Thesis for the Degree of Doctor of Philosophy

VOLATILE COMPOUNDS AS QUALITY INDICATORS IN CHILLED FISH: EVALUATION OF MICROBIAL METABOLITES BY AN ELECTRONIC NOSE

Guðrún Ólafsdóttir

Faculty of Science
University of Iceland
and
Icelandic Fisheries Laboratories

Reykjavík 2005

Academic Dissertation

University of Iceland, Faculty of Science,

Department of Food Science and Nutrition, Reykjavik, Iceland

and Icelandic Fisheries Laboratories, Reykjavik, Iceland

Supervised by

Dr. Kristberg Kristbergsson, Department of Food Science and Nutrition, University of Iceland

Ph D Committee

Dr. Jörg Oehlenschläger, Federal Research Centre for Fisheries (FRCF), Germany

Dr. Joop B. Luten, Netherlands Institute for Fisheries Research (RIVO), The Netherlands

Dr. Rögnvaldur Ólafsson University of Iceland

Dr. Ágústa Guðmundsdóttir, University of Iceland

Opponents

Dr. Ragnar L. Olsen, Norwegian College of Fishery Science, Norway

Dr. Saverio Mannino, Università degli Studi di Milano, Italy

Volatile compounds as quality indicators of fish

Printed in Iceland by Gutenberg, Reykjavik, 2005

ISBN 9979-70-052-1

VOLATILE COMPOUNDS AS QUALITY INDICATORS IN CHILLED FISH: EVALUATION OF MICROBIAL METABOLITES BY AN ELECTRONIC NOSE

Guðrún Ólafsdóttir
Department of Food Science and Nutrition, University of Iceland
Icelandic Fisheries Laboratories, Reykjavík, Iceland

ABSTRACT

Key determinants of chilled fish quality are characteristic odor changes caused by the formation of volatile compounds, like alcohols, aldehydes, ketones, esters, sulfur compounds and amines, by specific spoilage organisms (SSO). The objective of this thesis was to explore the potential use of electronic nose as a rapid technique to detect volatile compounds related to quality changes during chilled storage of different species of fish and fish products (capelin, cod, haddock, red fish and cold smoked salmon). The influence of various storage conditions (-1.5 to 15 °C) on the proliferation of SSO was studied and their spoilage potential was evaluated by electronic nose, chemical analysis of TVB-N and TMA and sensory analysis.

The results of measurements with electrochemical sensors (CO, NH_3, H_2S and SO_2), sensitive to the main classes of microbially produced compounds, demonstrated the spoilage potential of the SSO. The increased CO sensor response during early storage in all fish species, suggested the formation of alcohols, aldehydes and esters and the role of *Pseudomonas* spp. in the incipient spoilage changes. The response of the NH_3, H_2S and SO_2 sensors, sensitive to amines and sulfur compounds, respectively, indicated the importance of *S. putrefaciens* as a late spoiler in whole fish and in fillets stored under abusive temperature conditions. Production of ketones in high levels, contributed mainly by 3-hydroxy-2-butanone as identified by GC-MS, was associated with the active growth of *Photobacterium phosphoreum.* This bacterium was identified as the dominating SSO based on its growth and production of TMA as reflected by high levels of TVB-N in chilled cod and haddock fillets packed in styrofoam boxes.

Multivariate PCA and PLSR based models were used to predict the quality of the fish. Multiple quality indices based on the electronic nose sensors, SSOs and TVB-N were needed for classification or prediction of the complex quality changes of fish stored under different temperature conditions. However, when applying models adapted for each storage condition the ability of the electronic nose to classify samples was improved. For products like capelin with high concentration of volatile spoilage compounds a single sensor (NH_3) was sufficient to predict the TVB-N value by fitting a generalized linear model to the data and estimating parameters for each storage condition. Addition of sensors for the detection of ketones and increased sensitivity of sensors for TMA is suggested to improve the performance of the electronic nose to predict the quality of fish fillets.

KEYWORDS: Volatile compounds; quality indicators; electronic nose; gas chromatography; SSO; capelin, cod, haddock, red fish, cold smoked salmon, chilled, superchilled, MA

ROKGJÖRN EFNI SEM GÆÐAVÍSAR Í KÆLDUM FISKI:
MAT Á NIÐURBROTSEFNUM ÖRVERA MEÐ RAFNEFI

Guðrún Ólafsdóttir
Matvæla og næringarfræðiskor Háskóla Íslands
Rannsóknastofnun fiskiðnaðarins, Reykjavík

ÁGRIP

Ákvörðun gæða á kældum fiski tengjast lyktarbreytingum vegna myndunar á rokgjörnum efnum eins og alkóhólum, aldehýðum, ketónum, esterum, brennisteinsefnum og amínum af völdum sértækra skemmdarörvera (SSÖ). Markmið verkefnisins var að kanna mögulega notkun rafnefs, sem fljótvirkrar mæliaðferðar, til að mæla rokgjörn efni sem tengjast gæðabreytingum í mismunandi tegundum af fiski og fiskafurðum (loðnu, þorski, ýsu, karfa og reyktum laxi) við geymslu í kæli. Áhrif mismunandi hitastigs (-1.5 - 15 °C) og geymsluaðferða á vöxt SSÖ voru könnuð og skemmdarvirkni þeirra metin með rafnefi, TVB-N og TMA efnamælingum og skynmati.

Rafnef, sem þróað hefur verið hérlendis, var útbúið með raflausnanemum sem skynja helstu efnaflokka sem myndast við skemmd í fiski. CO nemi rafnefsins reyndist næmastur til að greina skemmdir á byrjunarstigi í öllum fisktegundum, vegna myndunar á alkóhólum, aldehýðum og esterum, af völdum SSÖ einkum *Pseudomonas* tegunda. Aukning í svörun NH_3, H_2S og SO_2 nema, sem greina amín og brennisteinsefni á síðari hluta skemmdarferils fisks benti til að *S. putrefaciens* væri virk skemmdarörvera í lok geymslutíma í heilum fiski (karfa og þorski) og í þorsk og ýsuflökum sem geymd voru við hátt hitastig eða urðu fyrir hitasveiflum á geymslutímanum. Myndun ketóna aðallega 3-hydroxy-2-bútanons, sem greint var með gasgreini, tengdist örum vexti skemmdarörverunnar *Photobacterium phosphoreum*. Þessi örvera reyndist vera helsti skemmdarvaldurinn vegna mikillar TMA myndunar, sem var einkennandi fyrir kæld þorsk og ýsuflök í frauðplastkössum.

Fjölbreytulíkön byggð á höfuðþáttagreiningum (PCA) og PLSR voru notuð til að spá fyrir um gæði fisks. Nauðsynlegt reyndist að nota fjölgæðavísa samsetta af örverutalningum, TVB-N og rafnefsmælingum til að flokka eða spá fyrir um þær margvíslegu gæðabreytingar sem verða í fiski við geymslu við mismunandi hitastig. Hinsvegar reyndust líkön, sem byggð voru eingöngu á rafnefi, hæf til að flokka afurðir eftir gæðum ef líkanið var aðlagað að hverri afurð og geymsluhitastigi. Fyrir gæðamat á loðnu til bræðslu reyndist NH_3 neminn gefa besta mat á TVB-N gildi með því að nota línulega aðhvarfsgreiningu og ákvarða stuðla til að taka tillit til einkennandi skemmdarferils við mismunandi geymsluaðstæður. Niðurstöðurnar benda til þess að sértækir nemar fyrir ketón og næmari skynjun á TMA myndi auka hæfni rafnefsins til að meta gæði fiskflaka

LYKILORÐ: rokgjörn efni, gæðavísar, rafnef, gasgreining, skemmdarörverur, loðna, þorskur, ýsa, karfi, kaldreyktur lax, kæling, undirkæling, loftskiptar pakkningar

LIST OF PUBLICATIONS

This thesis is based on the following papers, referred to in the text by their respective Roman numerals

I. Guðrún Ólafsdóttir, Áslaug Högnadóttir, Emilía Martinsdóttir, Helga Jónsdóttir, 2000. Application of an Electronic Nose to Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production, J. Agric. Food Chem. 48 ,6, 2353- 2359.

II. Corrado Di Natale, Guðrún Ólafsdóttir, Sigurdur Einarsson, Alessandro Mantini, Eugenio Martinelli, Roberto Paolesse, Christian Falconi, Arnaldo D'Amico, 2001. Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets. Sensors and Actuators B77, 572- 578.

III. Guðrún Ólafsdóttir, Xiuchen Li, Hélène L. Lauzon, Rósa Jónsdóttir. 2002. Precision and application of electronic nose measurements for freshness monitoring of redfish (*Sebastes marinus*) stored in ice and modified atmosphere bulk storage. JAFP 11, 3/4, 229-249.

IV. Guðrún Ólafsdóttir, Hélène Lauzon, Emilía Martinsdóttir, Kristberg Kristbergsson. Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction. Submitted.

V. Guðrún Ólafsdóttir, Hélène L. Lauzon, Emilia Martinsdottir, Joerg Oehlenschläger, Kristberg Kristbergsson. Shelf-life extension of superchilled cod (*Gadus morhua*) fillets and influence of temperature fluctuations on microbial growth and metabolites. Submitted.

VI. Guðrún Ólafsdóttir, Rósa Jónsdóttir, Hélène L. Lauzon, Joop Luten and Kristberg Kristbergsson. Characterization of Volatile Compounds in Chilled Cod (*Gadus morhua*) Fillets by Gas Chromatography and Rapid Detection of Quality Indicators by an Electronic Nose. Submitted.

VII. Guðrún Ólafsdóttir, Eric Chanie, Frank Westad, Rósa Jónsdóttir, Claudia R. Thalmann, Sandrine Bazzo, Saïd Labreche, Pauline Marcq, Frank Lundby, John-Erik Haugen, 2005. Prediction of Microbial and Sensory Quality of Cold Smoked Atlantic Salmon (*Salmo salar*) by Electronic Nose. Accepted for publication in J Food Sci

ABBREVIATIONS

ANOVA	analysis of variance
ATD	automated thermal desorber
ATP	adenosine triphosphate;
CBC	contact blast cooling
GC-MS	gas chromatography-mass spectrometry
GC-O	gas chromatography-olfactometry
Hx	hypoxhanthin
NPN	non-protein nitrogen containing compounds
OU	odor unit
PAR	peak area ratio
PCA	principle component analysis
PLSR	partial least squares regression
PUFA	polyunsaturated fatty acids
QIM	Quality Index Method
QDA	Quantitative descriptive analysis
RI	retention index
SIMCA	soft independent modeling of class analogy
SSO	specific spoilage organisms
TMA	trimethylamine
TMAO	trimethylamine oxide
TVB-N	total volatile basic nitrogen
TVC	total viable count

TABLE OF CONTENTS

1. INTRODUCTION

Currently, there is an increasing demand for traceability of food products in relation to the quality and safety of these products for the consumers. Techniques to verify the quality of food are essential to gain consumer trust and will facilitate the marketability of food products. The possibility to use rapid instrumental techniques like electronic noses to detect volatile compounds associated with odor and quality is in particular of interest for perishable products like fish.

Gas sensors commonly used in electronic noses are non-selective towards individual compounds, but show sensitivity towards certain classes of compounds. This property induces their potential for monitoring quality and the onset of spoilage associated with varying levels of different classes of volatile compounds produced in fish during storage. The main classes of compounds that are present in spoilage are similar for the different fish species stored under chilled conditions. However, the concentration of individual compounds varies depending on the dominant microflora in the products, causing different types of spoilage odors. The classification of fish odors according to main classes of compounds produced is useful to facilitate the interpretation of rapid measurements of the total headspace of fish using electronic noses. Characterization of the spoilage changes and the spoilage domain of the specific spoilage organisms (SSOs) based on their growth and ability to produce off odors is needed to determine which quality indicators are relevant for monitoring quality changes during storage under different conditions.

The overall objective of the project was to study the formation of volatile compounds in fish stored under chilled conditions and the influence of various storage conditions on the growth of the SSO contributing to the production off odors. The innovative approach in the research was to study simultaneously the proliferation of the microflora and its spoilage potential by analysing microbial metabolites contributing to the spoilage odors. The study gives a better understanding and explains the contribution of the different microflora to the spoilage odor development in naturally spoiling fish. Many of the earlier studies on the spoilage potential of the microflora have focused on inoculating bacteria strains into sterile fish extract or fish muscle. All the experiments in this study were performed on naturally spoiled products.

The study covers extensive storage trials of different fish species and products. The influence of different processing, packaging and storage conditions on the spoilage pattern of the fish products was studied and various temperature conditions selected, that typically occur during storage and transport of these products.

- Capelin stored whole in ice, chilled seawater and with and added acid as a preservative at 0 and 5 °C
- Redfish stored whole in ice and packed in vacuum and under modified atmospheres at 0 °C
- Cod stored whole in ice at 0 °C
- Haddock stored as fillets in Styrofoam boxes at 0°C and under abusive and fluctuating temperatures (0, 7 and 15 °C)
- Cod stored as fillets in Styrofoam boxes at 0°C and processed and stored under superchilling conditions (-1,5)
- Cold smoked salmon vacuum packed and stored at 5 and 10 °C

Based on gas chromatography analysis, quality indicators related to odor for the different fish species and fish products have been identified. Electronic noses based on different sensor technologies were applied in the studies and used as rapid techniques to monitor the volatile compounds during storage of fish. The performance of models based on e-nose data of fish and fish products stored under different conditions to predict the quality or classify products according to microbial, sensory and chemical criteria were evaluated.

The electronic nose is a multisensor approach since it is composed of different types of sensors of different selectivity and sensitivity detecting simultaneously several classes of compounds related to quality and freshness of fish. Therefore, the electronic nose can possibly give more information about the complex spoilage changes than the traditional methods based on detecting one single indicator like total volatile bases. Moreover, the electronic nose measurements are more rapid and have the potential to be used as a less costly alternative than the traditional methods for quality checks in the industry. The information gained is of practical value for the fish industry and can be used to select appropriate storage conditions to extend the shelf life of fish products by preventing or minimizing the development of the spoilage flora.

OBJECTIVES

The objectives of the thesis were to explore the potential use of electronic noses to monitor quality changes related to the formation of volatile microbial metabolites during chilled storage of fish and to achieve better understanding of the spoilage of fish. The following aims were established for the thesis work:

- To apply electronic noses based on different sensor technologies as rapid techniques to monitor the volatile compounds during storage of various fish species stored and processed under different conditions.
- To study the proliferation of specific spoilage organisms (SSO) and evaluate their spoilage potential by simultaneous analysis of microbial metabolites contributing to the spoilage odors.
- To explore the correlation of e-nose data and the reference methods and apply multivariate models to predict the quality or classify products according to microbial, sensory and chemical criteria.
- To monitor volatile compounds in fish during chilled storage and screen potential quality indicators by gas chromatography analysis.

The background chapter of the thesis is based on the following publications:

Olafsdottir G, Kristbergsson K. Electronic-Nose Technology: Application for Quality Evaluation in the Fish Industry. In: Nicolay, X. editor. Air Pollution Control: Odours Treatment in the Food Industry. Springer, NY, NY. (in print)

Olafsdottir G, Nesvadba P, Di Natale C, Careche M, Oehlenschläger J, Tryggvadóttir SV, Schubring R, Kroeger M, Heia K, Esaiassen M, Macagnano A., Jørgensen BM, 2004. Multisensor for fish quality determination. Trends Food Sci Technol 15:86-93.

Olafsdottir G. 2003. Developing rapid olfaction arrays for determining fish quality. In: Tothill IE, editor. Rapid and on-line instrumentation for food quality assurance. Cambridge, England: Woodhead Publishing Ltd. p 339-60.

Olafsdottir G, Fleurence J. 1998. Evaluation of fish freshness using volatile compounds - Classification of volatile compounds in fish. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to Determine the Freshness of Fish in Research and Industry. Proceedings of the Final meeting of the Concerted Action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 55-69.

Olafsdottir G, Martinsdóttir E, Oehlenschläger J, Dalgaard P, Jensen B, Undeland I, Mackie IM, Henehan G, Nielsen J, Nilsen H. 1997. Methods to evaluate fish freshness in research and industry. Trends Food Sci Technol 8:258-65.

2. BACKGROUND

2.1. Quality evaluation of fish

Quality assurance in the fish sector involves monitoring and documenting defined quality criteria as required by regulations (Anon., 1996), product specifications and consumer demands. These requirements may be of different importance to the various parts of the supply and distribution chains for fish, which vary greatly between countries and for different types of products. With the developments taking place in food law and in the marketing of food, the commercial participants are increasingly demanding a full range of information relating to fish quality and traceability of the products. Selection and supplement of relevant information, including parameters describing quality of fish is thus needed.

Fish quality is a complex concept involving a whole range of factors which for the consumer include for example: safety, nutritional quality, availability, convenience and integrity, freshness, eating quality and the obvious physical attributes of the species, size and product type (Bisogni *et al*., 1987; Botta, 1995; Olafsdottir *et al*., 1997a; Oehlenschläger and Sörensen, 1998; Bremner, 2000). Information about handling, processing and storage techniques, including time/temperature histories, which can affect the freshness and quality of the products, is very important for the partners in the chain. Additionally, seasonal condition, the effects of fishing grounds and catching methods and the occurrence of various quality defects influence the overall quality. One of the most unique characteristics of fish as food is that it is a highly perishable commodity. Consequently, time passed after catch and the temperature 'history' of fish is very often the key factor determining the final quality characteristics of a fish product.

It is well documented that packed fillets spoil more rapidly and have different spoilage pattern than whole fish (Lindsay *et al*., 1986; Jørgensen *et al*., 1988; Huss, 1995; Lauzon *et al*., 2002). The interest of the industry is to maintain the freshness of the fish fillets, by optimal handling and transport conditions to ensure the high quality of the products on the market. Temperature control during all stages of the production and in the distribution chain is emphasized in guidelines on good manufacturing practices.

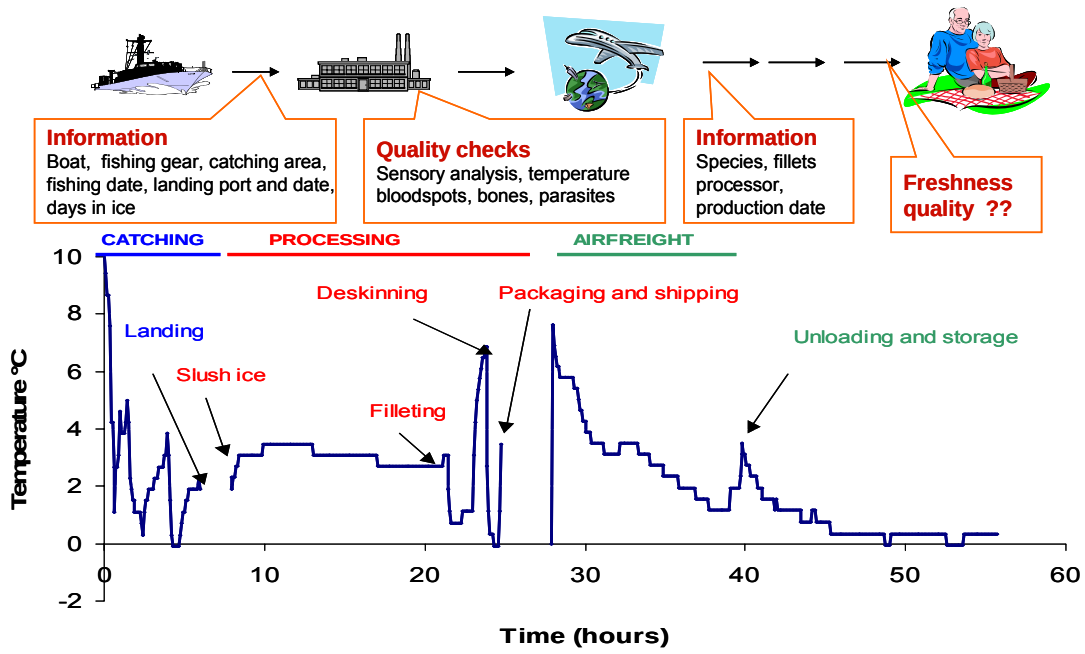


Figure 1. Typical temperature profile for the production of fillets and transport via air freight.

However, temperature fluctuations can occur in the production or in the distribution chain because of unforeseeable events such as improper icing of the raw material or delays in transport (Figure 1). In this case the rate of the deteriorative changes occurring in fish caused by microbial growth and oxidative changes will be influenced and therefore, the information on storage days or days from catch will not be reliable to determine the actual quality (Huss, 1995; Olafsdottir *et al.*, 2003a). Sensory assessment has always played a key role in quality and freshness evaluation in the fish industry. The various sensory characteristics, such as outer appearance, odor and color are still very important in quality control. Parameters related to origin, handling and defects are also considered important in the quality systems in the fish processing industry. Sensory inspection of processed fish is used in the fish industry to find defects that have occurred during handling and processing (Oehlenschläger, 1998).

Evaluation of the quality of whole wet fish: The evaluation of the raw material is done at the moment of landing, at fish auctions or in the reception area in the fish processing plants. Information about species, catching area and catching day has to be provided. Batches are evaluated by looking at handling practices on board: weight of fish and ice, how the fish is aligned in the tub, washing and icing, i.e. fish-ice layers and ice/fish ratio.

Evaluation of freshness is done at this stage using sensory assessment. For whole fish the EU quality grading scheme (Howgate *et al*., 1992), is used as required by EU regulation (Anon., 1996) but some initiatives have been taken to implement a new sensory method called the Quality Index Method (QIM) to standardize sensory assessment for each species (Bremner, 1985; Bremner *et al*., 1986; Larsen *et al*., 1992; Luten and Martinsdóttir, 1998; Martinsdóttir *et al*., 2003).

Evaluation of the quality of fillets: For the evaluation of fillets in fish processing, samples are taken randomly after trimming and checked for defects. Defects can be related to the condition of the fish flesh (e.g. gaping, watery), appearance, which includes color defects (bruises, bloodspots) and dehydration (frozen storage defects). Other defects such as improper packaging and cutting and trimming faults and oversights (remaining bones, parasites, foreign matters, skin and black membrane) are related to workmanship. Evaluation of defects is widely used in control of processes and to grade fish for selling or buying purposes.

For freshness determination of raw fillets, color and smell are evaluated, but for cooked fish schemes like the Torry scheme (Shewan *et al*., 1953) are in use. In many companies, however, sensory schemes tailor made for their special purposes have been developed. Evaluation of raw fillets is also done in secondary processing before further processing and in retail before packaging (e.g. MAP) and labeling for sale. Sensory evaluation of raw fillets is difficult and therefore, it is likely that the fish industry would welcome a reliable and easy to use multi-sensor device for the evaluation.

2.1.1. *Post mortem* changes in fish influencing quality

Odor is one of the most important parameters to evaluate fish freshness. The progression of characteristic odors in fish during chilled storage caused by microbial growth is well documented (Castell and Greenough, 1957; Shewan *et al*., 1953). The sensory descriptors for the progression of odor changes during storage of fish were described by Elliot (1947) as FRESH → FLAT → SWEET → STALE → PUTRID. The odor changes have been associated with the formation of volatile compounds produced by the main spoilage organisms (Miller *et al*., 1973a; b; c; Herbert and Shewan, 1976).

Complicated *post mortem* processes in the fish are responsible for the loss of freshness and the onset of spoilage. A combination of chemical, biochemical and microbiological

interactions occur which are species dependant and additionally various extrinsic factors such as handling and different storage conditions will further influence the spoilage pattern and the development of spoilage odors.

The *post mortem* changes in fish are initially dominated by autolytic activity, including degradation of nucleotides, formation of taste active inosine and accumulation of hypoxhanthin (Hx), lowering of pH and endogenous enzyme activity followed by the proliferation of the specific spoilage organisms (SSO) and development of volatile compounds contributing to spoilage changes and thus influencing the freshness and quality of the end product (Huss, 1995; Botta, 1995).

More knowledge is needed on the chemical characterization of spoilage processes of muscle food and the correlation with sensory and microbiological changes (Dainty, 1996). The *post mortem* changes influencing the development of odors in chilled fish are generalized in Figure 2. It is well established that enzyme mediated conversions of polyunsaturated fatty acids (PUFA) to volatile aroma compounds initiates the formation fresh fish odor compounds exhibiting fresh-plant-like aroma (Josephson *et al*., 1983; 1984; Hsieh *et al*., 1988). Further conversion and degradation of muscle constituents like sarcoplasmic proteins and membrane bound phospolipids by endogenous enzyme activity, autoxidation and microbial growth contributes to the changes in the aroma profile of fish during storage. The pool of components that are degraded and cause off flavors because of microbial growth and metabolism include soluble substances in the muscle, mostly composed of the various non protein nitrogenous components (NPN), including small peptides like carnosine and anserine, amino acids, guanidine compounds like creatine, TMAO and nucleotides (Ikeda, 1980). Some of these compounds influence the taste of fish like anserine giving “mouth satisfaction” (Jones, 1967) and the individual amino acids glycine, valine, alanine and glutamic acid are known to contribute to taste together with the the degredation components of the nucleotides like inosine.

Multi-compound indices based on gas chromatography analysis of the main volatile compounds produced by bacteria during spoilage have been suggested to evaluate the formation of spoilage odors occurring in fish products stored under different conditions (Lindsay *et al*., 1986; Olafsdottir and Fleurence, 1998; Jørgensen *et al*., 2000; 2001).

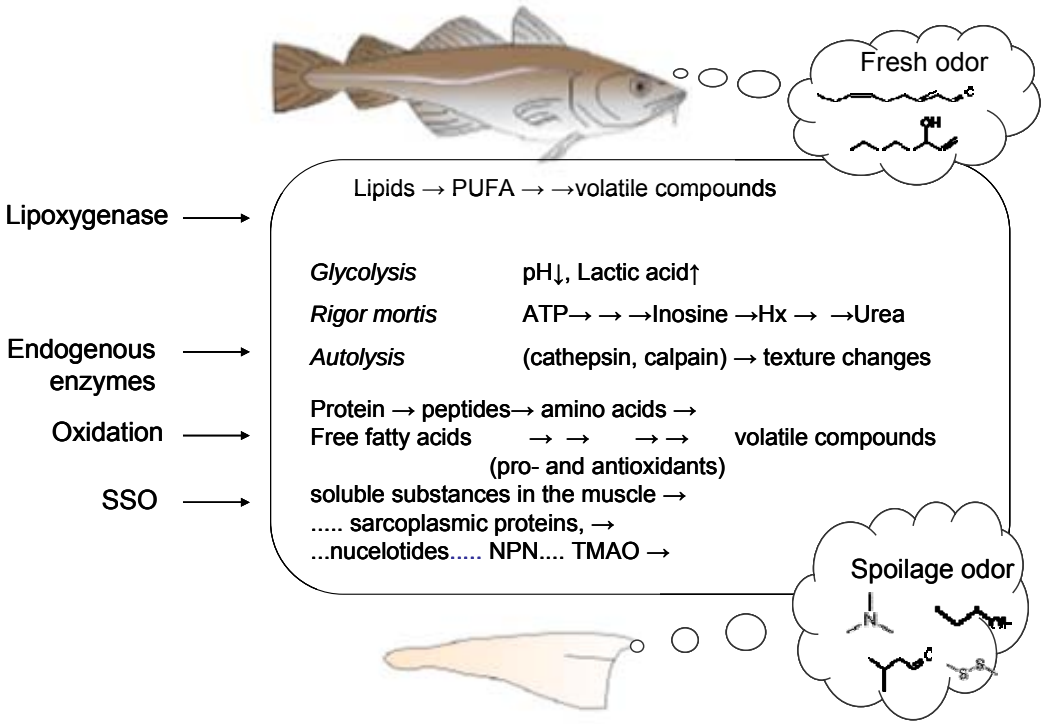


Figure 2. Overview of post mortem changes in fish influencing the development of odors (SSO: specific spoilage organisms, PUFA: plyunsaturated fatty acids; ATP: adenosinetriphosphate; Hx: hypoxhanthin; NPN: non-protein nitrogen containing compounds, TMAO: trimethylamineoxide)

Various pro- and antioxidants influence the stability of the muscle and have been studied in relation to the oxidative stability of phosopholipids (Hultin, 1994). Phospholipids are the main membrane bound lipids and because of their high unsaturation they are in particular sensitive to oxidation which is further enhanced by pre-processing and storage of the fish. Oxidative processes occurring during storage of fish will result in the accumulation of both saturated and unsaturated aldehydes that contribute to the development of rancid cold store flavors (McGill *et al*., 1974; Aro *et al*., 2003).

The role of antioxidants (α-tocopherol, ascorbic acid and glutathion peroxidase) and aqueous pro-oxidants in fish muscle including blood components like inorganic metals (iron (Fe) and copper (Cu)) have been studied to understand better the mechanisms of the oxidation in the muscle (Hultin and Kelleher, 2000; Undeland *et al*., 1999). Monitoring of the levels of pro- and antioxidants along with the traditional primary, secondary and tertiary degradation compounds of lipid oxidation in the muscle have been suggested as potential quality indicators for fish (Bandarra *et al*., 1998; Undeland, 1998).

Proteolysis plays a critical role in *post mortem* changes resulting in undesirable texture changes in fish. Endogenous enzyme activity influences the deterioration of fish muscle including calpains (neutral calcium-dependent proteases) and cathepsins (lysosomal proteases). The understanding of the mechanism of these reactions is still not clear but active research in this area is currently ongoing (Delbarre-Ladrat *et al*., 2004).

2.1.2. Instrumental techniques to evaluate quality

Various instrumental techniques to detect *post mortem* quality changes in muscle food have been developed (Olafsdottir *et al*., 1997a; 2004; Pau and Olafsson; 1997; Ellis and Goodacre, 2001). The state of freshness can be described by a variety of definite properties of the fish which can be assessed by different indicators (Bremner and Sakaguchi, 2000). Rapid, inexpensive and accurate instrumental and sensory methods have been developed, that can be correlated with time after catch or attributes related to fish freshness (Botta, 1995; Connell, 1995; Olafsdottir *et al*., 1997a). An estimate of freshness can be obtained by defining criteria related to changes in sensory attributes like appearance, odor, color and texture, which can be measured and quantified by sensory or instrumental methods. The future aims for fish freshness evaluation were established as the conclusions of the EU project "Evaluation of Fish Freshness" in the year 1997 (Olafsdottir *et al*., 1998a) and reflect the ongoing research in this area today (see Box 1). For accurate evaluation of quality no single index can encompass all the complex changes occurring during spoilage of fish (Martin *et al*., 1978). One means of achieving this is by developing an instrument which measures a set of attributes that together can give a better estimation of freshness or quality than with one technique alone (Olafsdottir *et al*., 1997a).

The possibility to develop a multi-sensor device to measure and/or estimate fish freshness with a combination of instrumental techniques (electronic noses, spectroscopic methods, texture-meters, image analyzers, color meters and devices measuring electrical properties) was investigated in a EU project called "Development of Multi-Sensor Techniques for Monitoring the Quality of Fish" (Nesvadba, 2003; Olafsdottir *et al*., 2004).

Box 1. Conclusions - Future aim for fish freshness evaluation

⇒ **Sensory evaluation** is the most important method today for freshness evaluation in the fish sector. The trend is to standardize sensory evaluation by improving methodologies and training of panels to make sensory evaluation an objective measurement.

⇒ It is suggested to develop **microbial methods** for evaluation of fish freshness together with mathematical models expressing the effect of storage conditions (temperature and atmosphere) on growth of the relevant spoilage microorganisms.

⇒ Rapid assessment of **volatile compounds** in fish using gas sensors to determine freshness is of increasing interest.

⇒ At the moment there are no rapid methods of determining changes in muscle **proteins** during *post mortem* storage.

⇒ Most of the techniques described to monitor **lipid** oxidation are only used in research, and very few are routinely applied in the fish industry. To monitor the progression of lipid oxidation, it is important to use more than one method especially when comparing various types of fish products

⇒ The use of **ATP** metabolites as freshness indices is a research technique not widely used in industry. The future trend is the development of rapid techniques for ATP metabolites.

⇒ The following **physical measurements** give information on parameters related to fish freshness. None of these methods however, give a unique and unambiguous answer to whether the fish is fresh or not.

* It is likely that **time / temperature indicators** will be gradually introduced into the wholesale and retail food chain, starting with temperature sensitive high value foods such as fish.
* **Texture measurements** of fish have been compared to sensory analysis and some results have shown good correlation
* Changes of **microstructure** of the fish muscle have been related to postmortem tenderization and liquid holding capacity of fish muscle.
* Changes in fish freshness can be measured by the **electrical properties** of the fish muscle. The advantage of electrical testers is their immediate response, the field use and the use without previous experience.
* **Color measurements** can be related to changes in fresh fish.
* **Spectroscopic methods** have recently gained importance in evaluation of food quality parameters.

From: Olafsdottir *et al*. (1997a) "Evaluation of Fish Freshness" (AIR3 CT94 2283)

The techniques used in the project complemented each other, supplying independent information about the state (quality, freshness) of the fish over the entire duration of chilled or frozen storage experiments. All techniques except the electrical testers and the VIS-spectroscopy have a counterpart in the sensory assessment as defined in the QIM scheme (Quality Index Method). The outputs of the instruments can therefore be calibrated with the corresponding QIM sensory scores (Figure 3). Moreover, the texture and electronic nose sensors complemented each other by being most sensitive indicators of freshness before and after day four of chilled storage, respectively. This means that combining the data from the various sensors improves the estimate of the freshness of fish. To demonstrate this Di Natale (2003) selected color, texture and electronic nose measurements and combined their calibrated outputs to construct the Artificial Quality Index (AQI) as illustrated in Figure 3. It was shown that the AQI can be as accurate and precise as the QIM score, with the uncertainty of the predicted storage time being less than 0.5 days (Di Natale, 2003).

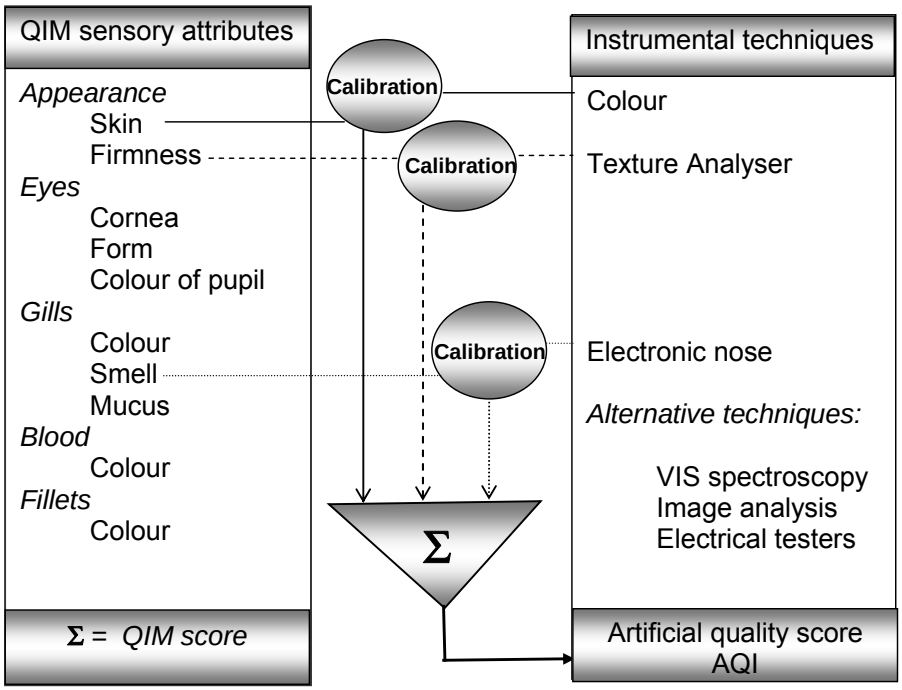


Figure 3. Construction of the Artificial Quality Index (AQI). After calibration with sensory data (Quality Index Method (QIM)) the instrumental readings are combined into Artificial quality score giving the AQI (adapted from Di Natale, 2003) (From: Olafsdottir *et al*., 2004)

The costs of labor and training of assessors are likely to increase and the cost of instrumentation such as the image analysis is set to decrease dramatically. Consumer and governmental pressures for better description of quality and traceability of fish products will also increase. All of these factors will increase the importance of instrumental techniques of monitoring the quality of fish.

The development of biosensors for fish quality was reviewed by Venugopal (2002). The main focus for biosensor development has been on measuring the nucleotide concentration which is a good indicator for the initial loss of freshness expressed as K value as suggested Saito (1959). Enzyme sensor systems based on detecting nucleotides in fish have been developed (Okuma and Watanabe, 2002). Commercial devices based on nucleotide degradation based on soluble enzymes and an oxygen electrode like "KV-101" and "Microfresh" and test kits for ammonia and ethanol are available for industrial use (Huss 1995). The development of "smart packaging" has received attention recently. The potential use of sensors based on pH sensitive films for monitoring spoilage volatiles released into packaged fish headspace were described by Byrne *et al*. (2003). The sensors were based on cresol red entrapped within a plasticized cellulose acetate matrix and a simple illumination source The TVB-N levels released from orange roughy and black scabbard, deepwater fish were monitored by color changes in the sensors.

2.1.3. Quality monitoring and labeling - View of the fish sector

A survey was done to gauge the European fish sector´s view on the importance of various quality attributes of fish and methods of measuring them. This survey also covered the attitudes of the fish industry towards the need of multi-sensor devices that can be used in quality monitoring and control (Jørgensen *et al*., 2003).

Sensory attributes influencing the freshness and quality of fish related to appearance, texture, smell, color, defects and handling were all considered very important. However, the views regarding the importance of instrumental techniques to measure these properties were contradictory. Instruments based on a single technique to measure individual properties were not considered important, but there was an agreement on the importance of the needs for rapid instrumental methods to measure the overall concepts freshness and quality (Jørgensen *et al*., 2003). The fish sector may have the perception

that complex concepts like freshness or quality cannot be quantified by single attribute measurements.

The long time tradition in the fish industry to have experienced people with many years of practice in control functions, performing sensory evaluation, is changing. Guidelines and reliable tools to perform the evaluation are now required, since personnel in fish industry is changing and new staff and younger people with little or no experience in evaluating fish quality come into fish business advice. Sensory schemes and instrumental methods to evaluate quality are therefore needed for use in the fish industry. However, the implementation of new sensory methods and instrumental methods which measure attributes related to freshness and quality has been very slow in the fish industry. The reason for the reluctance to use monitoring methods for freshness and quality may be that the fish industry is not familiar with the new sensory methods and instruments already commercially available. Perhaps there is no economical incentive for the industry to measure the quality because the demand for fish is greater than supplies and therefore all fish is sold at a high price despite of different quality. Another reason may be that in many cases the fish processors own the fishing vessels and are well informed about the quality of the catch. All information about origin, catching time and handling are well documented and the traceability of the products is assured giving the confidence that the quality is also known.

To maximize the value of seafood products, proper quality labeling and monitoring systems must be in place, which should be based on the pro-active attitude from the industry aiming at the development of self-regulation. This was the general view of the participants of the the EU project "Fish Quality Labeling and Monitoring", intergrating the opinion of both scientists and representatives from the fishery chain (fishermen, port auctions, processors, wholesalers, retailers) (Luten *et al*., 2003). A need for basic GMP guidelines on board of vessels was emphazised along with accessible information about the freshness of fish (expressed as storage time in ice, day of catch or best before date) to be implemented in a quality label. Lot of information of fish deriving from the fishery chain is available but it should be kept simple for the consumers. Quality standards based on well defined critera to evaluate freshness like the QIM method are well established in Europe and are expected to be implemented in the fish industry and expanded by the strategic alliance QIM Eurofish (Luten, 2003).

2.2. Volatile compound as indicators of fish quality - Classification of fish odors

The odor of fresh fish is one of the most important quality parameter used to determine whether fish is acceptable for consumption. The composition of volatile compounds in fish contributing to the characteristic odors can be determined and related to the quality. Characteristic odor and the progression of odor in fish during storage has been associated with varying levels of different volatile compounds present in the headspace of fish which can be measured to evaluate the freshness of fish. (Lindsay *et al*., 1986; Josephson *et al*., 1986; Lindsay, 1990; Kawai, 1996)

Fish odor can be classified as species related fresh fish odor, microbial spoilage odor, oxidized odor, processing odor and environmentally derived odor, based on the origin of the volatile compounds (Table 1). Kawai (1996) grouped fish based on their species related flavor and origin into three groups; saltwater fish, which are nearly odorless; freshwater fish that give off pyrrolidine an earthy-odor compound; and euryhaline fish which have a variety of unsaturated carbonyls and alcohols derived from enzymatic and non-enzymatic oxidation of polyunsaturated fatty acids (Figure 4).

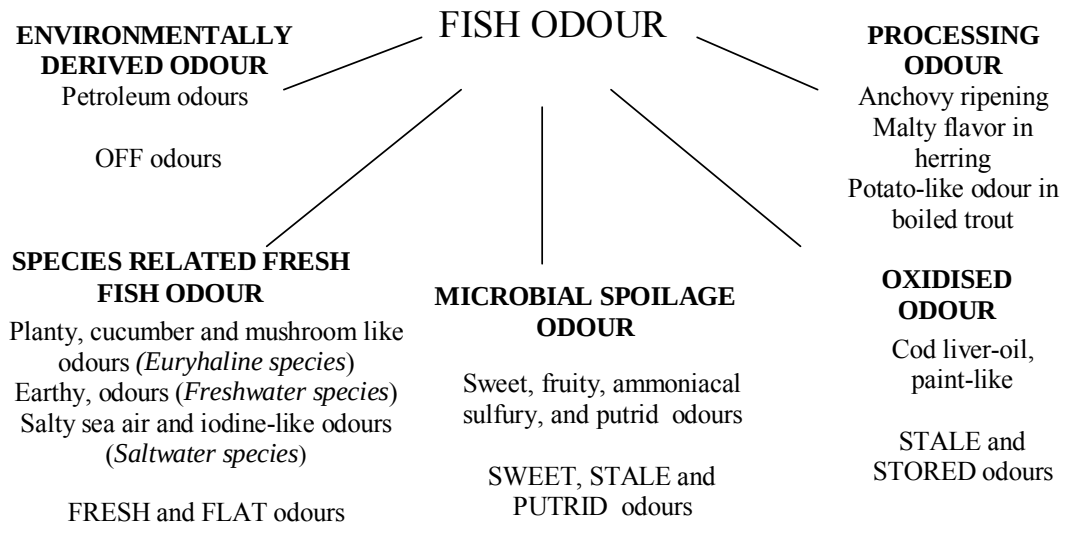


Figure 4. Classification of fish odors (From Olafsdottir and Fleurence, 1998)

Table 1. Classes of odors in fish and examples of compounds contributing to the odor. (From: Olafsdottir and Fleurence, 1998)

Fish odour	Class of chemical species	Examples of compounds	Aroma description	Odour threshold in water
SPECIES RELATED FRESH FISH ODOUR	C6-C9 alcohols and carbonyls	Hexanal / t-2-hexenal,	Green, aldehyde-like	4,5ppb / 17ppb[1]
		1-octen-3-ol, /1-octen-3-one	Mushroom	10ppb/ 0,009ppb[1]
		1,5-octadiene-3-ol	Heavy earthy, mushrooms	10ppb[1]
		1,5-octadiene-3-one	Geranium	0,001ppb[1]
		2,6-nonadienal	Cucumber	0,001ppb[1]
		3,6-nonadienol	Cucumber, melon-like	10ppb[1]
	Bromophenols	2,6-dibromophenol	iodine- and shrimp-like	0,0005µg/kg[2]
		2,4,6-tribromophenol	saltwater fish, brine-like.	0,6µg/kg[2]
		2-bromophenol	Sea, marine-like flavour	
	N-cyclic compounds	Pyrrolidine	Earthy	
		Piperidine		
MICROBIAL SPOILAGE ODOUR	Short chain alcohols	ethanol, propanol, butanol, 3-methyl-1-butanol	solvent like	1-100 ppm[3]
	Short chain carbonyls	acetone, butanone	solvent like	
		ethanal, propanal		
		3-methylbutanal	malty	0,06ppm[4]
		2-methylbutanal	malty	0,04ppm[4]
	Amines	ammonia,	ammoniacal	110 ppm[3]
		TMA	fishy, ammoniacal	30 ppm[3]
		DMA		0,6 ppm[3]
		histamine,		
		putrecine,	putrid, rotten	
		cadeverine		
	Sulphur compounds	hydrogen sulphide	sulphury, boiled eggs	5-40 ppb[5]
		methylmercaptan	rotten, cabbage	0,05 ppb[5]
		methyl sulphide	cabbage-like	0,9µg/kg[6]
		dimethyl disulphide	putrid, onion-like	12 ppb[7]
		dimethyl trisulphide	putrid, cabbage and onion-	0.01ppb[7]
		bis-methylthio methane	garlic like	0,3 µg/kg[6]
		thioesters		
	Aromatics	phenethyl alcohol	old roses	
		phenol,	phenolic,	2 ppm
		p-cresol	pigpen-odours , horse manure	300 µg/kg[6]
	N-cyclic compounds	indole	moth ball or faecal like	
		skatole		
	Acids	acetic acid,		34,2ppm[3]
		butyric acid	Sour, rotten, old socks	32,8ppm[3]
		isobutyric acid		
OXIDIZED ODOUR	Unsaturated aldehydes	hexanal	green, planty	4,5ppb[6]
		c4-heptenal	cardboard-like, potato-like	0,04ppb[8]
		2,4-heptadienal,	fishy oxidised flavour	
		2,4,7-decatrienal,	burnt, fishy, cod-liver oil-like	
PROCESSING ODOURS		2,4-heptadienal and 3,5-octadien-2-one	ripened anchovies	
		methional	boiled potato - like odour	
		2-methyl-3-furanthiol	meaty odour in canned tuna	
ENVIRONMENTAL ODOURS		methyl sulphide	petroleum odours	
		geosmin		
		2-methyl-iso-borneol	earthy, muddy odours	

[1]Josephson (1991); [2]Whitfield et al. (1988); [3]Kawai (1996); [4]Sheldon et al. (1971); [5]Fazzalari (1978); [6]Whitfield and Tindale (1984); [7]Buttery et al. (1976); [8]McGill et al. (1974)

Josephson *et al*. (1986) monitored the overall volatile pattern of whitefish during storage and grouped individual compounds into classes according to general odor quality and structural characteristics. The classes were fresh long chain alcohols and fresh long chain carbonyls (C6-C9) to index freshness and short chain alcohols and carbonyls, sulfur compounds, amines, sweet esters, aromatics and dienals were suggested as indicators of spoilage. By monitoring concentrations of different classes of compounds, they were able to make comparative assessments of the importance of the compounds during each phase of spoilage and were also able to select the components that are the most characteristic for the loss of freshness and onset of spoilage. Similar classification has been suggested for volatile compounds in beef to achieve better understanding of the spoilage odors (Stutz *et al*., 1991).

Rapid analysis of volatile compounds by electronic nose as a tool to monitor freshness or quality of various foods has gained increasing interest. The concept to classify compounds based on their structural and odor characteristics is the basis for the interpretation of electronic nose measurements. The gas sensors array can be designed based on the selectivity and sensitivity of the sensors to the quality indicating compounds. To facilitate and guide the future development of suitable sensors to monitor fish freshness and quality, examples of compounds belonging to the main classes of fish odors will be given herein.

Species related fresh fish odors, microbial spoilage odors and oxidized odors are most important to monitor freshness and spoilage of fish, however, to cover the range of different odors in fish the additional categories of odor will be mentioned also, but these are derived or processing odors and environmentally derived odors or flavors (Lindsay, 1990). While most of the research on spoilage compounds in fish has focused on fish stored in ice, recently researchers have also studied volatile compounds in boiled and processed fish products such as as smoked salmon and ripened products. Considerable efforts have also been on characterizing the volatile compounds in shellfish (Pan and Kuo, 1994). Numerous volatiles derived from amino acids degradation, contributing to seafood aromas, have been identified in spiny lobster (Cadwallader *et al*., 1995) and in cooked blue crab (Chung and Cadwallader, 1994; 1995).

2.2.1. Species related fresh fish odor

Fish odors are complex and the characteristic overall odor develops in accordance with the changes of the individual volatile compounds present in fish. Each species has a characteristic aroma and the changes of the aroma depend on various factors. Concentration of influential compounds and their odor thresholds along with combination of different components in the headspace are important factors, since some of the components in the headspace may exhibit synergistic effects and influence the overall odor quality. Some components are desirable at low levels but if their concentration increase they may contribute to off odors like the enzymically derived long chain alcohols and ketones that exhibit characteristic fresh- plant like notes to fresh fish but when accumulated in higher levels because of autooxidation they contribute to oxidized and fishy odors (Josephson, 1991).

Difference in the odor of saltwater, freshwater and euryhaline species appears to be because of various enzymatic activities (Kawai, 1996). Lipoxygenase enzyme activity has been found on the skin and gills of both freshwater and marine species (rainbow trout, river trout and sardines) and initiates the formation of the odorous volatiles contributing to green, pleasant aromas of fish (German and Kinsella, 1985; German *et al*., 1986; Hsieh *et al*., 1988; Hsieh and Kinsella, 1989; Zhang *et al*., 1992). The compounds that contribute to the characteristic plant-, cucumber-, melon- and mushroom- like odors, are unsaturated carbonyl compounds and alcohols with six, eight or nine carbon atoms which are present in low levels in the headspace above fish (ppb) (Josephson *et al*., 1983; 1984; Hirano *et al*., 1992; Milo and Grosch, 1993). In freshwater and euryhaline species the enzymic activity appears to be more than in saltwater species, resulting in characteristic green, plant-like odors of these species.

Fresh marine fish is nearly odorless, because it contains low levels of volatile compounds. Josephson *et al*. (1984) summarized the occurrences of volatile compounds in freshwater and saltwater species and concluded that the four common compounds found in saltwater species hexanal, 1-octen-3-ol, 1,5-octadien-3-ol and 2,5-ocatadien 1-ol were responsible for the moderate, faint odor of saltwater species. On the other hand the unsaturated C9 carbonyl compounds such as 2,6-nonadienal, which have potent green, plant-like, cucumber and melon-like odors were characteristic for freshwater and euryhaline fish. The odor threshold of the C8 alcohols common in the saltwater species is

much higher (1-10 ppb) than for the typical C9 compounds characteristic of the freshwater and euryhaline species like 2,6-nonadienal that has an odor threshold of 0.001 ppb and a strong cucumber like odor (Table1).

Bromophenols have been associated with the iodine like off-flavor in prawns (Whitfield *et al*., 1988), however Boyle *et al*. (1992a) found that bromophenols in nominal concentrations contributed to natural sea-, iodine, and marine-like flavors of seafood. Analysis of a few species of freshwater fish revealed that bromophenols were not present. The distribution of bromophenols in marine fish and seafood is probably linked to the feed chain (Boyle *et al*., 1993). It was suggested that by supplementing the feed of aquacultured fish, it might be possible to obtain flavor profiles of wild species. Attempts have been made to produce prawn feed with high bromophenol content and results indicate that direct addition of these components to the unprocessed feed is not successful and further studies on encapsulation are under investigation (Whitfield *et al*., 1997). Considerable variability in concentration and type of bromophenols has been found in seafood. Several studies on the odor character of bromophenols indicate that when evaluated in water the chemical, iodine or phenol-like flavor persists. However, when incorporated into fish, shrimp or triglyceride oils they exhibited different flavor characteristics, depending on isomers. 2,6-dibromophenol exhibited iodine- and shrimp-like notes while 2,4,6-tribromophenol was perceived as saltwater fish- and brine-like. 2-bromophenol generally enhanced rich marine seafood flavor quality. Fish-like flavors were perceived by sensory panellist when isomers were incorporated into vegetable oil, but when evaluated in deodorized menhaden oil, marine, herring-like oil flavor characteristics were perceived (Boyle *et al*., 1992b). Bromophenols are not present in high enough levels in the headspace of fish to be detected as part of the total headspace using available electronic nose techniques. The bromophenols are more important in influencing the "by mouth" or retronasal flavor perception of fish rather than influencing the nasal odor perception.

Environmental conditions and spawning can influence the odor quality of fish. The volatile pattern changes in mature salmon when migrating from the sea for spawning. C9 lipoxygenase derived compounds have been found in higher levels in spawning euryhaline and freshwater fish (Josephson *et al*., 1984). Capelin a saltwater species which belongs to osmeridae, has a very characteristic cucumber odor and 2,6-nonadienal was

found to be the most characteristic compound for the cucumber-like capelin odor, which was found in spawning capelin (Olafsdottir *et al*., 1997c).

Many species residing in ponds have characteristic earthy odor that has been associated with piperidine and its reaction products but the knowledge of the formation of these compounds is obscure. Piperdine levels have been reported to increase in spawning salmon and contribute to off odors (Yamanaka, 1989). The formation of pyrrolidine and piperidine has been linked with the presence of 1,4-diaminobutane (putrescine) and 1,5-diaminopentane (cadeverine) respectively but these compounds are only present in very low levels in fresh fish and can therefore not be expected to have great influence on the fresh odor (Kawai, 1996). Piperidine was tentatively identified in chilled cod fillets (Olafsdottir et al., VI) and Alasalvar *et al.,* (2005) suggested using piperidine along with other compounds showing increasing concentration with time as quality indicator of sea bream.

Oxidation of carotenoids also appears to generate characteristic aroma to fresh-like seafood flavor especially in algae (Josephson, 1991). β-ionone and β-cyclocitral were the most common compounds associated with algal blooms (Jüttner, 1981). 6-methyl-5-heptene-2-one derived from carotenoids was detected in chilled cod during storage and was described as spicy and flowery by GC-O and suggested to contribute along with other ketones and aldehydes to the characteristic sweet like odor of cod fillets (Olafsdottir et al., VI).

In general species related odor compounds in fresh fish are present in very low levels (ppb) in the headspace. Some of these compounds have very low odor thresholds and even though they are present in very low levels (ppb) they still affect the aroma of the fish and changes in their concentrations drastically affect the overall aroma (Josephson *et al*., 1986). However, it is not likely that the electronic nose techniques will detect these compounds in the total headspace of fish during storage.

2.2.2. Microbial spoilage odor

Microbial degradation of fish components mainly amino acids results in the formation of spoilage odors of fish. Ammonia, trimethylamine (TMA), ethanol, hydrogen sulfide, methyl mercaptan and sulfides are typical spoilage compounds that exhibit odors such as

fishy, stale, rotten and putrid and are present in the headspace above fish during spoilage at the parts per million levels (Herbert *et al*., 1975; Miller *et al*., 1973a; b) (Figure 5).

The concentration of the microbially formed compounds increases with time as the fish spoils and can be used as indices of spoilage (Lindsay *et al*., 1986). Both single compounds like TMA and a combination of compounds mainly alcohols, amines and sulfur compounds representing the different changes occurring during storage have been suggested as indicators for freshness and spoilage (Hebard *et al*., 1982; Oehlenschläger, 1992; Jörgensen *et al*., 2000; Lerke and Huck, 1977; Kelleher and Zall, 1983; Ahmed and Matches, 1983; Human and Khayat, 1981; Josephson *et al*., 1986; Lindsay *et al*., 1986; Alasalvar *et al*., 2005).

The detection of specific spoilage organisms (SSO) like *Shewanella putrefaciens*, *Pseudomonas* ssp. and *Photobacterium phosphoreum* is considered more reliable than total viable counts (TVC) to accurately evaluate the freshness or spoilage level of fish products because of their contribution to developing microbial metabolites causing spoilage of fish (Dalgaard, 2000; Gram *et al*., 2002).

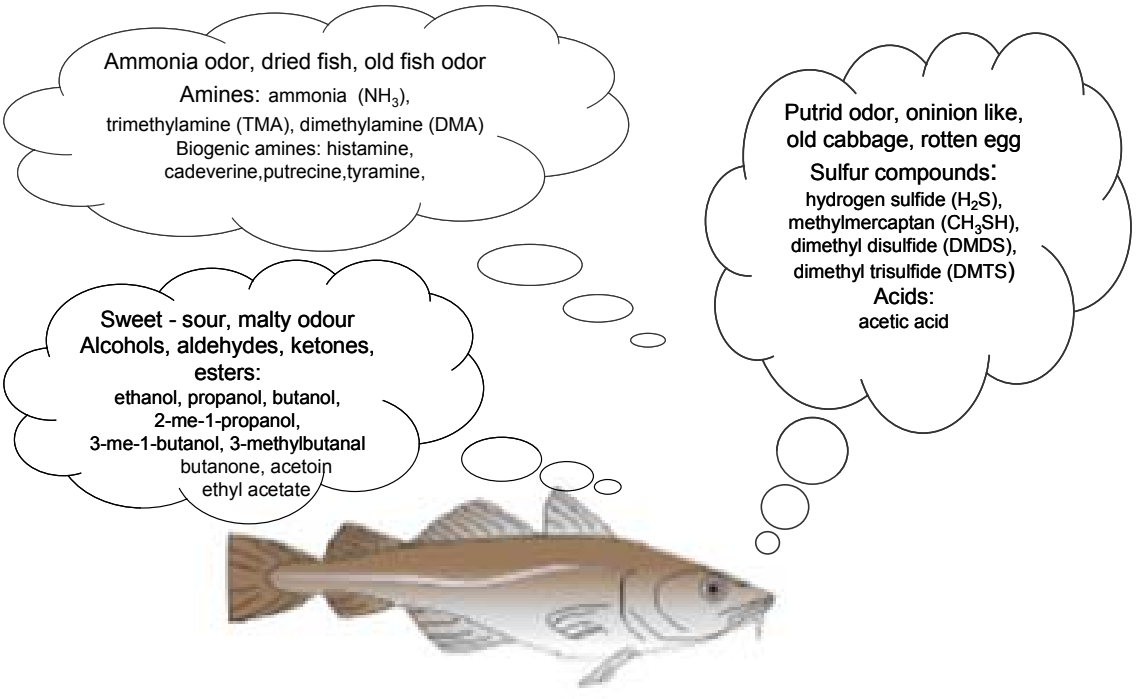


Figure 5. Quality indicators of chilled fish. Main classes of odors and characteristic compounds contributing to microbial spoilage odor of fish.

Short chain alcohols, carbonyls and esters accumulate in high concentration (ppm) in fish during chilled storage because of microbial growth. Ethanol has been suggested as an indicator of quality for canned fish (Lerke and Huck, 1977; Kelleher and Zall, 1983) and for raw tuna (Human and Khayat, 1981). Lindsay *et al*. (1986) suggested using short chain alcohols like ethanol, butanol and 3-methyl-1-butanol as potential indices of refrigerated fish spoilage. Propanol was suggested as a potential indicator when using modified atmosphere packaging techniques.

The initial production of ethanol in spoilage of fish has been related to the utilization of carbohydrate sources, while the formation of branched-chain alcohols and aldehydes like 2-methyl-1-propanol, 3-methyl-1-butanol and 3-methyl-butanal probably originate from degradation of valine and leucine, respectively. The branched chain aldehyde, 3-methyl butanal, is characterized by a malty and caramel like odors (Sheldon *et al*., 1971), which was perceived as a sweet, caramel and fish fillet like odor by GC-O in packed cod fillets (Olafsdottir *et al*., VI). The malty flavor of 3-methyl butanal was suggested to be mainly responsible for the malty off flavor defect of boiled cod by Milo and Grosch (1996). The corresponding alcohol 3-methyl-1-butanol, and 2-methyl-1-propanol which exhibit alcoholic and fruity odors were found to increase during storage of cod fillets (Olafsdottir *et al*., VI).

The formation of acetoin (3-hydroxy-2-butanone) was characteristic for the spoilage of chilled cod fillets packed in styrofoam boxes and was attributed to the growtn of *P. phosphoreum* (Olafsdottir *et al*., VI). Levels of acetoin increased earlier than TMA and therefore, it is more useful to monitor the loss of freshness as an early indicator of spoilage. The concentration of acetoin was much higher than the other lipid derived ketones detected like 2-butanone, 3-pentanone and the carotenoid derived 6-methyl-5-heptene-2-one that were present in cod fillets throughout storage but no obvious increase occurred until at the end of shelf-life and during continued storage. Ketones can influence the overall odor because of their typical odors and their low odor thresholds (Table 1).

