time:
Task 1, 6: REYKO participated in specification of system requirement and supplied information regarding their process.
Task 2, 3: REYKO provided samples for training sensory panel and all samples for storage study carried out at IFL. They also provided information and guidance in the development of the laboratory test programme.
REYKO supplied the RTD'S in the second half of the project with further raw material for prototype testing. They produced samples for storage studies including extreme handling conditions. Furthermore they participated in finalising the sensory reference methods used for the prototype testing.

3.5 Association Nacional de Fabricantes de Conservas de Pescados y Mariscos, Spain (ANFACO)

Participant number: A5

Address: ANFACO / CECOPESCA
Ctra. Colexio Universitario 16
E-36310 Vigo (Pontevedera)
Espania

Scientific Team: Mr. Juan M. Vieites, Mr. Carlos S. Ruiz,
Mr. José C. González, Mrs. Ana G. Cabado

Contractual link to other participants: SME contractor

Objectives:
The technical department of ANFACO was created in 1949 to provide analytical services for the canning industry in Spain. Since then, the laboratory has worked in the analytical control of fish and seafood products for more than 50 years, thus being one of the most important laboratories for the fishing industry in Spain.
The clients of the laboratory include not only canning industries, but also companies which produce frozen fish/seafood and semi-preserves (salted and smoked fish/seafood). In addition to the analytical services, the laboratory also carries out research projects mainly focused on the development and refinement of new techniques for the quality control of fish and seafood.
ANFACO expect to incorporate a new technology in the laboratory, which will expand the range of services available for the associated companies.
As end-user for laboratory application, ANFACO mainly will be involved in specification and testing of the FishNose Sensor to be developed. In this context ANFACO also will contribute their analytical services as standard methods as well as their experience in legislations of the fish sector.

Workplan:

Partner	A5									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	1	2	1	-	-	0,5	2	1	0,5	8
MM devoted Y 1	1	2	1	-	-	0,5	0,7	-	-	5,2
MM devoted Y 2	-	-	-	-	-	-	1,5	1,3	0,5	3,3

Deliverables:
ANFACO is responsible for the following deliverables:

- D04 Reference methods ready to use in the project partner labs (due after month 5)

The analysis methods have been established in time in June 2003. Corresponding up-date of memorandum is enclosed in this report, Annex A4.
In addition, ANFACO is not the main responsible of any deliverable, although according to the Technical Annex collaborates in those where its contribution has been required.

Research activities during project running time:
ANFACO contributed to the specification phase of tasks 1 and 6 by providing detailed information about their requirements as laboratory that is serving the fish industry.
In addition they established and offered chemical, microbial and sensorial reference analysis for RÜGEN-F and ARMORIC samples according to the laboratory programme described in chapter 2.3.2. On the kick-off meeting it was decided, in agreement with the partners as well as with the EC, not to perform the stability tests of the standard cocktail as described in the Technical Annex, but to focus on the reference analysis and also to supply the know-how in legislation rules within the fish sector. Thus ANFACO performed literature survey contributing to Deliverables D03 and D04 (Annex A03, A04).

In the second project year ANFACO provided established methods of reference analysis in context of laboratory and on-site tests to be performed. In addition ANFACO extended dissemination activities by promoting the project and the FishNose sensor at their network of clients out of the spanish fish processing industry.

3.6 Brødr Remø A/S, Norway (REMØ)

Participant number: A6

Address: Brodr Remø A/S
N-6035 Fiskarstrand
Norway

Scientific Team: Mr. Johnny Asbjorn Remo, Mr. Odd Skotheimvik, Ms. Kari Kjerstad, Ms. Kari Gutvik

Contractual link to other participants: SME contractor

Objectives:
REMO of Norway is a family-owned fish producer, founded in 1923 and specialised in shellfish and processed salmon (smoked, marinated, pepper). In addition to serving the Norwegian market, they also have a considerable export trade with the international market world-wide under their own brands “GoldFish” and “Stormy”. The company is located in Fiskarstrand outside the coastal city of Ålesund, in mid-west Norway.
The company has recently modernised its production facilities and have introduced a quality control system approved by the Norwegian Directory of Fisheries according to the regulations for fish and seafood products (HACCP).
As end-user, REMO mainly will be involved in specification and testing of the FishNose Sensor to be developed.

Workplan:

Partner	A6									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	0,75	0,5	1	-	-	0,25	-	2	0,5	5
MM devoted Y 1	0,75	0,5	1	-	-	0,25	-	-	-	2,5
MM devoted Y 2	-	-	-	-	-	-	-	2,1	0,5	2,6

Deliverables:
REMO is not the main responsible of any deliverable, although according to the Technical Annex collaborates in those where its contribution has been required.

Research activities during project running time:
REMO contributed to the specification phase of Tasks 1 and 6 by providing detailed information about their applied process technology and user’s requirements. They supplied sufficient amount of fresh smoked salmon fillets for performance of first storage trials at MATFORSK related to Task 2, 7 and 8. Thereby they actively have been involved in the planning of storage conditions. The samples additionally have been provided for detection of key compounds (Task 3) as well as for sensor-design (Task 5).
In the second year REMO provided further sample material for the prototype laboratory testing (Task 7) and was involved in the on-site tests to be performed within Task 8. Additionally, further dissemination activities were done by contacting and informing further clients about the new sensor and its potential application in their fish business.