In cultured and wild sea bream stored in ice for 23 days TMA, 3-methyl-1-butanol, 1-penten3-ol, piperidine, methanethiol, dimethyl disulfide, dimethyl trisulfide, and acetic acid were identified as spoilage indicators (Alasalvar *et al.,* 2005).

Earlier studies on the formation of odorous degradation compounds in fish (cod, haddock and rockfish) from the North Atlantic area showed that pseudomonads, in particular *P.*

fragi, were responsible for quality changes and development of sweet, fruity off-odors in chilled fish (Castell and Greenough, 1957; Castell *et al*., 1959; Miller *et al*., 1973a). Amines, sulfides ketones and esters were the main classes of volatile compounds associated with the growth of *Pseudomas fragi* and *S. putrefaciens* in prawns (Chinivasagam *et al*., 1998). *Pseudomonas* species have also been found responsible for the formation of volatile sulfides, alcohols (3-methyl-1-butanol, 1-penten-3-ol) and ketones (2-butanone) contributing to the stale and putrid off odors in fish because of amino acid and lipid degradation (Miller *et al*., 1973b; c). More recently the importance of pseudomonads in spoilage in fish species from the Mediterranean Sea (Koutsoumanis and Nychas, 1999; Koutsoumanis and Nychas, 2000) and American plaice (Lauzon, 2000) has been reported.

Alcohols, aldehydes and ketons in smoked salmon*:* Microbially produced ketones, aldehydes and alcohols are abundant in the headspace of cold smoked salmon products during storage (Jørgensen *et al*., 2001; Joffraud *et al*., 2001). Number of volatile compounds have been identified in vacuum-packed cold smoked salmon (*Salmo salar*), during cold storage at 5°C (Jørgensen *et al*., 2001). A multiple compound quality index based on 1-propanol, 2-butanone, and 2-furancarboxaldehyde was developed. 2-Butanone was the only ketone that increased during storage in cold smoked salmon. A few of the volatile compounds produced during spoilage of cold-smoked salmon contributed to the spoilage off-flavor of cold-smoked salmon as confirmed by gas chromatography-olfactometry. These were trimethylamine, 3-methyl butanal, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-penten-3-ol, and 1-propanol (Jørgensen *et al*., 2001). Semi-quantification of the main components detected in smoked salmon by GC-MS showed that compounds developed because of spoilage related processes (i.e. microbial growth and oxidation) were present in lower concentrations than compounds derived from the smoking process (Jónsdóttir *et al*., Forthcoming). Short chain alcohols, aldehydes and ketones were the main classes of compounds contributing to spoilage. 3-hydroxy 2-butanone was detected in high levels in all of the samples at the end of the storage time, even though some of them had no spoilage characteristics and low microbial counts (Jónsdóttir *et al*., Forthcoming). The role of 2-hydroxy butanone in smoked salmon products appears to be related to its contribution to the characteristic pleasant "butter"

like odor of the product but it is not considered important as contributing to the spoilage odor. Lactic acid bacteria were predominant throughout the storage in the smoked salmon products and may contribute to the formation of this compound. This supports what others have reported that lactic acid bacteria do not appear to be involved in the formation of spoilage off odors (Leroi *et al*., 1998; Paludan-Muller *et al*., 1998).

Stohr *et al*. (2001) studied the spoilage potential of various bacterial groups by inoculating pure cultures into sterile products. These conditions do not reflect the actual conditions when the various microbial groups may be competing, however, the results gave interesting information about the potential of each species to contribute to the sulfurous, acidic, rancid, rubbery, cheesy and acidic off-odors in spoiled smoked salmon.

Amines: The development of amines during fish spoilage is well known and measurements of the very volatile amines such as TMA or total volatile bases (TVB-N) have been used in the fish industry as indicators of quality for fish and fish products.

Development of ammonia-like and 'fishy' off-flavors has been related to fish spoilage bacteria like *Shewanella putrefaciens* and *P. phosphoreum* that can reduce TMAO to TMA (Jørgensen and Huss, 1989; Dalgaard, 1995a).

Photobacterium phosphoreum originating from the intestines of fish was identified as an important TMA producer in iced cod and in cod fillets (Van Spreekens, 1974; Van Spreekens and Toepoel, 1981) and has been suggested as the main spoilage organism in packed cod fillets, although this bacterium does not produce intensive off-odors (Dalgaard *et al*., 1993; Dalgaard, 1995a). Other well known spoilage bacteria like *Pseudomonas* spp. do not produce TMA from TMAO (Castell *et al*., 1959).

Studies of haddock and cod fillets packed in styrofoam boxes during chilled storage showed that *P. phosphoreum* dominated the spoilage microflora and high levels of TMA and TVB-N were found at sensory rejection, suggesting that this bacterium contributed considerably to the overall spoilage of aerobically packed cod and haddock fillets (Olafsdottir *et al.,* IV, V).

Enzymically produced DMA (dimethylamine), which forms very early after harvest of fish, has been suggested as a freshness indicator along with its precursor TMAO (trimethylamine oxide) (Oehlenschläger, 1992). TMA and TVB-N values are only useful

for advanced spoilage because they only begin to increase at later stages of storage (Oehlenschläger, 1998; Baixas-Nogueras *et al*., 2003).

It has been pointed out that TVB-N and TMA often give ambiguous information about the quality of the products as their levels are influenced by the storage method like in modified atmosphere packaging (Davis, 1990; Dalgaard *et al*., 1993; Debevere and Boskou, 1996; Lauzon *et al*., 2002) and in pre-frozen chilled fillets (Magnússon and Martinsdóttir, 1995; Guldager *et al*., 1998).

TMA is characteristic for the spoilage odors of fish, while DMA may influence the overall fresh flavor of fish in combination with the C8 unsaturated carbonyls and alcohols in marine fish. TMA has been noted for intensifying fishiness by a synergistic action with certain volatile unsaturated aldehydes derived from autoxidation of polyunsaturated fatty acids (Karahadian and Lindsay, 1989).

High levels of ammonia which are present in saltwater species contribute little to the odor but affect the pH. The pH can influence the odor character of some compounds and also their reactivity (Kawai, 1996). Basic environment influences the formation of aldehydes and when hydrogen sulfide is present these components can react to produce more complex flavor components such as S,N-heterocycles (Kawai, 1996).

Odor of saltwater species has often been related to the odor of TMA, however, the combination of TMA and other amines, acids and sulfur compounds (such as ammonia, DMA, acetic, formic and propionic acids, hydrogen sulfide and methyl mercaptan) contribute as well to saltwater fish spoilage odor rather than TMA alone.

Sulfur compounds: The volatile sulfur compounds, hydrogen sulfide, methyl mercaptan, methyl sulfide and dimethyl disulfide have been suggested as the main cause of putrid spoilage aromas in fish (Herbert *et al*., 1971). Dimethyl trisulfide has also been associated with spoilage in fish (Miller *et al*., 1973a; b; Lindsay *et al*., 1986), and it is responsible for a distinctive unpleasant onion flavor in prawns (Whitfield and Tindale, 1984). Milo and Grosch (1995) evaluated the headspace of boiled cod by gas chromatography olfactometry (GCO) and found that dimethyl trisulfide was the most potent odorant contributing to off-odors in cod formed when the raw material was inappropriately stored. During advanced spoilage of fish the concentration of the microbially produced sulfur compounds increases. The volatile sulfur compounds have

very low odor threshold (Table 1) and because of their odor characteristic they tend to predominate the putrid spoilage aroma, although the amines, acids and aromatics in addition, give the complete putrid spoilage aromas of fish. Shewan (1976) reported that the odor of methyl mercaptan varied from a pleasant odor below 0.05 ppb to a sour odor (0.5 ppb) to a cabbage water-onion like (50 ppb) and a metallic cooked meat odor (100 ppb).

The potential of H_2S-producing organisms, like *Shewanella putrefaciens,* to develop sulfur compounds contributing to off flavor described as onion, cabbage and putrid spoilage odors at advanced stages of storage in fish is well known (Miller *et al*., 1973a; Herbert *et al*., 1975). *Shewanella putrefaciens* has been identified as the main SSO in whole cod (Herbert and Shewan, 1976; Jørgensen *et al*., 1988) as well as in aerobically stored haddock fillets (Levin, 1968; Chai *et al*., 1971).

The origin of the sulfur compounds is from microbial degradation of cysteine and methionine to form hydrogen sulfide and methyl mercaptan respectively (Herbert *et al*., 1975). The proposed mechanism for forming oxidized volatile sulfur compounds includes the fast air oxidation of methyl mercaptan to form dimethyl disulfide (Kadota and Ishida, 1972; Miller *et al*., 1973a) and further oxidation of dimethyl disulfide and the incorporation of hydrogen sulfide yields dimethyl trisulfide (Miller *et al*., 1973b).

Strachan and Nicholson (1992) developed a method using Gastec detector tubes for the selective analysis of amines, hydrogen sulfide (H_2S) and ammonia in the headspace of gills and found that H_2S analysis gave the best result to predict storage period.

Acids: Some acids such as acetic, formic and propionic acids which are formed from amino acids or degradation of lipids are also involved in fish flavor. According to Kawai (1996), the acids with a short aliphatic chain (i.e. formic, acetic and propionic acids) can be associated with freshness flavor for several species like bonito (*Sarda sarda*), Spanish mackerel (*Scombrus japonicus*) and sea bream (*Pristimoides sieboldi*). Other authors, have reported that the production of volatile acids such as formic and acetic acids in tuna were associated with the spoilage (Hillig, 1956; Quaranta *et al*., 1984). Acetic acid was detected in increasing concentrations in cod fillets during chilled storage (Olafsdottir *et al.,* VI). The presence of acids in fish alters the odor quality of TMA and with increasing acid the volatility of TMA decreases and the odor quality changes.

Aromatics: Phenol and phenethanol have been found as the major high boiling volatile compounds in haddock during storage (Chen *et al*., 1974) derived from phenylalanine. Various benzene derivatives have been reported as a part of the total volatiles in chilled fish (Alasalvar *et al.,* 2005), in thermally processed crab (Cha *et al.,* 1993) and in fermented anchovy (Cha *et al.,* 1997). Their origin is not well understood but has been associated with environmental sources or oxidative processes. When studying volatiles in cod fillets styrene and chloroform were most abundant of the aromatics detected and were found to increase with storage (Olafsdottir *et al.,* VI). Styrene was also identified in wild and cultured sea bream packed in polystyrene boxes during chilled storage (Alasalvar *et al., 2005)*. The odor of styrene is described as kerosene like and has been associated with off odors in surimi based products related to the growth of yeasts (Koide *et al.* 1992).

Miscellaneous compounds: Although alcohols, aldehydes, ketones, acids, sulfur compounds, esters and acids are primarily of interest as spoilage indicators of fish, other classes of compounds may also have an impact when measuring the total headspace with electronic noses. The concentration of the straight chain alkanes (nonane, decane and undecane) appeared to be similar throughout storage in chilled cod fillets (Olafsdottir *et al.,* VI). Additionally, numerous branched chain alkanes were detected. The alkanes will not influence the responses of the electrochemical sensors of the electronic nose and are not considered of interest as quality indicators since they are not aroma active.
Several terpene derivatives have been identified in fish like limonene which was detected in sea bream during storage (Alasavar *et al.,* 2005). The origin of limonene in fish was related to the diet derived from algae or plant source. Limonene has low odor threshold and a fresh lemon odor was detected by GC-O analysis of cod suggesting that it may have an impact on the overall odor of fish fillets (Olafsdottir *et al.,* VI).

Malodors from fishmeal factories, fish drying and fish waste: During advanced spoilage of fish more complex spoilage odors are formed. This often causes odor pollution especially during summer in the neighborhood of fishmeal factories. The combination of acids, phenol, p-cresol, indole, skatole, sulfur compounds and amines contributes to the overall offensive odor of spoiled fish (Kamiya and Ose, 1984). Bulk stored capelin with high contents of feed degrades quickly because of high proteolytic

activity in the gut (Aksnes and Brekken, 1988). This results in liberation of peptides and free amino acids, which bacteria utilise for their energy demand resulting in the formation of bad smelling microbial metabolites. The amines, cadeverine, histamine and putrecine which are formed from lysine, histidine and arginine respectively are examples of such microbial metabolites. Sulfur compounds derived from cysteine and methionine also contribute to the offensive odors because of their low odor thresholds like dimethyl trisulfide with an odor threshold of 0.01 ppb (Table 1). Various thioester will also influence the overall putrid aroma.

Aromatics resulting from degradation of aromatic amino acids are typically present as the main contributing compounds in the characteristic malodors from animal waste and are also important in fish during advanced stages of spoilage (Kamiya and Ose, 1984). This type of odor is of concern in fishmeal factories and contributes to the air pollution in their neighborhood. Both indole and skatole which are formed during advanced spoilage of fish are very tenacious odorants, which tend to cling to clothing and other articles and to persist for long periods (Miner, 1977). Indole (2,3-benzopyrrole) with a mothball-like, burnt odor and skatole (3-methyl-1H-indole) exhibiting a mothball-like and faecal character (Acree, 1997) are found in animal waste and manure derived from tryphtophane. Similarly p-cresol derived from tyrosine contributes to pigpen odor. In animal waste and manure these compounds are presumed to be products of anaerobic microbial degradation constituents. Naphtalene has also been associated with similar offensive off odors which can cause odor taints in water and may originate from industrial and municipal sewage effluents or from biological activities of algae and micro-organisms. Studies of ventilation air of swine confinement buildings have shown that the components that cause the most offensive odors like indole and skatole are present in low concentrations because of their low vapor pressure. The sulfur compounds like hydrogen sulfide and methyl mercaptan which have high vapor pressure are on the other hand decomposed or oxidized, so they are not measured in high concentration in the ventilation air (Schaefer, 1977).

2.2.3. Oxidized odors

Unsaturated aldehydes: The fishy, cod liver oil-like, flavor in fish has been shown to be caused by two isomers of 2,4,7-decatrienal which are autoxidatively formed from long-chain polyunsaturated n-3 fatty acids in fish. In addition autoxidizing fish oils contain many other carbonyl compounds that contribute oxidized, painty and rancid aroma notes. The occurrence of c4-heptenal has been associated with the "cold storage flavor" of cod (McGill *et al*., 1974), however, some confusion exists about the role of c4-heptenal as the "cold-storage compound" (Lindsay, 1990). In fact, this aldehyde does not exhibit a fishy-type aroma by itself, but it rather participates in the expression of the overall fishy odor. Its odor has been described both as cardboardy, paint-like (Hardy *et al*., 1979) as well as boiled potato-like (Josephson and Lindsay, 1987a; b) and it has been found to potentiate the stale, burnt/fishy, cod liver oil-like flavor character of oxidizing oil that is caused primarily by 2,4,7-decatrienal (Karahadian and Lindsay, 1989).

Josephson *et al*. (1987) suggested a pathway that yields acetaldehyde and propanal via two sequential retro-aldol condensations of 2,4-heptadienal and 2-pentenal, respectively. During free radical oxidation of fish lipids 2,4-heptadienal can be formed along with 3,5-octadien-2-one from eicosapentaenoic acid. However, it is likely that most of the propanal in fish lipids results from a direct n-3/n-4 carbon chain scission during free radical oxidation of n-3 fatty acids. The level of the C7, C8 and C10 autoxidatively produced unsaturated carbonyls in the headspace of fish is typically low or at the ppb level. In spite of this low level, they influence the overall aroma as stated before since their odor threshold is low (see Table 1).

Pre-concentration techniques are necessary for the analysis of the unsaturated aldehydes, which is not practical for rapid determination of oxidation. On the other hand it is possible to detect the most volatile oxidation products like propanal and hexanal by rapid, static heasdpace sampling methods.

These compounds can be used as indicator compounds for oxidation as demonstrated by Boyd *et al*. (1992). They showed that direct analysis of propanal can provide a quick and economical method for the determination of oxidation of n-3 fatty acids and pentane and hexanal analysis can give an indication of the oxidation of linoleic acid.

2.2.4. Processing odors

Processing odors have been studied in various products such as ripened, pickled, cooked, smoked and hydrolyzed products like seafood flavorants. Volatile compounds characteristic for processed seafood odors like alkyl-pyrazines and sulfur-containing compounds have been found in cooked crustaceans, and furans have been found in spray-dried shrimp powder and shrimp hydrolysate (Pan and Kuo, 1994). Thermally generated aroma-active compounds via the Maillard reaction like pyrazines are characteristic for enzymatically hydrolyzed seafood products like crayfish processing byproducts (Baek and Cadwallader, 1996).

Josephson *et al*. (1987) have shown that reductions in intensity of the fresh fish flavor in pickled fish correlates with lower levels of fresh fish alcohols and carbonyls. The remaining fresh fish alcohols and carbonyls contribute to the mild but distinct flavor of pickled fish.

Ripening odor: Various proteolytic and lipolytic reactions influence the development of desirable flavor and texture of ripened products (Toldrá, 1998; Toldrá *et al*., 2000). The formation of the flavor is due to a complex combination of enzymatic or chemical reactions including lipid oxidation, Maillard reactions and Strecker degradation. Numerous volatile compounds have been detected in ripened products like dry cured ham, most of them generated from chemical or enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides and free amino acids. During the processing an intensive lipolysis has been observed. Free fatty acids are generated as a result of phospholipid hydrolysis, indicating a major role of phospholipases while the triglycerides remain almost intact. The free fatty acids accumulate during the process because of oxidative processes and volatile compounds such as hydrocarbons, aldehydes, alcohols and ketones are formed (Toldrá, 1998). The activity of proteases during the processing of raw ham leads to the formation of small peptides and free amino acids that can contribute to the taste (Toldrá and Flores, 1999). Free amino acids can also act as substrates for Strecker degradations and Maillard reactions with sugars (Toldrá, 1998). Similar processes have been reported in ripened seafood products where methional derived from methionine and 2,6-nonadienal from fatty acid oxidation were the main odorants in sugar salted ripend roe products (Jónsdóttir *et al*., 2004). Similarily, Triqui

and Reineccius (1995) found that 2,4-heptadienal and 3,5-octadien-2-one were associated with the development of typical flavor obtained after anchovy ripening and thus suggested that lipid autoxidation during ripening was primarily responsible for aroma development. However, manufacturers of ripened products have observed that some degree of proteolysis is necessary before flavor can develop. Methional and (Z)-1,5-octadien-3-one were also identified as potent odorants in ripened anchovy (Triqui and Guth, 1997) and aldehydes such as acetaldehyde, 2-methylpropanal and 3-methylbutanal were the key, highly volatile components of ripened anchovy, probably originating from amino acids.

The presence of malty flavor as a desirable characteristic in salt-ripened herring has been noted (Gudmundsdóttir and Stefánsson, 1997). The origin of this flavor in herring is not known, but in many foodstuffs the occurrence of branched chain aldehydes with low odor threshold has been associated with breakdown of amino acids (Amoore *et al*., 1976). Isobutyraldehyde that contributes a characteristic malty flavor is derived from valine by microbial action. Even though microbial action has not been given a major role in the ripening process of herring, it is however likely that degradation compounds from free amino acid may affect the ripening flavor.

Smoke odor: The typical smoke flavor results from a number of compounds originating in smoke, but is mostly attributed to the phenols (Miler and Sikorski, 1990). Phenolic compounds are important for preservation and flavor 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). Guaiacol and 4-methylguaiacol have been identified as the main phenolic compounds in smoked fillets of herring (*Clupea harengus*) fillets regardless of the smoke process used (Sérot *et al*., 2004).

During studies on the application of electronic nose to detect the quality of smoked fish products (Olafsdottir *et al*., VII) samples of different quality were selected to study both the characteristic volatiles related to the smoking and the development of the microbially produced volatiles by gas chromatography. The main classes of compounds and the individual compounds were identified and quantified to give an idea of the contribution of the respective classes to the total headspace in cold smoked salmon. Guaiacol

(2-methoxy phenol) was identified as the main compound contributing to the characteristic smokehouse odor. Furfural was also identified in high levels in some of the products (Jónsdóttir *et al*., Forthcoming). Furfural is a weak odorant and does therefore not contribute to characteristic smoked aroma.

Cooked odor: Various reactions occurring during heat treatment of muscle food have been related to the formation of meaty flavors and the involvement of ribose, amino acids and lipids in the formation of heat generated flavors have been reported (Lindsay, 1990). 2-methyl-3-furanthiol which exhibits a strong meaty character has been identified in tuna and is responsible for the characteristic meaty flavor in canned tuna (Withycombe and Mussinan, 1988).

Autoxidatively produced unsaturated carbonyl compounds were the most abundant components in boiled and canned fish, especially in trout and sulfur compounds are also present and contribute to off odors. In boiled trout methional with a characteristic boiled potato-like odor dominated the odor of the aldehyde fraction of the headspace volatiles (Milo and Grosch, 1993). On the basis of odor evaluation 3-methyl-butanal in combination with acetaldehyde, methional, (Z)-1,5-octadien-3-one, (E,Z)-2,6-nonadieanal and (E, E)-2,4-decadienal were determined as character impact odorants of boiled cod (Milo and Grosch, 1996).

2.2.5. Environmental odor

Environmental flavor taint in fish is for example petroleum type off odor resulting from high levels of methyl sulfide in canned salmon (Motohiro, 1962), mackerel, (Ackman *et al*., 1972) and Baltic herring, (Granroth and Hattula, 1976). The taint has been associated with high levels of dimethyl-β-propiothetin originating from the food plankton in the foods of fish, which has been suggested as the precursor for methyl sulfide. Shiomi *et al*. (1982) attributed off flavor in flat head (*Calliuricthys doryssus*) to microbially formed hydrogen sulfide and methyl mercaptan found in high levels on the skin. In contrast, they found methyl sulfide in higher levels in tissue than on skin. Therefore, methyl sulfide was associated with the precursor occurring in the stomach content or in the tissue.

The types of aromas associated with sulfur compounds are complex and dependent upon concentration. Volatile sulfides present in food in low concentrations are often

responsible for desirable odors i.e. methyl sulfide in low levels contributes to the characteristic aroma of fresh oysters (Ronald and Thomson, 1964) and soft-shell clams (Brooke *et al*., 1968). Methyl sulfide was also detected in fresh haddock and was present in similar levels throughout storage suggesting that this compound was not produced by microbial activity (Olafsdottir, 2003). Similarly, methyl sulfide was detected in fresh cod and was not found in samples during continued storage (Olafsdottir *et al.,* VI).

Geosmin and 2-methyl-iso-borneol have been associated with the earthy odor of freshwater species (Yurkowski and Tabacheck, 1980; Lovell, 1983). The formation of these compounds is related to mould-like bacteria (actinomycets) and blue green algae, which produce large quantities of these compounds in polluted freshwaters. Geosmin has a very low flavor threshold of 0.01 ppb in water and many freshwater fishes may contain low levels of these compounds as their characteristic flavor compounds. When present in high levels these compounds contribute to off odors in freshwater fish (Kawai, 1996).

2.3. Analysis of volatile compounds

Extensive literature on methods for sampling volatile compounds for gas chromatographic analysis from different kind of food is available (Jensen *et al*., 1998). In general it can be stated that it depends on the volatility (vapor pressure) of the components to be analyzed, what sampling methods are most suitable for their detection. Application of different sampling techniques is often necessary for the detection of the widest possible spectrum of volatile substances.

When detecting volatile compounds the general rule is that the sampling methods should be as mild as possible so the sample will represent the actual composition of the original sample. The benefits of using direct headspace analysis is the short time and simple equipment involved in sampling, also artefacts caused by heat and contact with solvent and sampling equipment are avoided and in addition the volatiles are collected in concentrations which represent their actual vapor pressure in the sample. Temperature has a direct influence on the volatility of the headspace components and sampling conditions should be chosen so that artefacts induced by temperature will be minimized. Sampling techniques for volatile compounds can in fact be used to select the components in the headspace based on their volatility (Viehweg *et al*., 1989). Both static and dynamic

headspace sampling systems have been used for the rapid detection of volatile compounds with electronic noses. Lengthy sampling procedures involved in the concentration of components present in low levels in foodstuffs are not considered practical for rapid measurement techniques. The use of gas chromatography to analyze volatile compounds requires laboratory equipment and trained personnel and is not suitable for industrial settings. The increased demand for rapid practical methods for industrial application has brought attention to the development of electronic noses.

Monitoring changes of fresh odor compounds as a part of the total headspace with an electronic nose is not possible using static headspace techniques. The fresh odor compounds are present in very low levels (ppb) and pre-concentration techniques are necessary to sample them in adequate concentrations for detection by the sensors. Concentration techniques are often time consuming, which is not practical for rapid analysis.

Moreover, because of the non-selective nature of electronic noses in general, it is likely that when analyzing total headspace of fish during storage, the changes in fresh odor compounds would not be noticed. The detection of low molecular weight, very volatile spoilage compounds such as amines (TMA), sulfur compounds and short chain alcohols that are present in much higher concentrations (ppm) in the headspace during storage of fish would overpower the fresh fish compounds.

Therefore, it can not be assumed that the gas sensors are measuring the overall odor, but rather detecting the components that are in the highest concentration in the headspace which can be of both odorous and non odorous nature. In some cases the compounds may have little or no effect on the human olfactory system, but they may be detected by the gas sensors.

Future research should focus on developing rapid concentration techniques for odorous components that are present in very low levels and development of selective sensors to use in electronic noses for their rapid detection.

Some of the influential odor compounds that have very low odor thresholds are often present in low levels and these are difficult to detect by rapid techniques. An alternative is to use instead indicator compounds, which are present in higher levels and can be detected by rapid methods. This is only possible if the pattern of the volatile compounds

is known and a connection has been verified between the indicator compound and the compounds that are responsible for the odors.

Classification of the volatile compounds occurring in fish, based on their structural characteristics is useful to guide future development and selection of gas sensors for fish applications. Information on the identity and quantity of the volatile compounds contributing to the spoilage pattern of each species will facilitate interpretation of measurements with electronic noses. Rapid measurements of volatile compounds with an electronic nose can thus detect rapidly the freshness stage of fish.

2.4. Development of the electronic nose technique:

The rapid detection of the quality of food has been one of the main application areas for the newly developed electronic nose technique. In the early nineties commercial instruments were launched on the market for this purpose, but some drawbacks to their immediate use in the industry occurred. The main reason was that the instruments were not fully developed and problems because of their sensitivities to humidity and environmental conditions led to misinterpretation of their performances. Active research in this area has since focused on characterizing better the overall technique. Improvements of the selectivity, sensitivity and reproducibility of the gas sensors have been the key issues. Identification of other important factors not directly related to the development of the sensor technologies have been brought into focus This includes sampling techniques and data analysis which are essential parts of the overall technique (Haugen and Kvaal, 1998; Haugen, 2001; Mielle,1996. Mielle and Marquis, 1999; Gardner and Bartlett, 1999).

Electronic noses have been suggested for various applications related to quality evaluation of different food like monitoring freshness and the onset of spoilage or bioprocesses of food. Many of these applications are based on detecting volatile compounds produced because of the growth of fungi, moulds or microbes or changes occurring in food because of oxidation (Olafsson *et al.,* 1992; Jonsson *et al.,* 1997; Namdev *et al*., 1998; Schnürer *et al*., 1999; Olsson *et al*., 2002; Keshri *et al*., 2002; McEntegart *et al*., 2000; Boothe and Arnold, 2002). Recently electronic nose technology has been used for quality assessment and monitoring ripening of Danish Blue Cheese

(Trihaas and Nielsen, 2005; Trihaas *et al*., 2005). Other application areas for electronic noses include medical applications, pharmaceutical industry applications, cosmetics, environmental monitoring, flavor and fragrances and monitoring tainting in packaging materials.

2.4.1. Electronic noses: key principles

The key principles involved in the electronic nose concept, is the transfer of the total headspace of a sample to a sensor array that detects the presence of volatile compounds in the headspace and a pattern of signals is provided that are dependent on the sensors´ selectivity and sensitivity and the characteristics of the volatile compounds in the headspace (Gardner and Bartlett, 1999). Feature extraction and pre-processing of the data are essential steps before applying pattern recognition techniques that are required to interpret the sensors signals characterizing the samples.

Qualitative discrimination power of the electronic nose technique has a resemblance to the subjective discrimination of odors by the human nose. The common approach in the multipurpose commercial electronic noses is to characterize odors by generating patterns with a sensor array using numerous sensors with partly overlapping selectivity. Another approach is the use of highly selective sensors for specific indicator compounds. For both approaches basic understanding of the composition and chemistry of the volatile compounds being measured is a key factor to ensure meaningful evaluation of the sensor responses.

More than 10,000 odorous compounds are known to exist in nature but only a few of these are likely to be important in solving any discrimination task by an electronic nose. Many studies have stressed the importance of comparing the electronic nose techniques to traditional analysis of volatile compounds by gas chromatography and obtain information about the most abundant volatiles in the headspace. This knowledge can be used further to select sensors in an array that are sensitive to some key indicator compounds in the headspace. For quantitative analysis a few sensors from the array can give adequate information, given that the selectivity of the sensors covers the different classes of compounds relevant for the particular application. It is unlikely that a universal electronic nose instrument, able to solve all odor detection problems will become a commercial reality, particularly since creating sensor diversity within an instrument is

expensive and most instruments are dedicated to certain applications. Numerous electronic nose instruments based on different types of sensors, sampling systems and data analysis procedures have been developed. Active research in the past years on different types of sensors has resulted in commercial gas sensors instruments with dedicated approaches for a wide range of applications from quality control of various food products to medical diagnosis (Craven *et al*., 1996; Pearce *et al*., 2003).

The leading companies manufacturing electronic nose instruments are from Europe, USA, and Japan. Instruments frequently used for food application in the scientific literature are from Alpha M.O.S. (France), Cyrano Science (USA), Neotronic Scientific, Inc (USA), Lennartz Electronic, Germany, Univ. Rome Tor Vergata, Italy, Nordic Sensor Technologies and S-SENCE, Sweden. Following review papers on the electronic nose technique are recommended for further reading Bartlett *et al*. (1997), Schaller *et al.* (1998), Gardner and Bartlett (1999), Harper (2001), Haugen (2001), Jurs *et al.* (2002), Garcia -González and Aparicio (2002). Information on the website of The NOSE II 2nd Network on Artificial Olfactory Sensing has the aim to stimulate information exchange between scientists, manufacturers, and end-users in Europe in order to develop synergy in the sensor community, improve the efficiency of R&D, encourage interdisciplinary research, and promote application of new ideas (http://www.nose-network.org/).

2.4.2. Sensor technologies

A comprehensive overview of the electronic nose technique was given by Gardner and Bartlett (1999), including sensor technology, data analysis and selected applications. A brief summary will be given here to introduce the different sensor technologies used in electronic noses.

Metal Oxide Semiconductors (MOS): A commercially available device called the Tagushi sensor is based on tinoxide (SnO2) sensors which are doped with precious metals to alter the response characteristics of the semiconductor. The gas analytes interact with surface absorbed oxygen and the conductivity of the tinoxide film changes. Typical operating temperatures are high (around 300-400°C) to ensure that the surface reactions are rapid and to decrease the chance that chemisorbed water will interfere. The Fishnose developed by AlphaMOS (Toulouse, France) for quality evaluation of smoked salmon discussed in paper VII is based on MOS sensors.

Metal Oxide Semiconducting Field Effect Transistors (MOSFETs) are related to the MOS sensors but the output signal derives from the change in potential when the gas molecules react at the catalytic surface. The operating temperature is 100-200°C.

Quartz Crystal Microbalance (QCM) devices measure the mass of molecules adsorbed on the sensor surface. The active element is piezoelectric crystal with a fundamental resonant frequency which decreases when mass is added to it. Bulk acoustic wave (BAW) sensors and thickness shear mode resonators are common types of these devices. They are operated at room temperature and have high stability over time. The Libra Nose developed by University of Rome (Tor Vergata) in Rome used in paper II is based on thickness shear mode resonators coated with various kinds of metalloporphyrins.

Surface Acoustic Wave (SAW) sensors have interdigitated electrodes on a piezoelectric substrate and a thin coating of a selective absorbing material on the surface. Different coatings include polymers, lipids, Langmuir –Blodgett films and self –assembled monolayers. A radio frequency voltage is applied which changes when molecules are absorbed on the surface. SAW sensors have higher sensitivities and faster response times than QCM devices and can be mass produced at low cost. They have good reproducibility, but their main limitation as for many types of sensors is their sensitivity to humidity.

Conduction Polymers (CP) chemiresistors are based on measurements of the resistance of a thin polymer film most often made from polypyrrole which has a conjugated π-electrone system. The sensors are made by electropolymerizing a thin polymer film across a narrow electrode gap. The devices can be operated at room temperature and the sorption of gas molecules changes the conductivity of the polymer.

Optical Sensors are based on a light source that excites for example the gas analyte and the signal measured is the resulting absobrance, reflectance, fluorescence or chemiluminescence

Electrochemical sensors contain electrodes and an electrolyte. The responses generated are dependent on the electrochemical characteristics of the molecules that will be oxidized or reduced at the working electrode and the opposite reaction will take place at the counter electrode. The output signal is the measured voltage between the electrodes generated by changes in current because of the reactions. Their advantages include long term stability, insensitivities to humidity and linear dependence on gas concentrations.

The electronic nose FreshSense based on commercial electrochemical gas sensors (CO, SO_2, H_2S and NH_3) was developed by IFL and the company Bodvaki-Maritech (Iceland). This instrument was mainly used in the study as described in papers I-VI

The inability to provide absolute calibration and reproducible result for gas sensors is often accounted for because of drift in sensor responses with time. This is of concern when renewing sensors in an array or when compiling data from different instruments with the same sensor array. Attempts have been made to compensate for sensor drifts using mathematical methods (Holmberg *et al*., 1996; Balaban *et al*., 2000; Tomic *et al*., 2002).

2.4.3. Sampling systems

Sampling in electronic nose systems involves the transfer of headspace from the sample to the sensor array. In general it depends on the volatility of the components to be analyzed, what sampling methods are most suitable. Closed sampling compartments are commonly used to allow the headspace to equilibrate and become enriched with the volatiles. Temperature directly influences the composition of volatiles in the headspace above the food sample and their concentration is also dependent on the sample size, in particular the exposed surface area of the sample and the ratio of sample to headspace in the sampling container.

Static headspace sampling methods are simple and low-cost. The benefit of using simple sampling systems and static headspace analysis is the short time and simple equipment involved in sampling. In addition the volatiles are collected in concentrations which represent their actual vapor pressure in the sample which allows more meaningful comparison to sensory methods.

Mielle and Marquis (1999) compared five different transfer methods irrelevant of the sensor techniques and concluded that the transfer techniques considerably influenced the system performance. Use of a carrier gas to transfer volatiles is common in electronic nose systems. The introduction of a carrier gas to transfer the headspace will result in lower concentration of volatiles reaching the sensors and less sensitive detection. Flushing of sensors with inert gas is often necessary to clean the sensors and speed up their recovery after exposure to samples.

Pre-concentration techniques: Solid-phase microextraction (SPME) and other more complicated pre-concentration sampling techniques commonly used in gas chromatography analysis have been used to increase the concentration of components that are present in low levels to allow detection by electronic noses. Marsili (2001) used an alternative electronic nose technique by using SPME, mass spectrometry (MS) and multivariate anlysis for assessing oxidation off-flavors in foods. By selecting peaks that contributed directly to the oxidation flavor and ignoring unimportant background chemicals it was possible to improve PCA groupings. They claimed that this technique was more robust than the new chemical sensor technologies currently used in e-nose instruments because of proven track record of mass spectrometry detectors.

Influence of sampling temperature: For improved performance of current e-nose systems it would be useful to take into account the equilibration kinetics of the headspace (Mielle and Marquis, 1999). The most important factor is the control of the sample temperature. Many of the commercial instruments today are equipped with automatic sampling systems and temperature controls.

The influence of temperature on the repeatability of measurements and discrimination efficiency of SnO_2 sensors has been studied by Roussel *et al*. (1999). They quantified the influence of the temperature of the headspace, the measurement cell and the way of injecting samples and concluded that for each application of aroma classification it is necessary to perform an optimization of the experimental conditions. For the experimental conditions studied "static" injection proved to be more discriminative and repeatable than the "dynamic" one for wine off-flavor volatiles. The lower temperature of the headspace studied (35°C) proved to be more discriminative for the headspace generated than the higher temperature (60°C) although the volatiles were more concentrated at higher temperature.

The temperature of the sample will influence the composition of the volatile compounds in the headspace. To increase the amount of volatiles in the headspace higher temperatures can be used, but at the same time the warming up of samples is often the time limiting factor in the analysis. At low temperatures, only very volatile compounds are present and slight changes in sample temperature will influence the vapor pressure of the volatiles and the headspace composition.

The influences of sample temperature on the response of the NH_3 sensor when measuring samples of haddock heads and fillets at different temperatures during a storage study of haddock in ice is illustrated in Figure 6. Haddock heads were used to represent the whole fish. Spoilage signs of whole fish will first appear on the gills and the skin, while the invasion of the microbial flora into the fillets is much slower. Therefore, the responses of the sensors to the heads are much higher than to the fillets. The temperature of the samples was monitored but not controlled during the measurements at room temperature. The temperature of the samples was 8-10°C before the first measurement, it increased to 10-12 °C before the second measurement started and had reached 14-15 °C before the third measurement started. The increasing response of the sensors with temperature illustrates clearly the importance of controlling the sample temperature during measurements. The influence of sample temperature has implications for the development of handheld devices. Sampling systems with temperature control will improve the repeatability of measurements. Tempering of the samples at room temperature before measuring may also give more reproducible results, but this is not practical for non destructive sampling.

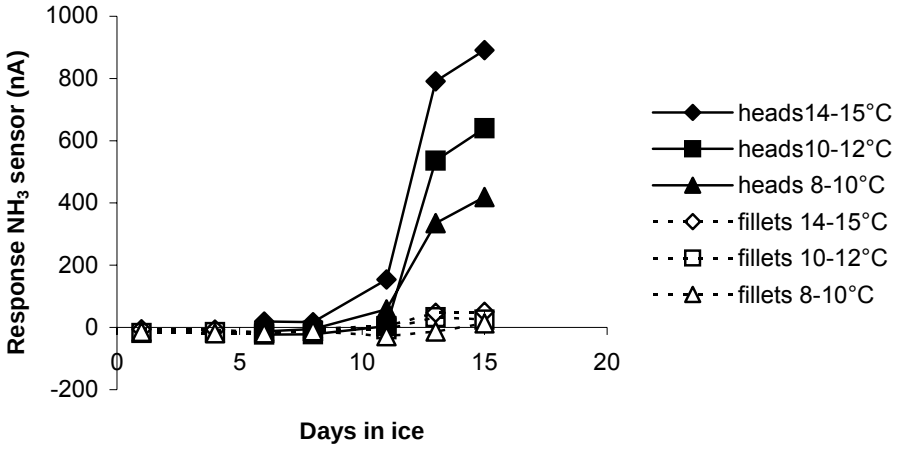


Figure 6. An example of temperature influences on the repeatability of e-nose measurements showing the response of the NH_3 sensor to haddock fillets and heads during repeated measurements of the same sample at different temperatures each day of sampling. (From Olafsdottir, 2003)

2.4.4. Data analysis

Jurs *et al*. (2000) give an excellent overview of methods for analysis of data from electronic noses including multivariate methods and neural networks. The first step is the assembling of the data set of the sensor array responses for the analyte of interest. Secondly, they describe the use of data pre-processing and normalization to provide more useful input for the mathematical tools like principal components analysis or neural networks selected for the data analysis. Thirdly, is the selection of the features to be used for the recognition of the pattern. This is often the steady – state response from each sensor as the input, but no general guidelines exist and it is necessary to explore different strategies for each application and sensor array. The fourth step is performing the data analysis and the techniques used can be categorized as statistical techniques or neural network based approaches. These various approaches can be used for classification while others can be used for quantification. The fifth step is the validation of the models preferably using data not used in creating the models.

The methods most often used for electronic noses are linear discriminant analysis (LDA), principle component analysis (PCA), principle component regression (PCR), partial least squares (PLS), cluster analysis and artificial neural networks (NN).

LDA has been used successfully to classify samples based on formulating boundaries between components of different classes and thus separate the samples in for example "good" and "bad" lots.

PCA is an efficient approach to reduce the dimensionality of a data set. Often two or three principle components provide an adequate representation of the data for a graphical output. Visual examination of the data can thus provide useful information about both samples and sensors. Loading plots can determine which sensors are providing similar information and which are providing unique information and can thus form a basis for selecting the useful sensors in an array. PC plots describe data variation and may or may not provide class separation. PCA is sometimes used to obtain quantitative information but normalisation of the sample responses may remove the concentration effects from the responses.

PCR provides a link between the response information and the concentration information and is especially useful for sensors with linear responses. The calibration information can

be used to find approximation to the regression coefficients and can be used to obtain quantitative estimates for analytes in the calibration samples.
PLS gives similar information as PCA and PCR and is frequently used for quantitative prediction of analytes based on sensor responses.
Cluster analysis requires a large number of training data and is used as an exploratory or a pattern recognition technique often associated with evaluating the performance of a sensor array to correctly cluster various analytes.
Artificial neural network techniques are popular for electronic nose data processing because of their ability to analyze complex and non-linear data. Various methods exists including feed forward neural networks and self organizing maps that apply various algorithms to adjust the weights and biases of the computational processes involved in the training to achieve pattern recognition.

2.5. Application of electronic noses for fish

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.
Electronic nose instruments based on various sensor technologies have been used for fish applications and compared with different reference methods like sensory analysis, chemical measurements of volatile compounds like TVN or TMA and microbial analysis.
Instruments based on different sensor technologies have been used like metal-oxide chemoresistor sensors (Ólafsson *et al*., 1992; Egashira *et al*., 1990; Ohashi *et al*., 1991), MOSFET sensors (Haugen and Undeland, 2003), amperometric sensors (Schweizer-Berberich *et al*., 1994; Olafsdottir *et al*., 1997b; c; 1998; 2000; 2002; Högnadóttir, 1999; Jónsdóttir *et al*., 2005), conducting polymer sensors (Du *et al*., 2001; 2002; Luzuriaga and Balaban 1999a; b; Newman *et al*., 1999) and quartz microbalance sensors (Di Natale *et al*., 1996; 2001; Zhao *et al*., 2002).

2.5.1. Selection of sensors to detect quality indicator compounds

The general approach when using electronic nose, is to analyze the total headspace of the sample without prior separation of individual compounds as is done in gas chromatography. The discrimination power of the electronic nose is based on utilizing an array of a large number of sensors of different but often overlapping selectivity and the interpretation of the data requires the use of pattern recognition techniques. Gas sensors that are currently used in electronic noses are non-selective towards individual compounds, but some of them are selective towards certain classes of compounds.

For quantitative analysis a few sensors from the array can give adequate information, given that the selectivity of the sensors cover the different classes of compounds relevant for the particular application (Di Natale *et al*., 1996).

When designing a sensor array for a dedicated application it is important to select a combination of sensors that are sensitive to the key components of interest and ideally the detection limit for each compound of interest should be specified as in any other analytical task. Adding a sensor with no sensitivity to the compounds of interest but with a significant noise will potentially degrade the performance of the array as a whole.

Research efforts have focused on developing membranes for selective detection of important quality indicating compounds like sensors aimed specifically at the detection of compounds contributing to freshness odors of fish (Deng *et al*., 1996). Many researchers have developed sensors for the selective detection of microbial metabolites like TMA (Storey *et al*., 1984; Egashira *et al*., 1990; Saja *et al*., 1999; Zhao *et al*., 2002). Ohashi *et al*. (1991) evaluated the performance of a semiconductive trimethylamine gas sensor (In2O3 -MgO) to detect the freshness of cod, sardine and yellow tail stored at 10 and 27°C. The same group reported further that even a single gas sensor was quite useful to monitor fish freshness by the selective detection of TMA, DMA and ammonia (Egashira *et al*., 1994). Volatiles associated with safety of fish products like histamine causing scombroid poisoning is the leading cause of finfish-borne illness. Histamine has traditionally been used as an indicator for safety. Selective detection of bioamines has been suggested using an amperometric detection of histamine with a methylamine dehydrogenase polypyrrole-based sensor (Zeng *et al*., 2000).

To determine the spoilage of capelin, quantitative analysis of the changes of volatile compounds in the headspace was done using only three gas sensors from an array of nine

electrochemical sensors in the electronic nose "FreshSense" (Olafsdottir *et al*., 1997b). The gas sensors were selected on basis of their sensitivity towards three classes of compounds that typically form during storage of fish, namely alcohols, sulfur compounds and nitrogen containing compounds. Analysis of standard compounds, namely ethanol, dimethyldisulfide and TMA showed that the gas sensors selected in the electronic nose "FreshSense" were selective and sensitive for their detection. FreshSense measurements, in particular the NH_3 sensor was found to be comparable to traditional TVB-N analysis to detect the spoilage of capelin (Olafsdottir *et al*., 1997c). Furthermore, the FreshSense instrument was applied to monitor changes in the headspace of herring and shrimp during storage at 0 °C and to measure sugar salted roe and fishmeal of different quality (Olafsdottir *et al*., 1998b). The development of ammonia is characteristic for shrimp during storage and the NH_3 sensor appeared to be promising to monitor changes in shrimp quality. The NH_3 sensor correlated well with sensory analysis (Olafsdottir *et al*., 1998b) and TMA measurements in shrimp (Zeng *et al*., 2005).

2.5.2. Quality indicators – volatile compounds in the highest amount

A study of haddock fillets kept in cold storage (0-2°C) for 3, 7, 10 and 14 days was performed to obtain more detailed information about the identity and the level of the most abundant volatile compounds present in the headspace during storage of fish (Table 2)

Analyzes of the headspace volatiles were conducted using air pump headspace sampling and collection of volatiles by a TENAX pre-concentration technique and analysis by gas chromatography (GC-MS) (Olafsdottir, 2003). The main classes of compounds identified in packed haddock fillets during storage were alcohols, aldehydes, esters, ketones, sulfur compounds and amines (Table 2). The quantitative GC-MS results of the main classes of spoilage compounds were compared to the responses of the FreshSense electronic nose sensors which are sensitive to the respective classes (Figure 7).

The sum of the GC responses of individual compounds belonging to each class of compounds like the alcohols, aldehydes and esters corresponded to the CO sensor responses of the electronic nose. The sum of the amines and the sulfur compounds detected by GC-MS corresponded to the responses of the NH_3 and SO_2 sensors, respectively. The response of the CO sensor was the highest and increased early in the storage while the responses of the NH_3 sensor and the SO_2 sensors increased later in

storage. Because of breakthrough of small polar, molecules on the TENAX traps the technique is not suitable for the quantification of compounds like ammonia, hydrogen sulfide, methyl mercaptan, and ethanol that are known to be present in abundance in the headspace of spoiled fish. However, the electrochemical sensors can detect these compounds and therefore the slopes and shapes of the curves are slightly different.

Table 2. Headspace volatiles of packed haddock fillets during storage at 0 °C collected by an air pump on a Tenax trap followed by thermal desorption, separation and detection by GC-MS. (From : Olafsdottir, 2003)

	RI DB-5ms[a]	**3 days**	**7 days**	**10 days**	**14days**
Alcohols					
ethanol	<173				
2-methyl-1-propanol	227		++	++	+++
1-penten-3-ol	263			+	
3-methyl-1-butanol	312	+	++	++	+++
2-methyl-1-butanol	314				++
2,3-butandiol	357				+
Aldehydes					
acetaldehyde	<173		+	+	
3-methyl-butanal	245			+	++
hexanal	376		+		
heptanal	494		+		
nonanal	703	++	++	++	++
decanal	803	+	+	+	+
Esters					
ethyl acetate	209	++	++	++	+++
propanoicacid-2-methyl,ethylester	333				++
acetic acid, 2-methylpropyl ester	348				+
butanoic acid, ethyl ester	381			++	+++
2-butenoic acid,ethyl ester	428				+
butanoic acid, 2-methyl, ethylester	433				++
butanoic acid, 3-methyl, ethylester	439				++
hexanoic acid, ethyl ester	595				++
Ketones					
2,3-butandione	207		++		
3-pentanone	273	+	+	+	
3-hydroxy-2-butanone	282		+++	+++	+++
Sulfur compounds					
dimethyl sulfide	182	++	++	++	++
dimethyl disulfide	319				++
dimehtyl trisulfide	562				+
Amines					
TMA	174	++	++	+++	+++

[a]Calculated ethyl ester retention index on DB-5ms capillary column

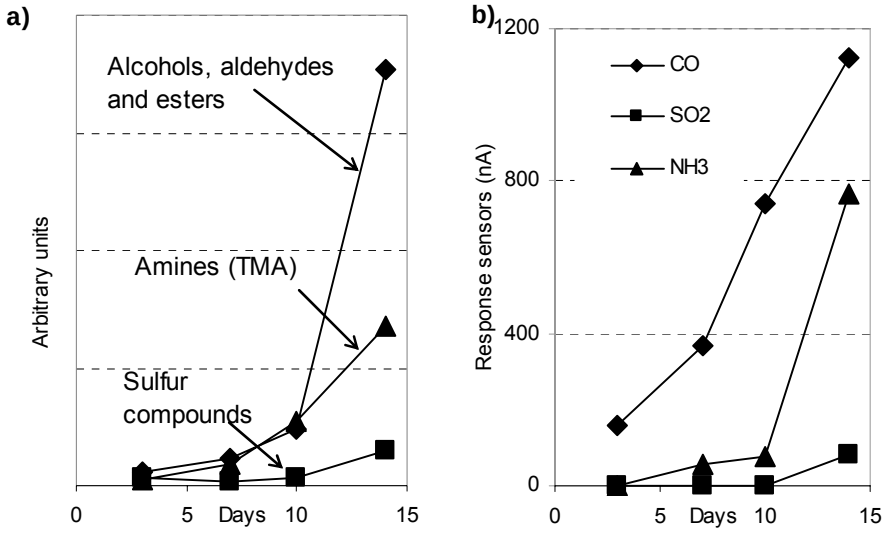


Figure 7. Sum of the peak areas of compounds representing the three different classes of compounds detected by GC in the headspace of haddock fillets during storage in ice (a) and responses of the CO, SO_2 and NH_3 sensors towards haddock fillets during storage in ice (b) (From: Olafsdottir, 2003)

TMA, 2-methyl-1-propanol, 3-methyl -1-butanol, 3-hydroxy- butanone, ethyl acetate and butanoic acid ethyl ester were found in the highest amount and increased with storage of haddock fillets. Dimethyl disulfide and dimethyl trisulfide were detected at the end of the storage time when the samples were spoiled, while dimethyl sulfide was detected in all samples and probably contributes to the typical potent odor of haddock fillets often described as innard-like.

Increasing concentrations of spoilage indicator compounds with storage time resulted in the development of characteristic odors and simultaneously increased responses of the electronic nose sensors were observed. On day 3 the odor of the fresh fillet was very little or neutral and low responses of the sensors were observed. The first spoilage odors of the fillets were sweet like odors that were contributed by the alcohols which give sweet, solvent like odors in combination with aldehydes giving sweet, rancid-like odors (day 7). The amines contributed to salted fish or stock fish odor and in combination with the sulfur compounds, cheesy and foul odors developed and the fillets became stale on day 10. Finally, esters were analyzed in high levels on day 14 giving characteristic sweet, fruity odors. When these sweet odors were mixed with the foul smell of sulfur compounds and ammonia- like stockfish character of the amines the odor of the fillet becomes TMA / ammonia-like and sour / putrid – like, signaling the overt spoilage

2.5.3. Selection of reference methods for freshness quality of fish

Direct comparison of the performance of the different e-nose systems is difficult because the results are strongly dependent on the choice of the sensor array and the sampling conditions used. Therefore, it is essential that the experimental conditions are carefully detailed so that the different systems can be compared to give advice and recommendations to future users. The selection of appropriate reference methods is important to validate the performance of electronic noses for different applications. The application of electronic noses to monitor the spoilage process as a function of storage days is a classical way to estimate the ability of the electronic nose to discriminate samples of different spoilage level. However, different handling and storage conditions in particular the temperature will influence the spoilage pattern of fish and consequently information about storage days may give ambiguous information about the freshness stage of the fish (Di Natale *et al*., 2001; Olafsdottir *et al*., 2003a). A more useful approach is to compare the electronic nose responses with a measurement that gives information about the microbial and oxidative processes influencing the freshness quality like sensory, microbial or chemical analysis.

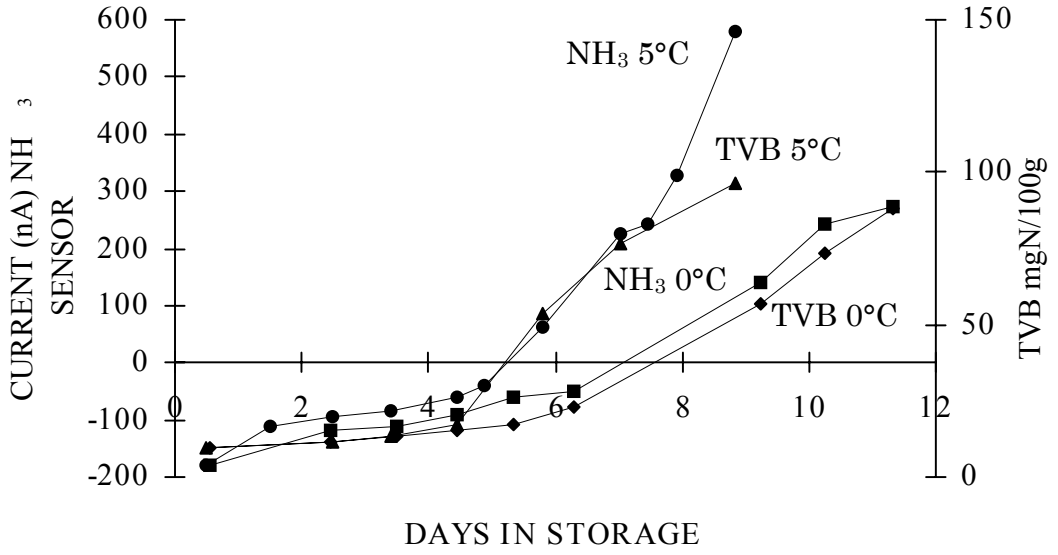


Figure 8. The results of TVB measurements(-◆-,-♦-) and measurements of the NH3 gas sensor (-■-,-●-) in capelin stored at 0 and 5°C for 11 and 8 days respectively.(From Olafsdottir *et al*., 1997c).

As an example the a study on capelin stored at 0 °C and 5 °C showed that the NH_3 sensor was comparable to the traditional TVB-N measurement (Figure 8) (Olafsdottir *et al.*, 1997c).

2.5.4. Seasonal variation and influence of processing (whole fish and fillets)

Raw material is often labeled with days from catch, however because of the effect of various extrinsic and intrinsic factors, the information about days from catch is not always reliable to determine the quality or freshness of the raw material. Sensory evaluation of fish fillets is more difficult than sensory evaluation of the whole fish. Therefore, instrumental techniques to evaluate the freshness quality of the fillets are of special interest. The most obvious spoilage signs of the fillets are development of spoilage odor, discoloration and decreased firmness of the flesh. Various instrumental techniques to detect these changes have been developed (Olafsdottir *et al.*, 1997c; 2004) but their implementation in the fish industry has been slow.

Several storage studies of haddock stored both as whole fish on ice and as fillets have been performed at IFL (Tryggvadottir and Olafsdottir, 2000; Olafsdottir *et al.*, 2003b). The main objective of the studies was to evaluate the possibility to use electronic nose to monitor spoilage changes of haddock fillets and to study the spoilage pattern of fish caught at different seasons (spring and autumn) using different fishing gear (long-line and Danish seine) and storage conditions .

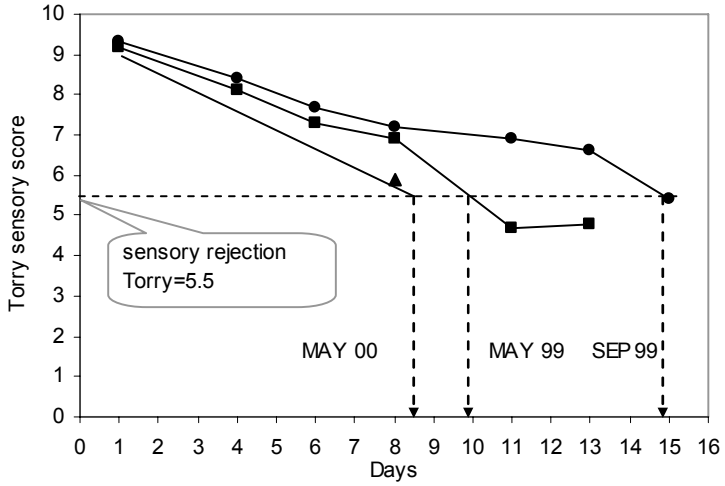


Figure 9. Sensory analysis of haddock fillets from storage studies of whole fish (May 99 -■-); Sept 99 (-●-) and fillets (May 00 (-▲-). (From: Olafsdottir and Kristbergsson, Forthcoming).

The fish was stored whole in ice during the first two experiments (MAY99 and SEP99), but in the third one (MAY00) the fish was stored as fillets in styrofoam boxes in cold room (0-2°C). Changes of various properties related to freshness quality of haddock stored in ice were monitored by the electronic nose FreshSense and compared to traditional chemical (total volatile bases (TVB-N)), sensory (Torry scheme) and microbial methods (total aerobic viable count (TVC) and texture measurements. Measurements were performed on fillets on days 1, 4, 6, 8,11,13 and 15 counted from the day of catch.

Sensory analysis of the fillets using evaluation of cooked fillets according to the Torry scheme showed that the spoilage pattern was different in the three studies (Figure 9). The limit of shelf life was defined as Torry score = 5.5. Fillets spoil faster than whole fish as expected and seasonal variation influenced the spoilage rate. Recently spawned fish caught by Danish seine and stored as whole fish in ice from the spring (MAY 99) had shorter shelf life in ice (10 days) than whole fish caught by long-line from the autumn season (SEP 99) (14-15 days). Fish stored as fillets at 0-2°C (MAY 00) spoiled faster than whole fish and had a shelf-life of only 8-9 days. The results of the electronic nose measurements and the microbial and chemical analysis were mostly in agreement with the sensory analysis (Figures 10 and 11).