3.7 Rügen-Feinkost GmbH, Germany (RÜGEN-F)

Participant number: A7

Address: Rügen-Feinkost GmbH
Am Stadthafen
D-18546 Saßnitz
Germany

Scientific Team: Mr. Andreas Berthold, Mrs. Dana Willmann

Contractual link to other participants: SME contractor

Objectives:
RÜGEN-F was founded in May 1991 and bought the facilities of Rügen Fisch Sassnitz in Lauterbach which were threatened with closure. The company produces and sells a complete range of delicatessen fish products. In 1996 Rügen-F took over Maro, a company in Rostock also threatened with closure, and built a new production plant for delicatessen fish products in which, with the exception of young herring, all product lines are located. Rügen Feinkost's product range encompasses:

- Fish and salads: Complete range of delicatessen fish products including smoked fish
- Convenience foods: Ready prepared fish and meat dishes
- Snacks: Fish and meat finger-food

As end-user, RÜGEN-F mainly will be involved in specification and testing of the FishNose Sensor to be developed.

Workplan:

Partner	A7									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	1	0,5	-	-	-	0,5	-	2,5	0,5	5
MM devoted Y 1	1	0,5	-	-	-	0,5	-	-	0,1	2,1
MM devoted Y 2	-	-	-	-	-	-	-	2,7	0,4	3,1

Deliverables:
RÜGEN-F is not the main responsible of any deliverable, although according to the Technical Annex collaborates in those where its contribution has been required.

Research activities during project running time:
RÜGEN-F contributed to the specification phase of tasks 1 and 6 by providing detailed information about their process technology and their idea of user's requirements. They supplied fresh salmon fillets for performance of first storage trials and reference analysis at ANFACO related to task 2. Additionally first dissemination have been performed by promoting the intended FishNose sensor at the international Fish Exhibition in Brussels, Belgium.
In the second year RÜGEN-F provided further sample material and was involved in the on-site tests to be performed within task 8. Additionally further dissemination activities were done by contacting and informing further clients about the new sensor and its potential application in their fish business.

3.8 Tønsberg Brygge AS, Norway (TBB)

Participant number: A8

Address: Tonsberg Brygge AS
Trelleborg veien 15
N-3112 Tonsberg
Norway

Scientific Team: Mr. Geir Naustvik, Mr. Tommy Kjellum, Ms. Rita Lund

Contractual link to other participants: SME contractor

Objectives:
TBB is a leading suppliers of fresh and smoked seafood. The company is located in the city of Tønsberg south of Oslo, Norway. Founded in 1999 TBB produces and selles seafood. The products are marketed and sold through the major Norwegian retail chains in the area of southern Norway. In the year 2000 the company finished their new smoking plant. The smoked salmon is produced and sold as vacuum-packed either as whole sides, whole sides sliced or bits under TBB own brand – Tønsberg Brygge.
As end-user, TBB mainly will be involved in specification and testing of the FishNose Sensor to be developed.

Workplan:

Partner	A8									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	0,75	0,5	1	-	-	0,25	-	2	0,5	5
MM devoted Y 1	0,75	0,5	1	-	-	0,25	-	-	-	2,5
MM devoted Y 2	-	-	-	-	-	-	-	2,1	0,5	2,6

Deliverables:
TBB is not the main responsible of any deliverable, although according to the Technical Annex collaborates in those where its contribution has been required.

Research activities during project running time:
TBB contributed to the specification phase of Tasks 1 and 6 by providing detailed information about their applied process technology and user's requirements. They supplied sufficient amount of fresh smoked salmon fillets for performance of first storage trials at MATFORSK related to Task 2, 7 and 8. Thereby they actively have been involved in the planning of storage conditions. The samples additionally have been provided for detection of key compounds (Task 3) as well as for sensor-design (Task 5).
In the second year TBB provided further sample material for the prototype laboratory testing (Task 7) and will be involved in the on-site tests to be performed within Task 8. Additionally, further dissemination activities were done by contacting and informing further clients about the new sensor and its potential application in their fish business.

3.9 Optotek d.o.o., Slovenia (OPTOTEK)

Participant number: A9

Address: OPTOTEK D.O.O.
Stegne 13a
SLO-1000 Ljublijana
Slovenia

Scientific Team: M.Sc. Boris Vedlin, M.Sc. Matjaz Zalar, Ph.D. Grisa Mocnik, B.Sc. Marjan Drasler, Mr. Viktor Pilko

Contractual link to other participants: SME contractor

Objectives:
OPTOTEK from Slovenia is specialised in optical and opto-electronic devices. Innovative development of new products, ISO 9000 standards, technical support to customers, competitive prices and strong interaction between research, engineering and manufacturing enable OPTOTEK to provide its customers with high-quality and reliable products.
Most of OPTOTEK's customers are in the European Union and the United States. OPTOTEK manufactures medical diagnostic and therapeutic devices such as the compact Nd:YAG laser system for photodisruption in the anterior eye segment, instruments for environmental pollution measurements (e.g. Aethalometer) and articulated arms for laser systems. The Aethalometer is an instrument for the measurement of pollution particulates in the air, using an optical absorption technique.
OPTOTEK will be responsible for development and generation of the simplified gas-sampling unit for the FishNose sensor array. Besides, advice during testing and optimisation of the FishNose prototype will be provided.

Workplan:

Partner	A9									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	0,5	-	-	5	-	-	0,5	0,5	0,5	7
MM devoted Y 1	0,5	-	-	5	-	-	0,5	-	-	6
MM devoted Y 2	-	-	-	-	-	-	-	0,5	0,5	1

Deliverables:
OPTOTEK is responsible for the following deliverables:

- D07 Optimised gas sampler (due after month 9)

Deliverables D07 has been completed in time in September 2003. The developed and established gas-sampling-unit prototype has been supplied to ALPHA-MOS. Corresponding specification is included in this report as Annex A7.