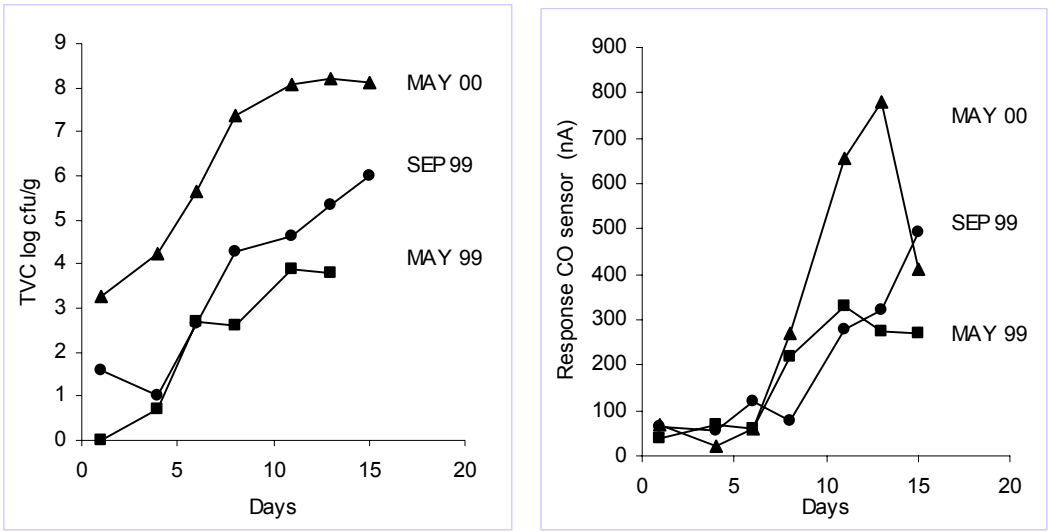


Figure 10. Microbial analysis (TVC) and electronic nose measurements (CO sensor) of haddock fillets from storage studies at 0-2 °C for up to 15 days of whole fish May 99 (-■-); Sept 99 (-●-) and fillets May 00 (-▲-).(From: Olafsdottir and Kristbergsson, Forthcoming)

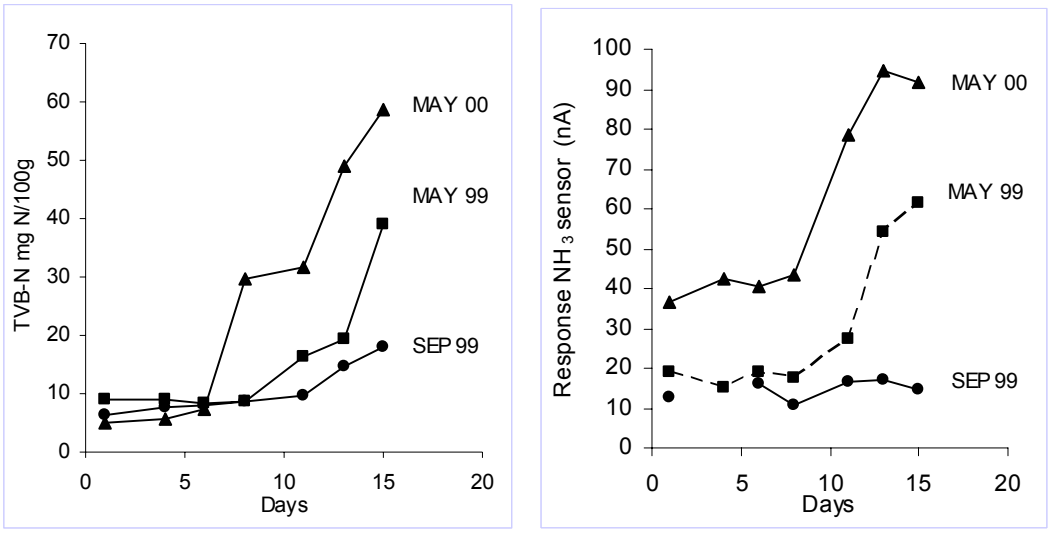


Figure 11. Chemical measurements of total volatile bases (TVB-N) and electronic nose measurements (NH_3 sensor) of haddock fillets stored at 0-2 °C for up to 15 days, from storage studies of whole fish May 99 (-■-); Sept 99 (-●-) and fillets May 00 (-▲-).(From: Olafsdottir and Kristbergsson, Forthcoming)

The traditional microbial analysis (TVC) and electronic nose measurements (CO sensor) showed a similar overall trend in the storage studies of whole fish (May 99 and Sept 99) and fillets (May 00) (Figure 10) and likewise the results of TVN chemical measurements and the NH_3 electronic nose sensor showed similar overall trend (Figure 11).

The electronic nose measurements showed that the responses of the sensors were highest for the (MAY00) samples indicating the most rapid spoilage for fish stored as fillets The results from sensory analysis, TVN (total volatile bases) measurements and the NH_3 sensor showed that recently spawned fish from the spring season (May 1999), stored as whole fish, had higher spoilage rate than fish from the autumn season (SEP 1999). Contradictory to these results the TVC and CO measurements indicated that the spoilage rate appeared to be higher in the fall (SEP99) than in the spring (MAY99). However, based on the results of sensory analysis it appears that the spoilage potential of the microflora was greater in the spring, probably because of the condition of the raw material favoring their growth. This emphasizes the role of the specific spoilage organisms and their spoilage potential suggesting the validity of TVC measurement may be questionable as has been suggested by other researchers (Gram *et al*., 2002).

At sensory rejection, increased responses of the NH_3, H_2S and SO_2 sensors detecting microbially produced amines and sulfur compounds was observed in all experiments.

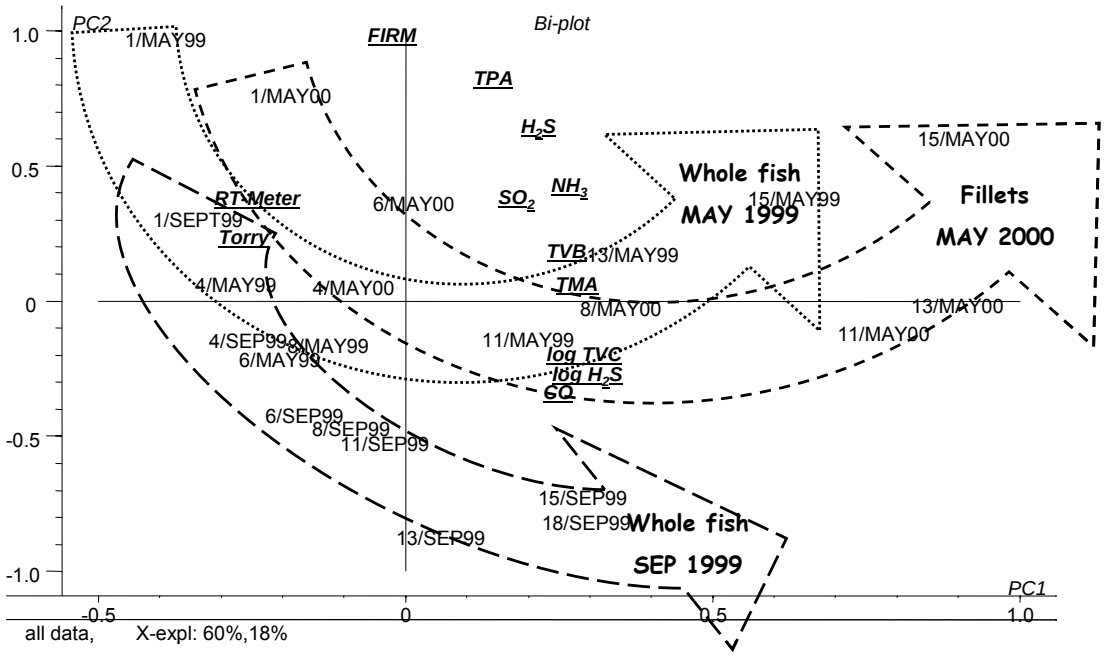


Figure 12. PCA showing a biplot of sample scores and variable loadings of all data from three storage studies of haddock as whole fish (May 99 and Sept 99) and fillets (May 00) stored at 0-2 °C for up to 15 days. The samples are labeled with storage days and time of the experiment and the variable loadings are labeled as follows: e-nose: CO, NH_3, SO_2, H_2S, chemical: TVB/TMA, microbial: log H_2S, log TVC, texture: Firm /TPA (-▲-). (From: Olafsdottir and Kristbergsson, Forthcoming).

Principal components analysis is useful to illustrate the overall results (Figure 12). The first PC explained 60% of the variation in the data set and the spoilage level of the samples increased from the left to right along PC1. The second PC explained 16% of the variation in the data and appeared to be discriminating samples based on the season. The variable loadings show that the CO sensor and the microbial counts (log H_2S and log TVC) are highly correlated and the positioning of the H_2S, SO_2 and NH_3 sensors on the upper half of the plot show the contribution of these sensors to discriminate samples from the spring season in combination with the texture measurement (FIRM). Fish spoils faster in the spring because the fish is recently spawned and the texture of the muscle is softer and more gaping than in the fall. Different spoilage patterns are evident depending on the season indicating, that days of storage is not a good estimate of freshness quality and fillets spoil faster than whole fish, as was to be expected. The e-nose sensor responses

appear to give information on the quality of fish related to sensory scores, microbial growth (TVC) and spoilage indicators like TVB-N.

2.6. Predictive models for shelf-life of fish

The development of models for shelf-life prediction of seafood has been based on various approaches like kinetic models to estimate relative rates of spoilage (RRS). Modeling of microbial growth requires that samples have to be taken frequently to make sure to detect the characteristic growth profile. Model studies using liquid media have therefore been used extensively to provide data for the development of models, because these are less costly and allow better control of the experimental conditions than studies done with naturally spoiling products. A few models to predict shelf-life have been developed that rely on data of naturally spoiling products (Koutsoumanis, 2001; Einarsson, 1992). Predictive microbiology as a tool to study the microbial ecology in food has gained considerable interest (Ross and McMeekin, 1994) and recently models based on enumeration of specific spoilage organisms (SSO) to determine the remaining shelf-life of fish products have been developed (Dalgaard, 1995b; Dalgaard *et al*., 1997; Koutsoumanis and Nychas, 2000; Koutsoumanis, 2001; Dalgaard, 2002). Because of the complexity of seafood spoilage, practical shelf-life models should take into accounts the different conditions and factors influencing the spoilage of each product (Dalgaard *et al*., 2002). The web enabled Seafood Spoilage and Safety Predictor (SSSP) software (http://www.dfu.min.dk/micro/sssp/) is based on kinetic models for growth of specific spoilage organisms and empirical RRS secondary models (Dalgaard *et al*., 2002). The software allows the practical use and validation of shelf-life models based on growth of *S. putrefaciens* and *P. phosphoreum*. The SSSP model is based on the initial concentration of the SSO in the product and it is only valid within a certain temperature range. As clearly explained by Dalgaard (2002) several assumptions are made regarding the growth of the SSO. For example it is assumed that other bacteria present in the microflora will not influence the growth of the SSO and that the SSO will grow without a lag phase and produce metabolites responsible for spoilage. It is also assumed that the SSO has to reach a minimal spoilage level (10^7 CFU/g) when the product is rejected by sensory analysis and micro-organisms other than the SSO should be of no importance for spoilage. SSSP

models have been found to predict SL of naturally contaminated seafood with an accuracy of 25% (Dalgaard *et al*., 2002). The SSSP model was used for predicting the shelf-life of haddock fillets based on the growth of *S. putrefaciens* but a high prediction error (0 to 46 %) was found (Olafsdottir *et al.,* IV). This was explained by the fact that *S. putrefaciens* was not the dominating SSO in the haddock fillets and had not reached a minimum spoilage level (10^7 CFU/g) when the fillets were rejected by sensory analysis. Therefore, this emphasizes the importance of identifying the SSOs and their spoilage domain in the respective products before applying a model based on only one SSO for shelf-life prediction. The drawback to the use of models based on enumeration of bacteria is that the microbial methods are lengthy, hence not very suitable for shelf-life prediction of perishable food products. However, efforts have been made to develop more rapid detection techniques (Dalgaard *et al*,. 1996; Koutsoumanis and Nychas, 2000; Skjerdal *et al*., 2005) and it is foreseeable that rapid PCR based technologies may be applied for the selective detection of spoilage bacteria.

Comparison of the chemical profiles of spoiled seafoods and of the metabolites produced by potential spoilage organisms has only been used to a limited extent for characterization of SSOs (Gram and Dalgaard, 2002). Detection of microbial metabolites by rapid electronic nose analysis would be useful as an alternative or supporting information to microbial enumeration along with the detailed time and temperature history of the products to use in models for a more rapid evaluation of the quality and shelf-life of fish products.

3. OVERVIEW OF THE STORAGE STUDIES

The thesis includes extensive storage studies of different species of fish and fish products (capelin, cod, haddock, red fish and cold smoked salmon). The aim was to apply electronic noses based on different sensor technologies as rapid techniques to monitor spoilage changes in fish and study the influence of preservation techniques and temperature conditions that would typically occur during storage and transport of these products. Different catching methods, handling and storage conditions, in particular the temperature, influence the spoilage pattern of fish and consequently information about storage time may give ambiguous information about the freshness stage of the fish Therefore, a more useful approach is to compare the electronic nose responses with reference measurements that give information about the microbial and oxidative processes influencing the freshness quality. Different sensory, chemical and microbial methods were selected in the studies based on the traditional techniques commonly applied for the different products. The innovative approach in the research was to study simultaneously the proliferation of the microflora and its spoilage potential by simultaneous analysis of the microbial metabolites contributing to the spoilage odors and in this way achieve better understanding of the spoilage processes.

Paper I An electronic nose was used as a rapid technique to monitor changes in the headspace of capelin (*Mallotus villosus*) during storage at 0°C and 5°C. At both temperatures, refrigeration was compared with ice/seawater cooling system. At 5°C, the effect of added 0.2% acetic acid was also tested. Electronic nose measurements were compared to measurements of total volatile bases (TVB-N), gas chromatography of volatile compounds and sensory analysis.

Paper II Freshness of cod (*Gadus morhua*) during storage in ice was measured with two electronic noses based on different sensor technologies and sampling systems. The LibraNose with TSMR (thickness shear mode resonators) sensors with metalloporphyrin coatings was based on sampling with a small metal capsule that is placed directly on the surface of the fish. The FreshSense has six electrochemical sensors and sampling is based on putting the whole fillet into a sampling container. The changes during storage of cod

expressed as storage days were measured and compared with trimethylamine (TMA) and total volatile bases nitrogen (TVB-N) as reference methods.

Paper III The aim of the study was to use an electronic nose with electrochemical gas sensors to monitor microbial metabolites produced during storage of redfish (*Sebastes marinus*) in ice and under MA (modified atmosphere) bulk storage and compare the results to traditional microbial, chemical and sensory methods. The precision of the electronic nose measurements was determined using standard compounds.

Paper IV Two storage experiments of haddock fillets were performed in the years 2001 and 2003. The objective was to characterize spoilage in haddock fillets and determine the spoilage domain of the specific spoilage organisms (SSO) by studying their growth and production of spoilage metabolites under a range of temperature abusive conditions (0, 7, and 15 °C) and temperature fluctuations.

Paper V The aim of the experiments was to study the spoilage characteristics of Combined Blast and Cooling (CBC)-processed cod fillets compared to traditionally processed fillets stored under superchilled (-1.5 °C) and/or chilled conditions (0.5 °C) and the effect of abusive temperature.

Paper VI The aim of the study was to screen the most abundant volatile compounds produced by SSOs that could be used as quality indicators for chilled fillets. Gas chromatography analysis of cod fillets stored in Styrofoam boxes under chilled conditions (0.5 °C) were performed and comparison was made with electronic nose analysis, TVB-N and pH measurements

Paper VII A prototype instrument called FishNose was developed and adapted for the measurements of smoked salmon. Storage studies were done at 5 and 10 °C to compare the microbial and sensory changes with the FishNose responses. The main objective of the studies was to select quality indicators related to microbial counts and sensory odor attributes and to establish quality criteria to use in models based on the FishNose responses to classify cold smoked salmon of different quality.

4. MATERIAL & METHODS

4.1. Storage experiments

- Capelin stored whole in ice, chilled seawater and added acid as a preservative at 0 and 5 °C (I).
- Cod stored whole in ice at 0 °C (II)
- Redfish (*Sebastes marinus*) stored whole in ice and packed in vacuum and under modified atmospheres at 0 °C (III)
- Haddock stored as fillets in Styrofoam boxes at 0°C and under abusive (7 and 15 °C) and fluctuating temperatures (IV)
- Cod (*Gadus morhua*) stored as fillets in Styrofoam boxes at 0°C (V, VI) and high fluctuating temperatures and processed and stored under superchilling conditions (-1,5) (V)
- Cold smoked salmon vacuum packed and stored at 5 and 10 °C (VII)

4.2. Electronic nose measurements

Three different types of electronic noses were used to monitor volatile compounds during storage of the different fish species and products. FreshSense developed in Iceland based on three to four electrochemical sensors (I – VI), FishNose from Alpha MOS in France based on six metal oxide sensors (VII) and LibraNose developed by University of Rome (Tor Vergata) based on eight Thickness Shear Mode Resonators with sensitive coating: tetrapyrrolics macrocycles (porphyrins sensors) (II). The sampling was based on static sampling systems and no sample preparation was needed. A pump was used in all cases to transfer the volatiles to the sensors.

The electronic nose FreshSense was developed by the Icelandic Fisheries Laboratories and the company Bodvaki-Maritech, Kopavogur (Iceland) (Figure 13).

Figure 13. FreshSense electronic nose (Iceland) based on electrochemical sensors with a large sampling container (2.3L) and a pump to transfer the headspace to the sensors (1-VI).

The instrument consists of an array of electrochemical gas sensors (CO, H_2S, SO_2 (Dräger, Lübeck, Germany) and NH_3 (City Technology, Portsmouth, Britain)), a glass container (2.3 L) closed with a plastic lid and a PC with a Labview measurement software. Although the sensors are designed to detect simple gases they are also able to detect other compounds that have similar electrical properties and are volatile enough to reach the sensors. The instrument has a closed sampling system to allow the sample to equilibrate and the volatiles to accumulate in the headspace. The headspace is then transported to the measurement chamber by using a pump. The measurement takes about 5 min. No sample preparation is needed. (I-VI)

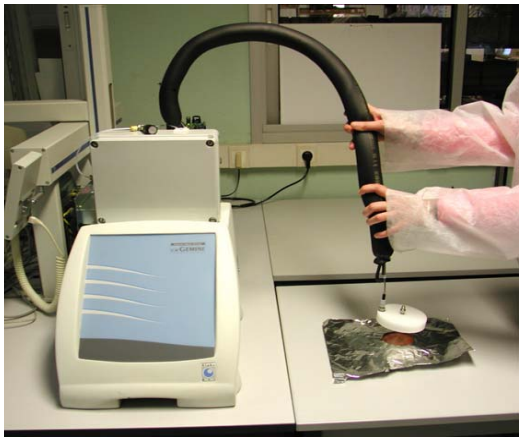


Figure 14. FishNose system from AlphaMOS (France) equipped with a sampling unit (10 mL) with a heated inlet tube and a pump (VII)

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 for monitoring quality of cold smoked salmon (Figure 15). 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 aluminum paper to prevent cross contamination of samples. Aluminum was used because of its inert property. Sampling temperature (headspace generation temperature) was 5 °C and loading time of 7 s was used. (VII)

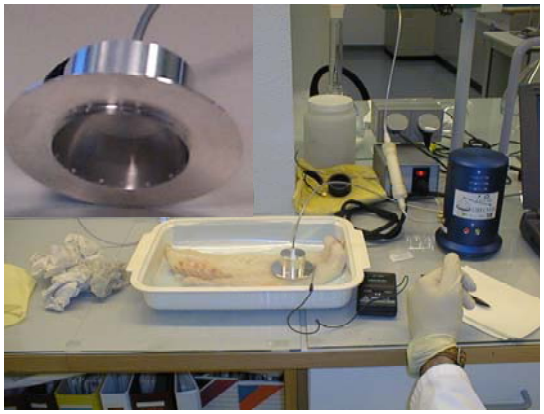


Figure 15. LibraNose (University of Rome, Tor Vergata) equipped with a small sampling capsule of metal, with an internal volume of 10 ml, and small holes for air refill. (II)

LibraNose is an instrument designed and fabricated at the University of Rome Tor Vergata in co-operation with the company Technobiochip (Figure 15). It is based on an array of Thickness Shear Mode Resonators (TSMR) also known as Quartz Microbalance sensors. The chemical sensitivity is given by a molecular film of metalloporphyrins and similar compounds. In the current configuration up to eight sensors are used (D'Amico *et al.*, 1998). Measurements of cod fillets were done using a metallic capsule with an internal volume of 10 ml approximately equal to the volume of the sensor chamber. The capsule is endowed with a series of small orifices for air refilling. It works in contact with the fish fillet, a stable and reproducible (from the point of view of sensors response) headspace is established in five minutes. (II)

4.3. Sensory analysis

The Icelandic Fisheries Laboratories sensory panel participated in the sensory assessments. They were all selected and trained according to international standards (ISO 1993). Different sensory methods were used in the studies. A sensory classification scheme based on 5 categories was used for the capelin (I).The QIM (Quality Index Method) was used for evaluation of redfish (II) (Martinsdóttir and Arnason, 1992). The Torry scheme for cooked fish (Shewan *et al.*, 1953) was applied for both haddock (IV) and cod (V). The smoked salmon quality was evaluated by QDA (Quantitative Descriptive Analysis) (Stone and Sidel, 1985) using a scheme based on 19 different attributes of appearance taste, odor, flavor and texture developed in the study (VII).

4.4. TVB-N, TMA and pH measurements

Total volatile basic nitrogen content (TVB-N) was measured by Struer steam distillation unit as described by Malle and Poumeyrol (1989) (IV,V)

Evaluation of TMA content was done by flow injection gas diffusion (FIGD) as described by Ruiz-Capillas and Horner (1999) (II, III)

The pH was measured in 5 grams of mince moistened with 5 mL of deionized water.

4.5. Microbial analysis

Total viable psychrotrophic counts (TVC, 15 °C) were evaluated in redfish, cod, haddock and smoked salmon by spread-plating aliquots onto modified Long & Hammer's medium (III, IV, V, VII); counts of H_2S-producing bacteria and presumptive pseudomonads (III, IV, V) were evaluated in redfish, cod and haddock on spread-plated Iron Agar (15 °C) and modified CFC medium (22 °C), respectively (Lauzon *et al*., 2002).

Counts of *Photobacterium phosphoreum* were estimated in cod and haddock fillets by using the PPDM-Malthus conductance method (Dalgaard *et al*., 1996), as described by Lauzon (2003) (IV, V).

Analysis of lactic acid bacteria (LAB) counts was done in smoked salmon using NAP (Nitrite Actidione-Polymyxin) medium slightly modified (Davidson and Cronin, 1973) (VII).

4.6. Gas chromatography analysis

Three different sampling methods were used for collection of the volatiles in whole capelin and cod fillets prior to gas chromatographic analysis based on different detectors. A static headspace sampling using a gas tight syringe to withdraw samples from sampling bags was used for analysis of volatile sulfur compounds in capelin (I) and separation by a GC-FPD (flame photometric detector). Sampling for GC-MS (gas chromatography mass spectrometry) and GC-O (gas chromatography – olfactometry) of cod fillets was based on

pre-concentration of the headspace samples onto TENAX traps (VI). The headspace was collected by an air pump sampling (ALPIN-2, Air sampler, METEK) by sweeping volatiles from the surface of the fillets, followed by thermal desorption of volatiles (ATD 400, Perkin-Elmer, Buchinghamshire, UK) from the sampling tubes prior to separation on a DB-5ms column (30 m × 0.25 mm i.d. × 0.25 µm, J&W Scientific, Folsom, CA, USA) using a GC-MS (HP G1800C GCD, Hewlett-Packard, Palo Alto, CA, USA). A purge and trap technique described by Olafsdottir *et al*. (1985) using Tenax in a Pasteur pipette and ether extraction prior to separation by a the same type of column where the end was split 1:1 between flame ionization detector (FID) and an ODO-1 olfactory detector outlet Measurements were performed on a GC (HP 5890, Hewlett-Packard, Palo Alto, CA)
Description of each odor and its duration in time was recorded and the intensity evaluated using a category scale with a description of intensity of odor at each score (*47-48*); 0 none; 0.5 thresholds or just detectable; 1 slight; 2 little; 3 moderate; 4 strong and 5 very strong.

4.7. Data analysis

Microsoft Excel 97 was used for data processing, to calculate means and standard deviations for all multiple measurements and to generate graphs. Analysis of variance (ANOVA) was applied to the sensory, chemical and electronic nose data using the Number Cruncher Statistical Software (NCSS 2000). Significant differences were determined by One way ANOVA and Duncan's Multiple-Comparison Test was used to determine the statistical difference between samples. An effect was considered significant at the 5% level ($p<0.05$).
Multivariate analysis was performed by the Unscrambler (version 7.8 and 9.2, CAMO A/S, Trondheim, Norway). The main variances in the data sets were studied using Principal Component Analysis (PCA) (III). The relationships between sensory, microbial, chemical and electronic nose measurements were explored by Partial Least Squares Regression (PLSR) (I). PLSR was performed on the data using average values of replicates standardized to equal variance (weighting with 1/standard deviation). The Jack-knife method (Martens and Martens, 1999) was used to determine significant variables in X with a significance level of 5% ($p<0.05$). Outlier detection is built into the

Unscrambler software and detects samples that are badly described by the models. All models were calculated with full-leave one-out cross validation (I, IV, V, VII).

Soft independent modeling of class analogy (SIMCA), a pattern recognition method relying on independent modeling of defined classes by means of PCA (Wold, 1976) was used to classify samples according to sensory quality (Torry score) based on the sensor responses, microbial and chemical data (V, VII).

Partial least square discriminant analysis (PLS-DA) was carried out using Matlab 5.0 to compare the performance of the FreshSense and the LibraNose and to combine their outputs to achieve class separation based on storage days of cod. PLS-DA is a supervised classification method where the search for optimal discriminant directions is performed using PLS. PLS-DA provides both a quantitative estimation of class discrimination and score and loading plots for a visual inspection of data separation and the contribution of single sensors to the array (II).

5. RESULTS & DISCUSSION

The results herein showed that electronic nose can be applied to monitor quality changes related to the formation of volatile microbial metabolites during chilled storage of fish and better understanding of the spoilage of fish was achieved.

5.1. Presicion of the electronic nose measurements

Electronic noses based on different sensor technologies were applied as rapid techniques to monitor the volatile compounds during chilled storage of fish. The electronic nose FreshSense was used in all the storage trials (I –IV) except for evaluating the quality of smoked salmon where the Fishnose was applied (VII). The LibraNose was applied in a storage study of cod along with the FreshSense (II).

The electrochemical sensors in the FreshSense instrument were selected based on their selectivity and sensitivity to the main classes of volatile compounds contributing to the spoilage odors in fish, like amines, sulfur compounds, alcohols, aldehydes and esters. Results from PLSR (partial least squares regression) analysis showed that models using only three gas sensors gave similar or better results than when using nine sensors to predict the quality of capelin as TVB-N value (I). Therefore, only three or four sensors out of nine initially were used in the continued studies with the FreshSense. The performance of the FreshSense was evaluated by measuring standard compounds representing the main classes of volatile spoilage compounds in fish (III). The repeatability for the standards selected (ethanol, acetaldehyde, TMA and dimethyldisulfide) evaluated measuring the same sample on the same day showed coeffiecent of variability (%CV) around 10%. The results of reproducibility measurements using the CO sensor responses to different concentrations of ethanol over one year period showed that the %CV was less than 30% (Olafsdottir *et al*., 2002) (III). The high variation of the electronic nose measurements can be explained by the influence of temperature during sampling and variation in sample size related to the surface area exposed (Olafsdottir, 2003).

Improvements were made in the sampling system for the Fishnose instrument (VII). A sampling unit with a heated inlet tube (55°C) and a pump was developed and sampling

was performed at 5 °C. Sensor readings of repeated measurements of calibration samples showed a repeatability for the 6 sensors of the array of 6.4 % (± 1.4%), and for repeated measurements of fish samples the repeatability was 4.3 % (± 2.6%) without purge of the system between the measurements (Olafsdottir *et al*, 2005a; Haugen *et al*., Forthcoming).

5.2. Overall trend in electronic nose measurements to chilled fish during storage

The electronic nose FreshSense showed a similar overall trend for the development of volatile spoilage compounds when monitoring changes during storage of capelin, redfish, haddock and cod that were handled and stored under different conditions (Olafsdottir *et al*., 2000; 2002; DiNatale *et al*., 2001) (I, II, III, IV, V).

The results indicated that the rapid electronic nose measurements give more information about the quality of the fish than each of the individual classical measurements like sensory evaluation, microbial counts and TMA/TVB-N analysis. This is based on the partial selectivity of the sensors towards the different classes of compounds produced by the dominating specific spoilage bacteria.

The electronic nose´s sensors appeared to give similar information as the traditional measurement techniques (QIM scores and TMA) as illustrated in Figure 16 for redfish as an example. The responses of all the sensors, microbial counts, and TMA production were higher in ice storage than in modified atmosphere storage (MA) of redfish indicating the hindering effect of MA on the spoilage microflora (Figure 16).

The CO sensor was most sensitive of the sensors showing increased responses earlier than the other sensors in all the studies and correlated well with sensory, chemical and microbial methods for redfish, haddock and cod (III, IV, V).

During ice storage of redfish the response of the CO sensor appeared to decline after 16 days of storage. The leveling off in the response of the CO sensor at the end of storage period in ice may be reflecting the depletion of certain substrates that the microflora utilizes (Lindsay *et al*., 1986; Gram and Huss, 1996). Responses of the NH_3, H_2S and SO_2 increased during late storage for capelin, redfish and cod that were stored whole on ice.

These sensors are sensitive to amines and sulfur compounds, respectively, that typically form at the end of the storage when the fish is spoiled

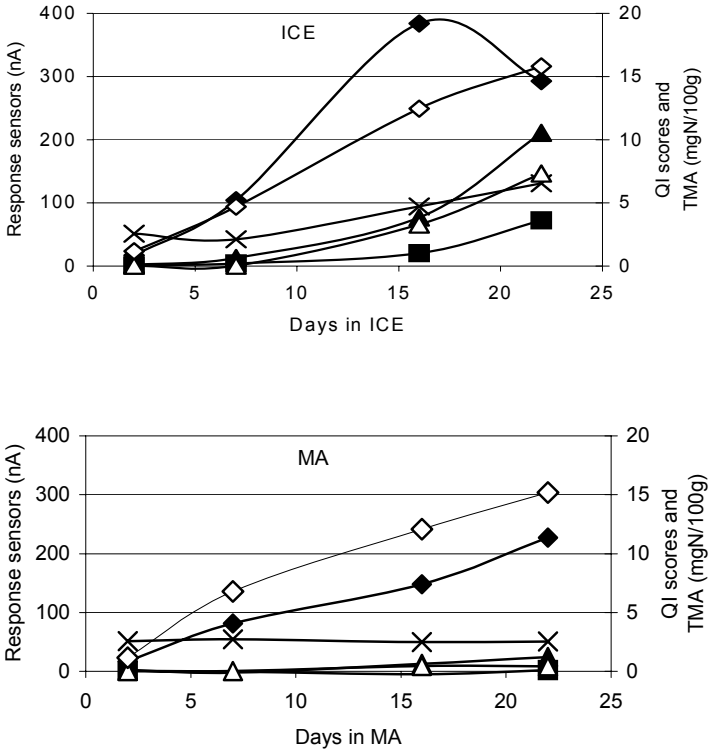


Figure 16. Electronic nose (FreshSense) measurements (◆CO, ■SO_2, ▲ NH_3, **x** H_2S), TMA (-△-), and QI scores (-◇ -) of redfish stored for 22 days in (a) ICE; (b) MA; Shelf life 20.5 days in ice and 22 days in MA. (From Olafsdottir *et al*., 2002) (III).

The responses of the the NH_3, H_2S and SO_2 sensors towards cod and haddock fillets were generally low and no increase in responses of these sensors was detected in MA-stored fish (Figure 16).

5.3. Specific spoilage organisms (SSO) and analysis of microbial metabolites

The proliferation of the SSO (*Photobacterium phosphoreum, Pseudomonas spp.* and H_2S-producers) and evaluation of their spoilage potential by simultaneous analysis of microbial metabolites contributing to the spoilage changes showed that the response of the electronic nose sensors appeared to reflect the production of microbial metabolites. At the same time as the increasing responses were observed for the CO sensor in iced redfish (Figure 16), indicating production of alcohols, aldehydes and esters, a continuous increase was seen in the growth of *Pseudomonas* sp. throughout storage (Figure 17).

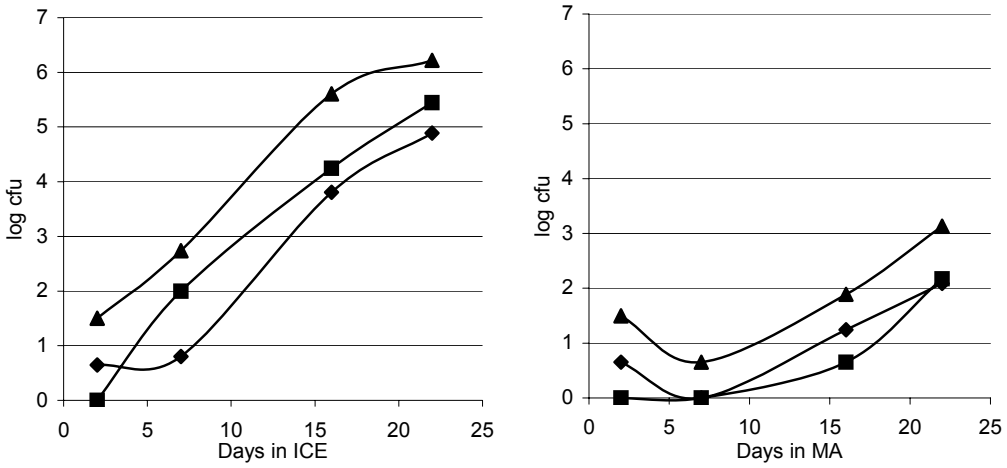


Figure 17. Microbial counts (■Pseud., ◆H_2S-prod. and ▲ TVC-15°C) of redfish stored for 22 days in Ice (left); MA(right) (From Olafsdottir *et al*., 2002) (III)

The low response of the sensors to MA-stored redfish corresponded to the slow development of the microflora under these conditions and a different spoilage profile appeared to develop in MA-stored fish (Figure 17).

The increased TMA production after day 16 coincided with the increased response observed for NH_3 sensor. Higher responses of the H_2S and SO_2 sensors were also noticed coinciding with the later development of H_2S-producers (Figure 17), possibly *S. putrefaciens,* which has been suggested as a late spoiler in iced stored fish (Lauzon, 2000).

The proportion of *Pseudomonas* sp. in iced redfish was about 9% of the total microflora on day 19, while that of H_2S-producers was 2.5% indicating the importance of *Pseudomonas* sp. in aerobically stored fish as has been reported previously (Lauzon, 2000). Based on the results of earlier studies on MA packed fish, higher levels of TMA were expected as a result of the growth of *Photobacterium phosphoreum*, which has been found to be an intensive TMA producer and the main SSO in MAP fish (Dalgaard, 1995a). Direct counts of *P. phosphoreum* were not obtained in the study of the redfish.

On the other hand studies on the proliferation of *P. phosphoreum* as well as *Pseudomonas* spp and H_2S-producers were included when studying the spoilage potential of the SSO in haddock and cod fillets packed in styrofoam boxes and stored under different conditions (IV, V). The different temperature conditions during storage (0, 7 15 °C) and temperature fluctuations were selected to study the influence of abusive conditions on the

proliferation of the microflora in haddock fillets. The effect of superchilled process and superchilled storage (-1.5°) compared to 0.5 °C storage and temperature abusive conditions were studied in cod fillets. The results showed that *P. phosphoreum* appeared to be very important based on their counts and growth rate in the spoilage of both fresh haddock and cod fillets packed in bulk (approximately 5 kg) under aerobic conditions in styrofoam boxes. High counts of *P. phosphoreum* coincided with high TVB-N values (IV, V). *P. phosphoreum* and *Pseudomonas* spp. were dominating under temperature abusive conditions in haddock fillets (IV). *Pseudomonas* spp. was suggested to contribute to the characteristic sweet, fruity spoilage odors of haddock fillets associated with high response of the CO sensor indicating the production of alcohols, aldehydes and esters (IV). Microbial metabolites were produced more rapidly and were detected in higher levels with increasing storage temperature as shown by the response of the electronic nose CO sensor and the TVB-N values at sensory rejection (Figure 18). Sensory rejection was on the following days: 4.5d (15 °C); 6.5d (7 °C); 9d (0 °C+abuse), 11.5d (0 °Ca) and 13.5d (0°C).

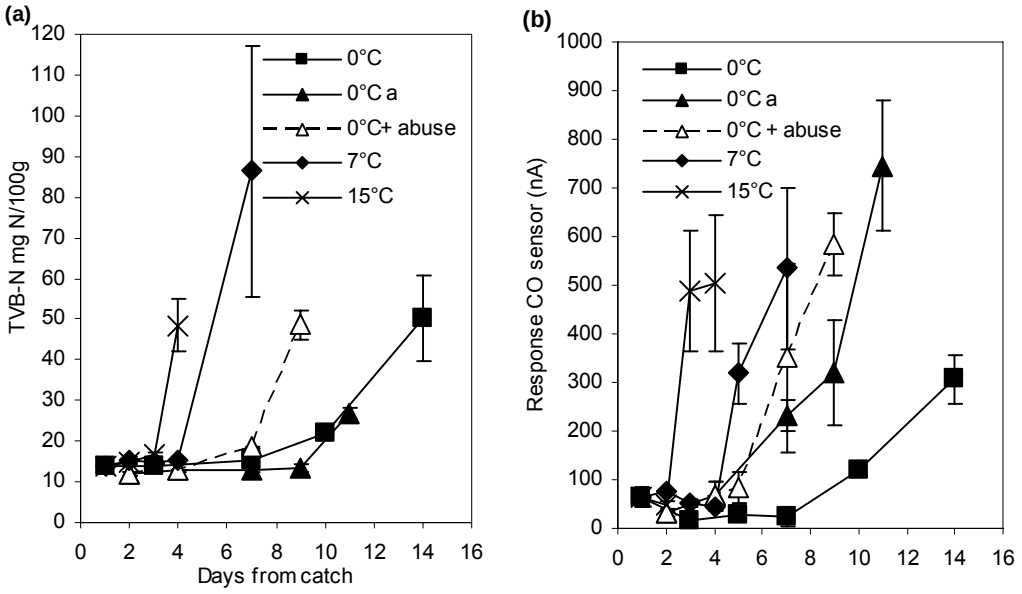


Figure 18. a) TVB-N values and b)CO sensor response in haddock fillets stored in styrofoam boxes at 0 °C (-■-), 7 °C (-♦-) and 15 °C (-x-) from experiment in 2001 and at 0 °C a (-▲-) (traditional process) and 0 °C +abuse (-Δ-) (traditional process and temperature abuse) in 2003. (IV).

5.4. Correlation of reference methods and electronic nose

Partial least squares regression (PLSR) models were used to explore the spoilage potential of the microflora by studying the correlation of the SSOs, volatile compounds measured by an electronic nose, TVB-N and sensory analysis. The correlation loading plot (Figure 19) for all the data from the haddock experiments showed that, microbial counts, TVB-N, pH and the CO sensor as response variables contributed significantly to the modeling of the Torry score as predictor variable. This indicates that these variables were measuring parameters related to spoilage as determined by sensory analysis. The SO_2 sensor did not contribute to the modeling of the data as seen by its location in the middle of the plot. The CO sensor was useful to detect incipient spoilage and characteristic spoilage of fillets stored at low temperatures while the H_2S and NH_3 sensors detected advanced spoilage and the influence of temperature abuse (7 °C and 15 °C) (IV).

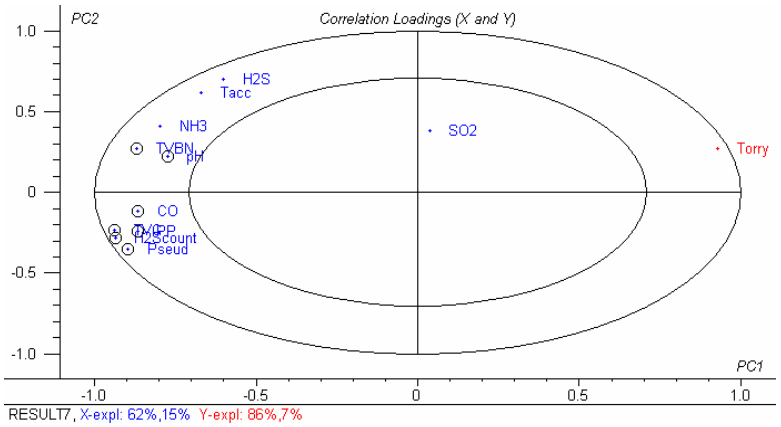


Figure 19. PLSR correlation loadings based on all the measured variables: microbial (TVC, pseudomonads, H_2S counts, *Photobacterium phosphoreum* (PP), chemical (TVB-N, pH) and electronic nose (CO, NH_3, SO_2 and H_2S sensors) and T_{acc} as predictors for the Torry scores as a response variable for the haddock samples stored at different temperatures. The outer and the inner ellipses indicate 100% and 50% explained variance, respectively. Significant variables are symbolized with small circles.(IV)

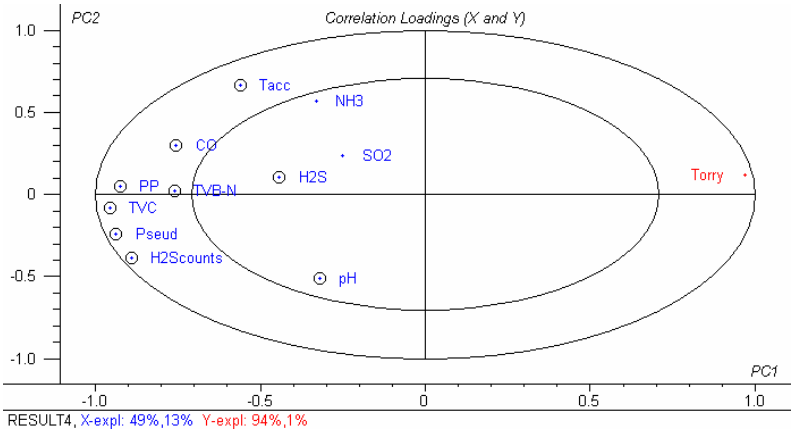


Figure 20. PLSR correlation loadings based on all the measured variables: TVC, pseudomonads, H_2S counts, *Photobacterium phosphoreum* (PP), TVB-N, pH, T_{acc} and electronic nose sensors (CO, NH_3, SO_2 and H_2S) as predictors (X) for the Torry scores as a response variable (Y) for the cod samples stored at different temperatures. (V)

All the SSO´s appeared to be of importance in cod fillets during storage under the different temperature conditions. PLSR correlation loadings based on all the measured variables showed that the spoilage characteristics of superchilled as well as chilled and temperature abused cod fillets were mainly explained by the SSO, TVB-N content and the CO sensor response. Because of the low response of the other sensors (NH_3, H_2S and SO_2) to chilled and superchilled fillets, they did not contribute to the modeling of data as seen by their location in the middle of the correlation loadings plot (Figure 20). (V)

The potential ability of the most important quality indicators, the CO sensor, TVB-N and all the SSO (Figure 20) to predict the Torry score was studied further by PLSR. The resulting PLSR model (r^2 = 0.97 and RMSEP = 0.30) showed that the overall spoilage pattern of the differently treated samples was similar, despite the differences in the spoilage rate of the groups of cod fillets as explained by the influence of the different temperature conditions and initial handling on the dominating SSO in the samples (Olafsdottir *et al*., V). Superchilled samples were described by H_2S-producer counts while *Pseudomonas* spp. characterized the spoilage of traditionally processed groups from one producer. Traditionally processed groups which had the highest temperature during storage had high *Photobacterium* counts and high values of the TVB-N and the CO sensor. The complexity of the dynamic spoilage changes reflected by the growth and

the spoilage potential of the dominating SSO can be explained by the interaction of the microorganisms as influenced by storage temperature, packaging and the inherent composition of available nutrients in the fish in agreement with other studies (Gram and Melchiorsen, 1996; Gram *et al*., 2002). Therefore, it appears that a multi-quality indicator based on the different SSO, TVB-N and the CO sensor response is necessary when using a global model to classify both traditionally processed and superchilled fish fillets (V).

5.5. Establishment of quality criteria for chilled fish

The complexity of the spoilage processes was obvious as seen by the different values observed for TVB-N, the sensor responses and the ratio of the different SSO to TVC at sensory rejection (Table 3). Although a similar spoilage pattern was observed for the differently treated products, the range of values observed for the quality indicators indicated differences depending on the dominating bacteria

The results from the various studies demonstrated that no single quality criteria for cod and haddock fillets can give adequate information about the quality of the products. Moreover, it appears that fixed values to determine the end of shelf-life or the quality of fish fillets based on electronic nose responses or values for the microbial and TVB-N reference methods will have to be developed for each product and the respective storage conditions. The need to establish relevant quality indices for products stored or processed using new preservation techniques has been emphasized. As a result of delayed or altered microbial growth the traditional spoilage signs may become distorted and therefore the commonly used quality indices may be of questionable value (Lindsay *et al*., 1986).

The storage temperature directly influenced the shelf-life of the packed cod and haddock fillets (Table 3). Superchilling of cod fillets with the new superchilling process (CBC contact blast cooling) was most effective in extending the shelf-life in particular when combined with superchilled storage (-1.5 °C) (V). Under superchilling conditions the growth rate of all the bacterial groups was slower and lower CO sensor response was observed compared to traditionally processed fillets stored at 0 °C (V). However, higher levels of TVB-N were observed at sensory rejection for superchilled fillets stored under chilled conditions (>0 °C) compared to the traditionally processed groups (Table 3).

Table 3. Overview of storage temperature, shelf-life estimation (based on Torry sensory score of 5.5) and experimental data showing ranges of values measured at or near sensory rejection for all experimental groups of cod and haddock fillets stored in styrofoam boxes at different temperatures (IV, V).

	Cod 0°C	Cod abused	Cod superchilled	Haddock 0 °C	Haddock abused
Average temperature of fillets during storage	0.4 - 1.9	1.9 - 3.9	-1.3 - 0.9	0.2	**1.7 - 12**
Estimated shelf-life (days)	**12 - 13.5**	**8-11.5**	**12.5 - >15**	**11.5 -13.5**	4.5 -9
% H_2S-producers / TVC	**7 -12.9%**	**1.4 - 3.7 %**	**1.0 - 36.3%**	**0.6 - 2.4 %**	**0.4 - 0.8%**
% *Pseudomonas* spp. / TVC	**4.9 - 37.5 %**	**0.04 - 25.4 %**	**0.2 - 2.9%**	**6.3 - 15.1%**	**1.6 - 15.2%**
% *P. phosphoreum* / TVC	**12.6 - 17.2 %**	**12.7 -24.0 %**	**0.9 -6.7%**	**79.4 - 100%**	**50.2 - 100%**
TVB-N (mg N / 100 g)	27 - 49	34 - 94	38 - 55	27 - 45	45 - 77
CO sensor (nA)	98 - 366	215 -439	136 - 300	280 -370	470 - 570

It has been pointed out earlier that TVB-N and TMA often give ambiguous information about the quality of the products as their levels are influenced by the storage method like in modified atmosphere packaging (Dalgaard *et al*., 1993; Lauzon *et al*., 2002). Similar total microbial counts were observed at sensory rejection in samples stored at different temperatures. However, the levels of the SSOs were different and the microbial metabolites were produced in higher levels with increasing storage temperature as shown by the increasing responses of the electronic nose sensors and increased TVB-N values at sensory rejection

The importance of *Photobacterium phosphoreum* in the packed fillets is especially of interest reaching high counts and being the dominating bacteria in haddock and cod stored at 0 °C and under temperature abusive conditions. Under superchilling conditions the *P. phosphoreum* became dominating in one group, but the dominance of H_2S-producers in the other groups was related to the initial handling of the raw material. (V).

Improper icing and handling of raw material, leading to higher temperature of the cod fillets, especially influenced the development of *P. phosphoreum*, but growth of H_2S-producing bacteria was also accelerated at higher temperatures contributing to a shorter shelf-life of temperature abused cod fillets (V).

The results emphasize the importance of studies performed with natural products and processes to accumulate information about spoilage domain of SSOs under actual conditions. The origin of the raw material, the initial handling and conditions in the processing factories including the time after catch before processing influenced the shelf-

life. When preservation techniques were used like chilled seawater system (I), superchilling conditions (V) or modified atmosphere packaging (III), the resulting spoilage pattern was delayed compared to storage in a traditional way at 0 °C. The proliferation of the specific spoilage bacteria was dependent on the temperature and environmental conditions during storage in agreement with other studies (Gram and Huss, 1996).

Therefore, as concluded before a multi-quality indicator based on the different SSOs, TVB-N and the CO sensor response is necessary for evaluating the quality of the differently stored fish fillets (IV, V). No single technique can replace sensory analysis which is still the best method to determine the consumer acceptability of the fish fillets. However, the sensory analysis was not useful for products like capelin used for fishmeal production. When the capelin turned putrid the odor profile became very complex and it was difficult to describe and distinguish between the various spoiled odor notes (I). The sensory classification scheme was developed for capelin intended for the production of high quality fishmeal (TVB-N<50 mg N/100g). Capelin will typically have TVB-N values around 80-100 mg N/100g to be classified as putrid. However, when producing standard fishmeal the raw material can exceed TVB-N 100 mg N/100g and in some cases sorting the raw material according to TVB-N values above 100 mg N/100g is required. The TVB-N value of the capelin when it is classified as putrid is close to 100 mg N/100g for the samples that were not kept in seawater. On the other hand, samples that were kept in seawater at 0°C and 5°C had much lower TVB-N values (50-60 mg N/100g) when they were classified as putrid by sensory analysis.

5.6. Application of multivariate models to classify or predict the quality of fish

Multivariate models were used to classify and predict the quality of fish based on electronic nose response and selected microbial, chemical and sensory critera

Cod: SIMCA pattern recognition was performed to classify the cod fillets stored at different temperatures (-1.5 – 0.5°C) according to sensory quality (Torry score >7 and <7). A multi-indicator based on the counts of SSOs (*P. phosphoreum, Pseudomonas* spp and H_2S-producing bacteria) in combination with the CO sensor and TVB-N value

resulted in 85% correct classification. The samples that were wrongly classified had sensory scores close to 7. This indicated that the combined quality indicators SSOs, TVB-N and CO sensor, were not sensitive enough to detect the incipient spoilage but they could discriminate fresh samples from spoiled samples (V).
Intergrating data from two electronic nose instruments LibraNose and FreshSense, improved the performance of a PLS-DA to classify cod based on storage days in ice (II). In this study the sensors correlated well with the results of TMA and TVB-N and interestingly the misclassification of samples based on the e-noses could be related to the fact that three batches were used in the design of the experiment. It was evident that the second batch had slower spoilage rate than the first batch, which was explained by slightly different initial handling of the batches. Therefore, it was concluded that information about storage days is not reliable to estimate the freshness quality. (II).

Haddock: Exploration of different PLSR models for haddock fillets stored at different temperatures (0.5 – 15°C) based on the FreshSense electronic nose data and the SSO´s as predictors and the Torry sensory scores as the response variable demonstrated that the spoilage characteristics of haddock fillets were best described by the pseudomonads counts in combination with the electronic nose responses (CO, NH_3 and H_2S sensors). Validation of the model based on a subset of the data showed an average of 4.8% difference between the predicted and the experimental data (IV).

Cold smoked salmon: When monitoring the spoilage of cold smoked salmon by the FishNose with metaloxide sensors the spoilage changes of the respective products where characterized by microbial and sensory methods. The quality indicators sweet/sour, off and rancid odor were selected from fourteen sensory attributes based on their ability to predict microbial quality (log TVC) using PLSR (Figure 21) (VII). The spoilage odors were directly related to the sensor responses, since the sensors in the FishNose were sensitive to spoilage related compounds like butanone contributing to the off odors, but they were not sensitive to the characteristic smoke flavor compounds like guaiacol which was detected in high levels in smoked salmon (Olafsdottir *et al*., 2005; Haugen *et al*., Forthcoming).

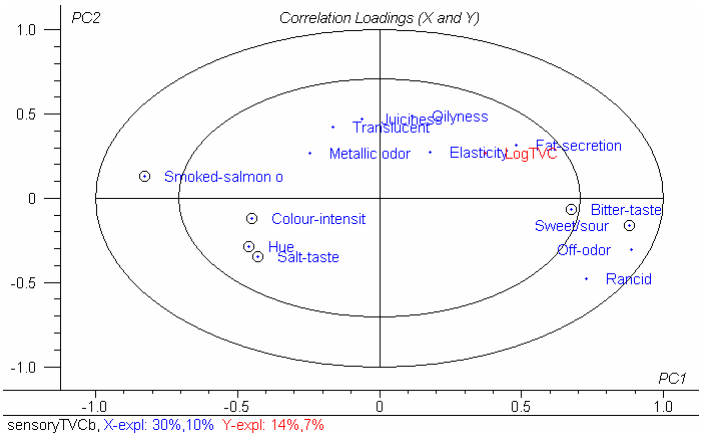


Figure 21. Correlation loadings for the first two components from PLS regression with fourteen sensory attributes as predictors and log TVC as response variable (VII)

Classification based on PLSR of all the smoked salmon samples (N=96) from three different producers in Europe in a global model resulted in 74 % of the samples correctly classified into their respective quality class and 26 % were classified wrongly. Local predictive models based on samples from individual processors using the same quality criteria appeared to generate more robust prediction of good and bad samples than the global model. High classification rates (100 %) were obtained for the FishNose prediction using both single and combined quality criteria. When evaluating local models with product from individual producers the optimal classification with regard to lowest number of "false positives" ("bad" samples predicted as "good") was achieved when using a combined criteria of TVC and sensory off- odor or sweet/sour - odor for the sample group with the highest number of expected bad samples.

This suggests that multiple quality indices are favorable to predict the complex spoilage changes occurring in smoked salmon products and a model based on the FishNose responses adapted for individual product may be useful for quality classification in the smokehouses related to microbial counts and sensory off- odor or sweet/sour - odor.

Capelin: For capelin stored at 0 °C and 5 °C using chilled seawater and acetic acid as preservation techniques, a single sensor in the FreshSense instrument, the NH_3 sensor,

appeared to be promising to predict the TVB-N value (Olafsdottir *et al*., 2000) (I). A generalized linear model with normal error was fitted to the data. (I).

$$TVB_i = \alpha_i + \beta_1 NH_3 + \beta_2 (NH_3)^2 + \varepsilon_i$$

Where α_i represents the intercept for condition level i, with i = storage conditions (0°C and 5°C; 0°C+sea and 5°C+sea; 5°C+acid) and ε_i the error term. α_i, β_1 and β_2 are parameters to be estimated for each storage condition (I).

Capelin is a pelagic species that is often used for fishmeal production and the level of spoilage was much higher than for the gadoid species (cod and haddock). TVB-N values for the packed fillets in the storage studies of haddock (IV) and cod (V) at sensory rejection were around 30-50 mgN/100g while the TVB-N values for capelin reached 100 mgN/100g at the end of the storage time (1).

5.7. GC analysis of volatile compounds in cod fillets

Volatile compounds in cod fillets were monitored by gas chromatography analysis to screen potential quality indicators during chilled storage. Identification of volatile compounds based on GC-MS analysis and quantification of the main classes of compounds based on the sum of the PAR for respective compounds in each class was done for cod fillets packed in styrofoam boxes during chilled storage (0.5 °C). The results showed that ketones were detected in the highest level (33 %), followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%) and a low level of esters (<1%) on day 12 when the fillets were rejected by sensory analysis (Figure 22) (VI). Selective sensors for the detection of ketones, amines (TMA), alcohols, aldehydes, acids and esters are suggested for monitoring quality changes of cod fillets during storage. The detection of sulfur compounds would be useful only when the products are spoiled. None of the sensors in the electronic nose FreshSense was sensitive to ketones and acids and therefore selective sensors for these components should be included in the instrument to monitor spoilage of cod fillets in addition to a more sensitive sensor for the detection of TMA.

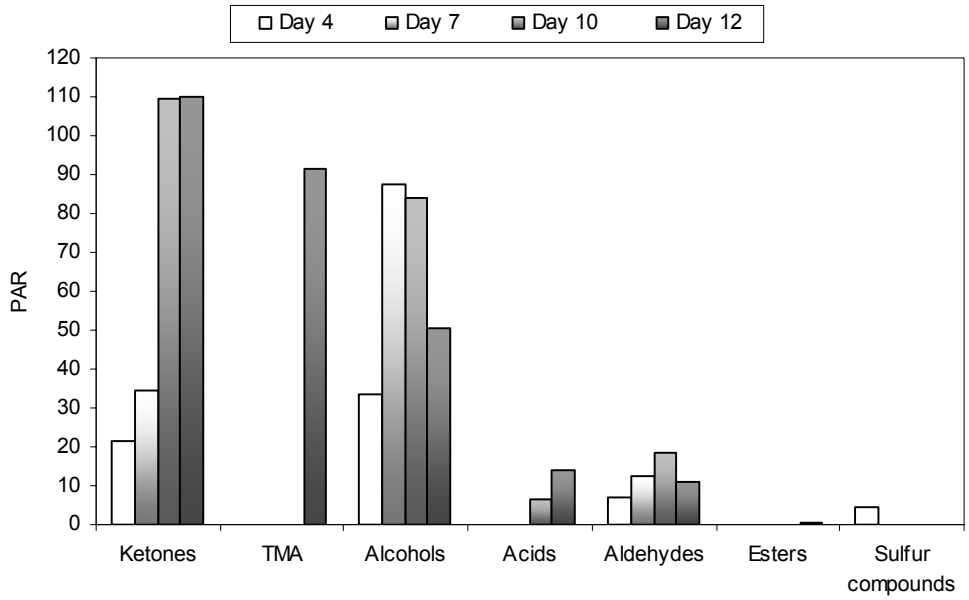


Figure 22. GC-MS analysis of volatile compounds showing changes in the levels (PAR: peak area ratio) of the main classes of compounds contributing to spoilage in cod fillets packed in styrofoam boxes during storage at 0.5 °C until sensory rejection on day 12.

5.7.1. Characterization of the spoilage potential of the SSO in cod fillets

At sensory rejection the spoilage microflora is composed of microorganisms that have contributed to the spoilage and others that have not caused unpleasant changes (Gram *et al.,* 2002). Quantitative evaluation of the spoilage is based on determining the cell count of the SSO and the metabolites causing off odors (Jørgensen and Huss, 1989; Dalgaard *et al*., 1993; Dalgaard, 1995a; Koutsoumanis and Nychas, 2000). *P. phosphoreum* was identified as the main SSO in the cod fillets based on its high counts throughout the storage time, reaching counts of log 7.2 CFU/g at sensory rejection (12.6 %). *Pseudomonas* spp. and H_2S-producing bacteria represented 4.9 % (log 6.6 CFU/g) and 7% (log 7 CFU/g), respectively, of the total microflora at sensory rejection (Figure 23).

P. phosphoreum appears to be the main contributor to the production of the ketones and amines detected in the highest level during storage. Acetoin (3-hydroxy-2-butanone) was the most abundant of the ketones detected in high levels throughout the storage (Figures 22 and 23).

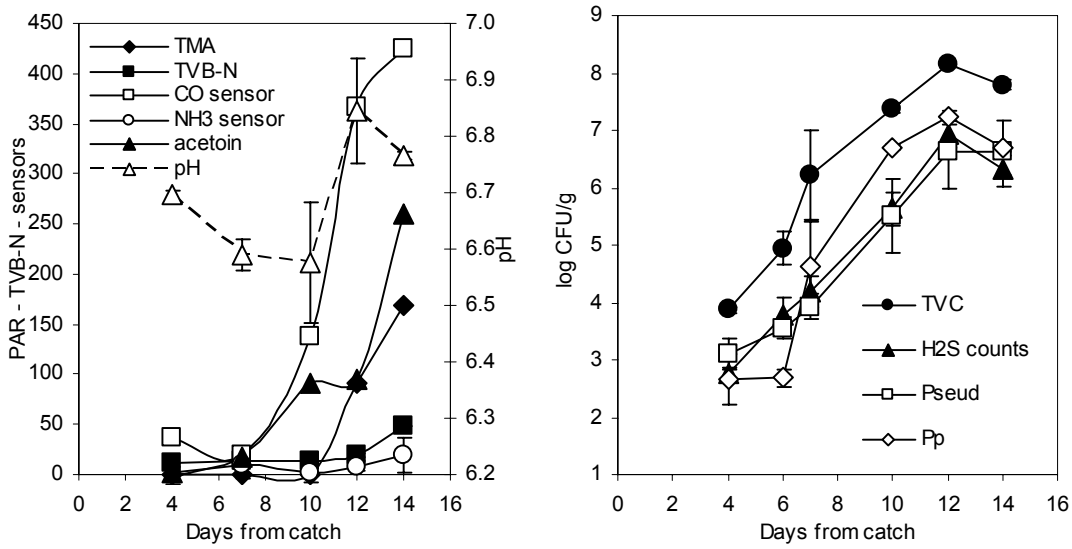


Figure 23. Response of the electronic nose CO and NH_3 sensor GC-MS analysis of TMA and acetoin (PAR), TVB-N and pH (left) and microbial counts (TVC, H_2S-producers, *Pseudomonas spp*. and *P. phosphoerum*) (right) in chilled cod fillets packed in styrofoam boxes during storage at 0.5 °C (Adapted from V, VI)

The formation of high levels of acetoin and TMA (VI) coincided with the growth of *P. phosphoreum* which was identified as the dominating SSO in the cod fillets (V) (Figure 23). TMA and acetoin have earlier been associated with dominating *P. phosphoreum* growth (Van Spreekens and Toepoel, 1981; Dalgaard, 1995a). Levels of acetoin increased earlier than TMA and acetoin is therefore more useful to monitor the loss of freshness as an early indicator of spoilage. TMA is a potent odorant with a characteristic fishy, dried fish, ammonia-like odor as detected by GC-O and received high odor scores (Table 4). The odor of acetoin has been described as butter- cream like (Acree, 1997), but a mild, sweet-sour like odor was detected by GC-O (Table 4).