Research activities during project running time:
OPTOTEK surveyed available gas sampling and concentration techniques. Thereby close contact was held to ALPHA and TTZ. Gas Sampling Method with the Sampling 6 Port Valve was chosen for the Fishnose application. After choosing the Method, a design of the sampling unit was made. Drawings for Measuring chamber, Heater and other plumbing were generated. Also special components for the unit were purchased. After finishing the design, a model of the suggested Sampling Unit prototype was constructed according to chapter 2.3.4 and deliverable D07 (Annex A07). The Model was tested and delivered to ALPHA for integration into the FishNose prototype.
In the second year OPTOTEK was involved in optimisation of prototype. Thereby they took care for the gas-sampling unit and guide modifications in case of need in context of laboratory and on-site tests. Furthermore additional dissemination activities to promote the FishNose sensor were done.

3.10 Verein zur Förderung des Technologietransfers an der Hochschule Bremerhaven e.V., Germany (TTZ)

Participant number: B1

Address: TTZ - Technologie Transfer Zentrum Bremerhaven
An der Karlstadt 10
D-27568 Bremerhaven
Germany

Scientific Team: Dr. Claudia Thalmann, Dr. Anja Noke, Dr. Sonia Rodriguez, Mr. Michael Langenhorst, Mr. Martin Schüring, Mr. Thomas Dietrich, Mrs. Iris Auffarth, Mrs. Nicole Schmid, Mr. Olaf Ortgies

Contractual link to other participants: RTD performer

Objectives:
The TTZ Bremerhaven was established in 1987 and comprises six institutes for applied research and development (R&D). More than 80 highly qualified employees (scientists and engineers) are working on business-related projects in the fields of Biotechnology, Environmental Technology, Energy and Process Engineering, Food and Bio Process Engineering, Manufacturing Technology, Analysis and Organisation.
TTZ's intention is to strengthen the regional economy through the development of new, innovative products and services. The goal is to help create new industrial work opportunities in the region.
One TTZ-institute, the Bremerhaven Institute for Food Technology and Bioprocess Engineering (TTZ-BILB) with its sensory evaluation laboratory, as well as the lately founded project-house "TTZ-BioNord", focusing on blue biotechnology and food technology / analysis, participate the project.
TTZ will be involved in specification, development and testing of the FishNose Sensor to be developed. Besides, sensorial, chemical, microbial and physical reference analysis will be provided for optimisation of the prototype. Among others, design of a web-page and generation of a project leaflet will be contributed for dissemination.

Workplan:

Partner	B1									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	1	3	1	3	3	2	5	2	2	22
MM devoted Y 1	1	3	1	2,6	3	1,7	2	-	0,7	15
MM devoted Y 2	-	-	-	0,4	-	0,3	3	2	1,3	7

Deliverables:
TTZ is responsible for the following deliverables:

- D04 Reference methods ready to use in the project partner labs (due after month 5)
- D18 Project Leaflet (due after month 21)

The analysis methods have been established in time until June 2003. The corresponding up-date version of the memorandum is enclosed in this report as Annex A4. After agreement between the partners and affirmation by the PTA, the deliverable D18, the project leaflet, has been generated earlier than estimated in the Technical Annex. It has been delivered in month 12 and is included in this report as Annex A12.

In addition, TTZ is not the main responsible of any further deliverable, although according to the Technical Annex it collaborates in those deliverables, where contribution are required.

Research activities during project running time:

TTZ established the questionnaire for the description of the end-user's fish processing facilities as well as for the investigation of the user's demands according to the gas sensor array to be developed. Inspections and extensive telephone conferences have been performed at FIEDLER, RÜGEN-F and ANFACO. The collected information has been filtered, sorted in tabular form and integrated into the definition of the Sensor's requirements and system specifications. Results are presented in chapter 3.2.1 (Task 1), as well as in the Deliverables D01 and D02, which are enclosed to this report as Annex A01 and A02.

Within Task 2, TTZ was intensively involved in planning, co-ordination and performance of the first round of storage trials. Chemical, microbial, physical and sensorial reference analysis methods have been established, as described in chapter 2.3.2. Since RÜGEN-F was not able to serve sample material for the estimated time period of storage experiments, more detailed investigations of FIEDLER samples were realised, such as additional storage times or the measurement of additional parameters, which were assumed to influence product quality. Detailed results and conclusions are summarised and included in the specified laboratory programme, representing the deliverable D03 (Annex A03). Furthermore, intensive literature survey about existing legislation rules for smoked and fresh fish have been performed, which have additionally been incorporated into Deliverable D03.

For contributing the detection of key compounds of Task 3, TTZ provided the generated FIEDLER samples of different quality to IFL for GC-MS measurement. The results and the correlation to reference analysis data as well as the performed literature survey led to the generation of Deliverable D05 as presented in chapter 2.3.3 and Annex A05. The establishment of a standard cocktail and mixture for sensor calibration was assessed as very critical due to a very different volatility of respective compounds and a thereof resulting lack of stability. Instead, a commercial liquid smoke and some specifically chosen single compounds were suggested for the utilization.

In the frame of Task 4, TTZ supported ALPHA and OPTOTEK in the design of the gas-sampling unit. Results are described in chapter 2.3.4. A detailed concept and specification is included in deliverable D07 (Annex A07). The intended validation, according to the Technical Annex, will be performed during the overall FishNose sensor prototype testing. Due to the loop size of 10 and 20 ml, respectively, flowmeter is anticipated to be used.

For contributing to the design of the sensor-unit and first prototype testing, TTZ delivered the generated and evaluated FIEDLER samples to ALPHA. Related to known sample specification the right configuration of sensor was chosen, as included in chapter 2.3.5. In this context a technical meeting was performed in Toulouse at ALPHA facilities.