Other compounds detected on day 12 at sensory rejection also contributed to the overall aroma. Lipid derived aldehydes, like hexanal, nonanal and decanal were detected in similar levels throughout the storage time and contributed to the overall sweet odors of cod fillets in combination with other carbonyls (3-hydroxy-2-butanone, acetaldehyde, 2-butanone, 3-pentanone and 6-methyl-5-heptene-2-one) (Table 4).

Table 4. Quality indicators in cod fillets - main classes of quality indicating compounds, PAR on day 12 at sensory rejection and odor analysis of compounds identified in cod fillets packed in Styrofoam boxes during storage at 0.5 °C (Adapted from VI)

Quality indicators	%PAR[a]		PAR		
Class	Day 12	Compounds	Day 12	Odor score[b]	Odor description[c]
Alcohols	15%	ethanol	-		
		2-methyl -1-propanol	35.0 ± 2.8		
		1-penten-3-ol	1.2 ± 0.0		
		3-methyl-1-butanol	8.5 ± 1.0		
		2-methyl-1-butanol	-		
		2,3-butandiol	4.0 ± 4.4		
		2-ethyl-1-hexanol	1.8 ± 0.2		
Aldehydes	3%	acetaldehyde	0.8 ± 0.5		
		3-methyl-butanal	1.3 ± 0.2	1.5 - 3.0	sweet, caramel, fish fillet
		hexanal	2.0 ± 0.1		-
		heptanal	0.7 ± 0.2	2.0 - 3.0	earthy, boiled potato
		octanal			-
		nonanal	3.7 ± 0.6		-
		decanal	2.4 ± 0.3	1.5	fresh, floral
		undecanal	0.4 ± 0.1	1.5	sweet, candy
Ketones	33%	2-butanone	-		-
		3-pentanone	13.6 ± 6.2	1.5 - 2.0	sweet, caramel
		3-hydroxy-2-butanone	95.3 ± 5.6	1.5 - 2.0	sweet, sour
		6-methyl-5-hepten-2-one	1.0 ± 0.1	1.5	spicy, flowery
Acids	4%	**acetic acid**	14.2 ± 13.0		-
Amines	27%	***TMA***	91.6 ± 28.5	3.0	TMA-like, dried fish
Esters	<1%	ethyl acetate	0.6		-
		ethyl butanoate	-	2.3	sickenly sweet, vomit
Sulfur compounds		dimethyl sulfide	-		-
		dimethyl disulfide	-	1.5 - 2.5	onion like
		dimethyl trisulfide	-	2.5	rotten, sulfur, cabbage

[a] PAR: peak area ratio based on comparison with an internal standard (GC-MS analysis); [b] Range of odor score in samples during storage, increasing with time (GC-O); [c] based on GC-O analysis (two panelists)

Aldehydes have generally low odor thresholds and therefore their odor impact was greater than the alcohols and the ketones although their overall levels were less (Table 4). The presence of ethyl acetate at sensory rejection suggested the role of *Pseudomonas fragi* in the development of the spoilage odors of chilled cod fillets. Low levels of sulfur compounds indicated that *S. putrefaciens* was not important in the spoilage of chilled cod fillets stored in styrofoam boxes.

The change in the pH value on day 12 may have influenced the overall odor perception because of possible synergistic effects causing more potent off odors leading to the

sensory rejection of the fillets. TMA has been noted for intensifying fishiness by a synergistic action with certain volatile unsaturated aldehydes derived from autoxidation of polyunsaturated fatty acids (Karahadian and Lindsay, 1989). The possible influence of other aroma active compounds present in lower levels like the unsaturated autoxidatively derived aldehydes (2,4-heptadienal and 2,4,7-decatrienal) should not be overlooked, but the sampling techniques used were not sensitive enough to allow detection of these compounds.

The CO sensor was useful for detecting incipient spoilage since its levels increased significantly between days 7 and 10 and was found to increase rapidly parallel to the increase in the pH value after day 10 of storage (Figure 23). The increasing response of the electronic nose's CO sensor was explained by the increasing level of alcohols during storage like ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2,3-butandiol in addition to the presence of aldehydes and the formation of esters at the end of the shelf-life (Table 4).

The early detection of alcohols by the CO sensor is of importance for monitoring incipient changes in quality during storage. The flavor thresholds of the alcohols are high compared with the carbonyls and they did not contribute to the odor of the fillets as evaluated by GC-O (Table 4).

5.7.2. Volatile compounds as quality indicators in different seafood products

It is of interest to compare the composition of the headspace of different fish products during storage. Similar volatile compounds as found in the cod fillets were identified in an earlier study of chilled haddock fillets stored in styrofoam boxes TMA, 2-methyl-1-propanol, 3-methyl -1-butanol, 3-hydroxy- butanone, ethyl acetate and butanoic acid ethyl ester were found in the highest amount and increased with storage of haddock fillets. Dimethyl disulfide and dimethyl trisulfide were detected at the end of the storage time when the samples were spoiled, while dimethyl sulfide was detected initially and throughout storage (Olafsdottir, 2003). Amines, sulfides ketones and esters were the main classes of volatile compounds in prawns associated with the growth of *Pseudomas fragi* and and *S. putrefaciens* (Chinivasagam *et al*., 1998). Microbially produced ketones, aldehydes and alcohols were abundant in the headspace of cold smoked salmon products during storage (Joffraud *et al*., 2001; Jörgensen *et al*., 2001). A few compounds mainly

alcohols were associated with the spoilage off-flavor as confirmed by gas chromatography-olfactometry. These were trimethylamine, 3-methyl butanal, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-penten-3-ol, and 1-propanol (Jörgensen *et al*., 2001).

A similar set of sensors with selectivity and sensitivity towards the main quality indicating classes of compounds like ketones, amines, alcohols, aldehydes, acids, esters and sulfur compounds can be used for a variety of fish species that are stored and processed by different techniques.

6. CONCLUSIONS

The studies provided better understanding and explained the contribution of the different microflora to the spoilage odor development in naturally spoiling fish. The electronic nose sensors with sensitivity towards the main classes of compounds produced during storage showed that the CO sensor was most sensitive to detect incipient spoilage in all fish species. This indicated the formation of volatile alcohols, aldehydes and esters coinciding with the growth of *Pseudomon*as spp. The increasing response during advanced storage of the NH_3, H_2S and SO_2 sensors, sensitive to amines and sulfur compounds, suggested the importance of *S. putrefaciens* as a late spoiler in whole redfish and cod, and in cod and haddock fillets stored under temperature abusive conditions (7 °C and 15 °C). These sensors are therefore useful for detecting the spoilage indicator compounds like sulfur compounds and amines which are only present in detectable amounts when products are overtly spoiled. No increase in the responses of these sensors was observed in MA-stored redfish, chilled haddock and superchilled cod fillets before the end of the sensory shelf-life, verifying that the spoilage process was slower or altered under these conditions. Only the CO sensor showed increasing responses in these products and appears to be a promising indicator of fish quality when stored under different conditions (III, IV, V). More studies are needed to characterize better the spoilage changes leading to sensory rejection of superchilled and MA packed fillets.

Quantitative evaluation of the spoilage potential based on determining the cell count of the specific spoilage organisms (SSO) and the quantity of metabolites causing off odors showed that *P. phosphoreum* appeared to be very important in the spoilage of both fresh haddock and cod fillets packed under aerobic conditions in styrofoam boxes. High counts of *P. phosphoreum* coincided with high TVB-N values at sensory rejection and *P. phosphoreum* appeared to be the main contributor to the formation of off odors based on its ability to produce TMA and 3-hydroxy-2-butanone (acetoin) which were detected by GC-MS in the highest concentration at sensory rejection. Levels of acetoin increased earlier than TMA and acetoin is therefore more useful to monitor the loss of freshness as an early indicator of spoilage, but high levels of TMA were detected at sensory rejection (VI). This is of importance when selecting sensitive sensors for the rapid detection of fish freshness.

The results of gas chromatography analysis showed that ketones, amines (TMA), alcohols, aldehydes, acids and esters were present in the highest concentration during chilled storage of fish fillets (VI). Therefore, selective sensors for the detection of these classes of compound are suggested in particular additional sensors are needed for ketones and acids when monitoring quality changes during storage of cod and haddock fillets. Increased sensitivity of sensors is required for monitoring the incipient quality deterioration and the early detection of compounds indicative of quality loss, including a more sensitive sensor for the detection of TMA.

Rapid measurements of volatile compounds in fish with an electronic nose appear to have a potential to detect the freshness or quality, if the sensor array can detect the respective indicator compounds that truly represent the condition of the fish. The studies showed that an overall similar pattern was observed in the electronic nose measurements of chilled fish indicating that similar volatile compounds were present during storage of different products (I –VI). This indicates that the same array of sensors with selectivity and sensitivity towards the main quality indicating classes of compounds of fish could be used for a variety of fish species and fish products processed by different techniques and stored under chilled condition. However, the studies clearly demonstrated that fixed values to determine the end of shelf-life or the quality of fish based on electronic nose responses or values for the microbial and TVB-N reference methods will have to be developed for each product and the respective storage conditions.

The extensive storage studies that have been performed on different fish species demonstrated the complexity of the spoilage changes caused by the diversity of the microflora and their different spoilage potential under the different temperature conditions. The microbial, sensory, chemical and electronic nose data including details on handling, processing and time and temperature history of the products provides a valueable overview of spoilage changes of chilled fish (I –VII). This data is important for recommendations on the validity of the reference methods implied in regulations like TVB-N and TVC when new preservation techniques like superchilling and MA packaging are used (V, III).

Classification of chilled fish fillets according to sensory criteria using multiple indices of quality (electronic nose; SSOs, TVB-N) gave 85% correct classification. The

misclassified samples were all samples of marginal quality. This indicated that the combined quality indicators SSOs, TVB-N and CO sensor, were not sensitive enough to detect the incipient spoilage, but they could clearly discriminate fresh samples from spoiled samples (V). Multivariate models adapted for individual products based on an electronic nose with metaloxide sensors gave up to 100% correct classification for cold smoked salmon according to microbial and sensory off- odor or sweet/sour -odor criteria (VII). The NH_3 sensor gave the best prediction of the TVB-N value in capelin intended for fish meal production by fitting a generalized linear model to the data and estimating parameters for each storage condition (I). Models based on electronic nose measurements, as well as any other technique to predict quality of fish products, have to be adapted for individual products and the respective storage conditions

The information gained is of practical value for the fish industry and can be used to select appropriate storage conditions to extend the shelf-life of fish products by preventing or minimizing the development of the spoilage flora. The studies showed that low temperature throughout the whole process from catch to the delivery of products to consumers is of utmost importance to preserve the quality of the products. Temperature fluctuations in the chain influenced the spoilage rate of the products and shortened the shelf-life (IV, V). Preservation techniques like MA and superchilling process combined with superchilled storage were effective in extending the shelf-life of fish products (III, V).

7. FUTURE PERSPECTIVES

The benefit of the electronic nose technique is the speed of analysis because of limited sample preparation and the fast data generation and data interpretation. The electronic nose technique provides a rapid detection of the volatiles in the headspace and the resulting pattern of the sensor responses gives qualitative or quantitative information about the headspace composition. Both sophisticated laboratory instruments and application specific simple instruments with selective sensors will find applications in the future for quality and safety monitoring in the food industry. The studies in the thesis showed that the electronic nose can be used to detect volatile compounds associated with microbial growth and can be used to classify or predict quality of fish products.

The information gained on the key quality indicating compounds like alcohols, ketones acids and esters can be exploited for the development of "smart" sensor technologies for example using microchips with imprinted sensors to include into the packaging to monitor the quality of fish products. The use of pH sensitive films or selective detection of TMA and ammonia are examples of techniques that have been suggested to include into the packaging for monitoring the quality fish. The criteria are that the tools should be readily implemented in the industry to monitor the freshness and quality of fish products to ensure high quality and safe products for consumers.

Development of a sampling system for the FishNose instrument (VII) improved its performance for at-line use in the smoked fish industry and stressed the importance of carefully controlling sampling conditions. On-line monitoring or continuous monitoring of food products is an interesting possibility that requires automated sampling options. For implementation of the electronic nose technique for monitoring quality of fish products in the industry, it is important to select temperature conditions to ensure reproducible sampling and prevent the possible interference of background air and contaminants by using closed sampling compartments. The fast development of sensor technologies and data processing techniques will improve the possibility of specific applications for quality monitoring in the food industry. More selective and sensitive sensors to detect the different quality indicators will improve the performance of the electronic noses.

The speed of the analysis of the electronic nose measurement and fast data generation opens up the possibility to use the data in predictive shelf-life models along with data on the time temperature history of the products. The studies on cod and haddock fillets showed that for accurate prediction of sensory quality and shelf-life of fish fillets a combined criteria of the SSOs and their metabolites is needed (IV, V). Additionally, the information on the time from catch, the handling and processing conditions and the temperature history is essential for accurate estimation of quality It is foreseeable that rapid PCR based techniques will become available in the near future for the detection of the different SSOs. Therefore, the information gained on the characteristic spoilage processes of the different products should stimulate continued studies on the characterization of spoilage processes of different fish species and products stored under

different conditions to further guide the development of the appropriate detection techniques to evaluate the quality.

Further research is needed on the possible involvement of other SSOs than have been studied here and also the role of endogenous enzyme activity and oxidation causing degradation of muscle components and the loss of quality. The degredation of the muscle results in more available nutrients for microbial growth leading to the formation of volatile compounds causing off odors. Future studies should include more sensitive detection of the volatile compounds causing off odors in fish. This includes the detection of ketones and other microbially, oxidatively or enzymically derived degradation compounds that possibly contribute to the sensory rejection of chilled, superchilled and MA packed fillets.

The sensitivity of the electronic nose sensors towards the very volatile degradation compounds like esters, sulfur compounds and amines signaling overt spoilage suggests that the e-nose may be promising for environmental monitoring of malodors. High concentration of these compounds often causes odor pollution from fish and animal waste and when processing fish meal or dried products when the raw material is not of optimal freshness.

The development of the liquid counterpart of electronic noses, namely array of sensors working in solution (the so-called electronic tongue) may be used in combination with the electronic nose for better characterization of the flavor of food (Winquist *et al*., 1999). Similarly, the simultaneous use of instrumental devices as counterparts of other sensory attributes like texture and appearance contributing to overall quality (to some extent mimicking the human senses) is a powerful approach for obtaining robust estimates of freshness and quality.

The results from the studies showed that multiple quality indices were favorable to predict the complex spoilage changes occurring in fish products. The development of sensors and instrumental techniques and advances in statistical and mathematical analyzes of the data, especially multivariate analysis and multi-sensor data fusion of instrumental results and correlation with the results of sensory analysis will give a more precise estimation of the quality of food. Sensory analysis was used in the studies as the main reference method and the ideal indicator of consumer acceptance of the products.

Sensory analysis is in fact a multidisciplinary approach taking into account various attributes like odor, taste, appearance and texture. Therefore, it can be expected that no single instrument relying on one attribute will encompass all the changes occurring in fish during storage. However, volatile compounds can give information about different degradation processes in the fish. Therefore, the electronic nose composed of selective sensors for detecting compounds characteristic for the growth of specific spoilage organism or oxidative processes is a multi-indicator device and has the potential to give more information than a single reference measurement for example TVB-N.

8. ACKNOWLEDGEMENTS

The background chapter in the thesis is based on book chapters and review papers which were the outcome of three European projects; Evaluation of Fish Freshness, MUSTEC and FQLM. The collaboration of my colleagues and friends in WEFTA in these projects and the participation in all the meetings in different locations throughout Europe has provided the necessary scientific and social stimulus to encourage me to complete this thesis. Participation in courses and workshops organized by the NOSE Network has been invaluable to keep up with the fast developing field of the electronic nose technology and to have the chance to meet my good colleagues in the NOSE community.

In particular I acknowledge the following:

Kristberg Kristbergsson my supervisor for believing in my work and providing support and guidance especially during the most challenging part in the end.

Doctoral Committee members: Joop Luten and Joerg Oehlenschläger for their friendship and for having continuously given me opportunities, guidance and encouragement throughout the years, Rögnvaldur Ólafsson for initiating the e-nose activities for fish applications and giving valuable comments to the thesis and Ágústa Guðmundsdóttir for support and encouragements. Also, Inga Þórsdóttir for her enthusiasm and support in planning the thesis work.

John Erik Haugen, Eric Chaine and other colleagues in the Fishnose project for good collaborations leading to the nomination of the Göpel Award at the ISOEN2005 meeting.

Corrado Di Natale and other MUSTEC friends for the long and fruitful "work-ins ".

Icelandic Fisheries Laboratories for providing the opportunity to carry on the work and Sjöfn Sigurgísladóttir and Helga Gunnlaugsdóttir for the motivation to finish the thesis.

The staff at IFL is thanked for their valued contribution in chemical, microbial and sensory analysis and for being good friends and problem solvers in all areas. Special thanks are due to Emilía for her everlasting interest in “aroma” and for pushing me forward and giving me support for many years, Rosa for her skilful way to master the puzzle of the GC data, Hélene for being the best organizer of storage studies and for her scientific discipline when it comes to microbiology and data interpretation, Soffia for all the joint experiments in the MUSTEC and for listening and sharing quality time and

Birna for always being there when needed and for inspiring me with her genuine interest in "cleaning and hygiene " especially during our visit in Vietnam.
Collaborations with students in the United Nations University Fisheries Training Program have been invaluable and given me insight to the world beyond Europe.
Jón Bæring Hauksson at Maritech for technical expertise and development of the electronic nose FreshSense.
Personnel at the participating companies Tros, Reykofninn, Tangi, SR-mjöl and Skaginn for their support.
Finally, my family Magnús, Siggi, Óli Torfi, Helga and Tommi for being around and sharing all the emotions involved in writing this thesis. Special thanks to Helga for carefully reviewing the reference list and helping with data handling.

Funding of the following research projects and network activities contributing to papers and background chapter of this thesis is acknowledged:
The Icelandic Centre for Research

- Notkun lyktarnema við mat á gæðum hráefnis í fiskimjölsiðnaði: Application of gas sensors for quality evaluation of capelin for fishmeal production (I)
- Nálkvæm geymsluþolsspálíkön: Precise predictive models to determine the shelflife of fish and fish products (IV, V, VI)

The Icelandic Centre for Research /AVS research fund of the Ministry of Fisheries

- Áhrif roðkælingar á gæði fiskflaka; The influence of the CBC superchilling process on the quality of fish fillets (V)

European Commission

- Implementation on board of systems of atmospheres with variable composition applied to fresh fish. Continuation on shore of the modified atmosphere chain. FAIR CT98-3833 (III)
- Development of multi-sensor techniques for monitoring the quality of fish FAIR4-4076 MUSTEC (II, Background)
- FishNose - Development of an electronic nose system for the automated quality control of smoked fish QLK1-CT-2002-71304 (VII)
- Fish Quality Labeling and Monitoring FAIR 4-4174 FQLM (Background)
- Evaluation of Fish Freshness AIR3 CT94 2283 (Background)
- NOSE I and NOSE II – Network on Artificial Olfactory Sensing / IST (Background)

9. REFERENCES

Ackman RG, Hingley J, MacKay KT. 1972. Demethyl sulfide as an odor component in Nova Scotia Fall Mackerel. J Fish Res Bd Can 29(7):1085–8.

Acree T, Arn H. 1997. Flavornet, http://www.nysaes.cornell.edu/fst/faculty/acree/flavornet/index.html, Cornell University.

Ahmed A, Matches JR. 1983. Alcohol production by fish spoilage bacteria. J Food Prot 46:1055-9.

Aksnes A, Brekken B. 1988. Tissue degradation, amino acid liberation and bacterial decomposition of bulk stored capelin. J Sci Food Agric 45:53-60.

Alasalvar C, Taylor KDA, Shahidi F. 2005. Comparison of volatiles of cultured and wild sea bream (*Sparus aurata*) during storage in ice by dynamic headspace analysis gas chromatography mass spectrometry. J Agric Food Chem 53:2616-22.

Amoore JE, Forrester LJ, Pelosi P. 1976. Specific Anosmia to Isobutyraldehyde: The Malty Primary Odor. Chemical Senses and Flavor 2:17-25.

Anonymous 1996. Council regulation (EC) No. 2406/96 of 26 November laying down common marketing standards for certain fishery products. Official Journal of European Communities No. L334/1-14, 23.12.96.

Aro T, Tahvonen R, Koskinen L, Kallio H. 2003. Volatile compounds of Baltic herring analysed by dynamic headspace sampling-gas chromatography-mass spectrometry. Eur Food Res Technol 216:483-8.

Baek HH, Cadwallader KR. 1996. Volatile compounds in flavor concentrates produced from crayfish-processing byproducts with and without protease treatment. J Agric Food Chem 44:3262-67.

Baixas-Nogueras S, Bover-Cid S, Veciana-Nogues MT, Vidal-Carou MC. 2003. Suitability of Volatile Amines as Freshness Indexes for Iced Mediterranean Hake. J Food Sci 68:1607-10.

Balaban MO, Korel F, Odabasi AZ, Folkes G. 2000. Transportability of data between elcetronic noses: mathematical methods. Sens Act B-Chem 71(3):203-11.

Bandarra NM, Undeland I, Nunes ML, Batista I, Empis JM. 1998. Lipid oxidation indices to evaluate sardine freshness. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to Determine the Freshness of Fish in Research and Industry. Proceedings of the Final meeting of the Concerted Action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 263-5.

Bartlett HN, Elliott JM, Gardner JW. 1997. Electronic noses and their application in the food industry. Food Technol. 51(12): 44-48

Bisogni CA, Ryan GJ, Regenstein JM. 1987. What is fish quality? Can we incorporate consumer perceptions? In: Kramer DE, Liston J, editors. Seafood Quality Determination. Amsterdam: Elsevier Science Publishers BV. p 547-63

Boothe AAJ, Arnold JW. 2002. Electronic nose analysis of volatile compoounds from poultry meat samples, fresh and after refrigerated storage. J Sci Food Agric 82(3):315-22.

Botta JR. 1995. Evaluation of seafood freshness quality. New York: VCH Publishers Inc. p. 1-67.

Boyd LC, King MF, Sheldon B. 1992. A rapid method for determining the oxidation of n-3 fatty acids. J Am Oil Chem Soc 69(4):325-30.

Boyle JL, Lindsay RC, Stuiber DA. 1992a. Bromophenol Distribution in Salmon and Selected Seafoods of Fresh and Saltwater Origin. J Food Sci 57(4):918-22.

Boyle JL, Lindsay RC, Stuiber DA. 1992b. Contributions of Bromophenos to Marine-Associated Flavors of Fish and Seafood. J Aquat Food Prod Technol 1(3/4):43-63.

Boyle JL, Lindsay RC, Stuiber DA. 1993. Occurrence and Properties of Flavor-Related Bromophenols Found in the Marine Environment: A review. J Aquat Food Prod Technol 2(2):75-112.

Bremner HA. 1985. A convenient easy-to-use system for estimating the quality of chilled seafoods. In: Scott DN, Summers G, editors. Proceedings of the fish processing conference, Nelson, 23-25 April 1985. Fish Processing Bulletin, 7. Wellington: DSIR. ISSN 0112-4633. p 59-70.

Bremner HA, Olley J, Vail AMA. 1986. Estimating time-temperature effects by a rapid systematic sensory method. In: Kramer DE, Liston J, editors. Seafood quality determination. Proceedings of an international symposium coordinated by the University of Alaska Sea Grant College Program. Amsterdam: Elsevier. p 413-35

Bremner HA. 2000. Toward practical definitions of quality for food science. Crit Rev Food Sci Nutr 40(1):83-90.

Bremner HA, Sakaguchi M. 2000. A critical look at whether 'freshness' can be determined. J Aquat Food Prod Technol 9(3):5-25.

Brooke RO, Mendelsohn JM, King FJ. 1968. Significance of dimethyl sulfide to the odor of soft-shell clams. J Fish Res Bd Can 25:2453-60.

Buttery RG, Guadagni DG, Ling LC, Seifert RM, Lipton W. 1976. Additional volatile components of cabbage, broccoli and cauliflower. J Agric Food Chem 24(4):829-32.

Byrne L, Lau KT, Diamond D. 2003. Development of pH sensitive films for monitoring spoilage volatiles released into packaged fish headspace. Irish J Agric Food Res 42(1):119-29.

Cadwallader KR, Tan Q, Chen F, Meyers SP. 1995. Evaluation of the aroma of cooked spiny lobster tail meat by aroma extract dilution analysis. J Agric Food Chem 43:2432-7.

Castell CH, Greenough MF. 1957. The action of *Pseudomonas* on fish muscle: 1. Organisms responsible for odour produced during incipient spoilage of chilled fish muscle. J Fish Res Board Can 12(4):617-25.

Castell CH, Greenough F, Dale J. 1959. The action of *Pseudomona*s on fish muscle: 3. Identification of organisms producing fruity and oniony odours. J Fish Res Board Can 16(1):13-9.

Cha YJ, Baek HH, Cadwallader KR. 1993. Volatile flavor components in snow crab cooker effluent and effluent concentrate. J Food Sci 58: 525-30.

Cha YJ, Lee GH, Cadwallader KR. 1997. Aroma-active compounds in salt-fermented anchovy. In: Shahidi F, Cadwallader KR, editors. Flavor and Lipid Chemistry of Seafoods. ACS Symp Series No. 674. Washington, D.C.: American Chemical Society. p 131-47.

Chai T, Chien C, Rosen A, Levin RE. 1971. Detection and incidence of specific species of spoilage bacteria on fish. Appl Microbiol 16(11):1738–41.

Chen TC, Nawar WW, Levin RE. 1974. Identification of major high-boiling volatile compounds produced during refrigerated storage of haddock fillets. Appl Microbiol 28(4):679-80.

Chinivasagam HN, Bremner HA, Wood AF, Nottingham SM. 1998. Volatile components associated with bacterial spoilage of tropical prawns. Int J Food Microbiol 42:45-55.

Chung HY, Cadwallader KR. 1994. Aroma extract dilution analysis of blue crab claw meat volatiles. J Agric Food Chem 42:2867-70.

Chung HY, Cadwallader KR. 1995. Cooked blue crab claw meat aroma compared with lump meat. J Food Sci 60:289-91.

Connell JJ. 1995. Control of Fish Quality. 4th ed. Oxford: Fishing News Books. 245 p.

Craven MA, Gardner JW, Bartlett PN. 1996. Electronic noses development and future prospects. Trends Anal Chem 15(9):486-93.

D'Amico A, Di Natale C, Macagnano A, Davide F, Mantini A, Tarizzo E, Paolesse R, Boschi T. 1998. Technologies and tools for mimicking olfaction: status of the Rome "Tor Vergata" electronic nose. Biosensors and bioelectronics 13(6):711-21.

Dainty RH. 1996. Chemical/biochemical detection of spoilage. Int J Food Microbiol 33(1):19-33

Dalgaard P, Gram L, Huss HH. 1993. Spoilage and shelf life of cod fillets packed in vacuum or modified atmosphere. Int J Food Microbiol 19:283-94

Dalgaard P. 1995a. Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int J Food Microbiol 26:319-33

Dalgaard P. 1995b. Modelling of microbial activity and prediction of shelf life for packed fresh fish. Int J Food Microbiol 26:305-317

Dalgaard P, Mejlholm O, Huss HH. 1996. Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. J Appl Bacteriol 81:57-64.

Dalgaard P, Mejlholm O, Huss HH. 1997. Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish. Int J Food Microbiol 38:169-79.

Dalgaard P. 2000. Fresh and lightly preserved seafood. In: Man CMD, Jones AA, editors. Shelf life evaluation of foods. 2nd ed. Maryland, USA: Aspen Publishing Inc. p 110-39.

Dalgaard P, Buch P, Silberg S. 2002. Seafood Spoilage Predictor—development and distribution of a product specific application software. Int J Food Microbiol 73:343–9.

Dalgaard P. 2002. Modelling and predicting the shelf-life of seafood. Chapter 12. In: Bremner HA, editor. Safety and quality issues in fish processing. Cambridge, England: Woodhead Publishing Ltd. p 191-219.

Davidson AP, Cronin F. 1973. Medium for the selective enumeration of lactic acid bacteria in foods. Appl Microbiol 26(3):439-40.

Davis HK. 1990. Some effects of modified atmosphere packaging gases on fish and chemical tests for spoilage. Chilling and Freezing of New Fish Products, International Institute of Refrigeration, Commission C2, Aberdeen 1990-3. p 201-7.

Debevere J, Boskou G. 1996. Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. Int J Food Microbiol 31:221-9.

Delbarre-Ladrat C, Verrez-Bagnis V, Noël J, Fleurence J. 2004. Relative contribution of calpain and cathepsins to protein degradation in muscle of sea bass (*Dicentrarchus labrax*). Food Chem 88:389–95.

Deng Z, Stone DC, Thompson M. 1996. Selective detection of aroma components by acoustic wave sensors coated with conducting polymer films. Analyst 121:671-9.

Di Natale C, Brunink JAJ, Bungaro F, Davide F, d´Amico A, Paolesse R, Boschi T, Faccio M, Ferri G. 1996. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. Meas Sci Technol 7:1103-14.

Di Natale C, Olafsdottir G, Einarsson S, Mantini A, Martinelli E, Paolesse R, Falconi C, D'Amico A. 2001. Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets. Sens Act B77:572-8. (II)

Di Natale C. 2003. Data Fusion in MUSTEC: Towards the Definition of an Artificial Quality Index. In: Luten JB, Oehlenschlager J, Olafsdottir G, editors, Quality of Fish from Catch to Consumer: Labeling, Monitoring and Traceability. The Netherlands: Wageningen Academic Publishers. p 273-80.

Du WX, Kim J, Huang TS, Marshall MR, Wei CI. 2001. Microbiological, sensory, and electronic nose evaluations of yellowfin tuna under various storage conditions. J Food Prot 64(12):2027-36.

Du WX, Lin CM, Huang TS, Kim J, Marshall MR, Wei CL. 2002. Potential application of the electronic nose for quality assessment of salmon fillets under various storage conditions. J Food Sci 67(1):307-13.

Egashira M, Shimizu Y, Takao Y. 1990. Trimethylamine sensor based on semiconductive metal-oxides for detection of fish freshness. Sens Act B-Chem 1(1-6):108-12.

Egashira M, Shimizu Y, Takao Y. 1994. Fish Freshness detection by semiconductor gas sensors, Olfaction and Taste XI. Proc Int Sym 11:715-9.

Einarsson H. 1992. Predicting the shelf life of cod (*Gadus morhua*) fillets stored in air and modified temperatures between -4 °C and +16 °C. In: Huss HH, Jacobsen M, Liston J, editors. Quality Assurance in the Fish Industry. Amsterdam, The Netherlands: Elsevier Science Publishers BV. p 479-88.

Elliot RP. 1947. Evaluation of surface pH as a freshness index for fish fillets. Food Research 12(2):87-91.

Ellis DI, Goodacre R. 2001. Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends. Trends Food Sci Technol 12:414-24.

Fazzalari FA. 1978. Compilation of Odor and Taste Threshold Value Data. Philadelphia, PA: American Society for Testing and Materials.

Gacrcía- González DL, Aparicio R. 2002. Sensors: From biosensors to the electronic nose. Grasas y Aceites, 53:96-114.

Gardner JW, Bartlett PN. 1999. Electronic noses. Principles and applications. Oxford, England: Oxford University Press.

German JB, Kinsella JE. 1985. Lipid oxidation in fish tissue. Enzymatic initiation via lipoxygenase. J Agric Food Chem 33:680-3.

German JB, Buckner GB, Kinsella JE. 1986. Lipoxygenase in trout gill tissue acting on arachidonic, eicosapentaenoic and docosahexaenoic acids. Biochim Biophys Acta 875:12-20.

Gram L, Melchiorsen J. 1996. Interaction between fish spoilage bacteria *Pseudomonas sp*. and *Shewanella putrefaciens* in fish extracts and on fish tissue. J Appl Bacteriol 80:589-95.

Gram L, Huss HH. 1996. Microbiological spoilage of fish and fish products. Int J Food Microbiol 33:121-37.

Gram L, Dalgaard P. 2002. Fish spoilage bacteria – problems and solutions. Current Opinion in Biotechnology 13:262-6.

Gram L, Ravn L, Rasch M, Bruhn JB, Christensen AB, Givskov M. 2002. Food spoilage - interactions between food spoilage bacteria. Int J Food Microbiol 78(1-2):79-97.

Granroth B, Hattula T. 1976. Formation of dimethyl sulfide by brackish water algae and its possible implication for the flavor of Baltic herring. Finn Chem Lett 148-50.

Gudmundsdóttir G, Stefánsson G. 1997. Sensory and chemical changes in spice-salted herring as affected by handling. J Food Sci 62(4):894-987.

Guldager HS, Bøknæs N, Østerberg C, Nielsen J, Dalgaard P. 1998. Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2 °C. J Food Prot 61:1129-36.

Hardy R, McGill AS, Gunstone FD. 1979. Lipid and autoxidative changes in cold stored cod (*Gadus morhua*). J Sci Food Agric 28:999.

Harper WJ. 2001. Strengths and weaknesses of the electronic nose. In: Rouseff and Cadwallader editors. Headspace Analysis of Food and Flavors: Theory and Practice. New York: Kluwer Academic / Plenium Publishers. p 59-71.

Haugen JE, Kvaal K. 1998. Electronic nose and artificial neural network. Meat Science 49:273-86.

Haugen JE. 2001. Electronic noses in food analysis. In: Rouseff RL, Cadwallader KR, editors. Headspace Analysis of Food and Flavors: Theory and Practice. New York: Kluwer Academic / Plenium Publishers. p 43-57.

Haugen JE, Undeland I. 2003. Lipid oxidation in herring fillets (*Clupea harengus*) during ice storage measured by a commercial hybrid gas-sensor array system. J Agric Food Chem 51(3):752-9.

Haugen JE, Chanie E, Westad F, Jonsdottir R, Bazzo S, Labreche S, Marcq P, Lundby F, Olafsdottir G. Rapid control of smoked Atlantic salmon quality by electronic nose: correlation with classical evaluation methods. Forthcoming Sens Act.

Hebard CE, Flick GJ, Martin RE. 1982. Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In: Martin RE, editor. Chemistry and Biochemistry of Marine Food Products. Westport: AVI Publishing Co., Inc. p 149–304.

Herbert RA, Hendrie MS, Gibson DM, Shewan JM. 1971. Bacteria active in the spoilage of certain seafoods. J Appl Bacteriol 34:41-50.

Herbert RA, Ellis JR, Shewan JM. 1975. Isolation and identification of the volatile sulphides produced during chill-storage of North Sea Cod (*Gadus morhua*). J Sci Food Agric 26:1195-202.

Herbert RA, Shewan JM. 1976. Roles played by bacterial and autolytic enzymes in the production of volatile sulphides in spoiling North Sea cod (*Gadus morhua*). J Sci Food Agric 27:89-94.

Hillig F. 1956. Volatile acids, succinic acid and histamine as indice of decomposition in tuna. Association of Official Agricultural Chemists. 39:773-800.

Hirano T, Zhang Chao-Hua, Morishita A, Suzuki T, Shirai T. 1992. Identification of volatile compounds in ayu fish and its feeds. Nippon Suisan Gakkaishi 58(3):547-57.

Högnadóttir, Á. 1999. Application of an electronic nose in the fish industry. MS thesis. Department of Food Science, University of Iceland

Holmberg M, Winquist F, Lundström I, Davide F, Di Natale C, D´Amico A. 1996. Drift counteraction for an electronic nose. Sens Act 35-36:528-35

Howgate P, Johnston A, Whittle KJ. 1992. Multilingual guide to EC freshness grades for fishery products. Torry Research Station, Food Safety Directorate, Ministry of Agriculture, Fisheries and Food, Aberdeen, Scotland, 32 p.

Hsieh RJ, German JB, Kinsella JE. 1988. Lipoxygenase in fish tissue: some properties of the 12-lipoxygenase from trout gill. J Agric Food Chem 36:680-5.

Hsieh RJ, Kinsella JE. 1989. Lipoxygenase generation of specific volatile flavor carbonyl compounds in fish tissue. J Agric Food Chem 37(2):279-86.

Hultin HO. 1994. Oxidation of lipids in seafoods. In: Seafoods: Chemistry, processing technology and quality. Shahidi F, Botta JF, editors. Glasgow: Blackie Academic and Professional. p 49-74.

Hultin HO, Kelleher SD. 2000. Surimi Processing from Dark Muscle Fish. In: Park JW, editor. Surimi and Surimi Seafood. New York: Marcel Dekker, Inc. p 59-78.

Human J, Khayat A. 1981. Quality Evaluation of Raw Tuna by Gas Chromatography and Sensory Methods. J Food Sci 46:868-73.

Huss HH. 1995. Quality and quality changes in fresh fish. FAO Fisheries Technical Paper, No.348, FAO, Rome

Ikeda S. 1980. Other organic components and inorganic components. In: Connell JJ, editor. Fish Science and Technology. Farnham, Surrey, England: Fishing News Books Ltd. p 111-23.

ISO 1993. Sensory analysis - general guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors, 8586-1. Geneva, Switzerland: The International Organization for Standardization. p 1-10.

Jensen B, Refsgaard HH, Olafsdottir G. 1998. Headspace and extraction methods for analysis of volatile and semivolatile compounds in fish - Chemical and sensory assessment of lipid- derived volatiles. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to determine the freshness of fish in research and industry. Proceedings of the Final Meeting of the concerted action "Evaluation of Fish Freshness"AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 70-91.

Joffraud JJ, Leroi F, Roy C, Berdagué JL. 2001. Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. Int J Food Microbiol 66(3):175-84.

Jónsdóttir R, Olafsdottir G, Martinsdottir E, Stefansson G. 2004**.** Flavor Characterization of Ripened Cod Roe by Gas Chromatography, Sensory Analysis and Electronic Nose. J Agric Food Chem 52:6250-6.

Jónsdóttir R, Olafsdottir G, Chanie E, Haugen JE. Variation in the Occurence of Volatile Compounds in Cold Smoked Salmon (*Salmo salar*) during Storage. Forthcoming.

Jones NR, 1967. Fish flavors. Schultz HW, Day EA, Libbey LM, editors. Symposium on food: The Chemisty and Physiology of Flavors. Westport, Connecticut: The AVI Publishing Co. p 267-95.

Jonsson A, Winquist F, Schnürer J, Sundgren H, Lundström I. 1997. Electronic nose for microbial quality classification of grains. Int J Food Microbiol 35:187-93.

Jørgensen BR, Gibson DM, Huss HH. 1988. Microbiological quality and shelf life prediction of chilled fish. Int J Food Microbiol 6:295-307.

Jørgensen BR, Huss HH. 1989. Growth and activtiy of *Shewanella putrefaciens* isolated from spoiling fish. Int J Food Microbiol 9:51-62.

Jørgensen LV, Dalgaard P, Huss HH. 2000. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. J Agric Food Chem 48:2448-53.

Jørgensen LV, Huss HH, Dalgaard P. 2001. Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar)* analyzed by GC-MS and multivariate regression. J Agric Food Chem 49:2376-81.

Jørgensen BM, Oehlenschläger J, Olafsdottir G, Tryggvadóttir SV, Careche M, Heia K, Nesvadba P, Nunes ML, Poli BM, Di Natale C, Pérez-Villarreal B, Ballo H, Luten JB, Smelt A, Denton W, Bossier P, Hattula T, Åkesson G. 2003. A study of the attitudes of the European fish sector towards quality monitoring and labelling. In: Luten JB, Oehlenschlager J, Olafsdottir G, editors. Quality of Fish from Catch to Consumer: Labeling, Monitoring and Traceability. The Netherlands: Wageningen Academic Publishers. p 57-71.

Josephson DB, Lindsay RC, Stuiber DC. 1983. Identification of compounds characterizing the aroma of fresh Whitefish (Coregonus Clupeaformis). J Agric Food Chem 31:326-30.

Josephson DB, Lindsay RC, Stuiber DC. 1984. Variations in the occurrences of enzymically derived volatile aroma compounds in salt- and freshwater fish. J Agric Food Chem 32:1344-7.

Josephson DB, Lindsay RC, Olafsdottir G. 1986. Measurement of Volatile Aroma Constituents as a Means for Following Sensory Deterioration of Fresh Fish and Fishery Products. In: Kramer DE, Liston J, editors. Proceedings of an International Symposium on Quality Determinations sponsored by the University of Alaska Sea Grant Program, Anchorage, Alaska, U.S.A. Amsterdam: Elsevier Science Publishers BV. p 27-47.

Josephson DB, Lindsay RC, Stuiber DA. 1987. Influence of processing on the volatile compounds characterizing the flavor of pickled fish. J Food Sci 52:10-14.

Josephson DB, Lindsay RC. 1987a. Retro-aldol degradations of unsaturated aldehydes: Role in the formation of c4-heptenal from t2,c6-nonadienal in fish, oyster, and other flavours. J Food Sci 64:1186-90.

Josephson DB, Lindsay RC. 1987b. c4-heptenal: An influential volatile compound in boiled potato flavor. J Food Sci 52:328-31.

Josephson DB. 1991. Seafood. In: Maarse H, editor. Volatile compounds in foods and beverages. New York: Marcel Dekker. p 179-202.

Jurs PC, Bakken GA, McClelland HE. 2000. Computational Methods for the Analysis of Chemical Sensor Array Data from Volatile Analytes. Chem Rev 100:2649-78.

Kadota H, Ishida Y. 1972. Production of volatile sulphur compounds by microorganisms. Ann Rev Microbiol 26:127-38.

Kamiya A, Ose Y. 1984. Study of the odorous compounds produced by putrefaction of foods. V. Fatty acids, sulphur compounds and amines. J Chromat 292:383-91.

Karahadian C, Lindsay RC. 1989. Evaluation of compounds characterizing fishy flavors in fish oils. J Amer Oil Chem Soc 66:953-7.

Kawai T. 1996. Fish flavor. Crit Rev Food Sci Nutr 36(3):257-98.

Kelleher SD, Zall RR. 1983. Ethanol accumulation in muscle tissue as a chemical indicator of fish spoilage. J Food Biochem 7:87-91.

Keshri G, Voysey P, Magan N. 2002. Early detection of spoilage moulds in Keshri bread using volatile production patterns and quantitative enzyme assays. J Appl Microbiol 92:165-72.

Koide K, Yanagisawa I, Ozawa A, Satake M, Fujita T. 1992. Studies on the kerosene-like off-flavour producing yeast in surimi-based products. Bull Jap Soc Sci Fish 58: 1925-30.

Koutsoumanis K, Nychas GJE. 1999. Chemical and Sensory Changes Associated with Microbial Flora of Mediterranean Boque (*Boops boops*) Stored Aerobically at 0, 3, 7, and 10°C. Appl Env Microbiol 65(2):698–706.

Koutsoumanis K, Nychas GJE. 2000. Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. Int J Food Microbiol 60:171–84.

Koutsoumanis K. 2001. Predictive modeling of the shelf life of fish under nonisothermal conditions. Appl Env Microbiol 67(4):1821-9.

Larsen E, Heldbo J, Jespersen CM, Nielsen J. 1992. Development of a method for quality assessment of fish for human consumption based on sensory evaluation. In Huss HH, Jakobsen M, Liston J, editors. Quality assurance in the fish industry. Developments in Food Science, 30. Amsterdam: Elsevier. p 351-8.

Lauzon HL. 2000. Shelf-life and bacteriological spoilage of American plaice (*Hippoglossoides platessoides*). In: Shahidi F, editor. Seafood in Health and Nutrition, Transformation in Fisheries and Aquaculture: Global Perspectives. NF, Canada: A Science Tech. Book, St. John´s. p 327-54

Lauzon HL, Stefánsson G, Jónsson GP, Sveinsdóttir K. 2002. Sensory quality and shelf life of MAP redfish (*Sebastes marinus*) fillets as affected by previous bulk storage of whole fish. J Aquat Food Prod Technol 11(3/4):251-73.

Lauzon HL. 2003. Notkun Malthus leiðnitækni til hraðvirkra örverumælinga. IFL project report 30-03, 30p (in Icelandic).

Lerke P, Huck RW. 1977. Objective determination of canned tuna quality: Identification of ethanol as a potentially useful index. J Food Sci 42(3):755-758.

Leroi F, Joffraud JJ, Chevalier F, Cardinal M. 1998. Study of the microbial ecology of cold-smoked salmon during storage at 8°C. Int J Food Microbiol 39(1/2):111-21.

Levin RE. 1968. Detection and incidence of specific species of spoilage bacteria on fish. Appl Microbiol 16(11):1734-7.

Lindsay RC, Josephson DB, Ólafsdóttir G. 1986. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In: Kramer DE, Liston J, editors. Proceedings of an International Symposium; University of Alaska Sea Grant Program, Anchorage, AK. Amsterdam, The Netherlands: Elsevier Science Publishers. p 221-34.

Lindsay RC. 1990. Fish Flavors. Food Rev Int 6(4):437-55.

Lovell RT. 1983. New off-flavors in pond-cultured channel catfish. Aquaculture 30:329-34.

Luten JB, Martinsdóttir E. 1998. QIM: a European tool for fish freshness evaluation in the fishery chain. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to determine the freshness of fish in research and industry. Proceedings of the Final Meeting of the concerted action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 287-96.

Luten JB, 2003. Introduction and outcome of the Concerted Action “Fish Quality Labelling and Monitoring” FAIR 98-4174). In: Luten JB, Oehlenschlager J, Olafsdottir G, editors.. Quality of Fish from Catch to Consumer: Labelling, Monitoring and Traceability. The Netherlands: Wageningen Academic Publishers. p 33-42.

Luten JB, Oehlenschlager J, Olafsdottir G, editors. 2003. Quality of Fish from Catch to Consumer: Labelling, Monitoring and Traceability. The Netherlands: Wageningen Academic Publishers. 456p.

Luzuriaga DA, Balaban MO. 1999a. Evaluation of Odor Decomposition in Raw and Cooked Shrimp: Correlation of Electronic Nose Readings, Odor Sensory Evaluation and Ammonia Levels. In: Hurst WJ, editor. Electronic nose and sensor array based system, Design and Applications. Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. USA: Technomic Publ. Co., Inc. p 177-84.

Luzuriaga DA, Balaban MO. 1999b. Electronic nose odor evaluation of salmon fillets stored at different temperatures In: Hurst WJ, editor. Electronic nose and sensor array based system, Design and Applications. Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. USA: Technomic Publ. Co., Inc. p 162-9.

Magnússon H, Martinsdóttir E. 1995. Storage quality of fresh and frozen-thawed fish in ice. J Food Sci 60:273-8.

Malle P, Poumeyrol M. 1989. A new chemical criterion for the quality control of fish: Trimetylamine / total volatile basic nitrogen (%). J Food Protect 52:419-23.

Marsili RT. 2001. SPMS-MS-MVA as a rapid technique for assessing oxidation off-flavors in foods. In: Rouseff RL, Cadwallader KR, editors. Headspace Analysis of Food and Flavors: Theory and Practice. New York: KluwerAcademic / Plenium Publishers. p 89-100.

Martens H, Martens M. 1999. Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). Food Qual Pref 11:5-16.

Martin RE, Gray RJH, Pierson MD. 1978. Quality assessment of fresh fish and the role of the naturally occurring microflora. Food Technol 5:188-92.

Martinsdóttir E, Arnason H. 1992. Redfish. In: Nordic Industrial Fund, Quality Standards for Fish. Final Report Phase II. p 21-35.

Martinsdóttir E, Luten J, Schelvis R, Hyldig G. 2003. Scientific developments of QIM: past and future. In: Luten JB, Oehlenschlager J, Olafsdottir G, editors. Quality of fish from catch to consumer: Labelling, monitoring and traceability. The Netherlands: Wageningen Academic Publishers. p 265-72.

McEntegart CM, Penrose WR, Strathmann S, Stetter JR. 2000. Detection and discrimination of coliform bacteria with gas sensor arrays. Sens Act B70:170-6.

McGill AS, Hardy T, Burt RJ, Gunstone FD. 1974. Hept-cis-4-enal and its contribution to the off-flavor of cold-stored cod. J Sci Food Agric 25:1477-81.

Mielle P.1996. Electronic noses: towards the objective instrumental characterisation of food aroma. Trends Food Sci Technol 7:432-8

Mielle P, Marquis F. 1999. An alternative way to improve the sensitivity of electronic olfactometers. Sens Act B58:526-35

Miler KBM, Sikorski ZE. 1990. Smoking. Florida: CRC Press. p 163-180.

Miller III A, Scanlan RA, Lee JS, Libbey LM. 1973a. Identification of the volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas fragi.* Appl Microbiol 25(6):952-5.

Miller III A, Scanlan RA, Lee JS, Libbey LM, 1973b. Volatile compounds produced by Pseudomonas putrefaciens, Pseudomonas fluorescens and an Achromobacter Species. Appl Microbiol 26:18–21.

Miller III A, Scanlan RA, Lee JS, Libbey LM, Morgan ME. 1973c. Volatile compounds produced in sterile fish muscle (Sebastes melanops) by Pseudomonas perolens. Appl Microbiol 25:257-61.

Milo C, Grosch W. 1993. Changes in the Odourants of Boiled Trout (Salmo Fario) as Affected by the Storage of the Raw Material. J Agric Food Chem 41:2076-81.

Milo C, Grosch W. 1995. Detection of Defects in Boiled Cod and Trout by Gas Chromatography-Olfactometry of Headspace Samples. J Agric Food Chem 43:459-62.

Milo C, Grosch W. 1996. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. J Agric Food Chem 44:2366-71.

Miner JR. 1977. Characterization of odors and other volatile emissions. Agric Environm 3:129-37.

Motohiro T. 1962. Studies on the petroleum odor in canned chum salmon. Mem Fac Fish, Hokkaido Univ. 10:1-65.

Namdev PK, Alroy Y, Singh V 1998. Sniffing out trouble: Use of an electronic nose in bioprocesses. Biotechnol Prog 14:75-8.

Nesvadba P. 2003. Introduction and outcomes of the project "Multi-sensor techniques for monitoring the quality of fish". In: Luten JB, Oehlenschlager J, Olafsdottir G, editors. Quality of fish from catch to consumer: Labelling, monitoring and traceability. The Netherlands: Wageningen Academic Publishers. p 175-187.

Newman DJ, Luzuriaga DA, Balaban MO. 1999. Odor and Microbiological Evaluation of Raw Tuna: Correlation of Sensory and Electronic Nose Data. In: Hurst WJ, editor. Electronic nose and sensor array based system, Design and Applications. Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. USA: Technomic Publication Company, Inc. p 170-6.

Oehlenschläger J. 1992. Evaluation of some well established and some underrated indices for the determination of freshness and/or spoilage of ice stored wet fish. In: Huss HH, Jakobsen M, Liston J, editors. Quality Assurance in the Fish Industry. Amsterdam: Elsevier Science Publisher. p 339-50.

Oehlenschläger J. 1998. Sensory evaluation in inspection. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to determine the freshness of fish in research and industry. Proceedings of the Final Meeting of the concerted action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 339-44.

Oehlenschläger J, Sörensen NK. 1998. Criteria of seafood freshness and quality aspects. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to determine the freshness of fish in research and industry. Proceedings of the final meeting of the concerted action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 30-5.

Ohashi E, Takao Y, Fujita T, Shimizu Y, Egashira M. 1991. Semiconductive trimethylamine gas sensor for detecting fish freshness. J Food Sci 56(5):1275-8.

Okuma H, Watanabe E. 2002. Flow system for fish freshness determinatio based on double multi-enzyme reactor electrodes. Biosens Bioelectron 17:367-72.

Olafsdottir G, Steinke JA, Lindsay RC. 1985. Quantitative Performance of a Simple Tenax-GC Adsorption Method For Use in the Analysis of Aroma Volatiles. J Food Sci 50:1431-6.

Olafsdottir G, Martinsdóttir E, Oehlenschläger J, Dalgaard P, Jensen B, Undeland I, Mackie IM, Henehan G, Nielsen J, Nilsen H. 1997a. Methods to evaluate fish freshness in research and industry. Trends Food Sci Technol 8:258-65.

Olafsdottir G, Martinsdóttir E, Jónsson EH, 1997b. Rapid gas sensor measurements to predict the freshness of capelin (*Mallotus villosus*). J Agric Food Chem 45(7):2654-9.

Olafsdottir G, Martinsdóttir E, Jónsson EH. 1997c. Gas sensor and GC measurements of volatile compounds in capelin (*Mallotus villosus*). In: Luten JB, Börresen T, Oehlenschläger J, editors. Seafood from Producer to Consumer, Integrated Approach to Quality. Amsterdam: Elsevier. p 507-20.

Olafsdottir G, Fleurence J. 1998. Evaluation of fish freshness using volatile compounds- classification of volatile compounds in fish. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to Determine the Freshness of Fish in Research and Industry. Proceedings of the final meeting of the concerted action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 55-69

Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. 1998a. Methods to Determine the Freshness of Fish in Research and Industry, Proceedings of the Final meeting of the Concerted Action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration 396p.

Olafsdottir, G.,. Högnadóttir, Á., Martinsdóttir, E. 1998b. Application of gas sensors to evaluate freshness and spoilage of various seafoods. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to Determine the Freshness of Fish in Research and Industry, Proceedings of the Final meeting of the Concerted Action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 100-9.

Olafsdottir G, Högnadóttir Á, Martinsdóttir E, Jónsdóttir H. 2000. Application of an electronic nose to predict total volatile bases in capelin (*Mallotus villosus*) for fishmeal production. J Agric Food Chem. 48(6):2353-9 (I)

Olafsdottir G, Li X, Lauzon HL, Jonsdottir R. 2002. Precision and application of electronic nose measurements for freshness monitoring of redfish (*Sebastes marinus*) stored in ice and modified atmosphere bulk storage. J Aquat Food Prod Technol 11(3/4):229-49. (III)

Olafsdottir G. 2003. Developing rapid olfaction arrays for determining fish quality. In Tothill IE, editor. Rapid and on-line instrumentation for food quality assurance. Cambridge, England: Woodhead Publishing Ltd. p 339-60.

Olafsdottir G, Di Natale C, Macagnano A. 2003a. Measurements of quality of fish by electronic noses. In: Luten JB, Oehlenschlager J, Olafsdottir G, editors. Quality of Fish from Catch to Consumer: Labelling, Monitoring and Traceability. The Netherlands: Wageningen Academic Publishers. p 225-234.

Olafsdottir G, Jonsdottir R, Tryggvadottir SV, Einarsson S, Lauzon HL. 2003b. Prediction of freshness quality of haddock fillets using electronic nose, texture and TVN measurements First Joint Trans-Atlantic Fisheries Technology Conference (TAFT) 33rd WEFTA Meeting and 48th Atlantic Fisheries Technology Conference, 11-14 June 2003, Reykjavik - Iceland 2003 p 148-150. (http://vefur.rf.is/TAFT2003/).

Olafsdottir G, Nesvadba P, Di Natale C, Careche M, Oehlenschläger J, Tryggvadóttir SV, Schubring R, Kroeger M, Heia K, Esaiassen M, Macagnano A, Jørgensen BM. 2004. Multisensor for fish quality determination. Trends Food Sci Technol 15:86-93

Olafsdottir G, Chanie E, Westad F, Jonsdottir R, Bazzo S, Labreche S, Marcq P, Lundby F, Haugen JE. 2005. Rapid Control of Smoked Atlantic Salmon Quality by Electronic Nose: Correlation with Classical Evaluation Methods. In: Marco S, Montoliu I, editors. Proceedings of the 11th International Symposium

on Olfaction and Electronic Nose, ISOEN2005, Electronic Department, Physics Faculty, Barcelona University, Barcelona, Spain. p 110-4.

Olafsdottir G, Lauzon H, Martinsdottir E, Kristbergsson K. Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction. Submitted (IV)

Olafsdottir G, Lauzon H, Martinsdottir E, Kristbergsson K. Shelf-life extension of superchilled cod (*Gadus morhua*) fillets and influence of temperature fluctuations on microbial growth and metabolites. Submitted (V)

Olafsdottir G, Jonsdottir R, Lauzon HL, Luten J, Kristbergsson K. Characterization of Volatile Compounds in Chilled Cod (*Gadus morhua*) Fillets by Gas Chromatography and Rapid Detection of Quality Indicators by an Electronic Nose. Submitted (VI)

Olafsdottir G, Chanie E, Westad F, Jonsdottir R, Thalman C, Bazzo S, Labreche S, Marcq P, Lundby F, Haugen JE. 2005. Prediction of Microbial and Sensory Quality of Cold Smoked Atlantic Salmon (*Salmo salar*) by Electronic Nose. Accepted for publication J Food Sci (VII)

Olafsdottir G, Kristbergsson K. Electronic-Nose Technology: Application for Quality Evaluation in the Fish Industry. In: Nicolay, X. editor. Air Pollution Control: Odours Treatment in the Food Industry. Springer, NY, NY. (Forthcoming)

Ólafsson R, Martinsdóttir E, Ólafsdóttir G, Sigfússon TI, Gardner JW. 1992. Monitoring of fish freshness using tin oxide sensors. In: Gardner JW, Bartlett PN, editors. Sensors and Sensory Systems for an Electronic Nose. Dordrecht, The Netherlands: Kluwer. p 257-72

Olsson J, Börjesson T, Lundstedt T, Schnürer J. 2002. Detection and quantification of ochratoxin A and deoxyinvalenol in barley grains by GC-MS and electronic nose. Int J Food Microb 72:203-14.

Paludan-Muller C, Dalgaard P, Huss HH, Gram L. 1998. Evaluation of the role of Carnobacterium piscicola in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5 °C. Int J Food Microbiol 39(3):155-66.

Pan BS, Kuo JM. 1994. Flavour of shellfish and kamaboko flavorants. In: Shahidi F, Botta JR., editors. Seafoods: Chemistry, Processing, Technology and Quality. Glasgow UK: Blackie Academic and Professional. p 85-114.

Pau LF, Olafsson R. editors. 1997. Fish quality control by computer vision., NY: Marcel Dekker Inc.

Pearce TC, Schiffman SS, Nagle HT, Gardner J.W Editors. 2003. Handbook of Machine Olfaction: Electronic Nose Technology. Weinheim: Wiley-VCH. ISBN: 3-527-30358-8, 592 p

Quaranta HO, Piccini JL, Perez S. 1984. Volatile acids content of irradiated tuna loins as a criterion of freshness. Dtsch Lebens Rundsch 4:106-7.

Ronald AP, Thomson WAB. 1964. The volatile sulfur compounds of oysters. J Fish Res Bd Can 21:1481-7.