TTZ assisted MATFORSK and ALPHA in software design and definition of a user-friendly interface within Task 6. Thereby related questions/aspects for the definition of end-user's demands have been integrated into the questionnaire, being developed and edited during specification phase in Task 1. Resulting key information are listed in chapter 2.3.6 and deliverable D11 (Annex A11). GOOD/BAD classification will be performed. Thereby good quality will be defined during training phase – bad samples are defined to be outside this class. According to the investigated demands, evaluation of existing FOX and Gemini software of ALPHA has been performed on a working meeting in Toulouse.

After collecting, correlating and evaluating the sensor signals and the reference analysis results of first round of storage trials, further laboratory tests have been set up as described in chapter 2.3.7. Thereby planning of on-site tests will be performed according to experience gained in laboratory tests in Task 7.

Regarding exploitation and dissemination activities, TTZ supported ALPHA in designing the project web-page. Furthermore TTZ established and released the project's leaflet, representing the Deliverable D18 in Annex A12 (edition: 1500 print-outs).

In context of intended laboratory and on-site tests, TTZ provided established methods of chemical, physical and microbial reference analysis. Additional storage and monitoring trials were set-up with samples of FIEDLER. For sensory reference analysis, development of a simplified method is anticipated, which later on allowed the end-user himself to perform needed sensory evaluation of fish quality and standards for sensor calibration. Thereby close contact was hold to IFL and the end-user SME's, especially to FIEDLER, RÜGEN-F and ANFACO.

Furthermore validation of gas-sampling unit were performed within the prototype testing and optimisation phase of the FishNose Sensor. Flowmeter was anticipated to be used for this purpose. Besides, TTZ was involved in FishNose optimisation by further testing with real samples and standard compounds.

During intended on-site testing phase, TTZ supervised the end-users FIEDLER and RÜGEN-F. In addition, experience gained out of lab- and on-site tests were included in a concept for staff-training and workshop for potential clients.

Concerning dissemination, TTZ represented on the FISH INTERNATIONAL 2004 in Bremen, Germany and further exhibitions and trade fairs regarding food and technology in 2004. Also information to the wide ranged network of regional, national and international partners of TTZ accelerated in the second year of the FishNose project.

3.11 Matforsk, Norway (MATFORSK)

Participant number: B2

Address: MATFORSK - Norwegian Food Research Institute
Osloveien 1
N-1430 As
Norway

Scientific Team: Dr. John-Erik Haugen, Dr. Frank Westad, Mr. Frank Lundby, Mr. Øistein Jakobsen, Per Lea, Elisabeth Olsen, Asgeir Nilsen, Tove Maugesten, Laura Blumlein, Solveig Le Divenah, Viggo Foss, Andreas Kolstad, Kristin Østby

Contractual link to other participants: RTD performer

Objectives:
MATFORSK of Norway is a centre for food quality expertise established in 1970. MATFORSK is an independent, non-profit making private foundation with the food industry represented on the company board. MATFORSK has approximately 160 employees with wide professional expertise.
MATFORSK has many years experience with seafood producers and exporters from Norway. One of their main activities has been to develop and apply chemical methods which correlate with the sensory analysis of rancid products and fish flavour properties. Gas-phase analysis based on traditional gas chromatography methods (mass spectrometry and olfactometry) has been used to identify key volatile compounds related to sensory qualities in combination with classical sensory methods with a trained accredited panel.
Research activities are concerned with meat, vegetables, cereals and seafood. Other research topics include consumer behaviour, sensory properties, oxidation/antioxidants, rapid instrumental methods, gene-technology, microbiology, flavour perception and release and chemometrics.
MATFORSK has 20 years experience in the use of rapid methods (in particular NIR) and multivariate analysis and has published more than 100 scientific papers in this field. Since 1995 the institute has gained experience with gas sensor array technology (electronic noses) and had close collaboration with commercial manufacturers of these systems. In particular, the institute has focused on the development of food applications and calibration algorithms. One of the present research activities is transferability and standardisation of electronic nose measurement data by means of univariate and multivariate algorithms.
MATFORSK mainly will be involved in specification, generation and optimisation of the FishNose-Sensor prototype, whereby they are responsible for the pattern recognition system, the software / interface as well as for the laboratory- and on-site-tests. Besides, sensorial, chemical, microbial and physical reference analysis will be provided for FishNose optimisation.

Workplan:

Partner	B2									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	1	3	3	2	2	3	3	2	1	20
MM devoted Y 1	1	1,6	2,8	0,3	1,4	3	0,7	-	-	10,8
MM devoted Y 2	-	-	-	-	2	-	3,3	3,3	1	9,6

Deliverables:
MATFORSK is responsible for the following deliverables:

- D04 Reference methods ready to use in the project partner labs (due after month 5)
- D10 Pattern recognition and compensation software (due after month 12)
- D11 User-friendly interface and control software (due after month 12)
- D12 Optimised prototype with specification of lab experiments (due after month 14)
- D13 test formats and protocol-schemes for laboratory and on-site sensor evaluation (due after month 12)
- D14 Pre-competitive, optimised industrial prototype with specification of in-site experiments (due after month 23)

MATFORSK has contributed with reference analyses according to the laboratory program including also GC analysis on the Norwegian SME's samples stored at MATFORSK as input to D04. Additionally, MATFORSK has carried out the data correlation computations combining the data generated during Task 2 and sensor measurements (Task 6). Deliverables D10 and D11 are completed by February 2004 (midterm meeting) and reported in Annexes A10 and A11. The deliverable D12 was finalised after month 14. The deliverable D13, test formats and protocol schemes for laboratory testing of prototype sensor system, were finalised on the basis of the experience and optimisation gained during the prototype laboratory testing and will be delivered together with D12 and D14 (appendix A2 – A3)as appendix of this report.

Research activities during project running time:
MATFORSK was involved in establishing the questionnaire for the description of the end-user's fish processing facilities as well as for the investigation of the user's demands according to the gas sensor array to be developed. Inspections, meetings and telephone conferences have been performed at REMO and TBB. The collected information has been filtered, sorted in tabular form and integrated into the definition of the Sensor's requirements and system specifications.
Within Task 2, MATFORSK was also involved in planning, co-ordination and performance of the first round of storage trials. Chemical, microbial, physical and sensorial reference analysis methods have been established (Annex A4).
For the contribution to Task 3, MATFORSK performed GC/MS analyses of REMO and TB samples from storage experiment. In addition the reference analysis program was performed on the sample set. For, MATFORSK slo provided the generated REMO and TBB samples of different quality to IFL for GC-MS measurement.
In the frame of Task 4, MATFORSK supported ALPHA and OPTOTEK in the design of the gas-sampling unit.
MATFORSK contributed with the data analysis and correlation computations on the reference and sensor data obtained during the initial storage experiment and prototype sensor testing, including also the sensor selection and optimisation.
For contributing to the design of the sensor-unit and first prototype testing, MATFORSK delivered the generated and evaluated REMO and TBB samples to ALPHA. In addition, MATFORSK contributed with its sampling and gas-sensor experience to the hardware development and specifications of the prototype sensor and sampling unit (Task 4 and 5).
MATFORSK with the assistance of ALPHA and TTZ defined and developed the software design and definition of a user-friendly interface within Task 6. According to the investigated demands, evaluation of existing FOX and Gemini software of ALPHA has been performed on a working meeting in Toulouse. The deliverables D10 and D11 were completed in due time (Annex 10 and 11).
With assistance by TTZ, IFL and ALPHA a draft of the prototype laboratory test program has been established (Task 7).
In context of intended laboratory and on-site tests, MATFORSK provided established methods of chemical, physical and microbial reference analysis. Additional storage and monitoring trials were set-up with samples from REMO and TBB.
Furthermore validation of gas-sampling unit were performed within the prototype testing and optimisation phase of the FishNose Sensor. Besides, MATFORSK was involved in FishNose optimisation by further testing with real samples and standard compounds.
MATFORSK was responsible for the laboratory and on-site testing (Task 7/8). In addition, experience gained out of lab- and on-site tests were included in a concept for staff-training and workshop for potential clients.

3.12 Icelandic Fisheries Laboratories, Iceland (IFL)

Participant number: B3

Address: IFL - Islandic Fisheries Laboratories
Skulagata 4
IS-121 Reykjavik
Island

Scientific Team: Mrs. Gudrun Olafsdottir, Mrs. Rosa Jonsdottir, Mrs. Kolbrun Sveinsdottir, Mrs. Hélène Lauzon

Contractual link to other participants: RTD performer

Objectives:
Established in 1934, IFL has the role of promoting the advancement of Icelandic fishing and fish processing. The institute is divided into three divisions: Analytical Services Division, R&D Division and Information Services Division The institute studies and assesses the chemical, microbiological, sensory and physical properties of fish and fish products. Methods included are: traditional microbial methods, proximate analysis of foods and feeds, assessment of traditional indicators of fish spoilage (TMA, TVB), determination of quality of fish oils and HPLC, GC, GC-MS based methods for analysis of trace elements and trace compounds in fish.
The institute has extensive experience in the sensory analysis of fish and fishery products and operates a sensory evaluation laboratory with a trained sensory panel for evaluating seafood freshness and spoilage of fish. The main research areas include: predictive modelling for shelf life of fish, distribution of pathogens in processing environments, sensors for rapid measurement of fish freshness, sensory evaluation of seafood, toxic chemicals in fishery products, new processing methods. Current and recent EU projects at IFL include further studies on valid methods to monitor fish freshness and projects with emphasis on microbiological aspects, bio-preservation and safety of fishery products.
IFL mainly will be involved in specification and optimisation of the FishNose-Sensor prototype, whereby they are responsible for the detection of key compounds as well as for organisation and coordination of the reference analysis programme. Besides, sensorial, chemical, microbial and physical reference analysis will be provided for FishNose optimisation.

Workplan:

Partner	B3									
Task	1	2	3	4	5	6	7	8	9	Total
MM total	1	3	5	1	1	1	3	2	1	18
MM devoted Y 1	0,6	2,3	3,5	-	-	-	-	-	-	6,4
MM devoted Y 2	-	1,1	1,7	1	0,4	-	6,4	-	1	11,6

Deliverables:
IFL is responsible for the following deliverables:

- D03 Specified laboratory programme (due after month 3)
- D04 Reference methods ready to use in the project partner labs (due after month 5)
- D05 List of characteristic key compounds for spoilage of smoked fish (due after month 5)
- D06 Standard cocktail of selected compounds for training of the pattern recognition system and calibration of the developed E-Nose system (due after month 5)

Research activities during project running time:
IFL was the leader for Tasks 2 and 3. For Task 2 the responsibilities of IFL included specifying the laboratory programme in collaboration with TTZ and MATFORSK (Annex 3) and selecting the reference methods to use in the project. The reference methods were established in all the laboratories (IFL, MATFORSTK, TTZ and ANFACO). Annex 4 includes a detailed list of available methods in all the laboratories to evaluate the composition and quality related characteristics. Moreover, methods to evaluate safety related parameters e.g. pathogens and histamine are also included. Development of the sensory scheme has been the main focus since the sensory analysis is the most important method for quality evaluation in the food industry. QDA was used to evaluate which