Ross T, McMeekin TA. 1994. Predictive Microbiology. Int J Food Microbiol 23:241-64.

Roussel S, Forsberg G, Grenier P, Bellon-Maurel V. 1999. Optimisation of electronic nose measurements. Part II: Influence of experimental parameters. J Food Eng 39:9-15.

Rozum J. 1992. Smoke flavouring in processed meats, 2nd edtion ed. London, UK: Blackie academic and professional. p 342-353.

Ruiz-Capillas C, Horner WFA. 1999. Determination of trimethylamine-nitrogen and total volatile basic nitrogen in fresh fish by flow injection analysis. J Food Sci Agric 79:1982-6.

Saja Rde, Souto J, Rodriguez-Mendez ML, de Saja JA. 1999. Array of lutetium bisphthalocyanine sensors for the detection of trimethylamine. Materials Science & Engineering C-Biomimetic and Supramolecular Systemes 8-9:565-8.

Saito T, Ari K, Matsuyoshi M. 1959. A new method for estimating the freshness of fish. Bull Jpn Soc Sci Fish 24:749-53.

Schaefer J.1977. Sampling, characterisation and analysis of malodours. Agricult Environ 3:121-7.

Schaller E, Bosse JO, Escher F. 1998. 'Electronic noses' and their application to food', Lebensm-Wiss Technol 31(4):305-16.

Schnürer J, Olsson J, Börjesson T. 1999. Fungal volatiles as indicators of food and feeds spoilage. Fungal Genetics and Biology 27:209-17.

Schweizer-Berberich PM, Vaihinger S, Göpel W. 1994. Characterisation of food freshness with sensor arrays. Sens Act 18-19:282-290

Sérot T, Baron R, Knockaert C, Vallet JL. 2004. Effect of smoke processes on the content of 10 major phenolic compounds in smoked fillets of herring (*Cuplea harengus*). Food Chem 85:111-20.

Sheldon RM, Lindsay RC, Libbey LM, Morgan ME. 1971. Chemical nature of malty flavour and aroma produced by *streptococcus lactis*. Appl Microbiol. 22:263-6.

Shewan JM, Mackintosh RG, Tucher CG, Ehrenberg ASC. 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J Sci Agric 6:183-9.

Shewan JM. 1976. The Biochemistry and Microbiology of Low Temperature spoilage. Food Tech Australia 11:409-10.

Shiomi K, Noguchi A, Yamnaka H, Kikuchi T, Iida H. 1982. Volatile sulfur compounds responsible for an offensive odor of the flat-head, Calliurichthys doryssus. Comp Biochem Physio 71B:29.

Skjerdal OT, Lorentzena G, Tryland I, Berg JC. 2004. New method for rapid and sensitive quantification of sulphide-producing bacteria in fish from arctic and temperate waters. Int J Food Microbiol 93(3). 325-33.

Stohr V, Joffraud JJ, Cardinal M, Leroi F. 2001. Spoilage potential and sensory profile associated with bacteria isolated from cold-smoked salmon. Food Res Int 34:797-806.

Stone H, Sidel JL. 1985. Sensory evaluation practices. Orlando FL: Academic Press, Inc. p 311.

Storey RM, Davis K, Owen D, Moore L. 1984. Rapid approximate estimation of volatiel amines in fish. J Food Technol 19:1-10.

Strachan NJC, Nicholson FJ. 1992. Gill air analysis as an indicator of cod freshness and spoilage. Int J Food Sci Technol 27:261-9.

Stutz HK, Silverman GJ, Angelini P, Levin RE. 1991. Bacteria and Volatile Compounds Associated with Ground Beef Spoilage. J Food Sci 56(6):1147-53.

Toldrá F. 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. Meat Science 49:101-10.

Toldrá F, Flores M. 1999. The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. Crit Rev Food Sci 38:331-52.

Toldrá F, Aristoy MC, Flores M. 2000. Contribution of muscle aminopeptidases to flavor development in dry-cured ham. Food Res Int 33:181-5.

Tomic O, Ulmer H, Haugen JE. 2002. Standardization methods for handling instrument related signal shift in gas-sensor array measurement data. Anal Chim Acta 474:99-111.

Trihaas J, Nielsen PV. 2005. Electronic Nose Technology in Quality Assessment: Monitoring the Ripening Process of Danish Blue Cheese. J Food Sci 70(1):E44-9.

Trihaas J, van den Tempel T, Nielsen PV. 2005. Electronic Nose Technology in Quality Assessment: Predicting Volatile Composition of Danish Blue Cheese During Ripening. J Food Sci 70(6):E392-400.

Triqui R, Reineccius GA. 1995. Changes in Flavor Profiles with Ripening of Anchovy (Engraulis encrasicholus). J Agric Food Chem 43:1883-9.

Triqui R, Guth H. 1997. Determination of potent odorants in ripened anchovy (*Engraulis encrasicholus L.*) by aroma extract dilution analysis and by gas chromatography-olfactometry of headspace samples. In: Shahidi F, Cadwallader KR, editors. Flavour and Lipid Chemisty of Seafoods. Washington, DC: ACS Symposium Series 674 American Chemical Society. p 31-8.

Tryggvadottir SV, Olafsdottir G. 2000. Multisensor for fish: Questionnaire on quality attributes and control methods -Texture and electronic nose to evaluate fish freshness. Project report for European Commission (MUSTEC CT-98-4076). RF report 04-00.

Undeland I. 1998. Lipid oxidation in fish – causes, changes and measurements. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to Determine the Freshness of Fish in Research and Industry, Proceedings of the Final meeting of the Concerted Action "Evaluation of Fish Freshness" AIR3 CT94 2283. Paris: International Institute of Refrigeration. p 241-57.

Undeland I, Hall G, Lingnert H. 1999. Lipid oxidation in fillets of herring *(Clupea harengus)* during ice storage. J Agric Food Chem 47(2):524-32

Van Spreekens KJA. 1974. The suitability of Long & Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. Arch Lebensmitt 25(10):213-9.

Van Spreekens KJA, Toepoel L. 1981. Quality of fishery products in connection with the psychrophilic and psychrotrophic bacterial flora. In: Roberts TA, Hobbs G, Christian JHB, Skovgaard N, editors. Psychrotrophic microorganisms in spoilage and pathogenicity. London, UK: Academic Press. p 283-94.

Venugopal V. 2002. Review. Biosensors in fish production and quality control. Biosens Bioelectron 17:147-57.

Viehweg SH, Schmitt RE, Schmidt-Lorenz W. 1989. Microbial Spoilage of Refrigerated Fresh Broilers. Part V. Evaluation of Suitable Methods for the Detection of the Volatile Aroma Compounds. Lebensm-Wiss Technol 22:334-45.

Whitfield FB, Tindale CR. 1984. The role of microbial metabolites in food off-flavours. Food Technol Australia 36:204-13.

Whitfield FB, Shaw KJ, Tindale CR. 1988. 2,6-dibromophenol: The cause of an iodoform-like off-flavour in some Australian crustacea. J Sci Food Agric 46:29-42.

Whitfield FB, Helidoniotis F, Shaw KJ, Svoronos D. 1997. Distribution of Bromophenols in Australian Wild-Harvested and Cultivated Prawns (Shrimp). J Agric Food Chem 45:4398-405.

Winquist F, Lundstrom I, Wide P. 1999. The combination of an electronic tongue and an electronic nose. Sens Act B-CHEM 58(1-3):512-7.

Withycombe DA, Mussinan CJ. 1988. Identification of 2-Methyl-3-furanthiol in steam distillates from canned tuna. J Food Sci 53:658.

Wold S. 1976. Pattern recognition by means of disjoint principal components models. Pattern recognition 8(3):127-39

Yamanaka H. 1989. Offensive odour of fish and shellfish. In: Koizumi C, editor. Odour of Marine Products. Tokyo: Koseisha-Koseikaku. p 53.

Yurkowski M, Tabachek JAL. 1980. Geosmin and 2-methylisoborneol implicated as a cause of muddy odor and flavor in commercial fish from Cedar Lake, Manitoba. Can J Fish Aquat Sci 37:1449-50.

Zeng K, Tachikawa H, Zhu ZY, Davidson VL. 2000. Amperometric detection of histamine with a methylamine dehydrogenase polypyrrole-based sensor. Anal Chem 10:2211-5.

Zeng QZ, Thorarinsdottir KA, Olafsdottir G. 2005. Quality Changes of Shrimp (*Pandalus borealis*) Stored under Different Cooling Conditions. J Food Sci Forthcoming

Zhang Chao-Hua, Hirano T, Suzuki T, Shirai T. 1992. Enzymatically generated specific volatile compounds in ayu tissues. Nippon Suisan Gakkaishi 58(3):559-65.

Zhao CZ, Pan YZ, Ma LZ, Tang ZN, Zhao GL, Wang LD. 2002. Assay of fish freshness using trimethylamine vapor probe based on a sensitive membrane on piezoelectric quartz crystal. Sens Act B-CHEM 81(2-3):218-222.

PAPER I

I

Application of an Electronic Nose to Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production

Guðrún Ólafsdóttir, Áslaug Högnadóttir, Emilía Martinsdóttir, Helga Jónsdóttir

Published in: J. Agric. Food Chem. 48, 6 (2000) 2353- 2359

Application of an Electronic Nose To Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production

G. Ólafsdóttir, Á. Högnadóttir, E. Martinsdóttir, and H. Jónsdóttir

Icelandic Fisheries Laboratories, P.O. Box 1405, 121 Reykjavík, Iceland

Journal of Agricultural and Food Chemistry®

Reprinted from
Volume 48, Number 6, Pages 2353–2359

Application of an Electronic Nose To Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production

G. Ólafsdóttir,* Á. Högnadóttir, E. Martinsdóttir, and H. Jónsdóttir

Icelandic Fisheries Laboratories, P.O. Box 1405, 121 Reykjavík, Iceland

An electronic nose was used as a rapid technique to monitor changes in the headspace gas above capelin (*Mallotus villosus*) during storage at 0 and 5 °C. At both temperatures, refrigeration was compared with an ice/seawater cooling system. At 5 °C, the effect of added 0.2% acetic acid was also tested. Electronic nose measurements were compared to measurements of total volatile bases (TVB), gas chromatography of volatile compounds, and sensory analysis. TVB analysis indicated less spoilage in seawater/ice systems than in refrigeration, but the other measurements indicated more spoilage in the seawater system compared to refrigeration. The possibility of using only a few sensors in the electronic nose to predict TVB was studied using partial least squares regression and a saturated generalized linear model. The results reported herein indicate that rapid electronic nose measurements, which require no sample preparation, can be used to predict the TVB value of the raw material stored under different conditions.

Keywords: *Electronic nose; total volatile bases; fish spoilage; fishmeal*

INTRODUCTION

Maintaining the freshness of the raw material used for fishmeal production is very important to ensure the nutritive value and quality of the meal. The rapid spoilage of capelin and insufficient cooling on board fishing vessels often result in poor quality of the raw material used for meal production. Recently, increased efforts have been put into maintaining the freshness of the raw material on board fishing vessels by cooling directly after catch. Various types of refrigerated seawater (RSW) and chilled seawater (CSW) systems have been used on board fishing vessels (Dagbjartsson et al., 1982; Hiremath et al., 1982; Kolbe et al., 1985). In CSW systems ice is used to maintain the low temperature. The amount of ice needed depends on the temperature of the seawater, 0 and 5 °C, and the fish and also the amount and the thickness of the fish layers. Therefore, during summer months more ice is required; many boats are inadequately insulated, thus requiring more ice due to heat leakage into the hold during a trip. An increased spoilage rate has been reported for capelin stored in seawater systems compared to similar capelin held in ice (Shaw and Botta, 1975). One of the disadvantages to the use of recirculating RSW for the storage of fish is the faster than normal buildup of aerobic bacteria in the water, due mainly to the high level of nutrients leached from the fish.

The formation of volatile compounds as a result of microbial activity and lipid oxidation during storage of fish is well-known, and many volatile compounds have been suggested as indicators of spoilage. Very rapid spoilage of capelin during summer often leads to complaints because of malodors in the neighborhood of fishmeal factories. Bulk stored capelin with high stomach contents is easily solubilized because of high proteolytic activity in the gut (Aksnes and Brekken, 1988). This results in liberation of peptides and free amino acids, which bacteria utilize for their energy demand, resulting in the formation of foul-smelling microbial metabolites such as hydrogen sulfide, methyl mercaptan, and dimethyl disulfide from cysteine and methionine (Herbert and Shewan, 1975). High levels of ammonia and trimethylamine are also formed in addition to amines such as cadeverine, histamine, and putrecine from the breakdown of lysine, histidine, and arginine, respectively (Aksnes and Brekken, 1988). The formation of short-chain alcohols, ketones, aldehydes, aromatics, and acids also occurs as a result of microbial degradation of amino acids and lipid oxidation during fish spoilage (Lindsay et al., 1986). The combination of various low molecular weight volatile compounds contributes to the overall spoilage odors in fish.

Various methods are available to evaluate fish freshness (Ólafsdóttir et al., 1997c, 1998). Sensory analysis is most often used in the fish industry to evaluate the freshness of fish, and odor is one of the most important factors in the analysis. Total volatile bases (TVB) analysis is traditionally used in the fishmeal industry to evaluate the quality of raw material. Gas chromatography (GC) can be used to measure volatile compounds as indicators of freshness (Josephson et al., 1986) and spoilage of fish (Lindsay et al., 1986) by monitoring changes in the headspace. However, GC is a laboratory technique, and recently electronic noses have been introduced as an alternative rapid technique to supplement or replace traditional quality control techniques in the food industry (Bartlett et al., 1997). Electronic noses have been developed to mimic the human sense of smell. This artificial olfaction is generally based on nonselectivity of the sensors, and usually an array of a large number of sensors is utilized and the interpretation of the data requires the use of pattern recognition techniques (Craven et al., 1996). For quantitative analysis it is possible to use only a few sensors and to obtain optimal performance given that the selectivity of the sensors covers the different classes of compounds relevant for the particular application (Di-Natale et al., 1996). This approach was used in the

10.1021/jf990322q CCC: $19.00
Published on Web 05/12/2000

development of the electronic nose FreshSense by the Icelandic Fisheries Laboratories and Element Sensor Systems. FreshSense is based on a closed, static sampling system and electrochemical gas sensors that are sensitive to the main classes of volatile compounds, namely, alcohols, carbonyls, sulfur compounds, and amines, which accumulate because of microbial growth during storage of fish (Ólafsdóttir and Fleurence, 1998).

Earlier work has shown that electronic nose measurements of capelin headspace correlate well with TVB measurements during storage at 0 and 5 °C and that acetic acid was effective in extending the shelf life of the capelin raw material (Ólafsdóttir et al., 1997a,b). This study was undertaken to confirm and investigate further the influence of different storage temperatures and added acetic acid and in addition the effect of adding an ice/seawater mixture to preserve capelin raw material during storage. The spoilage pattern of fish is altered when preservation techniques, such as seawater cooling systems, are used. Therefore, the fishmeal industry needs information regarding the validity of using traditional quality checks such as TVB for capelin stored in seawater systems. The application of an electronic nose (FreshSense) as a rapid technique to monitor volatile compounds in the headspace of capelin stored under different conditions was studied. Moreover, the potential to use electronic nose measurements instead of the traditional TVB method was examined, as was the possibility of using electronic nose measurements to predict TVB values of capelin stored under different conditions.

MATERIALS AND METHODS

Capelin was harvested in late February 1997 south of Iceland and transported by truck to the laboratory. The temperature of the capelin had reached 4 °C when the samples were prepared the following day (day1). Five groups of samples (20 kg each) were prepared and stored in 30 kg plastic barrels until analyzed. Samples were stored for 10 days both at 0−2 °C and at 4−5 °C, and the latter group was initially stored at 10 °C for 1 day to imitate temperature fluctuations that can occur during storage. At both temperatures, refrigeration was compared with an ice/seawater cooling system. At 4−5 °C, the effect of added 0.2% acetic acid was also tested. One liter of acetic acid in water solution (62.5 g of 96% acetic acid) was prepared and added to the 30 kg of capelin and agitated carefully to achieve the 0.2% acetic acid capelin sample. The seawater was prepared by using tap water with 3.5% added salt (NaCl), and ice was added. The ratio by weight was 10% ice/15% seawater/75% fish. The ice and seawater mixture was put in the bottom of the barrel, the fish was put on top, and the mixture was carefully agitated so as not to cause any damage to the fish but ensuring that the ice was evenly distributed. In this paper the series will be referred to as 0 °C, 0 °C + sea, 5 °C, 5 °C + sea, and 5 °C + acid. Samples from each series were analyzed with the FreshSense electronic nose, GC, TVB measurements, and sensory analysis on days 1, 3, 6, 8, and 10. The temperature of each group was recorded every hour throughout the storage using an EBi 125 temperature recorder (Ebro electronic GmbH, Germany).

Electronic nose measurements were performed using an electronic nose called FreshSense, developed by the Icelandic Fisheries Laboratories (IFL) and Element Sensor Systems (Artorg 1, 550 Saudarkrokur, Iceland). The instrument consists of a glass container (5.2L) closed with a plastic lid, an aluminum sensor box fastened to the lid, and a personal computer running a measurement program. The sensor box contains nine different electrochemical gas sensors (Dräger, Germany, CO, H_2S, NO, NO_2, and SO_2; City Technology, U.K., SO_2, H_2S, NH_3A7AM, and NH_3) and a temperature sensor. Electronics, an A/D converter, and a microprocessor to read the measurements and send them to the PC are also in the box. A fan is positioned in the container to ensure gas circulation. The measurement technique is based on a static headspace system as reported earlier by Ólafsdóttir et al. (1997a). One kilogram of capelin was analyzed; the measurement time was 20 min, which was sufficient time to allow the headspace gas to equilibrate in the 5.2 L container. In fact, 10 min was in all cases sufficient to reach the maximum value of the sensor's output. Therefore, shorter measurement times can be used for routine analysis in the fish industry.

TVB Measurements. Total volatile bases (TVB as milligrams of N per 100 g of muscle) were measured according to the method of Billon et al. (1979) with a Struer automatic distillation unit.

Sensory Analysis. Two members of the IFL sensory panel performed sensory analysis using a sensory classification scheme for capelin odor (fresh, flat, sweet, stale, and putrid) developed by Ólafsdóttir et al. (1997a,b). For multivariate analysis the classes were given scores from 1 to 5 with putrid as the highest score. Panelists at IFL are selected by a procedure described by Meilgaard et al. (1991). They have extensive experience in evaluating the freshness of various species of fish used for human consumption, and the Torry scheme (Shewan, 1953) is routinely applied. The limited number of panelists was considered to be sufficient for fish species such as the capelin that is not intended for human consumption. This is also the situation in the fish industry, where sensory analysis is commonly carried out by one or two trained personnel.

GC. Direct headspace analysis of volatile sulfur compounds from capelin was performed as reported earlier (Ólafsdóttir et al., 1997a). Headspace samples (1 mL) were injected splitless with a gastight syringe onto an HP-1 (cross-linked methyl silicone gum) capillary column (30 m; 0.32 mm i.d.; 4 μm film thickness) that was maintained at 50 °C for 4 min and then programmed to increase at 10 °C/min to 170 °C. The helium carrier gas flow rate was 32 mL/min. The gas chromatograph (HP5890) was equipped with an FPD sulfur sensitive detector. The temperature of the injector was 75 °C, and that of the detector was 250 °C.

Data Analysis. Multivariate analysis of the data was done using the software Unscrambler (Camo AS, Norway). Principal component analysis (PCA) for all of the samples was performed to study the main tendencies of variation among the measurement variables and to evaluate if the various analytical techniques applied were comparable to evaluate spoilage. PCA was also done to study the main trend in the data and to illustrate the effect of the different storage conditions on the spoilage level of capelin.

The essential relationships between TVB variables (Y block) and the electronic nose variables (X block) were studied by partial least squares (PLS) regression. PLS1 is an algorithm that relates a single TVB variable to the electronic nose variables by focusing the latter onto a few factors, which are used as regressors for Y (a vector of y values). The algorithm optimizes several partial submodels by minimizing the lack-of-fit residuals for each of them (Martens and Martens, 1986).

A Generalized Linear Model with normal error was also fitted to the data using S-Plus, version 4.0 (Data Analysis Products Division, MathSoft, Inc., Seattle, WA) to predict TVB values with electronic nose data.

RESULTS AND DISCUSSION

Temperature and Time. The first sampling day (day 1) was within 48 h from catch and at that time the temperature of the capelin was 4 °C. Figure 1a shows the temperature history of the samples throughout the storage period starting on day 1. Unfortunately, the temperature monitor for the 5 °C + sea sample was damaged during the experiment, and the temperature data could not be retrieved for that sample. However, it is assumed that the ice must have slowed the increase

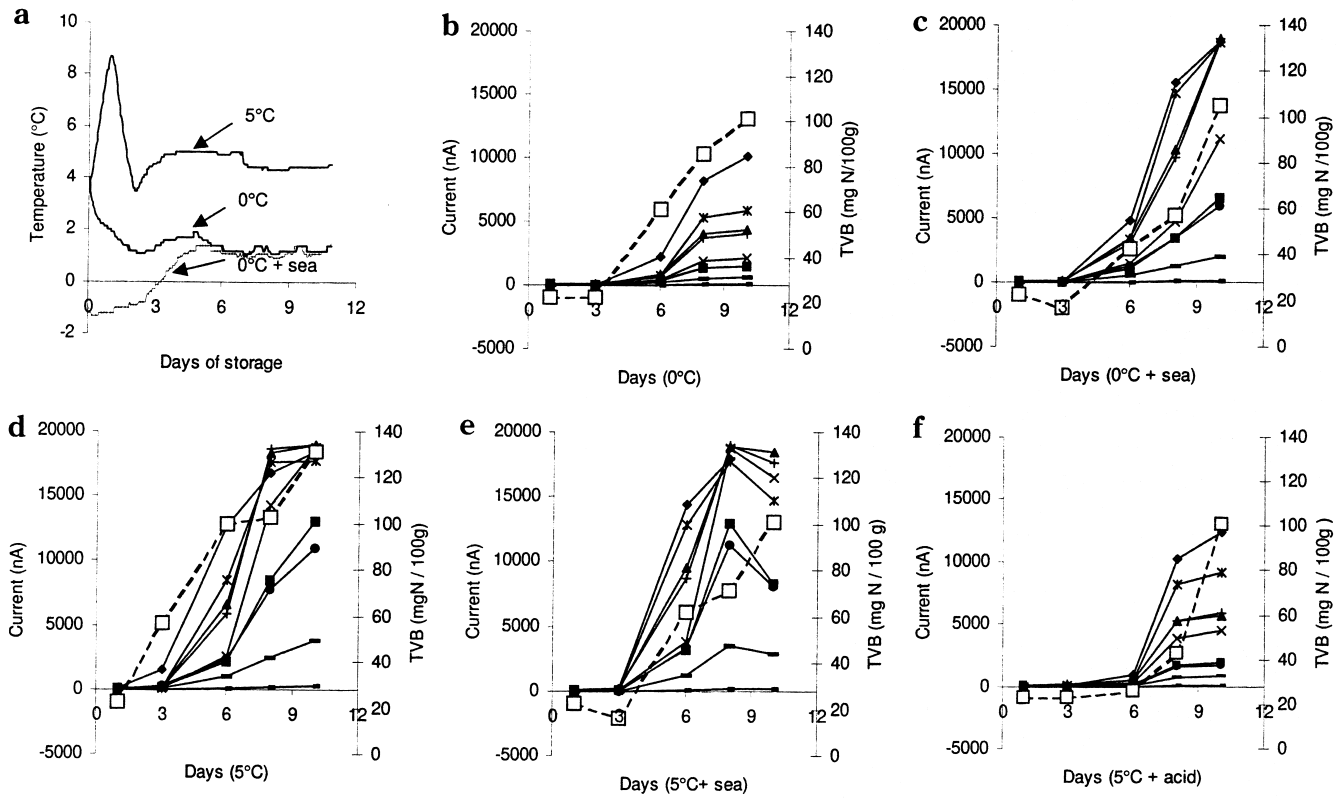


Figure 1. Temperature of samples (a) stored at 0 °C, 5 °C, and 0 °C + sea; TVB analysis and responses of electronic nose measurements of capelin samples stored under different conditions: (b) 0 °C; (c) 0 °C + sea; (d) 5 °C; (e) 5 °C + sea; (f) 5 °C + acid (TVB, □; CO, ◆; H_2Sa, ■; NO, ▲; NO_2, ×; SO_2, ∗; NH_3A7/AM, ●; H_2Sb, +; SO_2, - - -; NH_3b, —).

in temperature noticed in the other samples kept under the same conditions. On day 2 all of the ice had melted in the 5 °C + sea sample. The experimental conditions resemble CSW systems, where ice is not replenished during the storage time. On the other hand, when RSW systems are used, the effective cooling is throughout the storage period because cooling systems maintain the preset temperature.

Measurements of Capelin Headspace with Electronic Nose. Panels b, c, d, e, and f of Figure 1 show responses of the different gas sensors in the electronic nose to capelin during storage for 10 days under different storage conditions (0 °C, 0 °C + sea, 5 °C, 5 °C + sea, 5 °C + acid, respectively). The sensor responses indicate that the spoilage rates are different in these samples. The characteristic responses of individual sensors in all of the samples are all increasing with time. It is also evident from the graphs that the response increases logarithmically and then starts to level off, similar to traditional spoilage measurements.

The results show that on the same days, samples kept at 5 °C (Figure 1d) have considerably higher responses than those kept at 0 °C (Figure 1b). This is, however, not true for 5 °C + acid (Figure 1f), which has responses similar to those of the 0 °C sample because of the effect of the added acetic acid, which slows the spoilage rate as reported earlier (Ólafsdóttir et al., 1997a).

The results of the electronic nose measurements show that samples kept in ice/seawater have higher responses than those kept solely under refrigeration. This is especially clear for the 0 °C (Figure 1b) and 0 °C + sea (Figure 1c) samples, for which the difference in sensor responses is considerable, indicating increased spoilage in the seawater system. This is in agreement with the results of Shaw and Botta (1978), who reported an increased spoilage rate for capelin stored in a seawater system. However, when the sensor responses during the first days of storage are studied more closely, it is evident that on day 3 no noticeable changes are detected in the headspace of the samples stored at 0 °C, neither with nor without added seawater. Furthermore, there is no change in response in the 5 °C + acid sample. The sensors are, on the other hand, detecting changes in the samples stored at 5 °C. A lower response of the sensors in the 5 °C + sea sample than in the 5 °C sample indicates less spoilage because of the cooling effect of the added ice in the beginning. The added ice is effective only in cooling the samples during the first days, and in the 5 °C + sea sample the ice had melted on the second day.

The results during later stages of spoilage, on the other hand, show a rapid increase in the sensor responses for the 5 °C + sea sample as compared to the 5 °C sample. On day 6 of storage the increased response of sensors in the samples with added seawater is observed at both temperatures. This is similar to earlier reports for herring indicating that seawater systems are equally as effective as ice only during the first days of storage (Smith et al., 1980). However, during later stages of storage a more rapid spoilage rate has been reported for herring and oil sardine kept in seawater cooling systems (Smith et al., 1980; Hiremath et al., 1982; Perigreen et al., 1975). In those studies the seawater system was comparable to iced storage only during the initial stages (up to 2 days) of storage for oil sardine and during 4–5 days of storage for herring. Results of sensory evaluation showed that off-flavors developed in the seawater-stored fish ~1 day earlier than in the iced fish. This was explained by the fact that seawater was not replenished at periodic intervals, and

it was noticed that the seawater began to develop off-odors at an earlier stage than the fish.

TVB Measurements. The results of the TVB analysis are also shown in Figure 1. TVB is lowest in the 5 °C + acid samples (Figure 1f) but highest in the samples stored at 5 °C, indicating that those samples have the highest level of spoilage. The TVB value in the samples stored in seawater is lower than for the samples stored under refrigeration at the same temperatures. This is contradictory to the results of the electronic nose, where the samples stored in seawater (0 °C + sea and 5 °C + sea) appeared to be more spoiled than the 0 and 5 °C samples, respectively. The low TVB values in seawater samples may be because volatile nitrogen compounds can possibly leak from the fish when stored in seawater or ice (Magnússon and Martinsdóttir, 1995), resulting in lower TVB values of the fish. This is in fact observed on day 3 for the 0 °C + sea and 5 °C + sea samples when the TVB values are lower than on day 1. Another explanation may be that less TVB is being produced in the seawater system. The contradictory results of the TVB analysis and electronic nose measurement could also simply be because of the different sample preparation. In the TVB method the whole fish is homogenized, whereas in the electronic nose measurement the fish is measured whole. Therefore, the high levels of spoilage compounds detected by the electronic nose are most likely originating from the seawater on the surface of the fish. As mentioned before, the seawater contains soluble nutrients that are easily metabolized by spoilage bacteria. On day 3 the 0 and 5 °C + acid samples have the same TVB values as initially, but the TVB value for the 5 °C sample has increased considerably, and this sample appears to have the most rapid spoilage during the first 3 days. The transfer of all the 5 °C samples to 10 °C on the first day has without doubt increased the spoilage rate of those samples as compared to their being kept at 5 °C throughout the storage time.

GC and Sensory Analysis. Table 1 shows the results of the measurements of the volatile sulfur compounds in capelin. No sulfur compounds were detected in the samples until the first putrid spoilage odors had appeared on day 6 in the 5 °C and 5 °C + sea samples. Methyl mercaptan is detected at highest levels in the 5 °C, 5 °C + sea, and 0 °C + sea sample, and hydrogen sulfide is in higher levels in the samples stored at 5 °C than in those stored at 0 °C. The volatile sulfides contribute to the spoilage odors because their odors are very potent and the odor threshold low. Hydrogen sulfide has a sulfury and boiled eggs character and an odor threshold of 5−40 ppb (Fazzalari, 1978). The odor of methyl mercaptan is described as rotten and cabbage-like, and the flavor threshold is very low (0.05 ppb) (Fazzalari, 1978). Dimethyl disulfide has a putrid, onion-like odor and an odor threshold of 12 ppb (Buttery et al., 1976). External standards dimethyl disulfide and dimethyl sulfide were used to give semiquantitative information on the level of the sulfur compounds in the samples as described in Ólafsdóttir et al. (1997a). The levels of sulfur compounds when detected in spoiled samples (0.5−15 ppm) were much higher than their flavor thresholds.

Table 1 shows that for all samples the progression of odor is from fresh to flat, progressing to sweet and/or stale odors, and ending with putrid odor. When fish turns putrid, the odor profile is very complex, and it was difficult to describe and distinguish among the various odor notes. The sensory classification scheme was developed to classify capelin used for the production of high-quality fishmeal (TVB < 50 mg of N/100 g). Capelin will typically have TVB values around 80−100 mg of N/100 g to be classified as putrid. However, when standard fishmeal is produced, the raw material can exceed TVB 100 mg of N/100 g and in some cases sorting the raw material according to TVB values >100 mg of N/100 g is required. The TVB value of the capelin when it is classified as putrid is close to 100 mg of N/100 g for the samples that were not kept in seawater. On the other hand, samples that were kept in seawater at 0 and 5 °C had much lower TVB values (50−60 mg of N/100 g) when they were classified as putrid by sensory analysis. Therefore, the sensory scheme is not useful to grade such raw material. It was observed that the appearance of the samples stored in seawater was slightly different. The gills of the capelin kept in seawater lost their red color very early and were pale, because of the leaching out of slime and blood into the seawater. The 5 °C and 5 °C + sea samples had the most rapid spoilage; already on day 3 spoilage odor had developed, and both samples were classified as putrid on day 6. The 5 °C + acid sample had the slowest spoilage rate according to sensory analysis and was classified as putrid on day 10.

Table 1. Sensory Classification and Relative Concentration of Sulfur Compounds Analyzed by GC-FPD in Capelin Stored for 10 Days at 0 °C, 0 °C plus Seawater, 5 °C, 5 °C plus Seawater, and 5 °C plus Acid

sample (°C/days)	relative intensities (peak areas) of S compounds				sensory classification
	H_2S	CH_3SH	DMS	DMDS	
0/1	0	0	0	0	fresh
0/3	0	0	0	0	flat
0/6	0	1×10^5	0	0	sweet
0/8	0	11.2×10^5	0	0.1×10^5	putrid
0/10	1.2×10^5	80.5×10^5	0.1×10^5	0.4×10^5	putrid
0+sea/1	0	0	0	0	fresh
0+sea/3	0	0	0	0	flat
0+sea/6	0	110×10^5	0	0.1×10^5	stale
0+sea/8	2.2×10^5	425×10^5	0.2×10^5	0.2×10^5	putrid
0+sea/10	5.7×10^5	769×10^5	0.1×10^5	0.8×10^5	putrid
5/1	0	0	0	0	fresh
5/3	0	0	0	0	flat
5/6	12.6×10^5	182×10^5	0.1×10^5	0.3×10^5	putrid
5/8	14.4×10^5	548×10^5	0.1×10^5	1.0×10^5	putrid
5/10	648×10^5	1160×10^5	0.3×10^5	0.7×10^5	putrid
5+sea/1	0	0	0	0	fresh
5+sea/3	0	0	0	0	sweet
5+sea/6	3.5×10^5	561×10^5	0.1×10^5	0.3×10^5	putrid
5+sea/8	139×10^5	996×10^5	0.2×10^5	1.2×10^5	putrid
5+sea/10	412×10^5	208×10^5	0.3×10^5	2.2×10^5	putrid
5+acid/1	0	0	0	0	fresh
5+acid/3	0	0	0	0	flat
5+acid/6	0	0	0	0	sweet
5+acid/8	0.1×10^5	1.1×10^5	0	0	stale
5+acid/10	43.0×10^5	65.6×10^5	0.1×10^5	0.1×10^5	putrid

Data Analysis. The data from the various measurements used to monitor spoilage in capelin stored under different conditions was examined by principal component analysis (PCA). PCA describes the trend in the data and cannot be used to determine if there is a significant difference between samples. Figure 2 shows a PCA scores plot of all the samples and data from the electronic nose, sensory analysis, GC, and TVB. The x-axis is the first principal component, explaining 84% of the variance in the data. It can be seen that the first PC represents the spoilage of the samples. For each

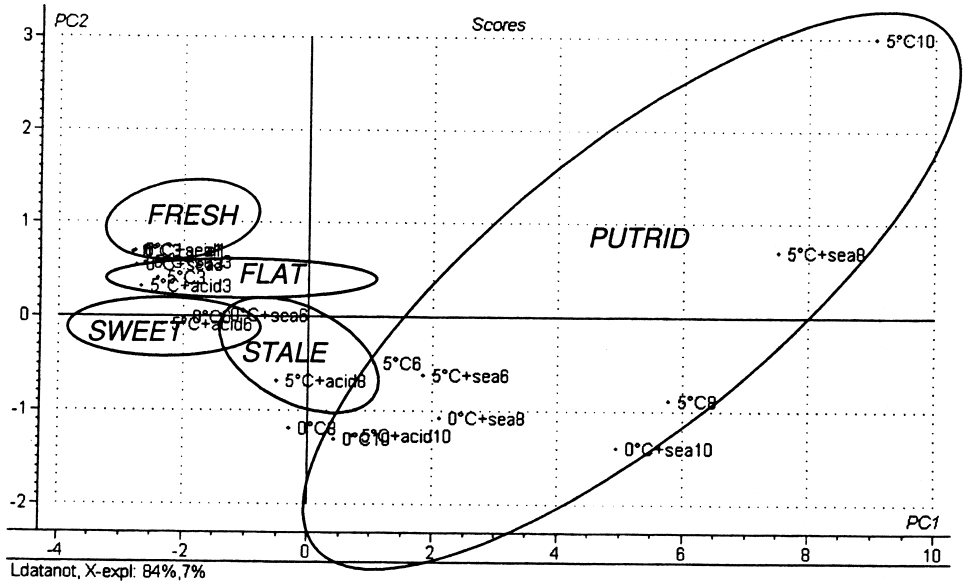


Figure 2. Scores plot for PCA of all the measurement variables [TVB, electronic nose (FreshSense), gas chromatography, and sensory analysis] and sample variables (0 °C, 0 °C + sea, 5 °C, 5 °C + sea, and 5 °C + acid).

group of samples the storage time increases from left to right. The days in the groups overlap, indicating that the samples kept in acid tend to spoil later than the other samples, the 5 °C samples spoil first, and the samples kept in seawater spoil sooner than the others. Samples of comparable freshness or quality are therefore located close to each other on the PCA plot and have been grouped together according to the sensory classification (fresh, flat, sweet, stale, and putrid). As mentioned before, it is clear that the sensory scale is not nearly wide enough and too many samples fall in the putrid category. Alternative schemes with more classes and detailed descriptions of spoilage odors may be more useful to classify raw material used for fishmeal production.

The loadings of all the variables were very similar in PC1, and because of its large explained variance it can be said that all of the different measurement methods are similar to monitor spoilage. After using PCA, error measures, and outlier detection, the 10th day measurement for the sample kept at 5 °C + sea was labeled an outlier and its results have been kept out of all calculations.

PLS regression and GLM were used to study the different types of spoilage measurements and to find a model to predict TVB from the electronic nose measurements. Also, the possibility of using fewer sensors in the electronic nose for this particular application was studied. The results of PLS regression are shown in Table 2. All of the sensors and two sets containing three of the highest responding sensors have been selected to predict TVB. The first set of sensors is CO Dräger, NO Dräger, and NH_3 A7AM, and the second set is CO Dräger, SO_2 Dräger, and NH_3 A7AM. The analysis was done using 15 samples (data from days 1, 3, and 6) and 24 samples (data from all days).

The root-mean-square error of prediction (RMSEP) indicates the accuracy of the model for prediction, and it is ~20 mg of N/100 g of fish for all samples (Table 2). The result when all sensors are used simultaneously is slightly better than in the case when only three sensors are used, but the results are fairly similar because the three sensors represent the whole array.

In Table 2 PLS regression parameters using only the samples from days 1, 3, and 6 are shown, as the TVB results did not agree well with electronic nose measurements in later stages of spoilage. The RMSEP is considerably lower in this case, and results using only three sensors are better than those using all sensors. This indicates that an instrument with only a few sensors could be used for this particular application. Furthermore, PLS was performed using only results from days 8 and 10, but a valid model could not be obtained.

Table 2. PLS Results Using Two PCs, Giving the *X* Variables, Their Explained Variance, the Slope and Correlation of the Predicted vs Measured Plot of the Model, and the RMSEP, in Milligrams of N/100 g of Fish, for the *Y* Variable TVB

	X	expl *X* (%)	expl *Y* (%)	slope	correlation	RMSEP
all days	all sensors	96	75	0.9487	0.8354	19.3
	CO, NO, NH_3	98	69	0.8775	0.7512	23.4
	CO, SO_2, NH_3	99	67	0.8965	0.7518	23.3
days 1, 3, and 6	all sensors	100	82	0.8396	0.8836	11.5
	CO, NO, NH_3	99	93	1.0623	0.9459	7.6
	CO, SO_2, NH_3	99	94	1.2115	0.9426	8.9

In addition to PLS regression, a GLM with normal error was fitted to the data. Three gas sensor variables, CO, SO_2, and NH_3, that had the highest factor loadings in PLS analysis were selected as covariates. The variables CO, SO_2, and NH_3 are all pairwise highly correlated. A saturated model therefore includes only one of the three variables. Best fit was found using the NH_3 sensor. To predict TVB, the following explanatory variables were chosen: NH_3 and condition, as a factor with three levels of storage (condition 1 = 0 and 5 °C; condition 2 = 0 °C + sea and 5 °C + sea; and condition 3 = 5 °C + acid). Storage temperature as a factor of two levels, 0 and 5 °C, did not give a better fit to the data and is therefore excluded from the model.

After error distribution, link function, and different transformations of covariates were altered, the following model was chosen as an estimate of TVB given the storage condition and the response of the NH_3 sensor:

$$TVB_i = \alpha_i + \beta_1 NH_3 + \beta_2 (NH_3)^2 + \epsilon_i$$

α_i represents the intercept for condition level i, with i = storage conditions (0 and 5 °C; 0 °C + sea and 5 °C + sea; 5 °C + acid), and ϵ_i the error term. α_i, β_1, and β_2 are parameters to be estimated. The results of the prediction of TVB are in Table 3. The reference level of the factor condition is chosen to be condition 1, which is storage in 0 and 5 °C. Thus, when TVB is predicted for 0 and 5 °C, the model is

$$\text{estimated } TVB_{0°C/5°C} = 43.15 + 0.02NH_3 - [2 \times 10^{-6}(NH_3)^2]$$

The predicted values of TVB for the other storage conditions are 29.56 lower for 0 °C + sea/5 °C + sea and 13.68 lower for 5 °C + acid. Therefore, taking also into account storage in ice/seawater systems (0 °C + sea/5 °C + sea) and the use of acid (5 °C + acid) the models are

$$\text{estimated } TVB_{0°C+sea/5°C+sea} = 43.15 - 29.56 + 0.023157NH_3 - [2 \times 10^{-6}(NH_3)^2]$$

$$\text{estimated } TVB_{5°C+acid} = 43.15 - 13.68 + 0.023157NH_3 - [2 \times 10^{-6}(NH_3)^2]$$

Results from analysis of deviance are shown in Table 4. The terms are added sequentially one at a time. Deviance residuals divided with the estimated scale parameter (307.47) are approximately F-distributed $F(2,24)$ for condition and $F(1,24)$ for the other terms. All terms are highly significant, with $p < 0.001$, with NH_3 as the most important term and as the best single variable to use in a linear model. Explained variance, r^2, is 0.80.

If storage time is known, an additional continuous term, time, may be included in the model and β_3 becomes an additional parameter to be estimated.

$$TVB_i = \alpha_i + \beta_1 NH_3 + \beta_2 (NH_3)^2 + \beta_3 (\text{time}) + \epsilon_i$$

The results of the prediction of TVB and analysis of deviance, including time as a continuous explanatory variable, are shown in Tables 5 and 6. In this case r^2 = 0.88 and the most important term is time.

CONCLUSIONS

According to the results of sensory analysis, GC, and electronic nose measurements, the ice/seawater systems were as effective as refrigeration to maintain the freshness of capelin during the first days of storage. After prolonged storage (>6 days), an increased spoilage rate was observed in seawater/ice systems. The added ice was effective in cooling the samples only during the first 2−3 days of storage. The question remains if TVB is the most appropriate method to evaluate spoilage of fish stored in seawater/ice systems. TVB analysis indicated less spoilage in seawater/ice systems than in refrigeration, which is contradictory to the other measurements. GC and electronic nose measurements give more information than sensory analysis about the quantity and the complex combination of the volatile degradation compounds during later stages of spoilage. The electronic nose and TVB measurements give more possibilities than the sensory scheme used to grade the raw material after the capelin has turned putrid. The electronic nose monitors several classes of compounds and can possibly give more information than the traditional TVB analysis. In addition to the detection of amines by the NH_3 sensor, the CO and SO_2 sensors can detect the presence of volatile alcohols and sulfur compounds, respectively.

Better understanding of the spoilage processes in ice/seawater systems is needed to establish useful quality criteria. Further research should include investigations using GC techniques to identify and quantify the various volatile compounds contributing to spoilage in capelin stored under different conditions. Furthermore, the possibility of using rapid electronic nose measurements to predict the presence of the various quality-indicating compounds is of interest for the fish industry.

Results from multivariate analysis indicate that the electronic nose can be used to predict capelin quality as TVB and models using only three gas sensors gave similar or better results than prediction with nine sensors. TVB is very well predicted by a saturated GLM (r^2 = 0.80) with the values from only one gas sensor [NH_3 and $(NH_3)^2$] and storage conditions (0 °C/5 °C; 0 °C + sea/5 °C + sea; 5 °C + acid) as linear predictors. Storage time can also be included in the model, and a

Table 3. Estimated Values, Standard Error, and Partial t Values for the Coefficients in a GLM Predicting TVB

coefficient	value (α_i, $\beta_{1,2}$)	SE	t value
$condition_{0and5°C}$	43.15	6.43	6.71
$condition_{0°C+sea/5°C+sea}$	−29.56	7.95	−3.72
$condition_{5°C+acid}$	−13.68	9.79	−1.40
NH_3	2.3157×10^{-2}	3.671×10^{-3}	6.31
$(NH_3)^2$	2×10^{-6}	3×10^{-7}	−4.41

Table 4. Analysis of Deviance for the Terms in the Prediction Model for TVB[a]

term	degrees of freedom	residual deviance	F value
null	24	31397.53	
condition	22	28284.46	10.12
NH_3	21	12137.74	52.51
NH_3^2	20	6149.36	19.47

[a] F values equal to deviance residuals divided by the estimated scale parameter are all significant.

Table 5. Estimated Values, Standard Error, and Partial t Values for the Coefficients in a GLM Predicting TVB, Including Time as a Continuous Explanatory Variable

coefficient	value (α_i, $\beta_{1,2,3}$)	SE	t value
$condition_{0and5°C}$	26.12	7.13	3.66
$condition_{0°C+sea/5°C+sea}$	−23.92	6.59	−3.63
$condition_{5°C+acid}$	−20.68	8.12	−2.55
NH_3	1.0×10^{-2}	4.7×10^{-3}	2.21
$(NH_3)^2$	-6.5×10^{-7}	3.8×10^{-7}	−1.73
time (days)	5.61	1.62	3.47

Table 6. Analysis of Deviance for the Terms in the Prediction Model for TVB Including Time as a Continuous Explanatory Variable[a]

term	degrees of freedom	residual deviance	F value
null	24	31397.53	
condition	22	28284.46	15.70
NH_3	21	12137.74	81.43
NH_3^2	20	6149.36	30.20
time	19	3767.71	12.01

[a] F values equal to deviance residuals divided by the estimated scale parameter are all significant.

better prediction of TVB is obtained ($r^2 = 0.88$). It is reasonable and can be expected that the NH_3 sensor gives the best prediction of TVB because it is selective and sensitive to volatile amines.

The electronic nose has potential as a rapid analysis tool to predict TVB of capelin raw material during fishmeal production. No sample preparation is required, and it is a rapid, easy-to-use technique. Data acquisition and analysis can be connected directly to information systems in fishmeal factories to be used in process control. The next step is to evaluate the performance of the electronic nose to measure the quality of the raw material during processing in fishmeal factories.

ABBREVIATIONS USED

CSW, chilled seawater; FPD, flame photometric detector; GC, gas chromatography; GLM, generalized linear model; IFL, Icelandic Fisheries Laboratories; PCA, principal component analysis; PLS, partial least squares regression; r^2, explained variance; RMSEP, root-mean-square error of prediction; RSW, refrigerated seawater; TVB, total volatile bases; SE, standard error.

LITERATURE CITED

Ahmed, A.; Matches, J. R. Alcohol production by fish spoilage bacteria. *J. Food Prot.* **1983**, *46*, 1055–1059.

Aksnes, A.; Brekken, B. Tissue degradation, amino acid liberation and bacterial decomposition of bulk stored capelin. *J. Sci. Food Agric.* **1988**, *45*, 53–60.

Bartlett, P. N.; Elliot, J. E.; Gardner, J. W. Electronic Noses and Their Application in the Food Industry. *Food Technol.* **1997**, *51* (12), 44–48.

Billon, J.; Ollieuz, J. N.; Tao, S. H. Etude d'une novelle méthode de dosage de l'azoto basique volatil total pour lévaluation qualitative des produits de la peche. *RTVA, Rev. Tech. Vet. Aliment.* **1979**, *1149*, 13–17.

Buttery, R. G.; Guadagni, D. G.; Ling, L. C.; Seifert, R. M.; Lipton, W. Additional volatile components of cabbage, broccoli and cauliflower. *J. Agric. Food Chem.* **1976**, *24*, 829–832.

Craven, M. A.; Gardner, J. W.; Bartlett, P. N. Electronic noses—development and future prospects. *Trends Anal. Chem.* **1996**, *15* (9), 486–493.

Dagbjartsson, B.; Valdimarsson, G.; Arason, S. Icelandic experience in storing fish in chilled seawater. In *Advances in the Refrigerated Treatment of Fish*; Proceedings of the International Institute of Refrigeration Conference, Boston, MA; IIR: Paris, France, 1982; pp 233–242.

DiNatale, C.; Brunink, J. A. J.; Bungaro, F.; Davide, F.; d'Amico, A.; Paolesse, R.; Boschi, T.; Faccio, M.; Ferri, G. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. *Meas. Sci. Technol.* **1996**, *7*, 1103–1114.

Fazzalari, F. A. *Compilation of Odor and Taste Threshold Value Data*; American Society for Testing and Materials: Philadelphia, PA, 1978.

Herbert, R. A.; Shewan, J. M. Precursors of the Volatile Sulphides in Spoiling North Sea Cod (*Gadus morhua*). *J Sci. Food Agric.* **1975**, *26*, 1195–1202.

Herbert, R. A.; Ellis, J. R.; Shewan, J. M. Isolation and identification of the volatile sulphides produced during chill-storage of North Sea Cod (*Gadus morhua*). *J. Sci. Food Agric.* **1975**, *26*, 1187–1195.

Hiremath, G. G.; Teutscher, F.; Nordheim, A. Experiments on Storage of Fish in Chilled Sea Water. *Myosore J. Agric. Sci.* **1982**, *16*, 329–337.

Josephson, D. B.; Lindsay, R. C.; Ólafsdóttir, G. Measurement of Volatile Aroma Constituents as a Means for Following Sensory Deterioration of Fresh Fish and Fishery Products. In *Proceedings of an International Symposium*; University of Alaska Sea Grant Program, Anchorage, AK; Kramer, D. E., Liston, J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1986; pp 27–47.

Kamiya, A.; Ose, Y. Study of the odourous compounds produced by putrefaction of foods. V. Fatty acids, sulphur compounds and amines. *J. Chromatogr.* **1984**, *292*, 383–391.

Kolbe, E.; Crapo, S.; Hilderbrand, K. Ice Requirements for Chilled Seawater Systems. *Mar. Fish. Rev.* **1985**, *47* (4), 33–41.

Lindsay, R. C.; Josephson, D. B.; Ólafsdóttir, G. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In *Proceedings of an International Symposium*; University of Alaska Sea Grant Program, Anchorage, AK; Kramer, D. E., Liston, J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1986; pp 221–234.

Magnússon, H.; Martinsdóttir, E. Storage Quality of Fresh and Frozen-Thawed Fish in Ice. *J. Food Sci.* **1995**, *60*, 273–278.

Martens, M.; Martens, H. Partial Least Squares Regression. In *Statistical Procedures in Food Research*; Piggott, J. R., Ed.; Elsevier Applied Science Publishers: Amsterdam, The Netherlands, 1986; pp 293–359.

Meilgaard, G.; Civille, V.; Carr, B. T. *Sensory Evaluation Techniques*, 2nd ed.; CRC Press: Boca Raton, FL, 1991; pp 142–147.

Ólafsdóttir, G.; Fleurence, J. Evaluation of fish freshness using volatile compounds—classification of volatile compounds in fish. In *Methods to Determine the Freshness of Fish in Research and Industry*; International Institute of Refrigeration: Paris, France, 1998; pp 55–69.

Ólafsdóttir, G.; Martinsdóttir, E.; Jónsson, E. H. Rapid gas sensor measurements to predict the freshness of capelin (*Mallotus villosus*). *J. Agric. Food Chem.* **1997a**, *45*, 2654–2659.

Ólafsdóttir, G.; Martinsdóttir, E.; Jónsson, E. H. Gas sensor and GC measurements of volatile compounds in capelin (*Mallotus villosus*). In *Seafood from Producer to Consumer, Integrated Approach to Quality*; Luten, J. B., Börresen, T., Oehlenschläger, J., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1997b; pp 507–520.

Ólafsdóttir, G.; Martinsdóttir, E.; Oehlenschläger, J.; Dalgaard, P.; Jensen, B.; Undeland, I.; Mackie, I. M.; Henehan, G.; Nielsen, J.; Nilsen, H. Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Technol.* **1997c**, 258–265.

Ólafsdóttir, G.; Verrez-Bagnis, V.; Luten, J. B.; Dalgaard, P.; Careche, M.; Martinsdóttir, E.; Heia, K. The need for methods to evaluate fish freshness. In *Methods to Determine the Freshness of Fish in Research and Industry*; International Institute of Refrigeration: Paris, France, 1998; pp 17–29.

Perigreen, P. A.; Pillai, S. A.; Surendran, P. K.; Govindan, T. K. Studies on preservation of fish in refrigerated sea water. *Fish Technol.* **1975**, *12*, 105–111.

Shaw D. H.; Botta, J. R. Preservation of Inshore Male Capelin (*Mallotus villosus*) Stored in Refrigerated Sea Water. *J. Fish. Res. Board Can.* **1975**, *32*, 2047–2053.

Shewan, J. M.; Mackintosh, R. G.; Tucker, C. G.; Ehrenberg, A. S. C. The Development of a Numerical Scoring System for the Sensory Assessment of the Spoilage of Wet White Fish Stored in Ice. *J. Sci. Agric.* **1953**, *6*, 183–189.

Smith, J. S. M.; Hardy, R.; MacDonald, I.; Templeton, J. The storage of herring (*Clupea harengus*) in ice, refrigerated sea water and at ambient temperature. Chemical and sensory assessment. *J. Sci. Food Agric.* **1980**, *31*, 375–385.

Received for review April 6, 1999. Revised manuscript received January 4, 2000. Accepted March 16, 2000.

JF990322Q

PAPER II

II

Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets

Corrado Di Natale, Guðrún Ólafsdóttir, Sigurdur Einarsson, Alessandro Mantini, Eugenio Martinelli, Roberto Paolesse, Christian Falconi, Arnaldo D'Amico

Published in: Sensors and Actuators B77, (2001) 572- 578

ELSEVIER

Sensors and Actuators B 77 (2001) 572–578

SENSORS AND ACTUATORS B CHEMICAL

www.elsevier.nl/locate/sensorb

Comparison and integration of different electronic noses for freshness evaluation of cod-fish fillets

Corrado Di Natale[a,b,c,d,*], Gudrun Olafsdottir[b], Sigurdur Einarsson[b], Eugenio Martinelli[a], Roberto Paolesse[a], Arnaldo D'Amico[a,b,c,d]

[a]*Department of Electronic Engineering, University of Rome (Tor Vergata), Via di Tor Vergata 110, 00133 Rome, Italy*
[b]*Icelandic Fisheries Laboratories, Skulagata 4, IS-101 Reykjavik, Iceland*
[c]*Department of Chemical Science and Technology, University of Rome (Tor Vergata), Via Della Ricerca Scientifica, 00133 Rome, Italy*
[d]*PSM-CNR, Via Del Fosso Del Cavaliere, 00133 Rome, Italy*

Abstract

For the instrumental analysis of fish freshness, several different methods have been proposed based on different principles (such as mechanical, electrical, and optical). Although, it is well-known that the headspace composition changes greatly during the spoilage process, the difficulties in sampling and measuring the headspaces with traditional analytical chemistry techniques makes the practical implementation of this principle not viable. Recently, the advent of sensor array technology has given the possibility to reconsider the headspace variation for instrumental applications. In this paper the use of measurements of two electronic noses, based on different sensor technologies and sampling methodologies, to detect freshness of cod-fish fillets is illustrated and discussed. Over a period of 17 storage days, the two sensor systems have shown different resolution, while the combination of them achieves the best performances allowing an almost complete evaluation of the freshness of samples. Trimethylamine (TMA) and total volatile basic nitrogen, measured with conventional techniques, show a non-monotonic behaviour, that induces the possibility of large errors in freshness estimation, as shown by the electronic noses.

Keywords: Cod-fish fillets; Electronic noses; Trimethylamine

1. Introduction

Among the numerous applications of electronic nose technology and methodology, the analysis of foodstuff is one of the most promising and also the most travelled road towards industrial applications. In this particular field, electronic noses can answer to the dilemma of freshness definition in product of limited shelf life, such as fish.

For fish, it is really important to determine the freshness degree, defined as the number of storage days at a certain temperature. Anyway for this kind of product, other issues such as the distinction between fresh and thawed samples and the maintenance of a constant temperature during storage are of great importance.

Currently, many methods based on different measurement principles are available to give a measure of fish freshness [1]. The physical properties of the fish such as the rheological characteristics (firmness and texture) and the electrical properties (impedance) can sometimes be correlated with storage days. For instance, the impedance of fish is, for many species such as cod and salmon, a good indicator of the time from catch. Nonetheless, this method is not effective in case of frozen and thawed fish.

The composition of the fish headspace is a source of information about the freshness degree of a sample. Spoilage in fish can be detected through the measure of the amount of amines, such as trimethylamine (TMA). Some methods, based on analytical chemistry procedures, are currently available to get information about the content of volatile TMA. Nevertheless, the formation of amines due to the decomposition starts only some days after the catch. Chemical investigations using gas chroamtographic techniques have shown that there are five sources of odours, which combined, give rise to the overall odour of fish [2]. The fresh fish odour is a characteristic related to the individual species. It is basically contributed by long chain alcohol and carbonyls, bromophenols, and N-cyclic compounds. Opposite to the fresh fish odour is the microbial spoilage odour — caused by compounds that are microbially formed during the spoilage processes. These compounds are short chain alcohol and carbonyls, amines, sulphur compounds, aromatics, N-cyclic

* Corresponding author. Tel.: +39-6-7259-7248; fax: +39-6-2020-519.
E-mail address: dinatale@eln.uniroma2.it (C. Di Natale).

0925-4005/01/$ – see front matter
PII: S0925-4005(01)00692-X

compounds and some acids. The concentration of these volatiles increase with time as the fish spoils, indeed some of these are often used as indicators of spoilage [2].

Other sources of odours can be environmental (such as petroleum odours), or due to the processing of fish, and from products of lipid oxidation [3]. Due to the high number of volatile compounds involved in the process, and to the fact that they also dynamically change, the measure of fish freshness over a long period of storage, can be achieved with a multicomponent approach. This is a typical electronic nose application, where a number of sensors non-selective and partially cross-correlated are utilised to get a qualitative analysis of samples.

Different electronic noses have been applied to the detection of fish freshness. Interesting results have been obtained with different sensor technologies such as metal-oxide semiconductor gas sensors [4,5], electrochemical sensors [6] and thickness shear mode quartz resonators [7]. Fish freshness sensors were also developed, based on metal-oxide semiconductors technology, with improved selectivity and sensitivity towards compounds such as dimethylamine and TMA [8].

In this paper, the application of two different electronic noses, based on different sampling procedures and sensor technologies is described. The sensors have been utilised to measure the freshness, expressed as storage days, of a number of samples of cod fillets.

As a reference method, TMA and total volatile bases nitrogen (TVB) have been measured in the same samples [9]. Each electronic nose has shown a certain way to classify the data, in particular, the higher resolution is achieved at different storage periods. The integration of both the instruments improved the performances of the single instruments and will be considered, together with experimental results in this paper.

2. Experimental

Experiments were performed at the Icelandic Fisheries Laboratory in Reykjavik from 15 to 20 November 1999. Three batches of Atlantic cod were collected for the experiment. Fish was obtained from small boats based in Reykjanes (Iceland), which fish in catching grounds in Faxaflòi, south-west of Iceland.

Fish was caught with longline, gutted and iced immediately after catch and brought to the Icelandic Fisheries Laboratories the following day. Fish was kept at 0°C before being analysed. Samples were filleted and deskinned prior to measuring on the following storage days: 1–4, 7, 9, 11, 15, and 17.

Eight samples per each storage day were measured in total 72 fishes. The measurements were performed on fillets. For each fish the right side fillet was measured, and the other side reserved for experiments not described here. Fillets were prepared about 1 h before the analysis and were held constantly on an ice-bed until measured. Two different electronic noses have been utilised: LibraNose and FreshSense. LibraNose is an instrument designed and fabricated at the University of Rome (Tor Vergata) in cooperation with the Technobiochip company.

The instrument is based on an array of eight thickness shear mode resonators coated with various kinds of metalloporphyrins. Details about the instrument have been previously published [10]. Fish odour measurements have been done using a suitably designed fish odour sampler. It is a metallic capsule with an internal volume of 10 ml approximately equal to the volume of the sensor chamber. The capsule is endowed with a series of small orifices for air refilling. It works in contact with the fish fillet, a stable and reproducible (from the point of view of sensors response) headspace is established in 5 min. Fig. 1 shows a typical run with two successive measurements.

During the experiment, the bone side of the right fillets were measured for each fish. The temperature of the fillet surface, monitored during the measurement, varied from 7 to 10°C, and no correlation of sensor responses with the fillet temperature has been observed.

FreshSense electronic nose is developed and distributed by the company Element-Bodvaki (Iceland) [6]. It is based on five electrochemical sensors each oriented towards a certain gas: CO, H_2S, NO, SO_2, and NH_3. The first four sensors are from Dräger (Germany) and the last one is from City Technology (UK). The sensors and a temperature sensor are fit into the lid of the sampling chamber that consists of a glass vessel (3 l). Each fillet (approximately 300–500 g) was placed in the glass vessel and temperature was measured before closing. The measurement technique is based on static headspace sampling, analysing directly the headspace of fish which accumulates in the closed glass container during measurements at room temperature. Measurements were taken every 10 s for 10 min. In the data analysis, the reported value (current) is the average of the last three measurements of the 10 measurements cycle [6].

TMA and TVB-N, extracted from fish muscles, were measured using a conventional "flow injection analysis — gas diffusion method" [11]. Electronic noses data have been analysed by partial least square discriminant analysis (PLS-DA). All calculations have been carried out in Matlab 5.0.

PLS-DA is a supervised classification method, where the search for optimal discriminant directions is performed using PLS. Class membership is numerically represented with a so-called one-of-many encoding. Namely, the y-block in PLS contains a number of variables equal to the number of classes and the membership of a single data is expressed putting to one the corresponding variable and to zero all the others. An unknown sample is assigned then to the class whose output is higher then the others. This procedure is standard when quantitative oriented classifiers are utilised, such as neural networks.

PLS-DA provides both a quantitative estimation of class discrimination and score and loading plots for a visual

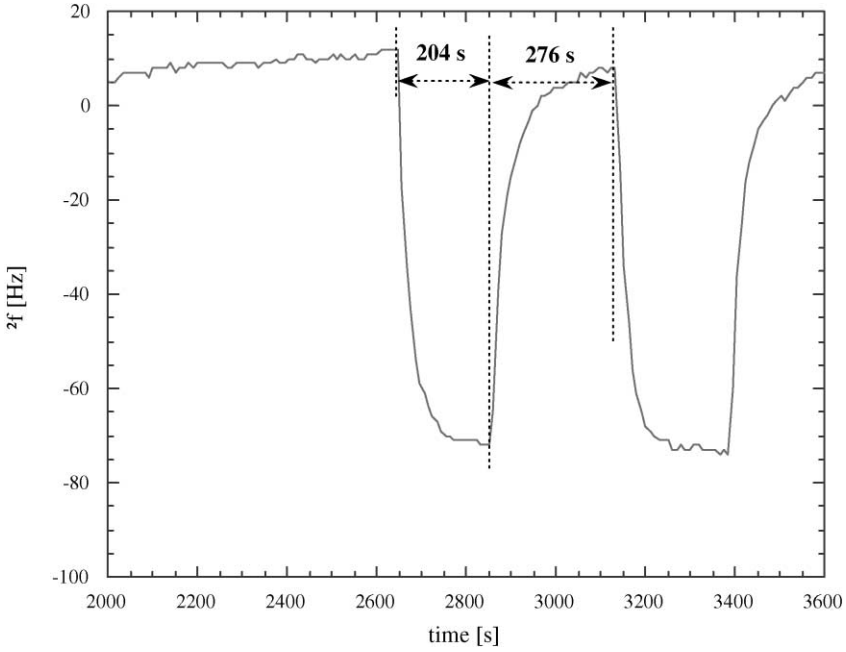


Fig. 1. Dynamic evolution of the signal during the LibraNose measurement using the fish odour sampler.

inspection of data separation and the contribution of single sensors to the array. The meaning of these plots is different from those obtained by a principal component analysis, in this case the latent variables are determined in a supervised procedure aimed at fitting the declared class membership. So that, even if the score plot of the first two latent variables may show class overlapping, the totality of all the latent variables can achieve a class separation. Nonetheless, these score plots, being linear projection over some basis, are indicative of the distribution of data in the sensor space. An

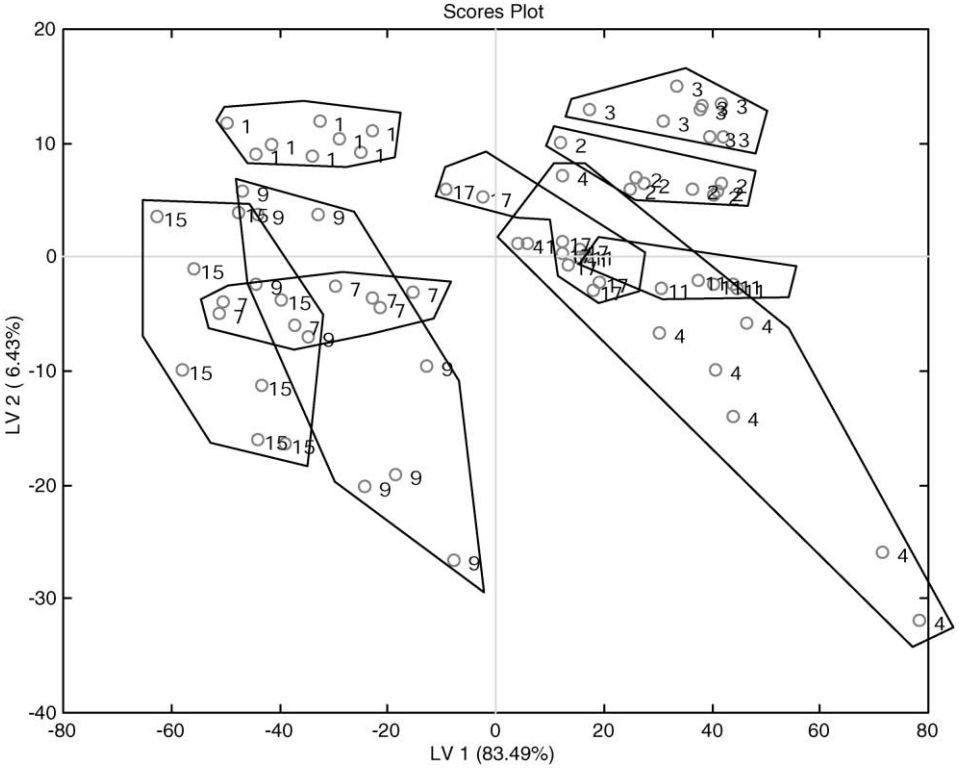


Fig. 2. Plot of the first two latent variables of the PLS-DA for the LibraNose data. Days 1–3 are separated, while days 4–11–17 and 7–9–15 forms grouped clusters.

evaluation of the classification properties of the respective electronic noses can be obtained through a training and validation procedure using the one-leave-out validation technique.

3. Results and discussion

Data collected with the two electronic noses have been analysed separately in order to study the behaviour of each sensor array, and then they have been fused together to investigate a possible improvement of the global performances.

Fig. 2 shows the LibraNose data plotted in a basis identified by the first two latent variables. Samples stored up to 3 days are clearly gathered in close clusters, the fourth day is overlapped with days 11 and 17, while the days 7, 9, and 15 are also overlapping. This tendency to overlap the last days of storage with the first days, namely, the tendency to mislead fish at two very different stages of storage will be shown to be consistent in this experiment. Here, we have to keep in mind that there were three batches of fish and evidently there was a slight variation in the spoilage rate of the different batches. There may have been slight variations in handling during the first 24 h after catch resulting in different spoilage rate of the batches. The results of sensory analysis (data not shown here) confirm this effect and in fact the spoilage rate of the second batch appears to be slower than the first one. To clarify, days 1–4 are from the first batch, days 7, 9 and 11 are from the second batch and finally days 15 and 17 are from the oldest batch.

Class identification is shown in Table 1 as a confusion matrix. The validation has been performed on the whole data set, since the one-leave-out validation technique has been used. Errors are numerically few, but some samples belonging to storage days from 7 to 15 are classified as belonging to the first day. The error is qualitatively not negligible.

Fig. 3 shows the same calculations performed for the FreshSense instrument data. In this case, the score plot does not show a net separation of the first days of fish storage, but rather the final storage days are separated from the rest. The score plot shows that the path leading from day 1 to 17 contains a clear inversion at days 9 and 11 that looks as folded back towards days 3 and 4. Plotting the data on a further dimension represented by the third latent variable, the elimination of this folding back effect is not obtained.

The confusion matrix for FreshSense is shown in Table 1(b). Errors are quantitatively greater compared to the LibraNose, meaning that more samples are wrongly classified, but qualitatively the errors are less influential. In particular, only two samples stored for more than 7 days are classified as belonging to the first day. And generally speaking, errors are almost always an overestimation of storage days. This avoids in practice an overestimation of the shelf life of the product. LibraNose errors, on the contrary, tended to underestimate the storage days.

Table 1
Confusion matrices of the LibraNose, FreshSense, and the two merged data[a]

	1	2	3	4	7	9	11	15	17
(a) LibraNose									
1	8								
2		7				1			
3			8						
4				8					
7	1				7				
9	2					5			1
11							8		
15	1							7	1
17									8
(b) FreshSense									
1	7					1			
2	2	3		2			1		
3			3	2			2		1
4				8					
7	1			1	1	4		1	
9						7		1	
11						1	6		1
15	1							8	
17								2	6
(c) LibraNose + FreshSense									
1	8								
2		8							
3			8						
4				8					
7	1				7				
9	2					6			
11							8		
15								8	
17									8

[a] Classification was calculated with a PLS-DA method with a leave-one-out validation technique. Class estimation is shown along the columns and the true classes on the rows.

Data was always autoscaled (zero mean and unitary variance) in all the PLS-DA calculation. Fig. 4 shows the PLS-DA score plot for a joint virtual instrument obtained fusing together the two sensor arrays in a unique array. The score plot is similar to that of the single instruments, the folding back effect of the classes is still present and the samples of days 15 and 17 are clearly separated from the others. The confusion matrix gives a minimal amount of misclassified samples (4% compared with 9% of LibraNose and 33% of FreshSense). But, the few errors remains qualitatively high being related to sample of 7 and 9 days classified as 1.

An interpretation of these qualitatively not negligible errors can be obtained considering the values of TMA and TVB-N. Fig. 5 shows the measured values of these two important indicators. As reported in the literature, TMA values become considerably different from zero only after 9 days of storage. While, TVB-N shows a non-linear and a non-monotonic behaviour with time. At the beginning of storage TVB-N increases to reach a maximum after 4 days approximately, then it reaches after 7 days the same levels as

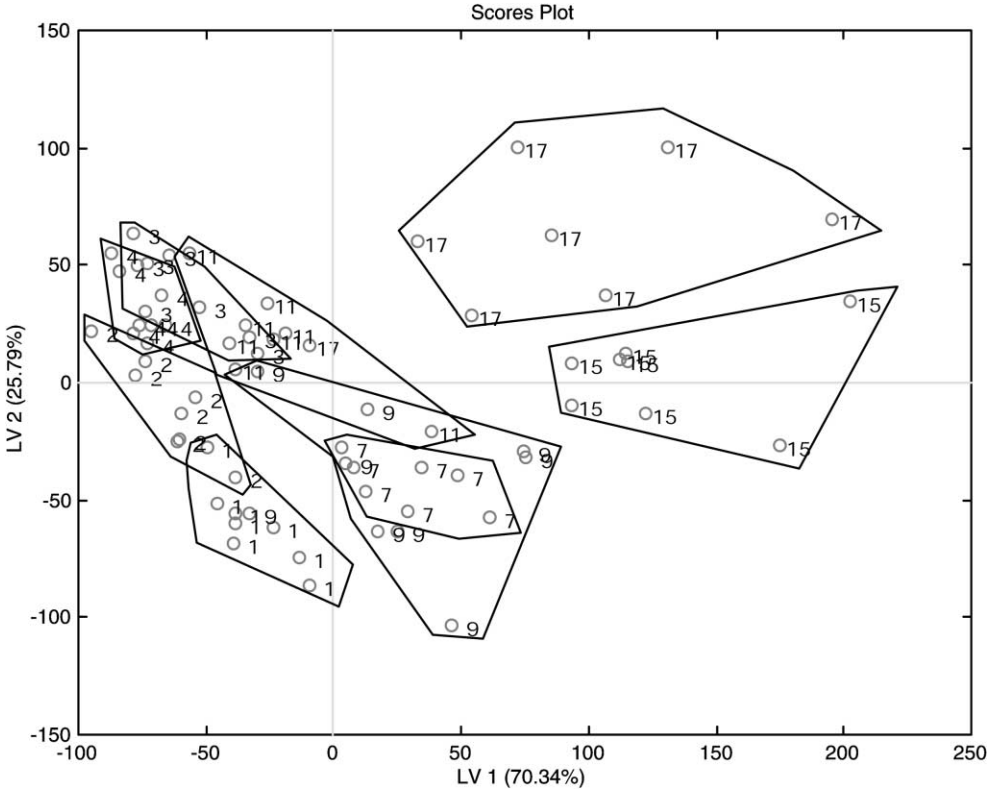


Fig. 3. Plot of the first two latent variables of the PLS-DA for the FreshSense data. The temporal evolution from the day 1 to 7 emerges, a folding back effect for days 9 and 11 is also shown.

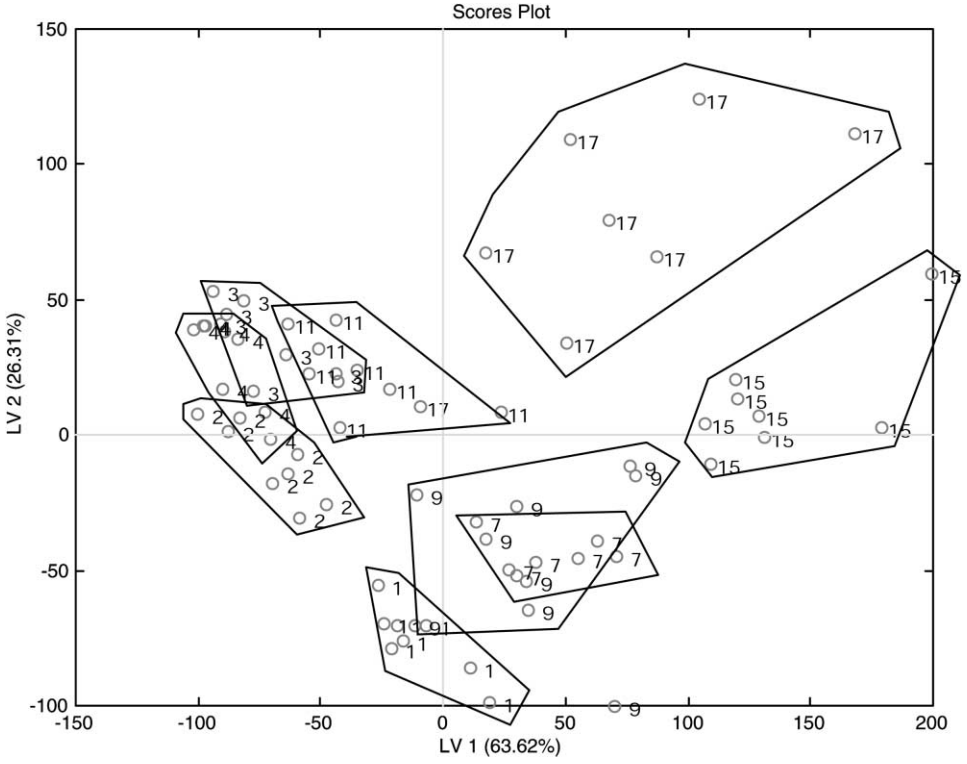


Fig. 4. The combination of the two instruments is shown through the plot of the first two latent variables of the PLS-DA. Features of both the instruments are preserved. For instance, the clustering of day 1 (from LibraNose), the separation of days 15 and 17 (from FreshSense) and the folding back effect.

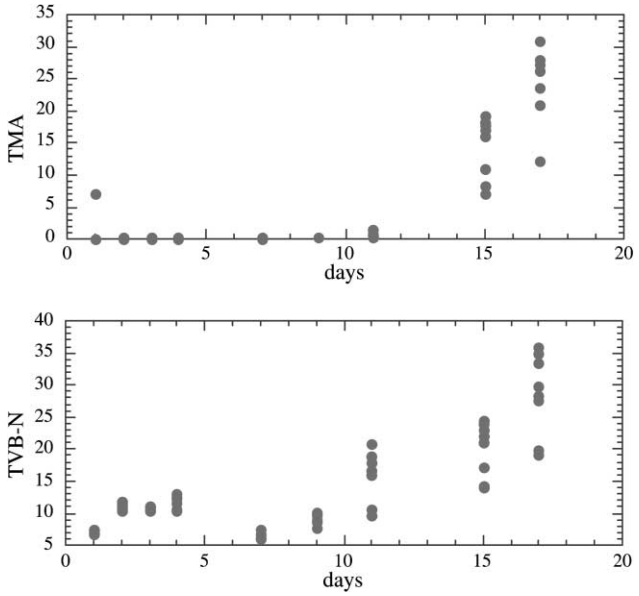


Fig. 5. Measure of TMA and TVB-N on the samples. TMA becomes important after day 11, TVB-N shows a non-monotonic behaviour during the first part of the evolution. In both the plots, the inter-class dispersion grows with the storage days.

the very fresh fish and then it increases following the behaviour of TMA.

Fig. 6 shows the plot of TMA versus TVB-N, a log–log scale has been chosen in order to avoid the different evolution of the two indicators. The plot show basically the same distribution exhibited by the electronic nose systems, namely, a straight evolution from days 1 to 4 and a folding back from 7 to 11 and then a net separation of the last days.

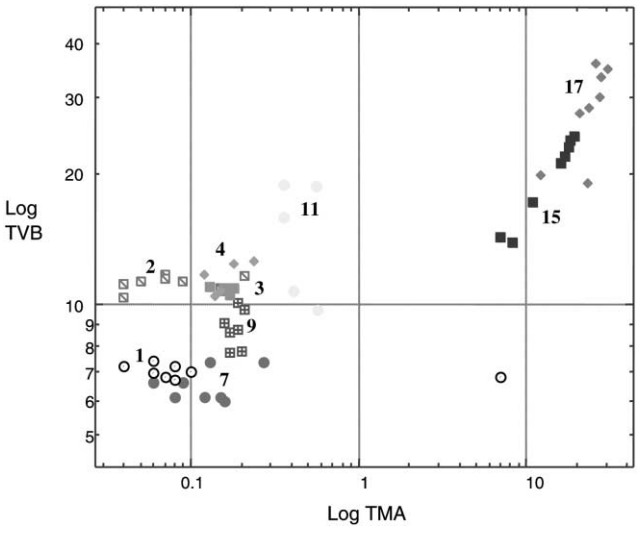


Fig. 6. The plot of TVB-N vs. TMA reveals a class distribution very similar to that achieved by the electronic noses. This result confirms that the class overlapping (a sort of folding back effect) may be considered as intrinsic to the examined samples. In order to put in evidence the different scales, a log–log plot has been utilised.

It is worth to mention that the similarity of the log–log plot with the electronic nose score plot (see Figs. 2–4) suggests that a logarithmic relationship between sensor response and volatile concentration should exists for the sensors here considered. The results of TMA and TVB-N show that the sensors are mostly correlated with these two parameters, and most of all that the evolution of the chemical composition (qualitative and quantitative) does not provide a straightforward indication of the freshness represented as storage days. This may be explained by slightly different spoilage rate of the three batches used, indicating that days of storage may not give the best information about the freshness status of the fish when different batches of fish are considered.

4. Conclusions

Freshness of cod-fish fillets has been measured with two electronic noses based on different sensors technologies and sampling systems. Both the instruments show sensitivity to the temporal variations of fish headspace. Nonetheless, the two instruments exhibited different resolution. The Libra-Nose, based on TSMR sensors with metalloporphrins coatings, gives rise to higher resolution during the first stages of evolution, while the FreshSense, based on electrochemical sensors, shows higher resolution at the end of the evolution process.

The two systems have been virtually joined together to form a unique sensor array. The merged electronic nose shows improved classification performances, reducing the amount of misclassified samples to 4%. On the other hand, this small error remains qualitatively not negligible, because samples of days 7–9 are classified as samples of day 1. This can partly be explained by the fact that different batches of fish may have slightly different spoilage rate. Measurements of TMA and TVB-N gave the evidence that this behaviour is related to the headspace composition as it evolves from the status of fresh to that of spoiled. A TMA versus TVB-N plot recovers the same class distribution given by the individual electronic noses and their combination.

The results here illustrated confirm that electronic nose systems based on different sensor technologies generally improve the performances with respect to a single technology. In this case, the better coverage of the whole spoilage processes has been achieved. It has also to be noted that non-linearity in the headspace composition evolution are responsible of the misclassification errors.

Acknowledgements

The work has been performed in the frame of the European Project ‘‘development of multi-sensor techniques for monitoring the quality of fish’’ FAIR CT98-4076.

References

[1] G. Ólafsdóttir, E. Martinsdottir, J. Oehlenschläger, P. Dalgaard, B. Jensen, I. Undeland, I. Mackie, G. Henehan, J. Nielsen, H. Nilsen, Methods to evaluate fish freshness in research and industry, Trend Food Sci. Technol. 8 (1997) 258–265.

[2] D.B. Josephson, R.C. Lindsay, G. Ólafsdóttir, Measurement of volatile aroma constituents as a means for following sensory deterioration of fresh fish and fishery products, in: D.F. Kramer, L. Liston (Eds.), Seafood Quality Determination Symposium, 10–14 November 1986, Elsevier, Amsterdam, 1986.

[3] G. Ólafsdóttir, J. Fleurence, Evaluation of fish freshness using volatile compounds — classification of volatile compounds in fish, in: Methods to Determine the Freshness of Fish in Research and Industries, Proceedings of the Final Meeting of the Concerted Action (Evaluation of Fish Freshness), AIR3CT94-2283, Nantes, France, 12–14 November 1997, Institute International du Froide Publication, Paris, 1997.

[4] R. Olafsson, E. Martinsdottir, G. Olafsdottir, T.I. Sigfusson, J.W. Gardner, Monitoring of fish freshness using tin dioxide sensors, in: J. Gardner, P. Bartlett (Eds.), Sensors and Sensory Systems for an Electronic Nose, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1992.

[5] M. Schweizer-Berberich, S. Vahinger, W. Göpel, Characterisation of food freshness with sensor array, Sens. Actuat. B 18/19 (1994) 282–290.

[6] G. Ólafsdóttir, E. Martinsdóttir, E.H. Jónsson, Rapid gas sensor measurements to predict the freshness of capelin (*Mallotus villosus*), J. Agric. Food Chem. 45 (1997) 2654–2659.

[7] C. Di Natale, J.A.J. Brunink, F. Bungaro, F. Davide, A. D'Amico, R. Paolesse, T. Boschi, M. Faccio, G. Ferri, Recognition of fish storage time by a metallophorphyrins coated QMB sensor array, Meas. Sci. Technol. 7 (1996) 1103–1114.

[8] Y. Takao, M. Nakanishi, T. Kawaguchi, Y. Shimizu, M. Egashira, Semiconductor dimethylamine gas sensors with high sensitivity and selectivity, Sens. Actuat. B 25 (1995) 380–382.

[9] J. Oehlenschläger, Suitability of ammonia-*n*, dimethylamine-*n*, trimethylamine-*n*, trimethylamine oxcide-*n*, and total volatile basic nitrogen as freshness indicators in seafoods, in: Methods to Determine the Freshness of Fish in Research and Industries, Proceedings of the Final Meeting of the Concerted Action (Evaluation of Fish Freshness), AIR3CT94-2283, Nantes, France, 12–14 November 1997, Institute International du Froide Publication, Paris, 1997.

[10] A. D'Amico, C. Di Natale, A. Macagnano, F. Davide, A. Mantini, E. Tarizzo, R. Paolesse, T. Boschi, Technology and tools for mimicking olfaction: status of the Rome Tor Vergata electronic nose, Biosens. Bioelectron. 13 (1998) 711–721.

[11] S. Sadok, R. Uglow, S. Haswell, Determination of trimethylamine oxide in seawater and small volumes of seafood tissue using flow injection analysis, Anal. Chim. Acta 334 (1996) 279–285.

PAPER III

Precision and application of electronic nose measurements for freshness monitoring of redfish (*Sebastes marinus*) stored in ice and modified atmosphere bulk storage

Guðrún Ólafsdóttir, Xiuchen Li, Hélène L. Lauzon, Rósa Jónsdóttir

III

Published in: JAFP 11, 3/4 (2002) 229-249

Precision and Application of Electronic Nose for Freshness Monitoring of Whole Redfish (*Sebastes marinus*) Stored in Ice and Modified Atmosphere Bulk Storage

Guðrún Ólafsdóttir
Xiuchen Li
Hélène L. Lauzon
Rósa Jónsdóttir

ABSTRACT. An electronic nose with electrochemical gas sensors (CO, NH_3, SO_2 and H_2S) was used as a rapid technique to monitor changes in the headspace of whole ungutted redfish (*Sebastes marinus*) stored in ice and under various conditions of modified atmosphere (MA) bulk storage (CO_2/N_2:60/40). The precision of the electronic nose measurements was determined using standard compounds (ethanol, trimethylamine, acetaldehyde and dimethyldisulfide). The response of the CO sensor, suggesting the formation of alcohols and carbonyls, increased with time for both

Guðrún Ólafsdóttir, MSc, Héléne L. Lauzon, MSc, and Rósa Jónsdóttir, MSc, are affiliated with the Icelandic Fisheries Laboratories, P.O. Box 1405, Skúlagata 4, 121 Reykjavík, Iceland (E-mail: gudrun@rf.is, or helene@rf.is, rosa@rf.is).

Xiuchen Li, PhD, is affiliated with Dalian Fisheries University, Heishijiao Street 52, Dalian, 116023 China (E-mail: zhlt@mail.dlptt.ln.cn).

The redfish storage experiment in this report was a part of the demonstration project funded by the EC (FAIR CT98-3833) called "Implementation on board of systems of atmospheres with variable composition applied to fresh fish. Continuation on shore of the modified atmosphere chain."

The evaluation of the precision of the electronic nose measurements reported herein is a part of the project "Precise predictive models to determine the shelflife of fish and fish products" funded by the Icelandic Research Council.

Journal of Aquatic Food Product Technology, Vol. 11(3/4) 2002
http://www.haworthpressinc.com/store/product.asp?sku=J030

aerobic and MA storage and correlated well with sensory analysis using the Quality Index Method. Slower spoilage rate reflected by lower intensities of sensors' responses, lower microbial counts and less TMA production were observed in MA-stored redfish compared with aerobic storage in ice.

KEYWORDS. Electronic nose, redfish (*Sebastes marinus*), modified atmosphere storage, freshness/spoilage

INTRODUCTION

Electronic noses have been introduced as rapid techniques to supplement or replace traditional odor evaluation techniques in food industries (Bartlett et al., 1997; Mielle, 1996; Jónsson et al., 1997). The concept of the electronic nose technique is to transfer the headspace of a product in a closed sampling system to a sensor array that detects the presence of specific volatile compounds in the headspace. Electronic noses based on various sensor technologies have been used for quality monitoring of fish. Ólafsson et al. (1992) used metaloxide sensors (MOS) to monitor the freshness of haddock and cod stored in ice. Changes in volatiles of cold stored trout have been monitored using amperometric sensors (Schweizer-Berberich et al., 1994). Luzuriaga and Balaban (1999a) used an electronic nose based on conducting polymers (CP) to measure decomposition in shrimp. The same authors demonstrated that an array of CP sensors could correlate the odor of salmon fillets with storage time (Luzuriaga and Balaban, 1999b). An array of metalloporphyrins-coated quartz microbalance (QMB) sensors were used to detect changes in volatile compounds in the headspace of cod fillets during storage (Di Natale et al., 1996). The electronic nose (FreshSense) used in the study reported herein is based on electrochemical gas sensors that are sensitive to volatile compounds present in high concentrations in the headspace above spoiled seafood such as alcohols, amines, and sulfur compounds. Earlier studies have shown that measurements using electrochemical sensors correlate well with classical methods to evaluate freshness and spoilage of seafood, i.e., TVB-N measurements and sensory analysis, for capelin (Ólafsdóttir et al., 1997a; 2000), herring and fresh roe (Ólafsdóttir et al., 1997b), and whole or peeled shrimp (Högnadóttir, 1999).

Sampling is a critical step in electronic nose measurements, influencing the selection of compounds detected by the sensors (Mielle and Marquis, 1999). The concentration or amount of compounds reaching the sensors is dependent

on extrinsic factors, i.e., temperature of the sample and the surroundings, ratio of sample to headspace in the sampling container, and whether static or dynamic transfer of the sample's headspace to the sensors is used. Additionally, intrinsic factors such as vapor pressure and solubility of the volatile compounds in the sample matrix and the composition or presence of other volatile compounds in the headspace influence which compounds are detected by the electronic nose. Static headspace sampling methods where no carrier gas is introduced are simple and low-cost and the main advantage is that the headspace reaching the sensors is neither distorted nor diluted and represents the composition of the original sample. Humidity is often problematic in electronic nose measurements especially for MOS and PC sensors. However, electrochemical sensors that consist of several electrodes and an electrolyte are not sensitive to humidity.

A common drawback to the long-term use of E-noses is the drift in their sensitivity with time. As the details of drift are in general not known, effective drift-compensating models can be difficult to develop for chemical sensors (Holmberg et al., 1996). Standard compounds that are typical for the samples being measured can be used for monitoring and calibration of the systems. Recent research efforts in the area of electronic nose technologies have focused on improving the selectivity, sensitivity and reproducibility of the gas sensors and on improving the hardware and software in electronic noses (Haugen and Kvaal, 1998) for their use in various areas, where monitoring of food quality is one of the most important applications. Various methods are available to monitor the freshness of fish and current research activities have focused on developing rapid methods for the fish industry (Ólafsdóttir et al., 1997c).

The use of preservation techniques to extend the shelf life of fish, such as modified atmosphere packaging (MAP), changes the microbial flora and spoilage patterns observed during storage of fresh fish (Dhananjaya and Stroud, 1994; Dalgaard et al., 1993). *Pseudomonas* sp., which produce aldehydes, ketones and esters, and *Shewanella putrefaciens*, a H_2S- and TMA-producing bacterium, are responsible for the spoilage of fish stored under aerobic conditions in ice. CO_2-packaging has a specific inhibitory effect on *S. putrefaciens* and most *Pseudomonas* sp. *Photobacterium phosphoreum*, a TMA-producing bacterium, has been identified as a specific spoilage organism in CO_2-packaged fish (Dalgaard, 1995a).

Redfish (*Sebastes marinus*) is a commercially important marine fish species. As the demand for high quality fresh redfish has increased in recent years, it is economically important to extend the shelf life of this fish species. Many studies have shown that cold storage and MA packaging storage of fish can meet this requirement (Dalgaard, 1995b; Huss, 1995). However, more knowledge is needed to understand the difference in the spoilage pattern caused by the growth of different microflora and the production of their metabolites con-

tributing to characteristic odor and quality loss in MA storage. During storage of fish the odor changes progressively through fresh, flat, sweet and stale odors and ends as putrid odor (Elliot, 1947). Numerous studies using gas chromatography have shown that during each phase of storage different volatile compounds characterize the odor (Ólafsdóttir and Fleurence, 1997). The use of electronic noses for detection of volatile compounds is of interest for rapid monitoring of food freshness.

The aim of the study reported herein was to use an electronic nose with electrochemical gas sensors to monitor microbial metabolites produced during storage of redfish (*Sebastes marinus*) in ice and under MA, and compare the results to traditional microbial, chemical and sensory methods. Furthermore, the aim was also to measure standard compounds representing the main classes of volatile spoilage compounds in fish to determine the precision of the electronic nose measurements.

MATERIALS AND METHODS

Redfish Storage Studies

Fresh whole redfish (*Sebastes marinus*) was caught south of Iceland in November 2000 and handled on board according to good practices. The fish was still *in rigor* when received at the laboratory 2 days post harvest. The fish was stored in boxes in ice aerobically or in MA bulk storage for up to 24 days at 0-2°C. Four different sample treatments of MA and ice storage were prepared. One sample group was stored in ice and another one in MA for the whole storage period. The two other groups were stored in ice or MA at the onset for 5 days and then (on day 7 after harvest) put in MA or in ice, respectively, for further storage. Approximately 20 fish were put in each box and the boxes were put into an insulated container (130 cm × 130 cm × 130 cm) and sealed. Mixture of gases (CO_2/N_2 : 60/40) were injected into the container to create the modified atmosphere (MA) storage environment for the fish. Further details relating to the experimental set-up are given by Lauzon et al. (2002).

Samples were analyzed upon receipt on day 2 and on days 7, 16, 22 and 24 post harvest by sensory evaluation, microbial and chemical analyses and by electronic nose. The fish samples used for sensory evaluation (QIM) were also evaluated by the electronic nose.

Electronic Nose Measurements

The electronic nose FreshSense (Bodvaki, Hlídarsmári 11, 200 Kópavogur, Iceland) was used for the electronic nose measurements. The instrument con-

sists of an array of electrochemical gas sensors [CO, H_2S, SO_2 (Dräger, Germany) and NH_3 (City Technology, Portsmouth, Britain)], a glass container (2.3 L) closed with a plastic lid and a PC with Labview measurement software. The static headspace sampling technique described earlier by Ólafsdóttir et al. (1997a) was slightly modified. A separate measurement chamber was installed and a pump was used to allow continuous circulation of the air between the measurement chamber and the sampling container while measuring. Measurements were taken every 10 seconds for 10 minutes. In the data analysis, the reported value (current) is calculated as the average of the 6 values of the final 1 minute measurement minus the base line that is the average of 6 values before the measurement starts.

Standard compounds measurement: Aqueous solutions of standard compounds from Merck: ethanol (1-100 mg/L), TMA(10-300 mg/L), DMDS (0.5-5 mg/L) and acetaldehyde (1-100 mg/L) were prepared in various concentrations. Twenty-five ml aliquots of four to five concentrations of each standard were put into a petri dish (diameter 8.8 cm), and placed into the sampling container (2.3 L) and closed with the plastic lid. Measurements were performed in triplicate.

Redfish measurements: The redfish samples taken from ice storage (0-2°C) were allowed to reach 7-10°C before measuring. Three fishes, approximately 400-700 g each, were measured for each storage condition, and each one was weighed and put into the glass container individually and the lid put on before measuring.

Microbiological Analyses

Upon receipt of the fish, microbial counts of the flesh were evaluated using 6 whole fish (2 fish per sample). Further sampling was done in duplicate (4 fish as 2 samples) as storage progressed. Each fish was aseptically skinned, pieces of flesh removed and comminuted in a mixer. Twenty-five grams of minced flesh were mixed with 225 mL of cooled Maximum Recovery Diluent (MRD, Oxoid) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required. Total viable counts (TVC) were assessed on modified Long and Hammer's medium (Van Spreekens, 1974) and H_2S-producing bacteria counts evaluated on Iron Agar (Gram et al., 1987), both containing 1% NaCl and spread-plated with aerobic incubation at 15°C for 4-5 days. Presumptive *Pseudomonas* counts (15°C, 4-5 days) were obtained using the modified CFC medium (Stanbridge and Board, 1994). After incubation, the plates were counted using a Darkfield Quebec Colony Counter (Spencer). The detection limit was 20 colony forming units (cfu)/g.

Chemical Analysis

Evaluation of TMA content was done with the rest of the flesh mince prepared for microbiological analyses, by flow injection gas diffusion (FIGD) as described by Ruiz-Capillas and Horner (1999).

Sensory Analysis

Eleven trained panelists at the Icelandic Fisheries Laboratory evaluated the freshness of whole ungutted redfish using the Quality Index Method (QIM) scheme for redfish described by Martinsdóttir and Arnason (1992).

Data Analysis

Microsoft Excel 97 was used to calculate means and standard deviation for all replicates and correlations. The data was also analyzed by principle component analysis (PCA) using The Unscrambler (CAMO A/S). In all PCA runs two principal components and full cross validation were used and the data was standardized with 1/sdev.

RESULTS AND DISCUSSION

Characteristic Response of the FreshSense Sensors to Standard Compounds

Ethanol, acetaldehyde, trimethylamine (TMA) and dimethyldisulfide (DMDS) were selected to represent the main classes of volatile spoilage compounds in fish to evaluate the sensitivity, selectivity, repeatability and reproducibility of the electronic nose.

Sensitivity and selectivity: Figure 1 shows typical response curves for the sensors. An example is given of the responses of the CO sensor to three different concentrations of aqueous solutions of ethanol during repeated measurements. The intensity of the responses of the sensors increased with increasing concentration of standard compounds and their responses were linear (response vs. concentrations) for the various standard compounds and concentration range measured.

The sensors showed different selectivities and sensitivities to the standard compounds selected. Table 1 shows the sensitivity (the slope of the line, nA/[mg/L]), intercept, and correlation of the linear best fit for ethanol, acetaldehyde, TMA and DMDS for the respective sensors that respond to these compounds. The CO sensor is the only sensor responding to ethanol, while TMA is only detected by the NH_3 sensor. The results also show that both the

FIGURE 1. Response of the CO sensor to three different concentrations (10, 50, 100 ppm [mg/L]) of aqueous ethanol solutions during repeated measurements.

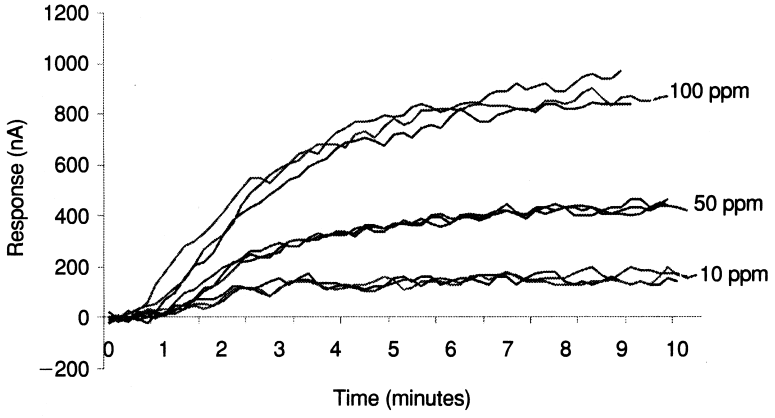


TABLE 1. The parameters of the best linear fit obtained from measurements of three replicates of each concentration of the standard compounds vs. the response of the sensors: (a) ethanol (1, 5, 10, 50, 100 mg/L); (b) acetaldehyde (1, 5, 10, 50, 100 mg/L); (c) TMA (50, 100, 200, 300 mg/L); (d) DMDS (0.5, 1, 5 mg/L).

Sensor	Compounds	Sensitivity (nA/[mg/L])	Intercept	Correlation (R^2)	n
CO	Ethanol	8.3	49	0.9899	3
CO	Acetaldehyde	39	−32	0.9898	3
H_2S	Acetaldehyde	8.3	93	0.9953	3
NH_3	TMA	6.0	−207	0.941	3
CO	DMDS -	152	68	0.966	3
H_2S	DMDS -	87	121	0.984	3
SO_2	DMDS -	41	2.3	0.981	3
NH_3	DMDS -	50	22	0.977	3

CO and H_2S sensors respond to acetaldehyde and all the sensors respond to DMDS.

The sensitivity of the sensors depends on the nature of the compounds and the respective sensors. For example the sensitivity of the CO sensor to acetaldehyde is higher than to ethanol as seen by the higher value for the slope (Table 1). Acetaldehyde has higher vapor pressure than ethanol and is thus present in higher concentration in the headspace. On the other hand the CO sensor has the highest sensitivity for DMDS although its vapor pressure is lower than for acetaldehyde. This may be explained by the low solubility of DMDS in water resulting in the formation of an undiluted DMDS droplet on the surface. All the sensors respond to DMDS and it could therefore be valid as a standard for simultaneous calibration of all the sensors (Högnadóttir, 1999).

The lowest concentration that the sensors can measure was evaluated for the CO sensor as an example by plotting %CV (percent coefficient of variability = (standard deviation/mean) %) vs. response (nA) for different concentrations of ethanol in water. With decreasing concentration the %CV increases as can be seen in Figure 2. The limit of detection (LOD) is defined as three times the signal-to-noise ratio of the blank signal (99% confidence level) (Keith et al., 1983). This means that %CV for LOD is 30%. In Figure 2 it can be seen that at 30%CV or higher the response of the CO sensor is less than 100 nA. This means that the LOD is less than 10 mg/L for ethanol (see Table 2). The LOD for all the sensors has not been evaluated precisely but further work is needed

FIGURE 2. Percent CV vs. response (nA) of the CO sensor for repeated measurements of different concentrations of aqueous ethanol solutions (10, 50, 100, 200 mg/L).

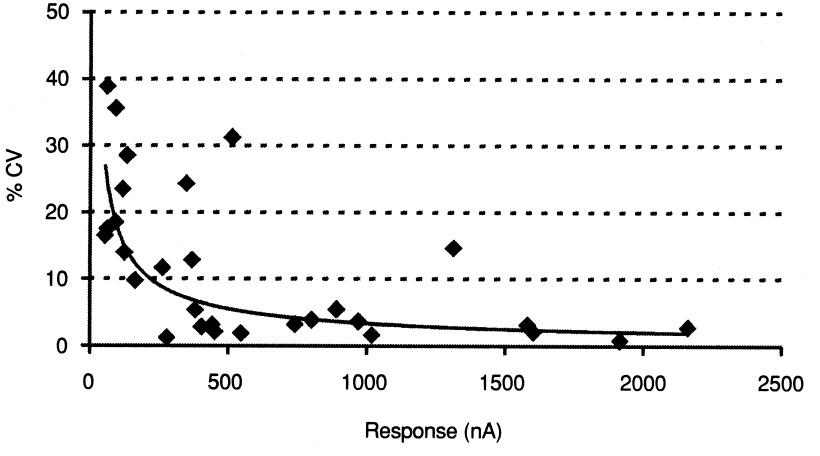


TABLE 2. Repeatability of the FreshSense sensors to different concentrations of standard compounds in aqueous solutions.

Compounds	Concentration (mg/L)	Sensor	Average response (nA)	SD	%CV	n
Ethanol	10	CO	164	16.0	9.8	3
	50	CO	442	14.1	3.2	3
	100	CO	891	48.7	5.5	3
Acetaldehyde	10	CO	364	16.7	4.6	3
	50	CO	1703	21.8	1.3	3
	100	CO	4016	239.8	5.9	3
TMA	100	NH_3	217	5.9	2.8	3
	200	NH_3	867	38.5	4.4	3
	300	NH_3	1767	40.5	2.3	3
DMDS	0.5	H_2S	168	11.2	6.6	3
	1	H_2S	212	26.5	12.5	3
	5	H_2S	74	13.6	2.4	3

measuring standards at lower concentrations. Previous studies done at the IFL show that the sensors can in general detect low ppm concentrations of aqueous solutions of both TMA and DMDS (Högnadóttir, 1999).

Precision: The precision of the electronic nose was studied by measuring the repeatability and reproducibility. The repeatability of the measurements was evaluated by calculating the %CV when measuring each concentration of the four standard compounds in triplicate. The %CV for repeated measurements is in all cases less than 10% (Table 2) except for the DMDS solutions which have slightly higher %CV, possibly because of the insolubility of DMDS in water.

Repeatability represents the short-term precision and is measured with the same sample the same day, while reproducibility gives the long-term precision and is determined by measuring different samples on different days. To evaluate the reproducibility of the electronic nose, the CO sensor was selected and measurements of different concentrations of ethanol was evaluated over one

year period (February 2000 to April 2001). The results show that the %CV is less than 30%.

Monitoring of the performance of the sensors over one year period showed that drift in sensitivity is only apparent at the end of the lifetime of the sensors, which is about one year. Measurements done over short period of time, like the study reported herein, have about 10% CV which is similar as repeated measurements done within the same day.

Determination of the precision of the redfish measurements was difficult because the replicates of redfish samples were different fishes which varied in weight (400-700 g). The temperature during sampling at ambient increased from 7°C-10°C, which can also introduce variability because of the high temperature influence on the vapor pressure of very volatile compounds. Despite those variations in sample weight and temperature, the %CV (% coefficient of variation) for the repeated measurements (data not shown) was < 60% for the redfish samples when the response was higher than 100 nA.

Redfish Storage Experiments–Results of Electronic Nose, TMA Measurements, Microbial Counts and Sensory Analysis

Redfish stored in ice and MA: The responses of all the sensors, TMA production and microbial counts, were highest in ice storage (Figures 3a and 4a), and the signals increased with time. The CO sensor had the highest response of the sensors for all storage conditions (Figures 3a, b, c, d). This suggests the production of short chain alcohols and carbonyls in all samples which is in agreement with gas chromatography analysis of fish stored both aerobically and carbon dioxide packed (Lindsay et al., 1986). The results of sensory evaluation showed that the QI scores increased linearly with storage time (R^2 = 0.97-0.99). The MA stored fish had the slowest spoilage rate as seen by the lowest value of the slope (0.68) for the line QI scores vs. days (Figure 3b). The slopes for the QI score vs. days for ice and ice-MA were very similar (0.75 and 0.78, respectively), but the MA-ice stored fish appeared to have the fastest spoilage rate (slope = 0.81).

During the first 7 days, the responses of all the sensors were low and no differences in response patterns were observed for redfish stored under the various conditions. The response patterns of the sensors changed with storage conditions after day 7 and the different response patterns for ice and MA-stored fish were obvious (Figures 3a and b). The overall responses of all the sensors in MA-stored fish were much lower than ice-stored fish which is in agreement with lower production of microbial metabolites in MAP stored fish as reported previously (Lindsay, 1986; Dalgaard, 1995). In ice storage the response of the CO sensor increases from the beginning but appears to decline after 16 days of storage. The levelling off in the response of the CO sensor at

the end of storage period in ice may be reflecting the depletion of certain substrates that the microbial flora utilizes, i.e., 5-6 carbon sugars (Lindsay et al., 1986; Gram and Huss, 1996). In MA-stored fish the response of the CO sensor was much lower than for the ice-stored fish, but increased continuously throughout the storage period, indicating that enough substrate was available for production of short chain alcohols and carbonyls. The increase in the response of the CO sensor suggests that the production of alcohols and carbonyls contribute to the characteristic sweet odor which is mainly causing the loss of quality observed in MA-stored fish. The low responses of the other sensors

FIGURE 3. Electronic nose (FreshSense) measurements (◆CO, ■SO_2, ▲NH_3, ×H_2S), TMA (dotted lines) (-△-), and QI scores (dotted lines) (-◇-) of redfish stored for 22 days in (a) Ice; (b) MA; (c) MA-Ice; (d) Ice-MA.

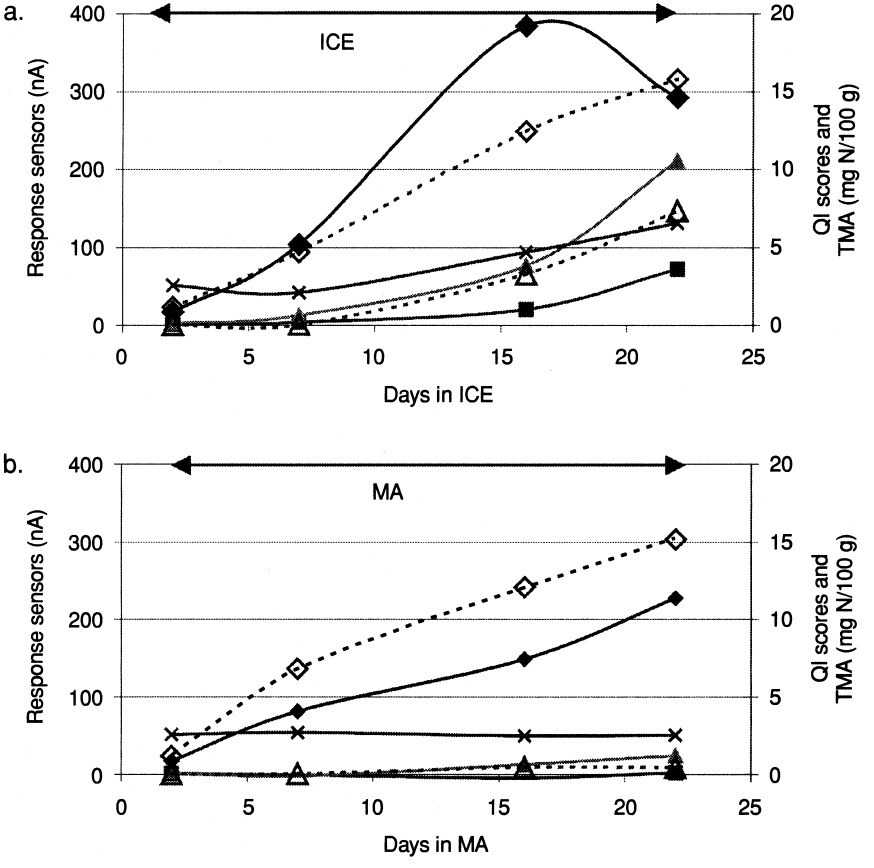
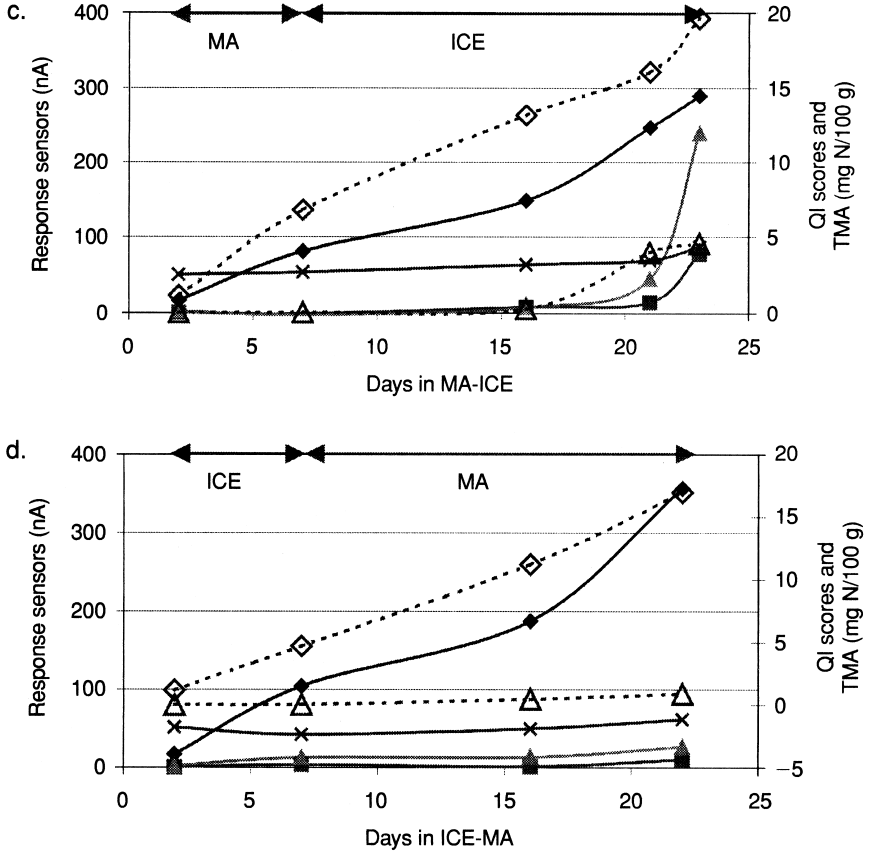


FIGURE 3 (continued)



suggest that sulfur containing compounds and amines are not important in MA. The increase in the response of the NH_3 sensor occurred after 5 days of storage but the values were much lower in MA-stored fish compared to those of iced fish (Figures 3a and b). The response of the NH_3 sensor and the TMA measurements had very good correlation in ice storage ($R^2 = 0.99$) while the correlation was lower in MA ($R^2 = 0.89$). The lower correlation in MA may be because the levels of amines were very low and the %CV is typically much higher for concentrations which are at the limit of detection for the sensors.

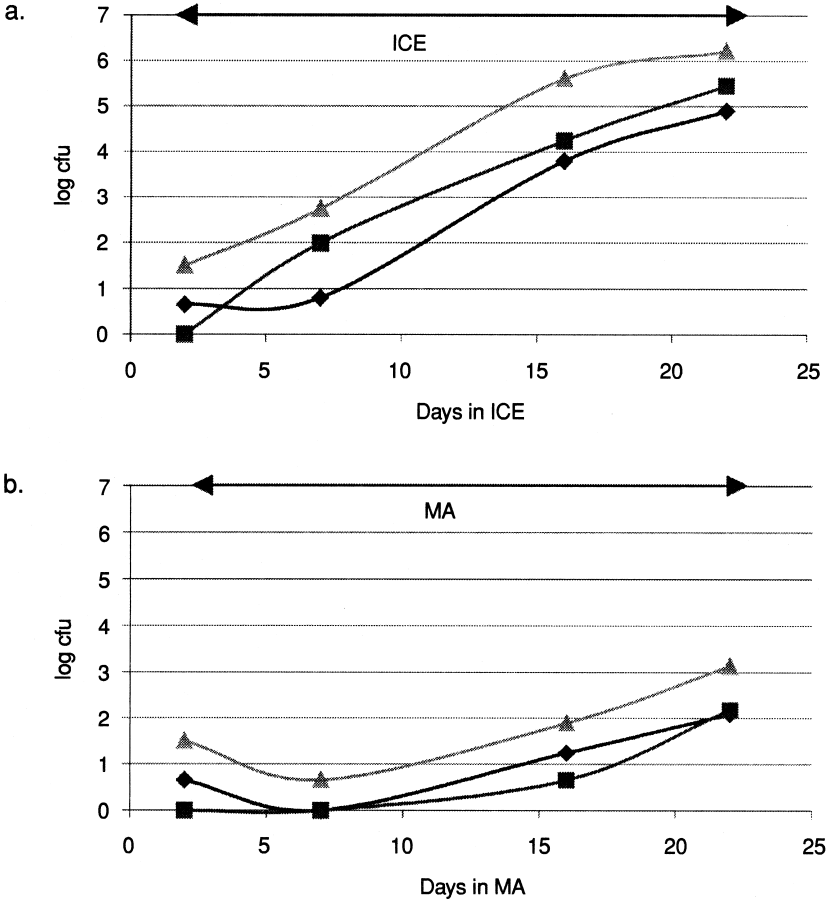
Considerable increases in the responses of the SO_2 and H_2S sensors were noticed in ice-stored fish after day 16 (Figure 3a). The increase in the response of these sensors in ice storage suggests the utilization of different amino acids

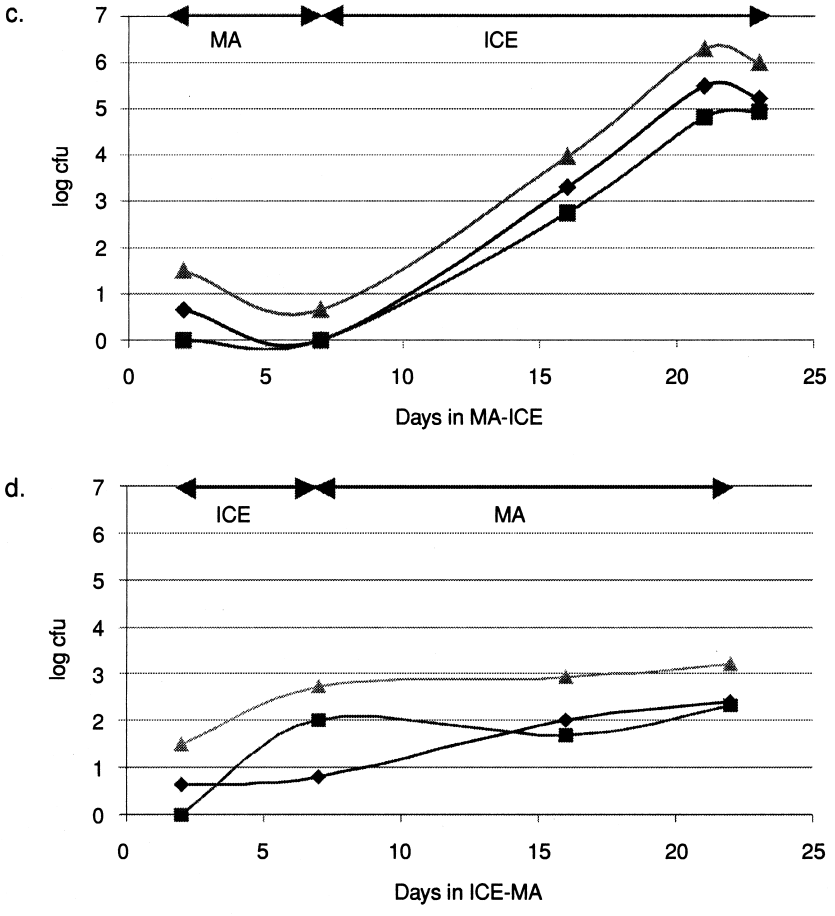
(i.e., cysteine and methionine) for microbial growth resulting in the formation of volatile sulfur compounds. No increase in the responses of SO_2 and H_2S sensors were observed for MA-stored fish at the end of the experiment (22 days) (Figure 3b) indicating that compounds contributing to putrid odor were not present or they were present in levels lower than the electronic nose can detect. In fact, additional measurements have been done in our laboratory using purge and trap sampling techniques and gas chromatography. The results show that low amounts of dimethyldisulfide and dimethyltrisulfide are present in MA stored redfish indicating that the H_2S producers or other bacteria may be part of the microbial spoilage flora in MA.

The responses of the sensors appear to reflect the production of microbial metabolites. At the same time as the increasing responses were observed for the sensors in iced fish, a continuous increase was seen in the growth of *Pseudomonas* sp. throughout storage (Figure 4a). Also, there was a slower development of H_2S-producers, expected to be mainly *S. putrefaciens*, during the first 5 days of storage, after which a steady proliferation occurred. Previous studies (Lauzon, 2000) on specific spoilage organisms (SSO) isolated from aerobically stored fish have been done using a sterile fish model (supplemented fish juice) inoculated with *Pseudomonas* sp. and *S. putrefaciens* strains. The electronic nose was used to determine the spoilage patterns obtained for the different bacterial mixtures. The results demonstrated that the addition of *Pseudomonas* II sp. to a co-mixture of *Pseudomonas* I sp. and *S. putrefaciens* caused an increase in the response of the CO, SO_2, H_2S and NH_3 sensors. This stressed the importance of this group of bacteria as a main spoiler of fresh fish stored aerobically at refrigerated temperatures, while *S. putrefaciens* was found to be a late spoiler. It should be mentioned that the proportion of *Pseudomonas* sp. in iced redfish was about 9% of the total microflora (TVC, 15°C) on day 19, while that of H_2S-producers was 2.5%. Interestingly, the increased TMA production after day 16, as measured by a chemical method, coincides with the increased response observed for NH_3 sensor and the later development of H_2S-producers (Figure 3a). The low response of the sensors to MA-stored fish (Figure 3b) corresponds to the slow development of the microflora under these conditions as seen in Figure 4b. Based on the results of earlier studies on MA packed fish, higher levels of TMA were expected as a result of the growth of *Photobacterium phosphoreum*, which has been found to be an intensive TMA producer and the main SSO in MAP fish (Dalgaard, 1995). Unfortunately, direct counts could not be obtained in our study and therefore the information about the importance of this bacterium in MA-stored redfish is not known. However, it is obvious that a different spoilage profile developed in MA-stored fish since very low counts were obtained at the end of the storage time.