quality attributes best describe the changes occurring in smoked salmon during storage. The next step is to further develop a simplified version of the sensory scheme. The IFL sensory panel will be responsible for this in collaboration with the other laboratories and taking into consideration the common practices to evaluate smoked salmon quality in the different smokehouses.
The harmonised test laboratory programme includes descriptions of standardised procedures for sampling, storage and transport. In addition it was considered necessary to carry out pre-trials to develop and finalise the laboratory programme. This was done by doing storage studies and monitoring the microbial, chemical and sensory changes. The pre-trials using the laboratory test programme was necessary to characterise the spoilage profile of typical smoked fish products in the different countries. Moreover, identification of common spoilage indicators was necessary to select the most appropriate reference methods.
IFL has compiled all the data from the storage trials from IFL, TTZ and MATFORSK, evaluated the data and written a detailed report (Annex 3)
The responsibilities of IFL in Task 3 included gas chromatography analysis of smoked salmon to determine key compounds for quality and spoilage evaluation of smoked fish during storage (Annex 5) and to develop a standard cocktail to calibrate the E-Nose prototype.
The laboratory programme (TASK2) was complete and used for the prototype testing and simultaneous measurement using the reference methods. The partners agreed to use a simplified programme of reference analysis for the future training and validation of the FishNose Sensor. The prototype testing involved storage trials and spot checks of smoked salmon products from the SME's.
TTZ, MATFORSK, IFL and ANFACO provided agreed chemical and microbial reference analysis during the FishNose laboratory and on-site testing of Tasks 7 and 8 in the second year. For better comparability of results the sensory analysis of all samples were performed by IFL. IFL carried out sensory analysis for the prototype testing to ensure comparable result. The FishNose QDA sensory scheme was used during the storage trials in Iceland but samples were also be taken and kept frozen. These samples were analysed by a modified FishNose scheme at the same time as samples from other producers. This allowed comparison of the FishNose scheme and a modified FishNose version with selected attributes. It was suggested to use mainly odour attributes, i.e. smoked salmon odour, rancid odour and spoilage odour (sweet /sour and off odour combined) and perhaps a few other attributes describing flavour and appearance (e.g. salt taste, fat secretion).
Further testing and validation of standard cocktail/key compounds, e.g. using standard addition in smoked salmon model system, were performed during prototype testing. The standard cocktail was also be tried out for calibrating the electronic nose. The stability tests of the standard cocktail and evaluation of the calibration procedure was carried out in collaboration with the project coordinator ALPHA.

4 Project Management and Coordination

Project management was carried out by the co-ordinator ALPHA. It was running well during the first year of the FishNose project. All partners demonstrated their full commitment to the project's progress and the partner's input in general has been as planned. Figure 24 shows again the project's management and consortium structure.

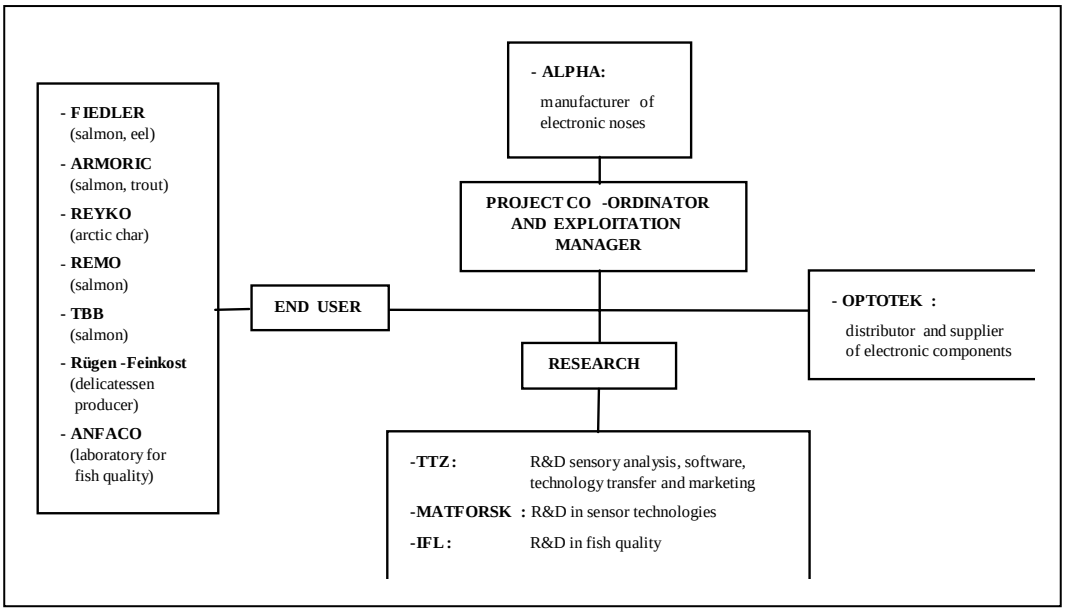


Fig. 24: Consortium Structure

4.1 Payment

The co-ordinator ALPHA received the advance payment by the EC (40% of total EC contribution) and transferred the payment (March 2003) to the partners according to the contract terms after they provided their banking information.
The first Cost Statements covering the first year of the project together with the Mid-Term report and required Deliverables and Milestones were submitted in February 2004. The Mid-Term report was accepted by the Scientific Officer Dr. Rosanna D'Amario in May 2004. The second payment was paid by the European Commission end of November 2004 and paid to the partners beginning of January 2005.

4.2 Contractual aspects

The co-ordinator ALPHA provided the project partners with copies of the following documents:

- The full paper version of the signed **EC-contract QLKI-CT-2002-71304** including general conditions (Annex 2)
- The signed **Model X contract**
- The **Technical Annex**
- The final version of the **Consortium Agreement (CA)** was signed by all the partners and submitted with the Mid-Term report to the European Commission. An original copy was handed out to the partners in June 2004.
- A draft version of the **Technological Implementation Plan (TIP)** was already submitted with the Mid-Term report to the European Commission. A final version of this document is submitted together with the Final Report.