Redfish stored in ice-MA and MA-ice: The ice-MA and MA-ice samples were stored in either ice or MA only during the first 5 days, and then stored in MA or ice, respectively, and sampled after 9 and 15 days (on days 16 and 22, post harvest). The trend in the responses of the sensors, TMA and QI scores (Figures 3c and d) and microbial counts (Figures 4c and d) depends on the storage conditions. The spoilage pattern for the MA-ice (Figure 3c) was similar to ice-stored fish (Figure 3a), but it was delayed. Similarly, the responses of the sensors in ice-MA stored fish (Figure 3d) showed the same tendency as

FIGURE 4. Microbial counts (■Pseud., ◆H_2S-prod. and ▲TVC-15°C) of redfish stored for 22 days in (a) Ice; (b) MA; (c) MA-Ice; (d) Ice-MA.





MA storage (Figure 3b). The CO sensor's response increased considerably during storage in the ice-MA fish (Figure 3d), and on day 22 the response of the CO sensor was almost as high as for iced-stored fish on day 16 (Figure 3a).

Lower responses of the sensors were observed at the end of storage in ice-MA stored fish (Figure 3d) compared with the MA-ice treatment (Figure 3c). Similarily, lower TMA content and bacterial loads (difference of 3 logs/g) were found in the flesh at the end of the storage time in samples of the ice-MA samples (day 22) compared with the MA-ice treatment on day 21 (Figures 4c and d). This demonstrates the fact that different spoilage patterns developed in the treatment with MA storage after a short aerobic storage in ice (ice-MA) compared with MA-ice. Therefore, the initial aerobic storage of the ice-MA

samples appears to contribute to the onset of the spoilage process. This can be seen by the rapid development of *Pseudomonas* sp. up to day 7, which was then hindered under the following MA storage. This early growth coincided with the increasing response of the CO sensor up to day 7, which slowed down when the fish was stored further under MA. On the other hand, the proliferation of H_2S-producers was delayed under initial aerobic storage, probably because of the hindering effect of *Pseudomonas* sp. towards *S. putrefaciens* (Lauzon, 2000; Gram and Melchiorsen, 1992). Progressive growth of H_2S-producers was observed under further MA storage. As demonstrated by the development of the microflora, growth of H_2S-producers contributed to 16% of the total microflora (TVC) at the end of the storage time on day 22. The increased response observed for the CO sensor after day 16 could be due to H_2S-producers as well as to the development of bacteria unhindered by MA, such as *P. phosphoreum*. However, based on earlier studies of Dalgaard (1995) *P. phophoreum* was expected to cause a noticeable increase in TMA but this was neither measured chemically nor by the electronic nose. To conclude it can be said that the inhibitory effect of MA was not sufficient to control the production of volatile compounds, such as alcohols and aldehydes, that typically contribute to the sweet spoilage odor of fish observed in MA-stored fish.

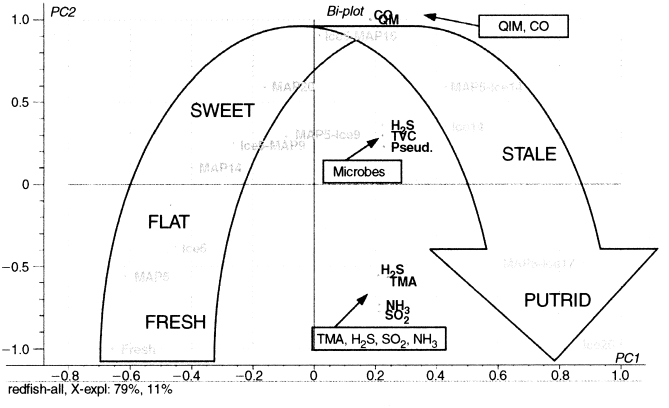
The initial MA storage in MA-ice stored fish has a hindering effect on *Pseudomonas* sp. and they were found in lower levels than H_2S-producers under further aerobic storage. Simultaneously, a lower response of the CO sensor was observed compared with the ice-stored fish. H_2S-producers proliferated steadily under aerobic storage and accounted for 16% of the total microflora at the end of the storage time. A sudden TMA production was detected from day 16, reaching levels comparable to those observed for iced fish after a similar storage period in ice. Similarly, a considerable increase in responses of the NH_3 and SO_2 sensors occurred after day 21. The correlation between TMA and the NH_3 sensor is high ($R^2 = 0.99$) for both ice and MA-ice storage conditions. It can be concluded that the initial MA storage mostly contributed to the reduced production of microbial metabolites measured by the CO sensor, which is probably related to the depressed growth of *Pseudomonas* sp. in the MA-ice treatment

PCA analysis of electronic nose, TMA measurements, microbial counts and sensory analysis of all samples: PCA can simplify complex and diverse relationships of observed variables by contracting information into a smaller number of principal components based on correlations among them. The application of the PCA to a data set provides two quantities, namely the score and the loading. The score plots, limited to the most significant PCs, give a visual image of the data set of an electronic nose. The loading is used to evaluate the contribution that each variable carries to the total information of the data set (Esbensen et al., 1996). PCA analysis of all the data from electronic nose mea-

surements (CO, H_2S, SO_2 and NH_3 sensors), TMA analysis, microbial counts (TVC, H_2S-producers and *Pseudomonas*) and sensory analysis (QI scores) for all storage conditions was done to study the main trend in the overall data (Figure 5). The first principle component (PC1) explains 79% of the variance in the data set and PC2 explains 11%. PC1 represents the spoilage of the samples and with increasing storage time from left to right along PC1, the odor of the redfish changes from fresh to putrid. The CO sensor showed high positive loading on PC2, and the other three sensors had certain positive loadings on PC1 and some negative loadings on PC2. The fresh samples are separated from the other samples because all the sensors have low values for the fresh samples and therefore the scores of the fresh sample is low. The samples ice20 and MA5-ice17 had high loadings for the H_2S, SO_2, NH_3 and TMA which indicate high levels of putrid spoilage compounds in these samples.

Similar to the CO sensor, QIM shows high loading on PC2 and is located close to the CO sensor on the plot (Figure 5). This demonstrates that the CO sensor gives similar information as QI scores which showed a linear increase with time for all the storage conditions. A high correlation between these variables was found for all the storage conditions (R^2 = 0.97-0.98) except for ice storage (R^2 = 0.91) which is lower because of the decline in the CO response at the end of the storage as mentioned before. The fresh samples are far from QIM loading on the plot because they have low QI scores. On the other hand the putrid samples are also far from QIM, therefore additional information

FIGURE 5. PCA bi-plot of the FreshSense measurements, TMA, microbial counts and QIM data from experiment of redfish stored in ice, MA, ice-MA and MA-ice. Sample scores are labelled with the storage condition and days of treatment. Loadings of variables (CO, NH_3, SO_2 and H_2S), microbial counts (TVC, H_2S-prod., Pseud.) and QIM are shown in boxes.



about the onset of putridity of the samples are given by the NH_3, SO_2 and H_2S sensors. The results also show that samples stored for 5 days in ice and MA are similar, but 9 days later the ice 14 and MA 14 samples are very different (Figure 5) indicating that MA is efficient in slowing down the spoilage rate. Samples stored in MA for longer period have a sweet-like character while samples stored in ice for longer time have a more stale and putrid-like character and are located on the right side of the plot.

The microbial methods have similar loadings, and the correlation of these variables is high for all storage conditions (R^2 = 0.92-0.99) except for the H_2S-producers in ice-MA which did not correlate well with neither TVC nor *Pseudomonas*. This can be explained by the slower development of H_2S-producers during the initial 5 days of storage in ice, after which a steady proliferation occurred as explained before. The loadings of the NH_3, H_2S and SO_2 sensors are similar and the correlation between the NH_3, H_2S and SO_2 sensors for the ice and MA-ice storage conditions is high ($R^2 > 0.91$). On the other hand the correlation between the H_2S and SO_2 sensors and all the other measurement variables in MA and ice-MA storage is not high because of the very low responses of the H_2S and SO_2 sensors under these conditions.

CONCLUSIONS

The electrochemical sensors showed good selectivity, sensitivity and repeatability when measuring standard compounds (TMA, ethanol, acetaldehyde and DMDS) that are representative of spoilage compounds in fish. The results indicate that these sensors can be used efficiently to monitor volatile compounds that contribute to the spoilage odor in fish. The results of measurements of redfish stored under various conditions showed that the electronic nose can detect the onset of spoilage of redfish. The response of the CO sensor increased earlier than the other sensors and it was most likely responding to short chain alcohols (i.e., ethanol) and aldehydes that form during early storage. The CO sensor appears to give similar information as the sensory evaluation (QI scores). The responses of the NH_3, H_2S and SO_2 sensors increased at later stages of storage. These sensors are sensitive to amines and sulfur compounds, respectively, that typically form in high concentrations at the end of the storage life.

The freshness of redfish depends on storage time and storage conditions. Slower spoilage rate reflected by lower intensities of sensors' response was observed in MA-stored redfish compared with other storage conditions (ice, ice-MA and MA-ice).

Each of the sensors of the electronic nose appear to give similar information as the traditional measurement techniques. For example, the CO sensor and the

QIM method are highly correlated for all storage conditions. The NH_3 sensor and TMA measurement give similar information while the combination of all the sensors can explain the growth of the *Pseudomonas* spp. and H_2S-producers. The SO_2 and H_2S sensors appear to give information about H_2S-producers possibly *S. putrefaciens* which has been suggested as a late spoiler in iced stored fish. Therefore, the results indicate that the rapid electronic nose measurements can give more information about the quality of the fish than each of the individual classical measurements, i.e., sensory evaluation, microbial counts and TMA analysis.

The electronic nose is promising for applications in food industries where rapid measurements with no sample preparation are needed to detect microbial spoilage. However, because of the sensitivity of the electrochemical sensors, the electronic nose needs to be located in an environment free of gases or organic solvents. In addition, careful control of the environment and monitoring and control of the temperature of samples are needed during measurements.

REFERENCES

Bartlett, H.N., Elliott, J.M., and Gardner, J.W. 1997. Electronic noses and their application in the food industry. Food Technol. 51(12):44-48.

Dhananjaya, S. and Stroud, G. 1994. Chemical and sensory changes in haddock and herring stored under modified atmosphere. Int. J. Food Sci. Technol. 29:575-583.

Dalgaard, P., Gram, L., and Huss, H.H. 1993. Spoilage and shelf life of cod fillets packed in vacuum or modified atmosphere. Int. J. Food Microbiol. 19:283-294.

Dalgaard, P. 1995a. Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int. J. Food Microbiol. 26:319-333.

Dalgaard, P., 1995b. Modelling of microbial activity and prediction of shelf life for packed fresh fish. Int. J. Food Microbiol. 26:305-317.

Di'Natale, C., Brunink, J.A.J., Bungaro, F., Davide, F., d´Amico, A., Paolesse, R., Boschi, T., Faccio, M., and Ferri, G. 1996. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. Meas. Sci. Technol. 7:1103-1114.

Elliot, R.P. 1947. Evaluation of surface pH as a freshness index for fish fillets. Food Research 12(2):87-91.

Esbensen, K., Schönkopf, S., and Midtgaard, T. 1996. Multivariate analysis in practice. Camo A/S, Norway.

Gram, L. and Huss, H.H. 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33:121-137.

Gram, L. and Melchiorsen, J. 1992. Interaction between fish spoilage bacteria *Pseudomonas* sp. and *Shewanella putrefaciens* in fish extracts and on fish tissue. J. Appl. Bacteriol. 80:589-595.

Gram, L., Trolle, G., and Huss, H.H. 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int. J. Food Microbiol. 4: 65-72.

Haugen, J.E. and Kvaal, K. 1998. Electronic nose and artificial neural network. Meat Science 49:273-286.

Högnadóttir, Á. 1999. Application of an electronic nose in the fish industry. MS thesis. Department of Food Science, University of Iceland.

Holmberg, M., Winquist, F., Lundström, I., Davide, F., Di Natale, C., and D´Amico, A. 1996. Drift counteraction for an electronic nose. Sensors and Actuators 35-36: 528-535.

Huss, H.H. 1995. Quality and quality changes in fresh fish. FAO Fisheries Technical Paper, No. 348, FAO, Rome.

Jonsson, A., Winquist, F., Schnürer, J., Sundgren, H., and Lundström, I. 1997. Electronic nose for microbial quality classification of grains. Int. J. Food Microbiol. 35: 187-193.

Keith, L.H., Crummett, W., Deegan J., Libby, R.A., Taylor, J.K., and Wentler, G. 1983. Principles of environmental analysis. Anal. Chem. 55:2210-2218.

Lauzon, H.L., Stefánsson, G., Jónsson, G.P., and Sveinsdóttir, K. (2002). Sensory quality and shelf life of MAP red fish (*Sebastes marinus*) fillets as affected by previous MA bulk storage of whole fish. J. of Aqua. Food Prod. Tech. 11(3/4):251-273.

Lauzon, H.L. 2000. Shelf-life and bacteriological spoilage of American Plaice (*Hippoglossoides platessoides*). *In:* Seafood in Health and Nutrition, Transformation in Fisheries and Aquaculture: Global Perspectives, A Science Tech. Book, St. John's, NF, Canada, pp. 327-354.

Lindsay, R.C., Josephson, D.B., and Ólafsdüttir, G. 1986. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. *In:* Proceedings of an International Symposium; University of Alaska Sea Grant Program, Anchorage, A.K., Kramer, D.E. and Liston, J. (Eds.) Elsevier Science Publishers: Amsterdam, The Netherlands, pp. 221-234.

Luzuriaga, D.A. and Balaban, M.O. 1999a. Evaluation of odor decomposition in raw and cooked shrimp: Correlation of electronic nose readings, odor sensory evaluation and ammonia levels. *In:* Electronic Nose and Sensor Array Based System, Design and Applications. Hurst, W.J. (Ed). Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. Technomic Publ. Co., Inc., USA, pp.177-184.

Luzuriaga, D.A. and Balaban, M.O. 1999b. Electronic nose odor evaluation of salmon fillets stored at different temperatures *In:* Electronic Nose and Sensor Array Based System, Design and Applications. Hurst, W.J. (Ed). Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. Technomic Publ. Co., Inc., USA, pp.162-169.

Mielle, P. 1996. Electronic noses: towards the objective instrumental characterization of food aroma. Trends Food Sci. Technol. 7:432-438.

Mielle, P. and Marquis, F. 1999. An alternative way to improve the sensitivity of electronic olfactometers. Sens. Act. B58:526-535.

Martinsdüttir, E. and Arnason, H. 1992. Redfish. *In:* Nordic Industrial Fund, Quality Standards for Fish. Final Report Phase II, pp. 21-35.

Ólafsson, R., Martinsdüttir, E., Ólafsdüttir, G., Sigfússon, T.I., and Gardner, J.W. 1992. Monitoring of fish freshness using tin oxide sensors. *In:* Sensors and Sensory

Systems for an Electronic Nose. Gardner, J.W. and Bartlett, P.N. (Eds.) Kluwer, Dordrecht, The Netherlands, pp. 257-272.

Ólafsdüttir, G., Martinsdüttir, E., and Jünsson, E.H. 1997a. Rapid gas sensor measurements to determine spoilage of capelin (*Mallotus villosus*). J. Agric. Food Chem. 45(7):2654-2659.

Ólafsdüttir, G., Högnadóttir, Á., and Martinsdüttir, E. 1997b. Application of gas sensors to evaluate freshness and spoilage of various seafoods. *In:* Methods to Determine the Freshness of Fish in Research and Industry; IIR/IIF. Paris, France. pp. 100-109.

Ólafsdüttir, G., Martinsdüttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I.M., Henehan, G., Nielsen, J., and Nilsen, H. 1997c. Methods to evaluate fish freshness in research and industry. Trends in Food Sci. and Tech. 8: 258-265.

Ólafsdüttir, G. and Fleurence, J. 1997. Evaluation of fish freshness using volatile compounds–classification of volatile compounds in fish. *In:* Methods to Determine the Freshness of Fish in Research and Industry; IIR/IIF. Paris, France. pp. 55-69.

Ólafsdüttir, G., Högnadóttir, Á., Martinsdüttir, E., and Jónsdóttir, H. 2000. Application of an electronic nose to predict total volatile bases in capelin (*Mallotus villosus*) for fishmeal production. J. Agric. Food Chem. 48(6):2353-2359.

Ruiz-Capillas, C. and Horner, W.F.A. 1999. Determination of trimethylamine-nitrogen and total volatile basic nitrogen in fresh fish by flow injection analysis. J. Food Sci. and Agric. 79:1982-1986.

Schweizer-Berberich, P.M., Vaihinger, S., and Göpel, W. 1994. Characterization of food freshness with sensor arrays. Sensors and Actuators 18-19:282-290.

Stanbridge, L.H. and Board, R.G. 1994. Letters in Appl. Microbiol. 18: 327-328.

Van Spreekens, K.J.A. 1974. The suitability of Long & Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. Archiv für Lebensmittelhygiene. 25 (10):213-219.

PAPER IV

Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction

Guðrún Ólafsdóttir, Hélène L. Lauzon, Emilía Martinsdóttir, Kristberg Kristbergsson

IV

Submitted for publication

Characterization of Volatile Compounds in Chilled Cod *(Gadus morhua*) Fillets by Gas Chromatography and Rapid Detection of Quality Indicators by an Electronic Nose

Gudrun Olafsdottir[*Ψ], *Rosa Jonsdottir*[Ψ], *Hélène L. Lauzon*[Ψ], *Joop Luten*[δ] *and Kristberg Kristbergsson*[γ]

[Ψ]Icelandic Fisheries Laboratories, Skulagata 4, 101 Reykjavík, Iceland

[δ] Norwegian Institute of Fisheries and Aquaculture Research, Muninbakken 9-13, 9291 Tromsö, Norway

[γ c]Department of Food Science, University of Iceland, Hjardarhagi 2 - 6, 107 Reykjavik, Iceland

TITLE RUNNING HEAD: Volatile compounds as quality indicators for cod fillets

[*] To whom correspondence should be addressed. E-mail: gudrun@rf.is. Tel.: +354 5308600, Fax: +354 5308601

ABSTRACT

Volatile compounds in cod fillets packed in styrofoam boxes were analyzed during chilled storage (0.5 °C) by GC-MS and GC-O to screen potential quality indicators present in concentrations high enough for detection by an electronic nose. On day 12 when the fillets were rejected by sensory analysis, ketones, mainly 3-hydroxy-2-butanone, were detected in the highest level (33 %), followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%) and a low level of esters (<1%). The electronic nose's CO sensor showed increasing response with storage time coinciding with the production of ethanol and 2-methyl-1-propanol that were produced early in the storage, followed by the production of 3-methyl-1-butanol, 3-methyl-butanal, 2,3-butandiol and ethyl acetate. Lipid derived aldehydes, like hexanal and decanal were detected in similar levels throughout the storage time and contributed to the overall sweet odors of cod fillets in combination with other carbonyls (3-hydroxy-2-butanone, acetaldehyde, 2-butanone, 3-pentanone and 6-methyl-5-heptene-2-one).

KEYWORDS: Volatile compounds; quality indicators; gas chromatography; electronic nose; cod fillets

INTRODUCTION

The use of electronic nose based on different sensor technologies has been suggested for the rapid detection of quality related volatile compounds for various food products (*1-3*) including monitoring the quality and spoilage processes in fish (*4-12*). Gas sensors that are commonly used in electronic noses are non-selective towards individual compounds, but show sensitivity towards certain classes of compounds. This property induces their potential for monitoring quality and the onset of spoilage associated with varying levels of different classes of volatile compounds produced in fish during storage (*13-14*). The progression of characteristic odors in fish during chilled storage caused by microbial growth is well documented (*15-17*) and has been associated with the formation of volatile compounds produced by the main spoilage organisms (*18-21*). Both single compounds and a combination of compounds representing the different changes occurring during storage have been suggested as indicators for freshness and spoilage (*22-31*).

Alcohols, aldehydes, ketones, amines and sulfur compounds have been identified in different seafood products during chilled storage and related to the growth of specific spoilage organisms (SSOs) (*32-34*). However, because of the interaction of the microorganisms (*35-36*) and the complexity of the dynamic spoilage changes the levels of the volatile compounds may vary and they are often not detected until the products are overtly spoiled. Storage temperature, packaging and the inherent composition of available nutrients in the fish influence the growth and spoilage potential of the dominating SSO. The SSO in chilled fish are mainly Gram-negative, psychrotrophic bacteria like *Pseudomonas* spp. and *Shewanella* spp. (*37*). Pseudomonads typically cause sweet, malty, fruity, and onion like odors contributed by alcohols, carbonyls, esters and sulfur compounds (*18,19*), while *S. putrefaciens* can produce more potent odors related to high levels of sulfur compounds and fishy odors because of reduction of TMAO to TMA (*38,21*). *Photobacterium phosphoreum*, is also of interest as SSO in chilled fish and has been identified as an active TMA producer in iced cod, and in cod fillets (*39-40*) and more recently in modified atmosphere packed fish (*41-43*). Oxidative processes occurring during storage of fish will also result in the accumulation of both saturated and unsaturated aldehydes that contribute to the development of rancid cold store flavors (*44-45*).

The information on the identity and quantity of volatile compounds present in the headspace during storage of fish is essential when selecting sensors in an array for quality monitoring of fish (*14*). It should be clearly stated that in many cases, the most abundant volatiles may have minimal odor significance. However, they may be indicative for the degradation processes occurring in the products like the production of metabolites by the SSOs. Therefore, the potential ability of the electronic nose to monitor quality is not necessarily directly related to detecting the most influential aroma active compounds contributing to off odors, since these may be present in too low concentrations.

The aim of the study was to screen the most abundant volatile compounds produced by SSOs that could be used as quality indicators for chilled fillets. This study was done in parallel to extensive storage studies on cod fillets (*46*) where the SSOs were monitored and the sensory shelf-life was determined. Herein are the results from gas chromatography analysis of cod fillets stored in Styrofoam boxes under chilled conditions (0.5 °C) and comparison was made with electronic nose analysis, TVB-N and pH measurements. The results obtained will be useful to characterize the spoilage potential of the SSO and to guide the future development of the electronic nose technique based on selecting sensors that are sensitive to the indicator compounds identified in chilled cod fillets.

MATERIALS AND METHODS

The fish was processed by a conventional process three days after catch as described earlier (*46*). The process includes mechanical filleting, deskinning and packing of fillets in styrofoam (EPS, expanded polystyrene) boxes (160 x 400 x 263 mm) lined with a plastic bag. The fillets were stored at 0.5 °C until analyzed. Gas chromatography analysis (GC-MS and GC-O), electronic nose, TVB-N and pH measurements were performed on days 4, 7, 10, 12 and 14 after catch

GC-MS measurements. The headspace from approximately 500 g of fish (1-2 fillets) in a glass container (2.3L, Ø 17 cm) was collected by an air pump sampling (ALPIN-2, Air sampler, METEK) by sweeping volatiles from the surface of the fish. Aqueous heptanoic acid ethyl ester solution (10 µL/L) was used as an external standard, using an amount of 1 mL in a 25 mL beaker (Ø 3.5 cm) located in the glass sampling container.

Quantification of volatiles PAR (peak area ratio) was based on comparison of peak area to the peak area of the external standard. Sampling time was 2 hours at room temperature (RT) (20 to 22 °C) using a flow rate of approximately 100 mL/min. Duplicate headspace samples were collected on 250 mg Tenax 60/80 (Alltech, IL) in stainless steel tubes (Perkin-Elmer, Buchinghamshire, UK). Volatiles were thermally desorbed (ATD 400, Perkin-Elmer, Buchinghamshire, UK) from the sampling tubes prior to separation on a DB-5ms column (30 m × 0.25 mm i.d. × 0.25 µm, J&W Scientific, Folsom, CA, USA) using a GC-MS (HP G1800C GCD, Hewlett-Packard, Palo Alto, CA, USA). Helium was used as a carrier gas and 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 injector temperature was 250°C and the detector temperature was 280°C. The mass detector ion range was 35-300 m/z.

GC-O measurements. Samples were prepared by weighing 100 ± 2 g of fish fillets and 100 ± 5 g of saturated aqueous solution of NaCl into a 250 mL and blended manually in a round bottom flask. Saturated NaCl solution (200 ± 5 g) was prepared as a reference sample. Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 1 mL of 10 µL/L aqueous solution of the standard to the 200 g fish and $NaCl_{sat}$ solution to evaluate the extraction based on the FID response. The sample was purged at RT with nitrogen at about 100 mL/min for 2.5 hours. Volatiles were collected on 150 mg Tenax in a Pasteur pipette. Each sample was prepared in duplicate. Volatiles were extracted from the Tenax traps with 1 mL diethyl ether. The sample was concentrated by passing nitrogen over the solution leaving a small amount of sample (20-30 µL) and 1µL was then injected splitless onto the column. 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 ionization detector (FID) and an ODO-1 olfactory detector outlet (SGE International Pty. Ltd, Australia). Nitrogen bubbled through water to add moisture was used to drive the sample to the sniffer port. Two trained panelist with former experience in describing seafood odor sniffed the effluent. Description of each odor and its duration in time was recorded and the intensity evaluated using a category scale with a description of intensity of odor at each score (*47-48*); 0 none; 0.5 thresholds or just detectable; 1

slight; 2 little; 3 moderate; 4 strong and 5 very strong. GC-O analysis were done on days 4, 10 and 17.

Identification of volatiles was done by matching retention indices (RI) of ethyl esters and mass spectra of samples with authentic standards (Sigma-Aldrich Chemical Co. St. Louis, MO, USA). Tentative identifications were based on the MS library data in the HP GCD ChemStation software (Hewlett Packard Co, 1997) and manually checked against literature sources and the database Flavornet (*49*).

Electronic nose measurements The electronic nose instrument (FreshSense, Bodvaki-Maritech, Kópavogur, Iceland) is based on four electrochemical gas sensors: CO, H_2S, SO_2 (Dräger, Luebeck, Germany); NH_3 (City Technology, Portsmouth, Britain). The measurements were performed at room temperature as described earlier (*9*). Approximately 500g of fish fillets were weighted and tempered for 30 minutes in the sampling container (2.3 L, Ø 17 cm). Measurement time was 5 minutes and temperature of fillets reached 8 to 12°C before measurements started. All measurements were done in duplicate.

TVB-N and pH measurements. Total volatile basic nitrogen content (TVB-N) was measured by the steam distillation method described by Malle and Poumeyrol (*50*). The pH was measured in 5 grams of mince moistened with 5 mL of deionized water. The pH meter was calibrated using buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

Data analysis. Statistical analysis was carried out with the Number Cruncher Statistical Software (NCSS) 2000, using ANOVA. In case of statistical significance, the Duncan's multiple range was performed. An effect was considered significant at the 5% level.

RESULTS AND DISCUSSION

Volatile compounds in cod fillets analyzed by GC – potential quality indicators

Volatile compounds detected in the highest level by GC-MS and dominating SSO: TMA, 2-methyl-1-propanol (isobutanol) and 3-hydroxy-butanone (acetoin) were detected in the highest amount in cod fillets and their levels increased with storage time (Table 1).

However, dynamic changes in their level were noticed, which could be related to the growth of the spoilage bacteria. An initial decline of the pH (Fig. 1) could be explained by the *post mortem* changes in chilled fish which are initially dominated by autolytic activity, including degradation of nucleotides, accumulation of hypoxhanthin, lowering of pH and endogenous enzyme activity (*51*). Proliferation of the microflora follows these changes and development of microbial metaboliltes contributing to spoilage changes as seen by increased pH value coinciding with the detection of trimethylamine (TMA) and higher TVB-N values on day 12 (Fig. 1). TMA has been suggested as a spoilage indicator since its level increases at later stages of storage (*29*), while DMA (dimethylamine) which forms enzymically very early after harvest of fish has been suggested as a freshness indicator along with its precursor TMAO (trimethylamine oxide) (*24*).

In the parallel study on sensory and microbiological changes of packed cod fillets (*46*) the shelf-life was determined as 12 days by sensory analysis using the Torry scheme (*16*). TMA and acetoin which were present in the highest concentration on day 12 (Table 1) have earlier been associated with dominating *P. phosphoreum* growth (*40*). Increasing levels of acetoin were detected on day 7 coinciding with a rapid *P. phosphoreum* growth reaching counts of log 7.2 CFU/g at sensory rejection. *P. phosphoreum* was identified as the main SSO in the cod fillets based on its high counts throughout the storage time and was found in the highest levels (12.6 %) at the end of shelf-life, while *Pseudomonas* spp. and H_2S-producing bacteria represented 4.9 % (log 6.6 CFU/g) and 7% (log 7 CFU/g), respectively, of the total microflora at sensory rejection. (*46*).

The variation in GC analysis for duplicate samples was high for the very volatile compounds like TMA and ethanol (Table 1), most likely because of their high breakthrough volume on the Tenax and also the fact that in some cases compounds with RI<173 coeluted and made both identification and quantification difficult. This was the case for ethanol, TMA and dimethyl sulfide as well as 2-methyl-1-propanol which was also identified as pentane in the MS library database (Table 1).

Characteristic odor of cod fillets: The overall odor of the fillets was observed as a mild and sweet odor that became sour, fruity and stale during storage and much less potent odors developed on the fillets than are generally observed on whole fish. Characteristic odor development during storage of the fillets could be explained by the odor description and odor scores for the individual compounds analyzed by GC-O (Table

1). The odor impact of the different compounds was also evaluated based on their amount in the samples measured by GC-MS and the reported odor threshold (OU (Odor Unit) = PAR / odor threshold x 1000) to determine the potential contribution of the individual compounds at sensory rejection on day 12. The GC-O scores were generally low but increased with storage time in agreement with the increased level of the compounds measured by GC-MS. The values and ranges of the GC-O odor scores detected on days 4, 10 and 17 are shown in Table 1. The most potent odor described as spicy, flowery, sweet, onion and mushroom-like, was detected at RI 457 in all samples during storage, with increasing odor scores with time of 3 (moderate) to 4.8 (very strong). This complex odor is most likely contributed by coeluting compounds in low concentrations which were not detected by GC-MS. Aldehydes appeared to influence the characteristic odors of the fresh fillets throughout the whole storage time and decanal had the highest odor impact as evaluated by OU (1200-24000), but 3-methyl-butanal and heptanal, probably in combination with methional and 2-heptenal that are known to coelute at the same retention time (*12*), received the highest odor scores of the aldehydes by GC-O (Table 1). TMA is a potent odorant with a characteristic fishy, dried fish, ammonia-like odor as detected by GC-O and received high odor scores. The odors of esters and sulfur compounds were detected by GC-O at advanced spoilage on day 17.

The calculated odor unit values for the volatiles detected by GC-MS on day 12 when the end of shelf-life was determined by sensory analysis are shown in Table 1. The reported odor threshold of TMA varies but values as low as 0.3 ppb have been reported (*52*) (Table 1). The calculated OU based on the different odor thresholds showed that TMA had by far the highest odor impact on day 12 with two to three orders of magnitude higher OU than the straight chain aldehydes (nonanal and decanal) that appeared to have a high odor impact as well, based on their odor threshold values. The high levels of acetoin suggested that this compound could contribute to the onset of spoilage odors in cod fillets (Table 1). The odor of acetoin has been described as butter- cream like (*49*), but a mild, sweet-sour like odor was detected by GC-O (Table 1). However, acetoin appeared to have less odor impact (OU=119) than the aldehydes (decanal and nonanal) because of its higher odor threshold (800 ppb), even though it was present in much higher levels. Aldehydes have generally low odor thresholds (~ 0.1 to 4.5 ppb) and therefore their odor impact was greater than the alcohols (odor thresholds range 0.4 to 270 ppm)

and the ketones (odor thresholds range 0.05 to 70 ppm) although their overall levels were less (Table 1). It appears that even though the alcohols like 2-methyl-1-propanol and aldehydes like decanal were detected in lower levels on day 12 than on day 10 ($p<0.05$), the presence of the more potent odorants like TMA on day 12 overpowered the odor and contributed to the sensory rejection of the fillets. Ethyl acetate was first detected on day 12 and probably also influenced the overall spoilage odor development based on its OU value (0.1-120) in combination with the other compounds detected on day 12. Additionally, the change in the pH value on day 12 may have influenced the overall odor perception and synergistic effects may have occurred. For example, TMA has been noted for intensifying fishiness by a synergistic action with certain volatile unsaturated aldehydes derived from autoxidation of polyunsaturated fatty acids (*53*). The possible influence of other compounds present in lower levels like the unsaturated autoxidatively derived aldehydes should not be overlooked, but the sampling techniques used in our study were not sensitive enough to allow detection of these compounds. The main aim was to detect the compounds present in the highest concentration in the headspace for evaluation of potential quality indicators for electronic nose detection.

Alcohols, carbonyls, esters and acids. The dynamic changes in the levels of the most abundant alcohols, esters, acids and carbonyls are demonstrated in Figs. 2 and 3. Ethanol was detected in high levels initially, followed by an increase in 2-methyl-1-propanol, 3-methyl-1-butanol, 2,3-butandiol, 3-methyl-butanal, and ethyl acetate (Fig. 2). The early detection of the ethanol and 2-methyl-1-propanol is of interest to monitor the initial changes related to the loss of freshness before the obvious spoilage signs appear. However, their levels did not increase continuously with time.

The initial production of ethanol in spoilage of fish has been related to the utilization of carbohydrate sources, while the formation of branched-chain alcohols and aldehydes like 2-methyl-1-propanol, 3-methylbutanol and 3-methyl-butanal probably originate from degradation of valine and leucine, respectively (*28*). The branched chain aldehyde, 3-methyl butanal, is characterized by a malty and caramel like odors (*54*), which was perceived as a sweet, caramel and fish fillet like odor by GC-O (Table 1). The corresponding alcohol 3-methyl-1-butanol, and 2-methyl-1-propanol exhibit alcoholic and fruity odours. 3-methyl -1-butanol was first detected on day 10 and significant continuous increases ($p<0.05$) were seen with time. Levels of 2-methyl-l-propanol were

much higher but as discussed above its level fluctuated with time. The flavor thresholds of the alcohols are high compared with the carbonyls and their odors were not detected by GC-O and therefore they probably did not contribute much to the overall odor of the fillets as seen by the low OU value on day 12 (Table 1). On the basis of odor evaluation 3-methyl-butanal in combination with acetaldehyde, methional, 1,5-octadiene-3-one, 2,6-nonadienal and 2,4-decadienal were determined as character impact odorants of boiled cod and the malty flavour of 3-methyl butanal was suggested to be mainly responsible for the malty off flavor defect of boiled cod by Milo and Grosch (*55*).

The formation of acetic acid and a decline in the ethanol level were observed on day 10 followed by the detection of ethyl acetate at the end of the shelf-life on day 12 and a sickenly sweet odor detected by GC-O on day 17 and identified as ethyl butanoate. The formation of esters suggested the activity of *Pseudomonas fragi* (*18*). At the end of the shelf-life on day 12 the counts of *Pseudomonas spp*. reached log 6.6 CFU/g (data from the parallel study) (*46*) and increasing level of ethyl acetate was seen on day 14 and much higher levels on day 17 at overt spoilage (Table 1). Esters have low odor thresholds and are known to contribute to sweet and fruity spoilage odors at advanced stages of spoilage. Similarly, ethanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 3-hydroxy-2-butanone, ethyl acetate and butanoic acid ethyl ester were the most abundant volatiles in the headspace of haddock stored in ice associated with the growth of *Pseudomonas* spp. in an earlier study on packed haddock fillets stored under chilled conditions (*56*). *Pseudomonas* species have also been found responsible for the formation of volatile sulfides, alcohols (3-methyl-1-butanol, 1-penten-3-ol) and ketones (2-butanone) contributing to the stale and putrid off odors in fish because of amino acid and lipid degradation (*18, 19*).

Much higher levels of 3-methyl-1-butanol, 2,3-butandiol, 2-methyl-1-propanol, TMA, ethyl acetate and acetoin were detected on days 14 and 17 compared to day 12 when the end of sensory shelf-life was reached (Table 1). The late development of the spoilage indicators is in agreement with studies on spoilage indicators for cultured and wild sea bream stored in ice for 23 days (*31*). TMA, 3-methyl-1-butanol, 1-penten3-ol, piperidine, methanethiol, dimethyl disulfide, dimethyl trisulfide, and acetic acid were suggested as spoilage indicators and the increase in the levels of most of the compounds were detected

around day 10 of storage, however, methyl mercaptan and dimethyl trisulfide appeared to accumulate later when the products were already spoiled.

Ketones: The formation of microbially derived acetoin was characteristic for the spoilage of chilled cod fillets as discussed above. Levels of acetoin increased earlier than TMA and therefore, it is more useful to monitor the loss of freshness as an early indicator of spoilage. Acetoin can be formed from carbohydrate sources via pyruvate and diacetyl, however the mechanism of its formation in fish is not well known but acetoin and diacetyl have been suggested as early indicators of spoilage in beef (*57*). The concentration of acetoin was much higher than the other lipid derived ketones detected like 2-butanone and 3-pentanone and carotenoid derived 6-methyl-5-heptene-2-one (Fig 3) that were present in cod fillets throughout storage but no obvious increase occurred until at the end of shelf-life and during continued storage (Fig. 3). Ketones can influence the overall odor because of their typical odors and their low odor thresholds (Table 1). Butanone has a butterscotch odor and pentanone a sweet fruity odor (*58*), but a sweet, caramel like odor was detected by GC-O for 3-pentanone (Table 1).

Aldehydes: The straight chain lipid derived aldehydes, hexanal, heptanal, nonanal, decanal and undecanal were detected in the cod fillets throughout the storage time. Their levels did not appear to increase until after the end of shelf life was reached (Table 1). Therefore, they did not appear to be useful as indicators of spoilage during chilled storage of the cod fillets, but aldehydes have been suggested as indicators of spoilage in fatty species (*45*). These compounds exhibit green, fatty, soapy, tallowy odors and hexanal is characterized by green odor (58). The aldehydes most likely contributed to the overall mild and sweet odors of the fillets since their odor threshold is low and therefore they are likely to have high odor impact as seen by the high odor unit for these compounds (Table 1). Based on the GC-O it is suggested that the lipid derived ketones in combination with 3-methyl-butanal and aldehydes like hexanal, nonanal and decanal, contributed to the characteristic sweet, caramel, and flowery odor of the cod fillets. Oxidation of fatty acids contributes to the rancid odors of fish with the formation of aldehydes like hexanal, 2,7-heptadienal, and 2,4,7-decadienal (*44, 59*). The unsaturated aldehydes were not detected by GC-MS in this study using the surface stripping sampling of the volatiles.

Odor of fresh cod fillets is typically characterized by a very mild and pleasant, marine like odor and aroma active compounds contributing to the fresh fish odors are present in

very low concentrations (*14*). Species specific odors of fresh fish are contributed by long chain alcohols and carbonyl compounds like 1,5-octadien-3-ol and 2,6-nonadienal, respectively, that are derived from polyunsaturated fatty acids (*60*). These compounds are more pronounced on the skin than in the muscle and were not detected in the fillets using the sampling conditions herein. However, an earthy and potato-like odor was detected by GC-O at the same retention time as heptanal which is most likely caused by low levels of methional and 4-heptenal eluting at the same retention time (*12*). These compounds were identified earlier as the most potent odorants in boiled cod (*55*).

Electronic nose and GC-MS analysis of the main classes of compounds in cod fillets during storage

Quality indicators: The sum of the peak area ratio (PAR) for all the volatile compounds detected in cod fillets by GC-MS showed that the total amount of volatiles increased with storage and alcohols, ketones and TMA (amines) were present in the highest amount (Table 2). On day 4 the alcohols were the most abundant of the volatiles (29%), mainly contributed by ethanol. Again on day 7 the alcohols were still the most abundant volatiles (45%) and 2-methyl-1-propanol was detected in the highest level. The ketones (36%) increased considerably on day 10 with the development of acetoin and acid (2%) was first detected on day 10. At sensory rejection on day 12 the ketones were detected in the highest level (33 %) followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%) and low level of esters (ethyl acetate) (<1%). Esters and acids were not detected during early storage and sulfur compounds were only detected in low levels (Table 2) as expected since these compounds are typically produced at advanced spoilage of seafood (*18-20, 27, 14, 32*). TMA was the most abundant volatile compound at advanced spoilage on day 17 and alcohols and ketones were also in high levels but in addition the level of esters had increased considerably comprising 8% of the total PAR (Table 1).

Miscellaneous classes of compounds: Although alcohols, aldehydes, ketones, acids, sulfur compounds, esters and acids are primarily of interest as spoilage indicators of fish, other classes of compounds were also present in the headspace, which may have an impact when measuring the total headspace with electronic noses. The concentration of the straight chain alkanes (nonane, decane and undecane) appeared to be similar throughout storage. Additionally, numerous branched chain alkanes were identified by

the MS library database, but their RIs (retention indices) were not confirmed and therefore, they were classified with the group "unknown" which represented compounds that remained unidentified. The alkanes will not influence the responses of the electrochemical sensors of the electronic nose and are not considered of interest as quality indicators since they are not aroma active.

Compounds classified as "others" appeared to increase with storage time. Among these compounds were some odorous compounds like piperidine which was tentatively identified. Piperidine has been reported earlier in low levels in fish and has been associated with 1,5-diaminopentane (cadeverine) (*61*). Piperidine was earlier suggested as quality indicator of sea bream (*31*). The terpene derivative limonene was also classified with "other" compounds but did not show an increasing trend with time. The origin of limonene in fish has been related to the diet derived from plant sources (*31*). Limonene has low odor threshold and a fresh lemon odor was detected by GC-O analysis, suggesting that it may have an impact on the overall odor of fish fillets (Table 1).

The aromatics detected were mainly benzene derivatives. Styrene and chloroform were most abundant and were found to increase with storage. Styrene was also identified in wild and cultured sea bream packed in polystyrene boxes during chilled storage (*31*). The odor of styrene is described as kerosene like and has been associated with off odors in surimi based products related to the growth of yeasts (*62*). However, it is speculated that both styrene and chloroform could be originating from the Styrofoam boxes, however this was not confirmed. Tainting from the packaging may be of concern when monitoring spoilage changes with an electronic nose, if the sensors are sensitive to the respective compounds. Therefore, it is very important to analyze the total headspace by gas chromatography and understand the origin of the suggested quality indicating compounds prior to the selection of suitable sensors for monitoring the relevant spoilage changes.

Electronic nose measurements: The results of the electronic nose measurements showed that the response of the CO sensor was much higher than the other sensors' responses (H_2S, SO_2 and NH_3) and significant increase ($p<0.05$) was first observed between days 7 and 10, and thereafter a continuous increase occurred (Fig. 4). Earlier studies have shown that the main classes of spoilage indicator compounds present in the headspace of fish can be estimated based on the individual sensor responses (*63, 9*).

Selected standard compounds representing the main classes of compounds causing spoilage odors showed that the CO sensor is sensitive to alcohols, aldehydes and esters. The NH_3 sensor is sensitive to amines and the H_2S and SO_2 sensors can detect sulfur compounds. The comparison of the electronic nose measurements and the GC analysis has some drawbacks related to the different sampling techniques used. Therefore, the results are only a semiquantitative approach to screen the major changes in the composition of the volatiles and the electronic nose responses can be partly explained by comparison to the compounds identified in the highest concentration by the GC-MS.

Based on the electronic nose responses it appears that only the CO sensor was useful to monitor the changes in volatiles during storage. The increasing PAR for alcohols, aldehydes and esters (Table 2) could explain the increasing CO sensor response with time based on its sensitivity towards these classes of compounds. It is of interest that much higher responses were observed for the CO sensor in an earlier study of haddock fillets stored under identical conditions as the cod fillets at the end of shelf-life. This can be explained by higher levels of alcohols and esters produced by the SSO in the haddock fillets (*56*). Although *P. phosphoreum* was also identified as the main SSO in the haddock fillets, high counts of *Pseudomonas* spp were observed at sensory rejection. The pseudomonads are not able to reduce TMAO to TMA (*64*) and therefore, they may have contributed to the higher levels of alcohols because of their metabolism and need for hydrogen acceptors, hence favouring the production of alcohols. Therefore, the dynamic spoilage changes and development of volatiles are dependent on the combination of the dominating SSO.

The detection of the high level of acetoin produced in cod fillets was not achieved by the electronic nose since none of the sensors is sensitive to ketones. The ketones, mainly acetoin, increased continuously with storage (Fig. 3) and appeared to be promising indicators for cod fillets packed in styrofoam boxes. Improvements should be made to include selective sensors in the electronic nose for the detection of ketones.

The NH_3 sensor's response increased first on day 14 although not significantly because of the high standard deviation. The high standard deviations for replicate samples for both the electronic nose and GC analysis, was partly caused by the influence of the temperature on the very volatile compounds during sampling (*56, 65*). Although high levels of TMA were detected by GC–MS on day 12 in agreement with high TVB-N

level (48.5 mgN/100g), the NH_3 sensor was not sensitive enough to detect this. Higher responses for the NH_3 sensor were observed in earlier studies for both shrimp and capelin but in these products TVB-N levels were higher than in cod fillets (*66-67, 63, 8, 68*).

The low response of the H_2S and SO_2 sensors suggested that the H_2S-producing bacteria were not important in the development of spoilage odors in packed cod fillets in agreement with the GC-MS analysis. Dimethyl sulfide was detected on day 4 suggesting that this compounds was most likely not associated with microbial spoilage but rather reflected the feeding conditions as has been suggested by others (*69*). Sulfur compounds like hydrogen sulfide and methylmercaptan, which are produced by microbial degradation of fish constituents (*70-71*) were not detected, but GC-O analysis on day 17 indicated that dimethyl trisulfide was present in the spoiled fillets (Table 2). Sulfur compounds contribute to potent spoilage odors because of their low odor thresholds (Table 2). Earlier studies using the FreshSense electronic nose have shown increasing response of the H_2S and SO_2 sensors at advanced spoilage of whole capelin and redfish (*66, 8-9*), but response to fillets of cod and haddock is generally low (*56, 72*).

CONCLUSION

Analysis of volatile compounds during storage of packed cod fillets by gas chromatography showed that acetoin and TMA were produced in the highest and increasing amounts coinciding with the growth of *P. phosphoreum* which was identified as the dominating SSO in the parallel study. The response of the electronic nose's CO sensor was explained by the increasing level of alcohols during storage like ethanol, 2-methyl-1-propanol and 3-methyl-1-butanol in addition to the presence of aldehydes and the formation of esters at the end of the shelf-life. At sensory rejection of the packed cod fillets on day 12, the ketones were detected in the highest level (33 %) followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%) and a low level of esters (ethyl acetate) (<1%). It is suggested that selective sensors for the detection of these classes of compounds could be used for monitoring spoilage changes in different fish products because similar volatile compounds emerge in the products. The CO sensor is useful for detecting incipient spoilage since its levels increased significantly between days 7 and 10. None of the sensors in the electronic nose was sensitive to ketones and

acids and therefore selective sensors for these components would be useful to monitor spoilage of cod fillets in addition to a more sensitive sensor for the detection of TMA. Increased sensitivity of existing methods is required for monitoring of quality deterioration and the early detection of microbially produced compounds. Low levels of sulfur compounds in the cod fillets suggested that *S. putrefaciens* was not very important in the spoilage of chilled cod fillets stored in styrofoam boxes.

However, because of the complexity of the spoilage processes caused by the diversity of the microflora and their different spoilage potential, it is likely that fixed values to determine the end of shelf-life or the quality of fish fillets based on electronic nose responses or amount of the main classes of compounds will have to be developed for each product and the respective storage conditions.

ABBREVIATIONS USED

GC-MS, gas chromatography-mass spectrometry; GC-O, gas chromatography-olfactometry; IFL, Icelandic Fisheries Laboratories; ATD, automated thermal desorber; RI, retention index; TMA, trimethyl amine; TVB-N, total volatile basic nitrogen; OU, odor unit; PAR, peak area ratio; SSO, specific spoilage organisms.

ACKNOWLEDGMENT

The authors thank The Icelandic Centre for Research for partly financing the project. The staff of IFL is thanked for their valued contribution in chemical, microbial and sensory analysis of samples.

REFERENCES

(1) Schaller, E.; Bosse, J.O.; Escher, F. 'Electronic noses' and their application to food. *Lebensm.-Wiss. Technol.* **1998**, *31*, 305-316.

(2) Gardner, J.W., Bartlett, P.N. Electronic noses. Principles and applications, Oxford University Press: Oxford, England, 1999.

(3) Haugen, J.E. Electronic noses in food analysis. In *Headspace Analysis of Food and Flavors: Theory and Practice*; Rouseff, R.L., Cadwallader, K.R., Eds.; Kluwer Academic / Plenum Publishers: New York, 2001; pp. 43-57.

(4) Ólafsson, R.; Martinsdóttir, E.; Ólafsdóttir, G.; Sigfússon, Þ.I.; Gardner, J.W. Monitoring of fish freshness using tin oxide sensors. In *Sensors and Sensory Systems for an Electronic Nose*; Gardner, J.W., Bartlett, P.N., Eds.; Kluwer Academic Publ.: Amsterdam, The Netherlands, 1992; pp. 257-272.

(5) Schweizer-Berberich, M.; Vahinger, S.; Göpel, W. Characterization of food freshness with sensor array, *Sens. Act.* **1994**, *B, 18-19*, 282-290.

(6) Di Natale, C.; Brunink, J.A.J.; Bungaro, F.; Davide, F.; d´Amico, A.; Paolesse, R.; Boschi, T.; Faccio, M.; Ferri, G. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. *Meas. Sci. Technol.* **1996**, *7*, 1103-1114.

(7) Di Natale, C.; Olafsdottir, G.; Einarsson, S.; Mantini, A.; Martinelli, E.; Paolesse, R.; Falconi, C.; D'Amico, A. Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets. *Sens. Act.* **2001**, *B77,* 572-578.

(8) Olafsdottir, G.; Högnadottir, A.; Martinsdottir, E.; Jonsdottir, H. Application of an electronic nose to predict total volatile bases in capelin (*Mallotus villosus*) for fishmeal production. *J. Agric. Food Chem*. **2000**, *48*, 2353-2359.

(9) Olafsdottir, G.; Xiuchen, L.; Lauzon, H.; Jonsdottir, R. Precision and application of electronic nose for freshness monitoring of whole redfish stored in ice and modified atmosphere bulk storage. *J. Aquat. Food Prod. Technol*. **2002**, *11*, 229-249.

(10) Zhao, C.Z.; Pan, Y.Z.; Ma, L.Z.; Tang, Z.N.; Zhao, G.L.; Wang, L.D. Assay of fish freshness using trimethylamine vapor probe based on a sensitive membrane on piezoelectric quartz crystal. *Sens. Act.* **2002**, *B81,* 218-222.

(11) Haugen, J.E.; Undeland, I. Lipid oxidation in herring fillets (*Clupea harengus*) during ice storage measured by a commercial hybrid gas-sensor array system. *J. Agric. Food Chem*. **2003,** *51*, 752-759.

(12) Jonsdottir, R.; Olafsdottir, G.; Martinsdottir, E.; Stefansson, G. Flavor Characterization of Ripened Cod Roe by Gas Chromatography, Sensory Analysis and Electronic Nose. *J. Agric. Food Chem*. **2004,** *52,* 6250-6256.

(13) Lindsay, R.C. Fish flavors. *Food Rev. Int.* **1990,** *6*, 437-455.

(14) Olafsdottir, G.; Fleurence, J. Evaluation of fish freshness using volatile compounds - Classification of volatile compounds in fish. In *Methods to Determine the Freshness of Fish in Research and Industry*; Olafsdottir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdottir, E., Heia, K., Eds.; International Institute of Refrigeration: Paris, France, 1998; pp. 55-69.

(15) Elliot, R.P. Evaluation of surface pH as a freshness index for fish fillets. *Food Research*. **1947**, *12 (2)*, 87-98.

(16) Shewan, J.M.; Macintosh, R.G.; Tucker, C.G.; Ehrenberg, A.S.C. The development of a numeric scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. *J. Sci. Food Agric.* **1953**. *4*, 283-298.

(17) Castell, C.H.; Greenough, M.F. The action of *Pseudomonas* on fish muscle: 1. Organisms responsible for odour produced during incipient spoilage of chilled fish muscle. *J. Fish. Res. Board Can*. **1957**, *12*, 617-625.

(18) Miller III, A.; Scanlan, R.A.; Lee, J.S.; Libbey, L.M. Identification of the Volatile Compounds Produced in Sterile Fish Muscle (*Sebastes melanops*) by *Pseudomonas fragi. Appl. Microbiol.* **1973**, *25*, 952-955.

(19) Miller III, A.; Scanlan, R.A.; Lee, J.S.; Libbey, L.M. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas putrifaciens, Pseudomonas fluorescens,* and an *Achromobacter* species. *Appl. Microbiol.* **1973**, *26*, 18-21.

(20) Miller III, A.; Scanlan, R.A.; Lee, J.S.; Libbey, L.M.; Morgan, M.E. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas perolens. Appl. Microbiol*. **1973**, *25*, 257-261.

(21) Herbert, R.A.; Shewan, J.M. Roles played by bacterial and autolytic enzymes in the production of volatile sulphides in spoiling North Sea cod (*Gadus morhua*). *J. Sci. Food Agric*. **1976**, *27*, 89-94.

(22) Lerke, P.; Huck, R.W. Objective determination of canned tuna quality: Identification of ethanol as a potentially useful index. *J. Food Sci*. **1977**, *42*, 755-758.

(23) Human, J.; Khayat, A. Quality Evaluation of Raw Tuna by Gas Chromatography and Sensory Methods. *J. Food Sci*. **1981**, *46*, 868-873.

(24) Hebard, C.E.; Flick, G.J.; Martin, R.E. Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In *Chemistry and Biochemistry of Marine Food Products*; Martin, R.E., Ed.; AVI Publishing Co., Inc.: Westport, Connecticut, 1982; pp. 149–304.

(25) Kelleher, S.D.; Zall, R.R. Ethanol accumulation in muscle tissue as a chemical indicator of fish spoilage. *J. Food Biochem*. **1983**, *7*, 87-91.

(26) Ahmed, A.; Matches, J.R. Alcohol production by fish spoilage bacteria. *J. Food Prot*. **1983,** *46*, 1055-1059.

(27) Josephson, D.B.; Lindsay, R.C.; Olafsdottir, G. Measurement of Volatile Aroma Constituents as a Means for Following Sensory Deterioration of Fresh Fish and Fishery Products. In *Seafood Quality Determination*, *Proceedings of an International Symposium*, University of Alaska Sea Grant Program, Anchorage, Alaska, U.S.A Kramer D.E.; Liston J. Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1986; pp. 27-47.

(28) Lindsay, R.C.; Josephson, D.B.; Olafsdottir, G. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In *Seafood Quality Determination, Proceedings of an International Symposium*, University of Alaska Sea Grant Program, Anchorage, Alaska, U.S.A. Kramer D.E.; Liston J. Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1986; pp. 221-234.

(29) Oehlenschläger, J. Evaluation of some well established and some underrated indices for the determination of freshness and/or spoilage of ice stored wet fish. In *Quality Assurance in the Fish Industry*; Huss, H.H., Jakobsen, M., Liston, J.; Eds.; Elsevier Science Publisher: Amsterdam, The Netherlands, 1992; pp 339-350.

(30) Jørgensen, L.V.; Dalgaard, P.; Huss, H.H. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *J. Agric. Food Chem*. **2000**, *48*, 2448-2453.

(31) Alasalvar, C.; Taylor, K.D.A.; Shahidi, F. Comparison of volatiles of cultured and wild sea bream (*Sparus aurata*) during storage in ice by dynamic headspace analysis gas chromatography mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 2616-2622.

(32) Chinivasagam, H.N.; Bremner, H.A.; Wood, A.F.; Nottingham, S.M. Volatile components associated with bacterial spoilage of tropical prawns. *Int. J. Food Microbiol.* **1998**, *42*, 45-55.

(33) Joffraud, J.J.; Leroi, F.; Roy, C.; Berdague, J.L. Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. *Int. J. Food Microbiol*. **2001**, *66 (3)*, 175-184.

(34) Jørgensen, L.V.; Huss, H.H.; Dalgaard, P. Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar)* analyzed by GC-MS and multivariate regression. *J. Agric. Food Chem*. **2001,** *49*, 2376-2381.

(35) Gram, L.; Melchiorsen, J. Interaction between fish spoilage bacteria *Pseudomonas sp.* and *S. putrefaciens* in fish extracts and on fish tissue. *J. Appl. Bacteriol*. **1996**, *80*, 589-595.

(36) Gram, L.; Ravn, L.; Rasch, M.; Bruhn, J.B.; Christensen, A.B.; Givskov, M. Food spoilage – interactions between food spoilage bacteria. *Int. J. Food Microbiol.* **2002**, *78,* 79-97.

(37) Huss, H.H.; Dalgaard, P.; Gram, L. Microbiology of fish and fish products. In *Seafood from producer to consumer; intergrated approach to quality*; Luten, J.B., Börresen, T., Oehlenschlager, J., Eds.; Elsevier: Amsterdam, The Netherlands, 1997; pp. 413-430.

(38) Shewan, J.M. The bacteriology of fresh and spoiling fish and some related chemical changes. In *Recent Advances in Food Science;* Hawthorn, J., Leitch, J.M., Eds.; Butterworths: London, 1962; 1, 167-193.

(39) Van Spreekens, K.J.A. The suitability of Long & Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. *Arch. Lebensmitt.* **1974**, *25 (10)*, 213-219.

(40) Van Spreekens, K.J.A.; Toepoel, L. Quality of fishery products in connection with the psychrophilic and psychrotrophic bacterial flora. In *Psychrotrophic microorganisms in spoilage and pathogenicity*; Roberts, T.A., Hobbs, G., Christian, J.H.B., Skovgaard, N., Eds.; Academic Press: London, UK, 1981; pp. 283-294.

(41) Dalgaard, P.; Gram, L.; Huss, H.H. Spoilage and shelf life of cod fillets packed in vacuum or modified atmospheres. *Int. J. Food Microbiol*. **1993**, *19*, 283-294.

(42) Dalgaard, P.; Mejlholm, O.; Christiansen, T.J.; Huss, H.H. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. *Lett. Appl. Microbiol*. **1997,** *24*, 373-378.

(43) Dalgaard, P. Qualitative and quantitative characterization of spoilage bacteria from packed fish. *Int. J. Food Microbiol.* **1995**, *26*, 319-333.

(44) McGill, A.S.; Hardy, R.; Burt, J.R. Hept-*cis*-4-enal and its contribution to the off-flavour in cold stored cod. *J. Sci. Food Agric.* **1974**, *25*, 1477-1489.

(45) Aro, T.; Tahvonen, R.; Koskinen, L.; Kallio, H. Volatile compounds of Baltic herring analysed by dynamic headspace sampling-gas chromatography-mass spectrometry. *Eur. Food Res. Technol*. **2003**, *216*, 483-488.

(46) Olafsdottir, G.; Lauzon, H.L.; Martinsdóttir, E.; Oehlenschläger, J.; Kristbergsson, K. Shelf-life extension of superchilled cod (*Gadus morhua*) fillets and influence of temperature fluctuations on microbial growth and metabolites. In press.

(47) ISO 1985. Sensory analysis – methodology - genereral guidance. ISO 6658. Geneva, Switzerland: The International Organization for Standardization. 14 p.

(48) ISO 1987. Sensory analysis – methodology – evaluation of food products by methods using scales. ISO 5124. Geneva, Switzerland: The International Organization for Standardization. 7 p.

(49) Acree T.;Arn, H. http://www.flavornet.org/flavornet.html; Flavornet, Cornell University, 1997.

(50) Malle, P.; Poumeyrol, M. A New Chemical Criterion for the Quality Control of Fish: Trimethylamine/Total Volatile Basic Nitrogen (%). *J. Food Prot*. **1989,** *52*(*6)*, 419-423.