The **Consortium Agreement (CA)** has been established and signed by all partners, which is included in the current report as Annex A17. In this context a Project Board was founded whereby the composition was set as the following table 12:

Tab. 12: Composition of the Project Board

Company	Member of the Project Board
ALPHA	Eric Chanie
FIEDLER	André Fiedler
ARMORIC	Jean-Francois Feillet
REYKO	Kari P. Olafsson
ANFACO	Carlos Ruiz Blanco
REMO	Johnny A. Remo
RÜGEN-F	Andreas Berthold
TBB	Geir Naustvik
OPTOTEK	Matjaz Zalar
TTZ	Claudia Thalmann
MATFORSK	John-Erik Haugen
IFL	Emilia Martinsdóttir

4.3 Meetings

General Meetings:

Five general project meetings have been organised during the project running time:

- The **Kick-Off Meeting** took place on 17th February 2003 in Toulouse, France, at the co-ordinator ALPHA-MOS. The minutes of the meeting were included as Annex A6 in the First 6 months progress management report.
- The **6-Months Meeting** took place on 23‘~‘ and 24t1~ June 2003 in Alesund, Norway, at Hotel Bryggen and REMO. The minutes of the meeting were included as Annex A7 in the First 6 months progress management report.
- The **Midterm Meeting** took place on 12th and 13th February in Bremerhaven, Germany, at TTZ and FIEDLER. The minutes of the meeting were included as Annex A 15 in the Mid-Term Report.
- The **18-Months Meeting** took place on 18th and 19th June 2004 in Reykjavik, Iceland at IFL and REYKO. The minutes of the meeting are included as Annex 6 in this 18 months progress management report.
- The **Final Meeting** took place on the 13th December 2004 in Bremerhaven, Germany, host by TTZ. The minutes of the meeting are included as Annex 3 in this 24 months progress report.

Technical Meetings:

Several technical meetings took place between project partners during the first 18 months reporting period:

- Meeting between MATFORSK and ALPHA concerning software design being held in parallel to the kick-off-meeting
- Meetings between TTZ and FIEDLER concerning end-user‘s process specification and requirements
- Meetings between MATFORSK and TBB concerning end-user‘s process specification and requirements
- Meetings between IFL and REYKO concerning end-user‘s process specification and requirements
- Meetings between TTZ and RÜGEN-F concerning end-user‘s process specification and requirements
- Meeting between ALPHA, MATFORSK and TTZ in Toulouse, France, on 27th and 28th October 2003 concerning software and interface development as well as sensor design.

In addition numerous extensive telephone conference have been held, e.g. between TTZ and RÜGEN-F, TTZ and ANFACO, ALPHA and ARMORIC as well as between MATFORSK and REMO concerning end-user‘s process specification and requirements.

4.4 Reporting

The 6-month management report (deliverable D15a), has been generated and submitted in time in June 2003.
The midterm report was represented was submitted with 1 month delay which was conceded by EC due to the date of Midterm Meeting in month 14. As affirmed by the project's PTA, it combines deliverable D 15 b and D16 as well as Milestone M 04.
The 18-month management report (deliverable D15c), has been generated and submitted in time.
The 24-month progress report (deliverable D15d) as well as the Final report (deliverable 20) were generated and submitted together with the Final TIP (deliverable 19) and the final cost statements after the end of the project.

4.5 Manpower and financial situation

The following table summarises the manpower allocation from month 1 to month 24 (01/01/2003 until 31/12/2004):

Partner	Total hours planned for the project *	Hours spent on the project during 24 month period
ALPHA	1890	2411
FIEDLER	675	675
ARMORIC	810	746
REYKO	675	668
ANFACO	1080	1150
REMO	675	697
RÜGEN-F	675	695
TBB	675	685
OPTOTEK	945	945
TTZ	2970	2970
MATFORSK	2700	2339
IFL	2430	2427

* 135h/MM

There was no major deviation observed regarding the manpower allocation.

The following figure summarises the manpower allocation from month 1 to month 12 (01/01/2003 until 31/12/2004):

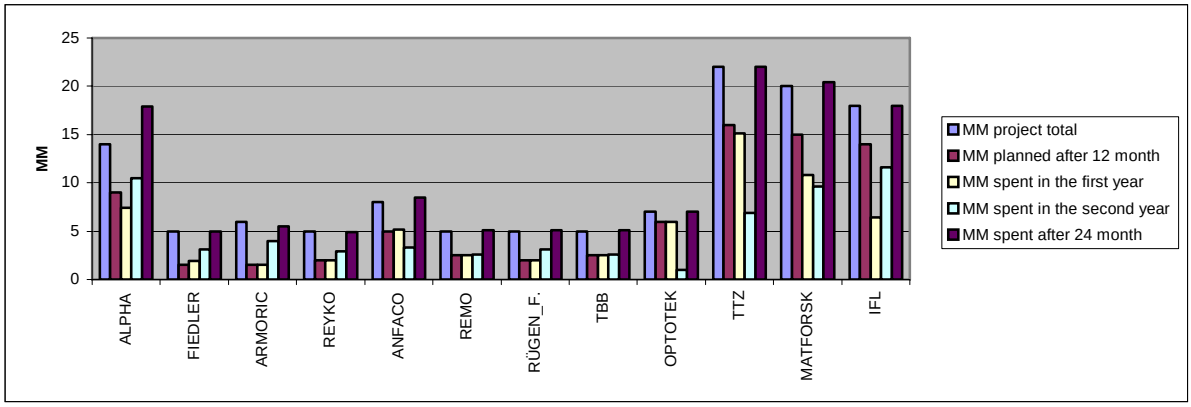


Fig. 25: Overview of manpower

There were no major deviation observed regarding the manpower allocation. Most partners contributed according to the Technical Annex. IFL was about 7,5 MM behind time table in the first year, but on reason that they performed all the sensory analysis and further GC measurements during prototype testing they gained on in the second year period.

5 Exploitation and Dissemination Activities

Regarding exploitation and dissemination activities, all the partners take part in this work and assume the own responsibilities.

A project web-page has been designed and established by ALPHA with support of TTZ:

http://www.alpha-mos.com/projects/public_form.php

Detailed information about project status and meetings are available to the partners in a password controlled section.
On the kick-off meeting the partners agreed to generate a leaflet earlier than planed in the Technical Annex (Deliverable D18 - due Month 21). To promote the FishNose Sensor already during the operating time of the project, the leaflet was released in month 12 by TTZ (edition: 1500). Several copies of this leaflet were distributed between the partners and handed out to the Commission on the Midterm Meeting for dissemination purposes. Besides, one example is enclosed to the current report as Annex A12.

The FishNose project has been introduced at several occasions for dissemination:

- ALPHA presented the FishNose on the SISQA fair, Toulouse, France (Traceability in food industry, December 2003)
- FIEDLER promoted the intended FishNose Sensor at “Bundesverband der deutschen Fischindustrie“ in Hamburg, Germany
- RÜGEN-F promoted the project on the Fish exhibition 2003 in Brussels, Belgium
- TTZ introduced the project on the Fish International exhibition 2004 in Bremen, Germany.
- Reportage in Norwegian TV 2 channel new 13th February 2005

Additionally, an article about FishNose objectives and progress was published in “Fisch Magazin 3/2004 (p.55), Germany - Special edition bout the “Fish international exhibition in Bremen 2004“.

In the last 6 months of the project the FishNose partners intend to make several publications including some of the results obtained during the project. During the 18 months progress meeting, the consortium discussed the potential topics of those publications taking into account the very promising results gained until now. A preliminary list of the foreseen publications is listed below:

- Sensory correlations of gas sensor array for QC of smoked salmon
- An on/at line sensor array system for quality control of smoked salmon
- Characterisation of key volatile compounds in smoked salmon and correlation with solid state based gas sensor array
- Three draft manuscripts have now been prepared
  - Rósa JÓNSDÓTTIR, Guðrún ÓLAFSDÓTTIR, Frank WESTAD, Erik CHANIE, 2005. Variation in the Occurence of Volatile Compounds in Cold Smoked Salmon during Storage will be submitted for publication in J Sci Food & Agric.
  - Gudrun OLAFSDOTTIR, Eric CHANIE, Frank WESTAD, Rosa JONSDOTTIR, Sandrine BAZZO, Saïd LABRECHE, Pauline MARCQ, Frank LUNDBY, John-Erik HAUGEN, Rapid Control of Smoked Atlantic Salmon Quality by Electronic Nose: Correlation with Classical Evaluation Methods. Proceedings of the ISOEN 2005 in Barcelona on April 13th-15th 2005 (short version)
  - Gudrun OLAFSDOTTIR, Eric CHANIE, Frank WESTAD, Rosa JONSDOTTIR, Sandrine BAZZO, Saïd LABRECHE, Pauline MARCQ, Frank LUNDBY, John-Erik HAUGEN, Rapid Control of Smoked Atlantic Salmon Quality by Electronic Nose: Correlation with Classical Evaluation Methods will be submitted for publication in Sensors and Actuators or in a Food/Agricultural Science Journal. (extended version)
- ISOEN 2005 Conference
  Gudrun OLAFSDOTTIR, Eric CHANIE, Frank WESTAD, Rosa JONSDOTTIR, Sandrine BAZZO, Saïd LABRECHE, Pauline MARCQ, Frank LUNDBY, John-Erik HAUGEN, Rapid Control of Smoked Atlantic Salmon Quality by Electronic Nose: Correlation with Classical Evaluation Methods. Will be presented at ISOEN 2005 in Barcelona on April 13th-15th 2005

6 Ethical Aspects and Safety Provisions

The new sensor system of FishNose will be introduced during a time of increasing demand of rapid, reliable and automated quality control in the fish processing industry. Fish - both fresh and smoked - is a very sensitive foodstuff with regard to spoilage and food poisoning is often the result. Inappropriate storage or transportation can lead to a spoilage of the fish meaning the development of pathogenic micro-organisms such as E coli, Listeria Salmonella etc.

The FishNose project totally fits to current and annual recurring discussion about smoked fish quality offered to the consumer in high-seasons, like Christmas-time. Among others, public studies about smoked salmon freshness took place in Germany: Stiftung Warentest (December 2001), ÖKO-TEST (December 2003). Thereby main criticising parameters have been the microbial contamination as well as sensorial attributes like consistence, taste and smell.
By using the FishNose sensor, smoke fish producers will be able to prove and justify the excellent product quality delivered to the clients and retailers. On the other hand companies who purchase smoked fish will be able to establish an objective, quick and reliable incoming inspection. Thereby the FishNose has got the potential for monitoring and traceability of the whole trade chain - from producer to the consumer. Should official standards for quality and freshness be approved as a result of the sensor values, as is anticipated, this will enable standardised product categorisation for trading and survey performed by official or private food monitoring institutions.

7 Annex

- A1: Deliverable D09: FishNose prototype with specification regarding the standard mixture
- A2: Deliverable D12: Optimised prototype with specification of laboratory tests
- A2: Deliverable D13: Test formats and protocol-schemes for on-site sensor evaluation
- A3: Deliverable D14: Pre-competitive, optimized industrial prototype with specification of on-site experiments
- A4: Minutes of the 18 Months Meeting
- A5: Minutes of the Final Meeting