(51) Huss, H.H. Quality and quality changes in fresh fish. FAO Fisheries Technical Papers - T348. Rome: Food & Agriculture Organisation of the United Nations & Danish International Development Agency (FAO/DANIDA). 1995; 195 p.

(52) Fazzalari, F.A., Ed. Compilation of Odor and Taste Threshold Data, ASTM Data Series DS 48A, 1978, Philadelphia, PA.

(53) Karahadian, C., Lindsay, R.C. Evaluation of compounds contributing characterizing fishy flavors in fish oils. *J. Am. Oil Chem. Soc.* **1989**, *66*, 953-960.

(54) Sheldon, R.M.; Lindsay, R.C.; Libbey, L.M.; Morgan, M.E. Chemical nature of malty flavour and aroma produced by *streptococcus lactis*. *Appl. Microbiol.* **1971**, *22*, 263-266.

(55) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366-2371.

(56) Olafsdottir G. Developing rapid olfaction arrays for determining fish quality. In *Rapid and On-Line Instrumentation for Food Quality Assurance*; Tothill, I.E., Ed.; Woodhead Publishing Ltd: Cambridge, England, 2003; pp. 339-360

(57) Dainty, R.H.; Edwards, R.A.; Hibbard, C.M. Time course of volatile compound formation during refrigerated storage of naturally contaminated beef in air. *J. Appl. Bacteriol.* **1985,** *59*, 303-309.

(58) Curioni, P.M.G.; Bosset, J.O. Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int. Dairy J.* **2002**, *12*, 959-984.

(59) Hardy, R.; McGill, A.S.; Gunstone, F.D. Lipid and autoxidative changes in cold stored cod (*Gadus morhua*). *J. Sci. Food Agric.* **1979**, *30*, 999-1006.

(60) Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. Variations in the occurrences of enzymically derived volatile aroma compounds in salt- and freshwater fish. *J. Agric. Food Chem*. **1984**, *32*, 1344-1347.

(61) Kawai T. Fish flavor. *Crit. Rev. Food Sci. Nutr*. **1996,** *36, 3*, 257-298.

(62) Koide, K.; Yanagisawa, I.; Ozawa, A.; Satake, M.; Fujita, T. Studies on the kerosene-like off-flavour producing yeast in surimi-based products. *Bull. Jap. Soc. Sci. Fish*. **1992,** *58*, 1925-1930.

(63) Olafsdottir, G.; Högnadottir, A.; Martinsdottir, E. Application of gas sensors to evaluate freshness and spoilage of various seafoods. In *Methods to Determine the Freshness of Fish in Research and Industry*; Olafsdottir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdottir, E., Heia, K., Eds.; International Institute of Refrigeration: Paris, France, 1998; pp 100-109.

(64) Castell, C.H.; Greenough, F.; Dale, J. The action of *Pseudomona*s on fish muscle: 3. Identification of organisms producing fruity and oniony odours. *J. Fish. Res. Board Can.* **1959**, *16*, 13-19.

(65) Mielle, P.; Marqui, F. An alternative way to improve the sensitivity of electronic olfactometers. *Sensors and Actuators* **1999**, *B-Chemical 58(1-3)*, 526-535.

(66) Olafsdottir, G.; Martinsdottir, E.; Jonsson, E.H. Rapid gas sensor measurements to predict the freshness of capelin (*Mallotus villosus*). *J. Agric. Food Chem.* **1997**, *45*, 2654-2659.

(67) Olafsdottir, G.; Martinsdóttir, E.; Oehlenschläger, J.; Dalgaard, P.; Jensen, B.; Undeland, I.; Mackie, I.M.; Henehan, G.; Nielsen, J.; Nilsen, H. Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Technol.* **1997**, *8*, 258- 265.

(68) Zeng, Q.Z.; Thorarinsdottir, K.A.; Olafsdottir, G. Quality changes of shrimp (*Pandalus borealis*) stored under different cooling conditions. In press *J. Food Sci*. 2005, accepted for publication.

(69) Ackman, R.G.; Hingley, J. Dimethyl sulfide as an odor component in Nova Scotia fall mackerel. *J. Fish. Res. Board Can.* **1972**, *29*, 1085-1088.

(70) Herbert, R.A.; Ellis, J.R.; Shewan, J.M. Isolation and identifiaction of the volatile sulphides produced during chill-storage of North Sea cod (*Gadus morhua*). *J. Sci. Food Agric.* **1975**, *26*, 1187-1194.

(71) Kamiya, A.; Ose, Y. Study of the odorous compounds produced by putrefaction of foods. V. Fatty acids, sulphur compounds and amines. *J. Chromat*. **1984**, *292*, 383-391.

(72) Tryggvadóttir, S.V.; Olafsdottir, G.; Einarsson, S. Multisensor for fish: Storage studies of cod in Reykjavik and Tromsö. Texture, electronic nose (FreshSense), FIGD analysis, RT-Freshmeter and sensory analysis. RF report 02-01. 2001; pp 19-24.

(73) Buttery, R.G.; Guadagni, D.G.; Ling, L.C.; Seifert, R.M.; Lipton, W. Additional volatile components of cabbage, broccoli and cauliflower. *J. Agric. Food Chem.* **1976**, *24*, 829-832.

(74) Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem*. **1988**, *36*, 1006-1009.

(75) Sheldon, R.M.; Lindsay, R.C.; Libbey, L.M.; Morgan, M.E. Chemical nature of malty flavour and aroma produced by *streptococcus lactis*. *Appl. Microbiol.* **1971**, *22*, 263-266.

(76) Whitfield, F.B.; Tindale, C.R. The role of microbial metabolites in food off-flavours. *Food Technol. Australia*, **1984**, *36*, 204-213.

(77) Buttery, R.G.; Teranishi, R.; Ling, L.C.; Turnbaugh, J.G. Quantitative and sensory studies on tomato paste volatiles. *J. Agric. Food Chem.* **1990**, *38*, 336-340.

FIGURE CAPTIONS

Figure 1. Changes in pH (--Δ--), development of TMA (-♦-) measured by GC-MS (PAR: peak area ratio) and TVB-N (mgN/100g) (-■-) in cod fillets packed in styrofoam boxes during storage at 0.5 °C. Vertical line indicates the end of shelf-life determined by sensory analysis.

Figure 2. PAR (peak area ratio) for alcohols (ethanol (-♦-), 3-methyl-1-butanol (-■-), 2,3-butandiol (-▲-) and isobutanol (-Δ-) detected in highest concentration and ethyl acetate (-x-)-in cod fillets packed in styrofoam boxes during storage at 0.5 °C. Vertical line indicates the end of shelf-life determined by sensory analysis.

Figure 3. PAR (peak area ratio) for ketones (6-methyl-5-hepten-2-one (-■-), 2-butanone (-●-), 3-pentanone (-▲-), acetoin (-Δ-)) and acetic acid (-x-) in cod fillets packed in styrofoam boxes during storage at 0.5 °C. Vertical line indicates the end of shelf-life determined by sensory analysis.

Figure 4. Response of the electronic nose sensors (CO, H_2S, NH_3 and SO_2) towards cod fillets during storage in styrofoam boxes at 0.5 °C on days 4, 7, 10, 12, and 14 after catch.

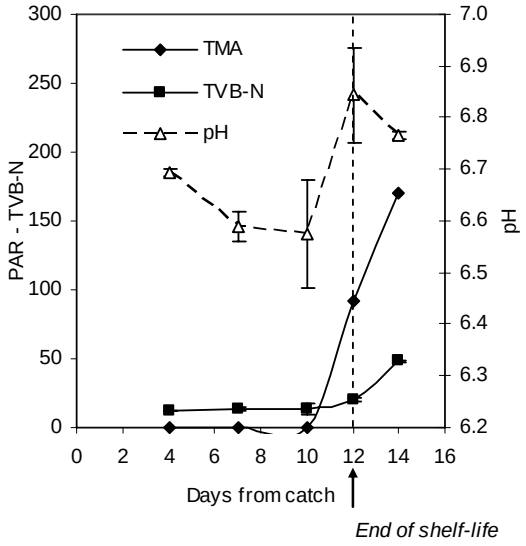


Figure 1

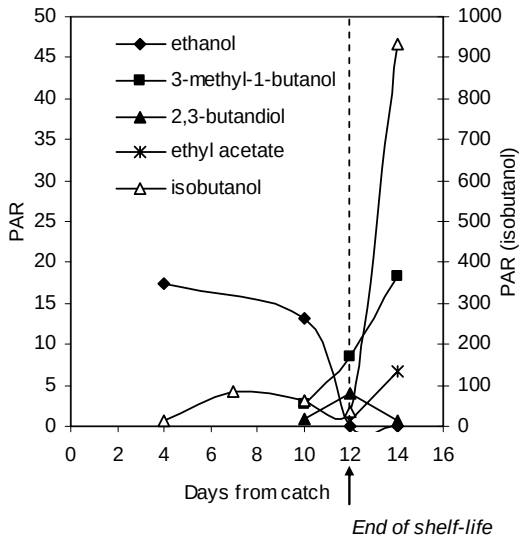


Figure 2

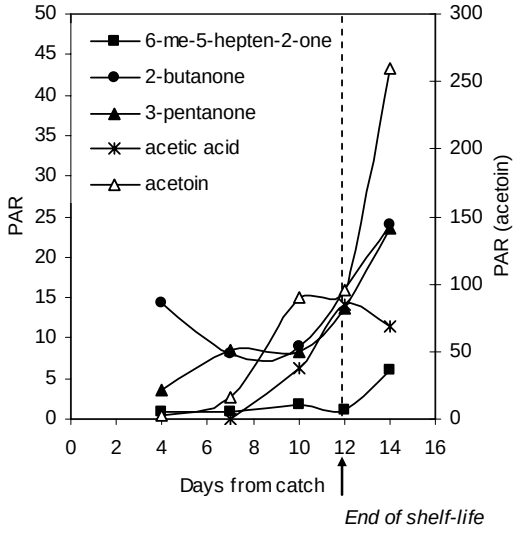


Figure 3.

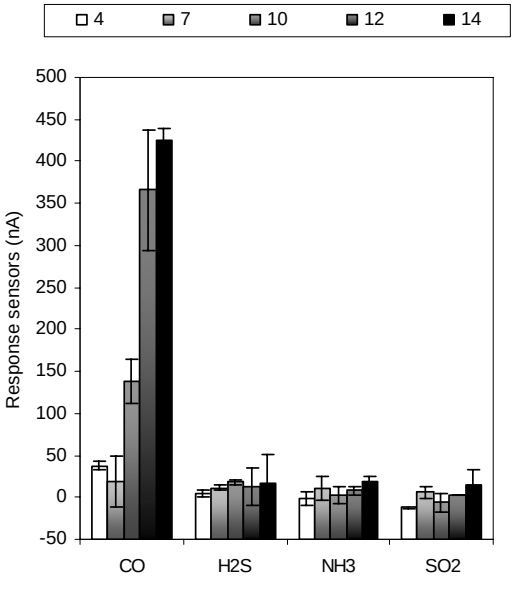


Figure 4.

Table 1. Volatile compounds associated with spoilage in cod fillets during storage in styrofoam boxes at 0.5 °C for 17 days. PAR (peak area ratio) of compounds analyzed by GC-MS, odor descriptions and odor scores based on GC-O analysis and OU (odor unit) value of potent odorants at sensory rejection on day 12.

Compounds	RI[a]	PAR[b]						Odor description[c]	Odor score[d]	OU[e]	Odor threshold[f]	ID means[g]
		Day 4	Day 7	Day 10	Day 12	Day 14	Day 17			Day 12	in water	
Alcohols												
ethanol	<173	17.4 ± 20.7	-	13.1 ± 3.8	-	-	-	-			100000 ppb [1]	MS, RI
2-methyl -1-propanol/ pentane[h]	<173	14.0 ± 1.4	85.4 ± 36.5	64.4 ± 13.2	35.0 ± 2.8	934.6 ± 1096.8	451.9	-		5	7000 ppb [1]	MS, RI
1-penten-3-ol	245	2.3	0.4	0.8 ± 0.1	1.2 ± 0.0	2.5 ± 1.7	-	-		3	400 ppb [1]	MS, RI
3-methyl-1-butanol	300	-	-	2.7 ± 2.4	8.5 ± 1.0	18.3 ± 6.2	31.6 ± 5.6	-		28-34	250-300 ppb [2]	MS, RI
2-methyl-1-butanol	306	-	-	-	-	5.3 ± 2.7	-	-				MS, RI
2,3-butandiol	359	-	-	0.9 ± 1.2	4.0 ± 4.4	0.7	21.3 ± 30.1	-				MS, RI
2-ethyl-1-hexanol	633	-	2.0 ± 2.1	2.3 ± 1.0	1.8 ± 0.2	1.4	2.5 ± 1.1	-			270000 ppb [1]	MS, RI
Aldehydes												
acetaldehyde	<173	2.8 ± 0.8	1.4 ± 0.4	0.9 ± 0.6	0.8 ± 0.5	5.8 ± 4.4	7.5 ± 6.4	-		7-160	15-120 ppb [4]	MS, RI
3-methyl-butanal	227	-	-	1.7	1.3 ± 0.2	2.6	1.2	sweet, caramel, fish fillet	1.5 - 3.0	22	60 ppb [5]	MS, RI, GC-O
hexanal	378	1.9	2.1 ± 2.0	3.2 ± 1.2	2.0 ± 0.1	8.7 ± 6.2	-	-		444	4.5ppb [6]	MS, RI
heptanal	501	-	2.4	1.7 ± 0.8	0.7 ± 0.2	2.9 ± 2.2	3.5 ± 1.8	earthy, boiled potato	2.0 - 3.0	233	3 ppb [1]	MS, RI, GC-O
octanal	606	-	-	1.4 ± 0.6		-	-	-			0.7 ppb [1]	MS, RI
nonanal	710	-	4.3 ± 3.7	6.3 ± 1.1	3.7 ± 0.6	10.2 ± 4.4	-	-		3700	1 ppb [1]	MS, RI
decanal	807	2.3 ± 0.2	2.5 ± 1.9	3.5 ± 0.4	2.4 ± 0.3	8.0 ± 4.2	14.9 ± 5.7	fresh, floral	1.5	1200 - 24000	0.1-2 ppb [1]	MS, RI, GC-O
undecanal	910	-	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	-	2.0 ± 1.1	sweet, candy	1.5	100	4 ppb [1]	MS, RI, GC-O
Ketones												
2-butanone	191	14.3 ± 10.2	8.1 ± 1.1	9.0	-	24.0 ± 13.9	-	-			50000 ppb [1]	MS, RI
3-pentanone	255	3.7 ± 3.2	8.5 ± 5.2	8.2 ± 3.9	13.6 ± 6.2	23.5 ± 17.7	34.6 ± 18.0	sweet, caramel	1.5 - 2.0	0.2	70000 ppb [1]	MS, RI, GC-O
3-hydroxy-2-butanone	273	2.6 ± 1.3	16.8 ± 20.8	90.3 ± 51.6	95.3 ± 5.6	259.2 ± 177.5	341.8 ± 129.3	sweet, sour	1.5 - 2.0	119	800 ppb [7]	MS, RI, GC-O
6-methyl-5-hepten-2-one	588	0.8 ± 0.2	0.9 ± 0.2	1.9 ± 0.4	1.0 ± 0.1	6.1 ± 4.1	5.4 ± 1.8	spicy, flowery	1.5	20	50 ppb [2]	MS, RI, GC-O
Acids												
acetic acid	191	-	-	6.3 ± 4.2	14.2 ± 13.0	11.4 ± 4.3	15.5	-		0.4	34200ppb[3]	MS, RI
Amines												
TMA	<173	-	-	-	91.6 ± 28.5	922.2 ± 1064.2	1721.4 ± 47.6	TMA-like, dried fish	3.0	91600-305333	0.3-1 ppb [1]	MS, RI, GC-O
Esters												
ethyl acetate	200	-	-	-	0.6	6.6 ± 1.4	258.9 ± 330.0	-		0.1-120	5-5000ppb [1]	MS, RI
ethyl butanoate	377	-	-	-	-	-	13.9 ± 2.2	sickenly sweet, vomit	2.3			MS, RI, GC-O
Sulfur compounds												
dimethyl sulfide	173	4.5	-	-	-	-	-	-			900 ppb [6]	MS, RI
dimethyl disulfide	312	-	-	-	-	-	1.2	onion like	1.5 - 2.5		12 ppb [2]	MS, RI, GC-O
dimethyl trisulfide	564	-	-	-	-	-	-	rotten, sulfur, cabbage	2.5		0.01ppb [2]	GC-O
Unknown	457	-	-	-	-	-	-	spicy, fish, flowery, sweet, onion, mushroom	3.0 -4.8			GC-O

[a]RI: calculated retention index on DB-5ms capillary column; [b]PAR: peak area ratio, average of duplicate analysis ± standard deviation when detected in both samples or " - " if not detected; [c]odor evaluated by GC-O; [d]GC-O odor scores (average value of two panelists) giving the range of scores on days 4, 10 and 17; [e]OU: odor unit calculated by dividing the PAR by the odor threshold value x 1000; [f]odor thresholds from literature: 1. Fazzalari, 1978 (52); 2. Buttery et al., 1976 (73); 3. Kawai 1996 (61); 4. Buttery et al., 1988 (74); 5. Sheldon et al., 1971 (75); 6. Whitfield and Tindale, 1984 (76); 7. Buttery et al., 1990 (77); [g]identification on the basis of MS database,retention index and odor evaluation; [h]coeluting peaks.

Table 2. PAR of main classes of compounds identified by GC-MS in cod fillets packed in styrofoam boxes during storage at 0.5 °C for 17 days

	PAR [a]					
Class	Day 4	Day 7	Day 10	Day 12	Day 14	Day 17
Quality indicators						
Alcohols	34 (29)	88 (45)	84 (28)	51 (15)	963 (39)	507 (15)
Aldehydes	7 (6)	13 (6)	19 (6)	11 (3)	38 (2)	27 (1)
Ketones	21 (18)	34 (18)	109 (36)	110 (33)	313 (13)	382 (11)
Acids	-	-	6 (2)	14 (4)	11 (<1)	15 (<1)
Amines	-	-	-	92 (27)	922 (37)	1721 (51)
Esters	-	-	-	1 (<1)	7 (<1)	273 (8)
Sulfur compounds	5 (4)	-	-	-	-	1 (<1)
Miscellaneous						
Alkanes	3 (3)	4 (2)	6 (2)	3 (1)	4 (<1)	3 (<1)
Aromatics	17 (15)	39 (20)	40 (13)	22 (7)	80 (3)	76 (2)
Other	5 (4)	5 (3)	3 (1)	12 (4)	97 (4)	351 (10)
Unknown	26 (22)	10 (5)	36 (12)	17 (5)	28 (1)	44 (1)
Total volatiles	118	194	306	335	2464	3402

[a] PAR (peak area ratio) and percentage of total volatiles each day shown in parenthesis

PAPER V

Shelf-life extension of superchilled cod (*Gadus morhua*) fillets and influence of temperature fluctuations on microbial growth and metabolites

Guðrún Ólafsdóttir, Hélène L. Lauzon, Emilia Martinsdóttir, Joerg Oehlenschläger, Kristberg Kristbergsson

V

Submitted for publication

Shelf-life extension of superchilled cod *(Gadus morhua*) fillets and influence of temperature fluctuations on microbial growth and metabolites

Gudrun Olafsdottir [a,c], Hélène L. Lauzon [a], Emilía Martinsdóttir [a], Joerg Oehlenschläger [b] Kristberg Kristbergsson [c]

[a]Icelandic Fisheries Laboratories, Skulagata 4, 101 Reykjavik, Iceland
[b] Federal Research Centre for Nutrition and Food, Department for Fish Quality (FRCNF), Palmaille 9, D-22767 Hamburg, Germany [c]Department of Food Science, University of Iceland, Hjardarhagi 2 - 6, 107 Reykjavik, Iceland

Contact information for Corresponding Author:
Gudrun Olafsdottir
Icelandic Fisheries Laboratories, Skulagata 4, 101 Reykjavik, Iceland
Fax: +354 530 8601, Phone: +354 530 8600, gudrun@rf.is

ABSTRACT

Quality changes of aerobically packed cod fillets stored under different temperature conditions were characterized by the growth of specific spoilage organisms (SSO) and the production of microbial metabolites measured by an electronic nose along with traditional sensory and chemical analysis (TVB-N, pH). A new process based on quick contact freezing and cold air blasting was used to achieve superchilling of fillets, prior to chilled (0.5 °C) or superchilled (-1.5 °C) storage. Additionally, the effect of temporarily storing conventionally processed chilled cod at higher temperatures to imitate possible scenarios of abusive conditions during initial handling and transport were studied. *Photobacterium phosphoreum* dominated under temperature abusive conditions coinciding with high levels of TVB-N and increased electronic nose responses at sensory rejection. CO sensor responses indicated that alcohols and aldehydes contributed to the spoilage odors under all storage conditions. *Pseudomonas* spp. was dominating when chilling of fillets was employed in the process and longer shelf-life was achieved. The superchilling process followed by superchilled storage (-1.5 °C) extended the sensory shelf-life of the fillets for at least 3 days compared to traditional process, resulting in a shelf-life of 15 days. Microbial growth and traditional spoilage signs were delayed or altered under superchilled conditions. In particular high content of TVB-N was observed in superchilled fillets at sensory rejection. *P. phosphoreum* counts were lower under superchilling conditions (log 6 to 6.8 CFU/g), compared to the traditionally processed chilled fillets (log 7.2 CFU/g). However, H_2S- producing bacteria appeared to grow steadily under superchilling conditions reaching counts higher than 10^7 CFU/g at sensory rejection.

Keywords: superchilling, cod fillets, sensory analysis, specific spoilage organisms, electronic nose

Introduction

The extension of shelf-life of chilled fish fillets is of importance to allow the transport of products to distant markets at lower cost. Superchilling has proven to effectively delay bacterial growth and prolong the shelf-life of chilled fish (Huss 1995; Chang and others 1998). Various types of cooling systems have been used for superchilling (-4 to 0°C) of

seafood products including chilled and refrigerated seawater (Smith and others 1980; Shaw and Botta 1975; Olafsdottir and others 2000), liquid-ice and brine solutions (Lee and Toledo 1984; Huidobro and others 2002), flake ice or slurry ice (Losada and others 2004; Zeng and others 2005), subzero temperatures during storage (-2 and -3) (Riaz-Fatima and others 1988; Sivertsvik and others 2003) and the use of cooling agents like CO_2 snow (LeBlanc and LeBlanc 1992; Jeyasekaran and others 2004). Chilling of fillets is traditionally done in the fish industry by immersing the fillets in ice/water or brine solutions (Lee and Toledo 1984). Subsequent chilling in a freezer after packaging has proven useful to store refrigerating capacity into the product (Magnussen and others 1998). However, slow freezing can occur causing undesirable ice crystal formation, inducing tissue damage. By using quick freezing, less damage may result (Fennema and others 1973). A new technique, "Combined Blast and Cooling" (CBC) (Skaginn, Iceland), is based on superchilling by lowering the temperature of the fillets quickly to -1 °C thus allowing initial phase transition to occur. The liquid phase becomes viscous and cooling of the fillets is then based on the cooling capacity stored in the skin side surface layer thus minimizing ice crystal formation.

The rate of freezing and the size of ice crystals are important factors influencing the survival of microorganisms. Gram-negative bacteria, which include many of the specific spoilage organisms (SSO) in fish, are considerably more sensitive to freezing conditions (Ingram and Mackey 1976; Olson and Nottingham 1980).

The SSO concept is an approach to find the most important microorganism among the total microflora that reaches a high cell count and causes spoilage before it is inhibited by the dominating microflora (Dalgaard 2000). *Shewanella putrefaciens* and *Pseudomonas* spp. are well established as the main SSO in fresh chilled aerobically stored fish caught in temperate waters (Shewan 1962; Herbert and Shewan 1976; Jørgensen and Huss 1989; Huss and others 1997; Koutsoumanis and Nychas 2000). The influence of high temperature during storage on the proliferation of the SSOs in fish model system have shown the importance of *S. putrefaciens* at high temperature and as a late spoiler (Lauzon 2000). *Photobacterium phosphoreum* the main SSO in packaged cod fillets (Dalgaard and others 1993; Dalgaard 1995) originates from the intestines (Van Spreekens 1974) and was earlier identified as an important TMA producer in iced cod, and in cod fillets (Van Spreekens and Toepoel, 1981; Larsen and others 2003). Recently, *P. phosphoreum* was

identified as the dominating microorganism in aerobically packaged haddock fillets and was along with *Pseudomonas* spp. suggested as the main SSO in haddock fillets stored under chilled and temperature abusive conditions (Olafsdottir and others Forthcoming). The knowledge on the effect of superchilled storage on the proliferation of SSOs and their production of metabolites in cod fillets is limited. Generally, an initial reduction in microbial load has been observed after the first day of chilled or superchilled storage as a result of a cold shock of the microorganisms (Ingram 1951; Ingram and Mackey 1976; Lakshmanan and others 2002). The resulting spoilage pattern and proliferation of the specific spoilage bacteria depend on the temperature and environmental conditions during further storage (Gram and Huss 1996). As a result of delayed or altered microbial growth when preservation techniques are used the traditional spoilage signs may become distorted and therefore the commonly used quality indices may be of questionable value (Lindsay and others 1986). Detection of the main classes of volatile degradation compounds (alcohols, aldehydes, esters, sulfur compounds and amines) produced during chilled storage of fish by an electronic nose has proven useful to determine the spoilage level of different fish species (Olafsdottir and others 1997, 2000, 2002; Di Natale and others 2001). The rapid detection of microbial metabolites related to odor or quality changes is therefore ideal for characterization of the spoilage activity of the dominating SSOs.

The aim of the experiments reported herein was to study the spoilage characteristics of CBC-processed cod fillets compared to traditionally processed fillets stored under superchilled and/or chilled conditions and the effect of abusive temperature on the proliferation of specific spoilage organisms and their metabolites. Spoilage changes were monitored by sensory, microbial and chemical analyses and by an electronic nose. Characterization of the spoilage changes and the spoilage domain of the SSOs in superchilled cod fillets is needed to determine which quality indicators are relevant for monitoring quality changes of cod fillets stored under superchilled and temperature abusive conditions. Furthermore the knowledge can be applied to optimize the chilling conditions to extend the shelf-life of cod fillets.

Materials and Methods

Preparation of samples

Three extensive storage studies were performed with products from two different fish factories in Iceland. The raw material originated from different catching areas (Southwest and Northeast of Iceland), using different fishing gear (longline and bottom trawl) and varying processes in the factories.

Traditional process Factory I - Experimental groups A, B and D: The fish was caught by the ice fish trawler Brettingur east off Iceland in the Berufjardarall catching zone in October 2003 by bottom trawl. The fish, selected from one haul (batch 1) was gutted on board and iced in tubs (400L). Landing was three days after catch at the processing plant. Approximately 170 fishes from batch 1 were processed into fillets to prepare the sample groups. The traditional process in factory I involves mechanical filleting and deskinning and packing of fillets in styrofoam (EPS, expanded polystyrene) boxes (160 x 400 x 263 mm) lined with a plastic bag. Each box contained 8 to 12 fillets, an absorbing pad at the bottom and a cooling mat (230 x 160 mm; 146 g) placed on top. The samples were transported by a refrigerated truck (4 to 5°C) to Reykjavik and the samples arrived in the morning on day 4 after catch (groups A, B and D). Group A was then stored constantly at 0.5 °C while group B was stored at 15°C for 8 hours on day 6 and then transferred back to the cold store (0.5 °C). Thirty fishes were selected from the same batch to prepare sample group D that represented bad handling. Abusive temperature conditions were simulated by keeping the fish without ice in a tub in the reception area for approximately 8 hours at 2 °C and then transferring the tub into the processing area (15 to 18°C) until the following morning when the fish was filleted, deskinned, packed and transported to the laboratory. The same temperature conditions during storage were used as for group B.

Traditional process Factory II - Storage groups H and I: The fish was caught by longlining close to Sandgerði south west off Iceland in November 2003. The cod was gutted and iced on board the boat. Landing was in the afternoon the same day and the fish was stored iced overnight. The fish was hand filleted the following morning and cooled by placing the fillets on aluminum pans floating in ice-water for 10 to 20 minutes before being mechanically deskinned and packaged into plastic bags in styrofoam (EPS) boxes as described above. Each box contained 11 fillets and were transferred to the laboratory the same day. Two groups of samples were prepared (groups H and I). Both sample groups were initially stored at 0.5 °C, but on the fourth day of storage, group I

was removed from the cooler and stored overnight (16 hours) at room temperature (RT) and then moved back to the cooler.

Superchilling process Factory I - Experimental groups C, E, F and G: Group C was prepared from batch 1 in October 2003 (labeled "C new" indicating that this was the first trial with the new superchilling technique). After filleting, eighty fillets were immersed in an ice-water cooling solution containing 0.85% NaCl for 45 minutes until further processed with a new superchilling CBC (Combined Blast and Contact) cooling technique (Skaginn, Iceland). The CBS technique involves superchilling of the surface of the skin side of the fillets by moving them through a freezing tunnel on a teflon covered aluminum conveyor belt (- 35 til – 40°C) and simultaneously blasting cold air over the fillets. The superchilling process facilitates handling of the fillets, in particular the removal of the skin and effective cooling of the fillets results in a temperature around – 1 °C when packed. Group C was stored at 0.5 °C at the laboratory.

The second trial with the new superchilling technique was done in December 2003. Three experimental groups (E, F and G) were prepared from about 190 fishes (batch 2). The fish was caught by the same trawler as batch 1, but ice slurry was used on board for cooling. Landing and processing were on day 1 after catch.

The fish was hand filleted and the fillets were cooled in ice water (0.85% NaCl) for 20 min before being superchilled and deskinned by the new technique. Nine to eleven fillets were packed in each styrofoam box (160 x 400 x 263 mm; Borgarplast, Iceland) and a cooling mat put on top (230 x 160 mm; 146 g). The samples were transported by a refrigerated truck (4 to 5°C) to the laboratory and arrived the following morning on day 3 post catch. The sample groups were stored under different conditions, group E at 0.5 °C, group F at -1.5 °C and group G was initially stored at – 1.5 °C but transferred to 0.5 °C on day 8 post catch.

Sampling

At each day of sampling one box containing 11 to 12 fillets was used for the various analyses. Three or four fillets were used for sensory analysis. Other four fillets were pooled into 2 samples and used for microbial analysis (TVC and SSO counts), chemical analysis of total volatile bases nitrogen (TVB-N) and pH. Four fillets were then used for the analysis of volatile compounds with the electronic nose. The first samples were

measured on the day following processing and duplicate samples were analyzed regularly until sensory rejection.

Temperature monitoring.

Selected boxes contained automatic temperature data loggers (Stow Away®, Onset Computer Corporation, USA) to monitor the temperature of fillets during storage. Temperature was recorded at 5 minutes intervals. The loggers were inserted underneath, in between and above the fillets in one box for each temperature treatment, as well as on top of the box to follow the environmental temperature. Data are only shown from loggers located underneath the fillets.

Sensory analysis

The Icelandic Fisheries Laboratories (IFL) sensory panel evaluated the freshness of the fillets to determine the shelf-life of differently treated products. The sensory panel was trained according to international standards (ISO 1993), including detection and recognition of tastes and odors and training in the use of scales. Sensory assessments were carried out by eight to twelve assessors (age range 30 to 55). Fish from each treatment was portioned into an aluminum box and cooked in a steam oven (98 to 100 °C for 5 min). Each treatment was assessed in duplicate and the samples were anonymously coded. A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystèmes, France) was used for data recording of cooked samples and further data processing. Average scores were calculated for each treatment and significant differences between corresponding treatments evaluated.

The Torry scheme (Shewan and others 1953) was used to assess the freshness (odor and flavor) of cooked pieces. The scheme ranges from 10 = very fresh to 3 = very spoiled, with a rejection level at 5.5.

Microbial analysis

Fillets were aseptically minced, assessing 2 pooled fillets for each sample. Minced flesh (25 g) was mixed with 225 mL of cooled Maximum Recovery Diluent (MRD, Oxoid) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required. Total viable psychrotrophic counts (TVC, 15 °C) were evaluated by spread-plating aliquots

onto modified Long & Hammer's medium; counts of H_2S-producing bacteria and presumptive pseudomonads were evaluated on spread-plated Iron Agar (15 °C) and modified CFC medium (22 °C), respectively (Lauzon and others 2002).
Counts of *Photobacterium phosphoreum* were estimated by using the PPDM-Malthus conductance method (Dalgaard and others 1996), as described by Lauzon (2003).

TVB-N and pH measurements

Total volatile basic nitrogen content (TVB-N) was measured by the steam distillation method described by Malle and Poumeyrol (1989). The pH was measured in 5 grams of mince moistened with 5 mL of deionised water. The pH meter was calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

Electronic nose

Electronic nose measurements were performed using a gas sensor instrument called FreshSense (Maritech, Kópavogur, Iceland). The instrument is based on four electrochemical gas sensors: CO, H_2S, SO_2 (Dräger, Germany) and NH_3 (City Technology, Britain). The measurements were performed using a static headspace sampling as described earlier (Olafsdottir and others 2002). Duplicate analyses were done using approximately 500g of fish fillets. The samples were tempered at RT for approximately 30 minutes until the temperature of the fillets reached 8 to 12°C before recording the sensors' responses for 5 minutes.

Data analysis

Multivariate analysis was performed using the Unscrambler Version 9.1 (CAMO Process, Trondheim, Norway). The main variance in the data set was studied using Principal Component Analysis (PCA). Partial Least Squares Regression models (PLSR) were used to explore the correlation of the sensory, microbial, TVB-N, pH and electronic nose variables. The effect of temperature of fillets and time was also included as an independent variable by calculating, for all samples at each sampling day, the accumulative influence of temperature (T) and time (t): $T_{accumulative} = \sum (T - T_{min}) \times dt$; T_{min} was defined as the minimum temperature of fillets in storage.

Soft independent modeling of class analogy (SIMCA), a pattern recognition method relying on independent modeling of defined classes by means of PCA (Wold 1976) was used to classify samples according to sensory quality (Torry score) based on the sensor responses, microbial and chemical data. Average scores of assessors were used for the sensory data and average of sample replicates for each sample. Values were standardized to equal variance. Full leave-one-out cross validation was used in the validation method. The Jack-knife method (Martens and Martens 1999) was used to determine significant variables in X with a significance level of 5% ($p<0.05$).

Microsoft Excel 97 was used to calculate means and standard deviations for all multiple measurements and to generate graphs.

Analysis of variance (ANOVA) was applied to the data using the Number Cruncher Statistical Software (NCSS 2000). Significant differences were determined by One way ANOVA and Duncan's Multiple-Comparison Test was used to determine the statistical difference between samples. An effect was considered significant at the 5% level ($p<0.05$).

The bacterial growth data collected were fitted to determine maximum specific growth rates (h^{-1}) by using DMFit (http://www.ifr.ac.uk/safety/DMFit/), an in-house program of IFR (UK) which is based on a reparameterized version of the model of Baranyi and Roberts (1994). DMFit is an Excel add-in program to fit curves where a linear phase is preceded and followed by a stationary phase.

Results and Discussion

Effect of handling and temperature on shelf-life determined by sensory analysis

Initial average temperature of the fillets varied based on the processing practice used (Fig. 1). In a conventional process the temperature of fillets will typically increase during filleting, skinning and trimming. The process in factory I (batch 1) was a conventional process that did not include cooling of the fillets before packaging, resulting in a much higher initial temperature of the fillets (4 to 6 °C for groups A and B) than in factory II where cooling of the fillets was applied (0.5 °C for groups H and I) (Fig. 1). The temperature fluctuations of the fillets that were simulated firstly by storing the raw material without ice until processed (group D) as seen by the highest initial temperature

and secondly, by exposing the fillets to temperature fluctuations during storage to simulate possible scenarios of abusive conditions during transport (groups B, D and I) are shown in Fig. 2a. The superchilling process effectively cooled the fillets and much lower initial temperature was observed (-1.0 to -1.4°C) than for the traditionally processed groups (Fig. 1).

The shelf-life determined by estimating the day of sensory rejection (Torry score of 5.5). from the sensory data (Fig. 2b and 3b) was much shorter for the traditionally processed groups that were severely temperature abused (8 and 10.5 days for groups D and I, respectively), compared to the corresponding groups (A and H from batches 1 and 3) stored continuously at the same temperature with a shelf-life of 12 and 13.5 days, respectively (Fig. 1). The extended shelf-life of group H was due to the lower initial temperature of batch 3 (Fig. 1) compared to batch 1 because of the initial cooling of the fillets. It should also be pointed out that the processes differed in the initial handling since batch 3 was processed one day after catch, while batch 1 had been stored whole on ice for three days onboard the trawler prior to processing. The average storage temperature of groups A and B was similar, but the temperature abuse of group B (15 °C for 8h) (Fig 2a) resulted in its slightly more rapid quality deterioration, as seen by a lower Torry score ($p<0.05$) on day 7 compared to group A (7.6 and 8.0, respectively). However, the resulting shelf-life was similar (11.5 to 12 days) for both groups (Fig. 2b). Similar shelf-life of 10 to 12 days was reported in an earlier study on conventionally processed cod fillets, filleted one day after catch or a total shelf-life of 11 to 13 days post catch (Magnússon and Martinsdóttir 1995).

The superchilling process (CBC) extended the shelf-life of batch 1 slightly by 0.5 day (12.5 d for group C), while the second trial (batch 2: groups E, F and G) demonstrated that further storage of CBC-processed fillets under superchilled conditions (-1.5 °C) was most effective in prolonging shelf-life, resulting in a shelf-life of groups G and F of about 15 days compared to groups C and E with shelf-life of 12.5 and 14.5 days, respectively (Fig. 1). Additional sensory analysis was performed (data not shown) to evaluate the texture of the superchilled fillets. Only little difference was observed between groups except on day 8 when the fillets stored at -1.5 °C (group F) exhibited softer texture ($p<0.05$) than fillets stored at 0.5 °C (group E).

Extension of marketable shelf-life and effect of processing and storage techniques

It is of interest to compare the actual storage time of the fillets when the sensory score was 7, because this score is more realistic to evaluate the marketability of the fillets (Figs.2b and 3b). At this stage the fillets are described as neutral in odor and taste and have lost their characteristic initial fresh, sweet taste. Shelf-life extension of fresh fish can be reached by different processing and/or storage methods, but usually the neutral phase is extended rather than the earlier stage where freshness characteristics are important. It is therefore important to evaluate whether superchilling processing and storage is extending this earlier phase, since this would provide an improved market value. For batches 1 and 3, the traditionally processed fillets had reached score 7 on day 9 (groups A and H) while the CBC-processed fillets (group C) reached this score after 11 days. For batch 2 (CBC-processed), group E (0.5 °C) reached this score on day 11, but groups F and G (superchilled storage) after 13 days. This shows that CBC-processed fillets will have an extended marketable shelf-life. Furthermore, storage under superchilled conditions (-1.5 °C) will prolong the time of marketability for at least two days. This could contribute to improved quality of fillets for consumers in distant markets

Microbial analysis

The initial microbiological quality of the raw material of the different batches varied when evaluated on the first sampling day, one day after packing in styrofoam boxes (Table 1). The initial total viable psychrothrophic counts (TVC) were lowest (log 3.9 CFU/g) for traditionally processed fillets (batch 1, Factory I) and highest for the CBC-processed fillets in batch 2 (log 5 CFU/g). A higher TVC (log 4.8 CFU /g) was also observed for CBC-processed fillets (group C) from batch 1 compared to traditionally processed groups which could be related to poorer hygienic handling of the former (contaminated chilling brine or processing equipment) as well as to the fact that the traditionally prepared fillets underwent a rinsing step while being deskinned mechanically, hence slightly decontaminated. Also, a high TVC was observed for batch 2 despite the fact that the raw material was processed one day after catch while batch 1 was processed 3 days after catch. It should be pointed out that because of the continuing process in the factory, an older batch had been processed earlier the same day as the experiment was carried out with batch 2. Apparently, the processing lines were not

cleaned appropriately before the experimental groups were processed and therefore cross-contamination from the older raw material may have occurred. This was unfortunate, but emphasizes the need for thorough cleaning procedures between processing different batches. This may have influenced the outcome of the study, resulting in a shorter shelf-life of the superchilled experimental groups than could have been obtained if hygienic conditions would have been optimal.

Initial counts of SSOs showed that slightly higher levels of H_2S-producing bacteria and *P. phosphoreum* (*Pp*) were found in batch 1 (3 days post catch) than batch 2 (1 day post catch) and that batch 3 (1 day post catch) had the lowest counts of all (Table 1). Pseudomonads counts were found in highest numbers on the first sampling day for all batches. Comparison of bacterial development under normal chilled conditions (Figs. 4a,b) explains the shorter shelf-life of group A than group H, as best demonstrated by the maximum specific growth rate (μ_{max}) of *Pp* being higher for A (0.377 h^{-1} after a lag phase of 60h) than H (0.055 h^{-1}) (Table 2), probably due to higher initial *Pp* load (Fig. 4b) and higher temperature of processed fillets for batch 1 (Fig.2a). Abusive temperature conditions (groups B, D and I) triggered all SSOs evaluated, but post-processing temperature abuse usually led to a lag phase (32 to 60h) of SSOs before rapid growth (Table 2). It is quite clear that such undesirable environmental conditions will affect the physiological state of bacterial groups since lag phases were observed under abused temperatures for most groups. This behavior could be attributed to the fact that bacteria need to readjust to current environmental conditions, independent to how beneficial these are to their development. Under abusive conditions (8 and 16 h at RT), *Pp* was the fastest developing SSO (groups B and I: 0.153 and 0.442 h^{-1}) and the longer abusive treatment (I) affected its growth rate tremendously. Similarly, the maximum specific growth rate of H_2S-producing bacteria was much higher after having undergone the longer treatment, doubling in value (groups B and I: 0.062 and 0.124 h^{-1}). However, similar growth rates were observed for pseudomonads following either treatment (groups B and I: 0.085 and 0.089 h^{-1}). Dominance of *Pp* (log 7.2 to 7.8/g) over other SSOs was usually observed under chilled and abused conditions, representing 12.6 to 17.2% (A and H) and 16.2, 24.0 and 12.7% of the total microflora (B, I and D, respectively). *Pp* load was considerable in group D (log 6.0/g, representing about 10% of microflora) on the first sampling day (day 6) compared to that of group B (log 2.7/g) analysed on day4 (Table 1). The apparent

slower growth rate observed for group D, reflected the declining growth during late exponential growth phase while approaching the stationary phase (Table 1). This indicates that unproper icing and handling of raw material, leading to higher temperature of the fillets, may especially influence the development of *Pp* in the raw material. However, extreme temperature abuse (groups I and D) obviously stimulated H_2S-producing bacteria over pseudomonads, as seen by a faster growth rate and the levels reached by the former (log 6.2/g) compared to the latter (log 4.4/g) on the last sampling day (group D). Interestingly, dominance of pseudomonads was only observed in spoiled fillets originating from the factory II (groups H and I, batch 3) where cod was caught in the Southwest of Iceland. High counts of pseudomonads in fillets from batch 3 are in agreement with recent studies on haddock fillets from the same factory (Olafsdottir and others Forthcoming). In that study, *Pp* was the predominant SSO based on their counts, but the characteristic sweet, fruity spoilage odors were associated with the growth of *Pseudomonas spp*. The study of Jørgensen and others (1989) showed on the contrary that H_2S-producers were the main SSO in aerobically stored cod fillets from different origin, but this can be explained by the different conditions in the factories, since the origin of the H_2S-producers is mainly from the filleting plants (Van Spreekens and Toepoel 1981). The CBC-superchilling process was efficient in cooling the newly processed fillets (groups C, E, F and G) (Fig. 3a), resulting in slower bacterial development (Figs. 5; Table 2) which was further decreased under superchilled storage (groups F and G). The total superchilling effect of each group, ranking the most to the least as F, G, C, E is illustrated in Fig. 1 and reflected by the corresponding *Pp* growth rates of 0.059 (lag phase of 105.6h), 0.034, 0.042, 0.072 h^{-1} (lag phase of 75.2h), respectively (Table 2), exemplifying the slower growth of *Pp* caused by the degree of superchilling. *Pp* had therefore reached much lower counts (log 6 to 6.8/g) than in traditionally processed fillets (log 7.2/g) at sensory rejection (Table 1). This difference is probably explained by the sensitivity of this bacterium to sudden cooling/freezing, which can lead to a cold shock, damaging the cells and resulting in slower initial growth. Pseudomonads had the slowest growth rates (Table 2) and reached similarly low levels at sensory rejection (Table 1), indicating their sensitivity to superchilled conditions. H_2S-producing bacteria grew quite steadily in fillets from batch 2 (Fig. 5d) and dominated (24-36%) over other SSOs (Table 1). However, it cannot be confirmed whether this was due to the effect of the superchilling process on

other SSOs which may have decreased competition for H_2S-producing bacteria or to the cross-contamination from the older raw material occurring during processing. H_2S-producing bacteria were found at lower levels (log 5.9/g) in superchilled fillets of batch 1 (group C). Nevertheless H_2S-producing bacteria seemed to tolerate better the superchilling conditions.

TVB-N analysis

The formation of TVB-N was delayed in the superchilled groups (Fig. 6b) compared to the traditionally processed groups (Fig. 6a). At sensory rejection different levels of TVB-N were found in the experimental groups (38-55 mgN/100g) (Table 1). The highest TVB-N value (94 mgN/100g) was measured for the severely abused group D (Table 1), but this value is overestimated since the measurement was performed on day 10 while the estimated value at sensory rejection (day 8) seen in Fig 6a is about 35 mgN/100g. The influence of temperature abuse was seen in a more rapid production of TVB-N for groups B and I compared to groups A and H, respectively, stored at constantly low temperature (Fig. 6a).

The highest TVB-N values at sensory rejection for the superchilled groups (Fig. 6b) were observed in groups C and E that were stored at 0.5 °C (53 and 56 mg N/100g, respectively). Both H_2S-producing bacteria and *P. phosphoreum* are known to be able to produce TMA (Van Spreekens 1974; Jørgensen and others 1988; Dalgaard 1995). The higher levels and more rapid growth of *P. phosphoreum* in groups C and E compared to groups G and F stored at -1.5 °C (Fig.5b) suggest their importance in contributing to high TVB-N values throughout the storage time for groups C and E (Fig. 6b). It is likley that both H_2S-producing bacteria and *P. phosphoreum* were actively producing TMA in group E based on their highest counts in that group. Although the growth rate of these bacteria was slower in groups F and G, high levels of TVB-N (~38 mg/100 g) were formed and an increased growth rate of *P. phosphoreum* was observed for group G between days 10 and 12 after the group had been transferred to higher storage temperature (Fig. 5b). The influence of low temperature on overall decreased growth rate of H_2S-producing bacteria and *P. phosphoreum* appears not to hinder their ability to produce TMA under superchilling conditions.

Based on the findings of Dalgaard (1995) that *P. phosphoreum* was a 30 times more active TMA producer than *S. putrefaciens* and cell counts of 10^7 CFU/g of *P. phosphoreum* corresponded to 30 mgN/100g TMA in packed cod fillets, it is likely that *P. phosphoreum* is very important in the formation of TVB-N at least in the traditionally processed groups where *P. phosphoreum* dominated and reached the highest counts of $>10^7$ CFU/g at sensory rejection. However, based on their lower counts in the superchilled groups (log 6.0 to 6,8) (Table 1) the role of the H_2S-producers in contributing to TVB-N can not be overlooked since their counts reached $>10^7$ CFU/g in the groups E, F and G. According to Jørgensen and Huss (1989) in a study with fish juice inoculated with *S. putrefaciens* and Dalgaard (1995) who studied TMA production in packed cod, TMA was detected when counts of *S. putrefaciens* were about 10^7 CFU/g and off odours when counts reached 10^8 CFU/g. Similarily, Koutsoumanis and Nychas (2000) reported that higher counts (10^8 CFU/g) were observed in sterile fish inoculated with *S. putrefaciens* when TVB-N started to increase, followed by off odors detected by the sensory panel. Therefore, based on the H_2S-producers count ($>10^7$ CFU/g) it is likely that they could contribute to some of the TVB-N levels found in this study, but most likely in combination with *P. phosphoreum* to account for the high levels measured in the superchilled groups (38 to 55 mgN/100g).

It has been pointed out that TVB-N and TMA often give ambiguous information about the quality of the products and levels only increase at late storage when spoilage signs are obvious (Oehlenschläger 1997, 1998). Varying levels of TVB-N have been found at sensory rejection in whole cod and vacuum packed fillets (Jørgensen and others 1988). TVB-N levels are also influenced by the storage method (Dalgaard and others 1993; Magnússon and Martinsdóttir 1995; Debevere and Boskou 1996; Guldager and others 1998; Lauzon and others 2002).

Despite this controversy, fixed TVB-N limit (35 mg N/100 g) for acceptability of consumption for gadoids as a confirmation of a prior sensory assessment have been set in EU regulations (Anon 1995). Based on these limit the shelf-life of the experimental groups was evaluated (Fig. 6). A slightly shorter shelf-life was estimated, than when using the sensory Torry score criteria of 5.5, for the traditionally processed and temperature abused experimental groups A, B, D from batch 1 and the superchilled groups C and E. The estimated shelf-life based on TVB-N criteria for the superchilled

groups F and G was in agreement with shelf-life according to the sensory rejection limit (Table 1). On the other hand longer shelf-life was estimated for sample groups H and I processed from batch 3 and they had not exceeded the TVB-N limit of 35 mgN/100g at sensory rejection. Interestingly, the predominance of *Pseudomonas* spp. (log 7.4 to 7.5 CFU/g) in these groups may explain the lower level of TVB-N because pseudomonads do not produce TMA (Castell and Greenough 1959). However, considerable levels of TVB-N (27 to 34 mgN/100g) were found at sensory rejection which can be accounted for by the high levels of *P. phosphoreum* or 17 to 24% of the pyschrotophic counts, although the pseudomonads were dominating (25 to 38 %). Koutsoumanis and Nychas (2000) suggested pseudomonads level of 10^7 CFU/g as a limit for the end of shelf-life coinciding with a TVB-N level of about 26 mg N/g in a Mediterranean species.

pH measurements

The effect of the different catching techniques and time elapsed from catching until processing is seen by the difference in the initial pH value (Table 1). Higher pH value ($p<0.05$) was observed for batch 3 than batch 1 initially (6.85 and 6.70, respectively), this may be explained by the different catching methods, longline and bottom trawl, respectively. Similar difference in pH for cod was observed for longline and gillnet by Esaiassen and others (2004). The age of the raw material when processed can also explain the difference in initial pH measured, because of the autolytic post mortem changes (Huss 1995). Therefore, the lower pH value for batch 1 (6.70) can also be related to the fact that it was processed 3 days post catch while batch 3 was processed one day post catch (6.85). The difference in the initial pH value because of the effect of different chatching methods is a drawback when using pH to monitor quality. However, changes are seen with time in some groups although the end value is not the same at sensory rejection. Increase in pH values ($p<0.05$) were noticed with storage time for sample groups A, D, the superchilled group C from batch 1 and sample group G from batch 3 (Table 1). The initial pH value of group D measured on day 6 post catch was the lowest (6.55) possibly because of the extent of the autolytic changes under high temperature storage (Huss 1995). A lower initial pH for group C (6.58) compared to the traditionally processed groups may be related to the chilling process. The highest increase in pH with storage time was observed for groups C and D with the lowest inital pH. High correlation of pH

and the CO sensor were observed for groups C and D (r^2 = 0.81 and 0.87, respectively) and the TVB value for groups C and D (r^2 = 0.94 and 0.97, respectively). The correlation of pH to microbial counts was observed for the H_2S –producer´s counts (r^2 = 0.83) in group D, while there was a correlation with all the SSOs (r^2 >0.88) in the superchilled processed group C indicating the difference in the spoilage at abusive temperatures compared to the superchilled process despite similar pH levels.

Electronic nose measurements

CO sensor - detection of alcohols, aldehydes and esters: Spoilage of the fillets was characterized by higher response of the CO sensor than the other sensors (Fig. 7). The CO sensor can detect alcohols, aldehydes and esters (Olafsdottir and others 2002) which are common metabolites of spoilage bacteria like *Pseudomonas* ssp. (Miller and others 1973a, 1973b). Increase was noticed earlier for the traditionally processed (Fig.7a) groups than for the superchilled groups (Fig.7b) showing that the spoilage process was slower at lower temperature, which was in agreement with the other data. Increased CO response was first observed for the temperature abused groups D and I, followed by group B (seen as open data points in Fig 7a) in agreement with the TVB-N analysis. The superchilled group F had the lowest CO response (Fig 7b) suggesting the slowest spoilage rate for that group. The influence of higher temperature storage of group G is seen as slightly higher CO response on day 12 in agreement with microbial analysis in particular *P. phosphoreum* showing increasing counts (Fig. 5b) and TVB-N measurements (Fig.6b).

The fact that the CO sensor responses correlated well with the TVB-N values for both the traditionally processed groups (r^2=0.87) and the superchilled groups (r^2=0.83) indicates that TMA and metabolites like alcohols and aldehydes were being produced simultaneously. Based on the higher counts of the *P. phosphoreum* and H_2S producing bacteria (Fig. 4 d) it is likely that these bacteria could be contributing to the development of metabolites that the CO sensor can detect. *S. putrefaciens* is known to produce alcohols like 3-methyl-butanol (Miller and others 1973b) and could contribute to the CO sensor response. It is likely that other volatile compounds not detected by the sensors may also contribute to the spoilage of the samples, in particular it should be mentioned that the CO sensor can not detect ketones, which are produced by pseudomonads and

Photobacterium in fish during storage (Miller and others 1973c; Van Spreekens and Toepoel 1981) and may also contribute to the spoilage odors.
Large standard deviation observed for the CO sensor is explained by the effect of the varying temperature (8-12 °C) during measurements of samples influencing the volatility of the compounds measured and causing deviations. Better control of temperature during sampling would improve the performance of the system (Olafsdottir 2003).

NH_3 sensor – detection of TMA and NH_3: At the end of the storage time, increasing response was observed for the NH_3 sensor for sample groups B, D, and I stored under abusive temperature conditions and the superchilled group C (Table 1). The response of the NH_3 sensor was higher ($p<0.05$) for the superchilled group C than the traditionally processed group A from the same batch, although the overall spoilage appeared to be slower in C. As discussed before it is likely that *P. phosphoreum* which is known to be an active TMA producer (Van Spreekens, 1974; Dalgaard 1995) may be contributing to the high TVB-N content and NH_3 sensor response under superchilled conditions. Offensive ammonia-like, dried fish odor was observed at sensory rejection of the superchilled fillets, but when tasted the spoilage was less noticeable indicating that odor and volatile compounds were probably limiting their shelf-life.
H_2S and SO_2 sensors – detection of sulfur compounds: Very low response of the H_2S and SO_2 sensors were observed in all samples but slight increase was observed for the H_2S sensor at the end of the storage time for traditionally processed and abused sample groups B, D, H and I although not significant (Table 1). This suggests the influence of H_2S-producing bacteria under abusive temperature conditions as reported by Lauzon (2000). Slightly higher H_2S sensor values were also noticed in the superchilled groups E and F which can be explained by dominance of the H_2S -producers in these groups (25% and 36%, respectively) (Table 1). At advanced spoilage of fish, onion-like, sulfide and rotten vegetable-like, putrid spoilage odors develop because of microbially produced sulfur compounds (Herbert and others 1975; Shewan and others 1953; Miller 1973a). Earlier studies using the FreshSense electronic nose have shown increasing response of H_2S and SO_2 sensors at advanced spoilage of whole capelin and redfish (Olafsdottir and others 1997, 2000, 2002), but response to fillets of cod and haddock is generally low (Olafsdottir 2003; Tryggvadóttir and others 2001). The overall insignificant response of

the SO_2 sensor indicated that sulfides were not present or in very low levels in the samples, below the detection limit of the sensors (data not shown). Based on the low responses of the H_2S and SO_2 sensors in this study, it appears that volatile sulfur compounds do not contribute significantly to the development of off odors of fillets at sensory rejection.

Correlation of microbial counts, TVB-N, pH and electronic nose sensors and prediction of sensory quality

Partial least squares regression (PLSR) was used to study the correlation of the variables and their contribution to predict the Torry sensory score (Fig. 8). A PLSR model based on all the samples (N = 41) and all the measured variables had a high correlation ($r^2 = 0.97$) with a RMSEP of 0.29 (Table 3). Because of the low responses of the NH_3, H_2S and SO_2 sensors they explained less of the variance in the data than the other variables, as seen by their location in the middle of the plot (Fig 8). The same was observed for pH, since the influence of the different initial pH values of the samples precludes the use of pH as a spoilage indicator as explained above. The contribution of the significant ($p<0.05$) variables (TVC, SSO, TVB-N and CO sensor) to predict the sensory score was studied further by exploring different PLSR models (Table 3). A model based on the microbial variables had high correlation ($r^2 = 0.96$) and low prediction error (RMSEP = 0.32) indicating that microbial counts were well suited to predict the sensory quality of the overall data set. This was expected since the growth of the SSO characterized the spoilage pattern of the samples. A model based on the classical spoilage indicators TVC, TVB-N and pH had a lower correlation ($r^2 = 0.92$) and higher error (RMSEP = 0.41), indicating that the SSO gave better information about the sensory quality. PLSR model based on the electronic nose variables was inferior with much lower correlation ($r^2 = 0.70$) and higher error (RMSEP = 0.80).

Because of the significance of the CO sensor variable to predict the Torry score and the importance of TVB-N (Fig 8) it was of interest to study further a model including these variables in combination with the SSOs. The resulting PLSR model ($r^2 = 0.97$ and RMSEP = 0.30) showed that the overall spoilage pattern of the differently treated samples was similar as indicated by the arrow, tracking the increasing spoilage level of samples along PC 1 from right to left (Fig 9). The second PC explained the influence of

the temperature conditions on the spoilage pattern in the different experimental groups. The superchilled samples E, F and G tended to be located lowest on the plot and were best described by the H_2S-producer counts. The traditionally processed groups H and I were placed in the middle and described by the *Pseudomonas* spp. while the traditionally processed groups with the highest temperature during storage were situated on the upper half of the plot showing that the temperature abused samples D6 and D7 were most affected and described by high *Photobacterium* counts and high values of the TVB-N and the CO sensor Despite the differences in the spoilage rate of the groups explained by the influence of the different temperature conditions and initial handling on the dominating SSO in the samples (PC2), the main changes in their spoilage level was explained by their different location along PC 1. Therefore, it appears that these quality indicators could be used to classify both the traditionally processed and the superchilled samples.

Classification based on sensory criteria

Classification of the differently treated samples based on sensory criteria (Torry score >7 and Torry score < 7) using five selected variables (TVB-N, CO sensor, H_2S-producers, pseudomonads and *P. phosphoreum*) was studied using SIMCA. The Torry score of 7 was selected because at this point the samples are at the last stage of acceptable marketable quality and it is of interest to determine if samples are still acceptable for distribution. The results showed that 85% of the samples were correctly classified into respective classes (34 samples) while 15% were identified in both classes (6 samples). The samples that were wrongly classified in both classes were samples A10, C10, D7, H9, E10 and F12 that were located in the middle of the plot and not well described by the variables (Fig 9). All these samples had sensory scores close to 7. For example, the superchilled samples (E10 and F12) with Torry scores <7 had at this point low values for the quality variables suggesting that other indicators are needed to describe better their sensory characteristics. The role of endogenous enzymatic degradation in the fillets may explain partly the quality deterioration (Ashie and others 1996) and should be studied further in particular in relation to superchilled conditions when microbial growth is delayed.

Conclusions

The spoilage characteristics of cod fillets were influenced by the dominating bacteria during storage in the experimental groups. The results emphasize the importance of studies performed with natural products and processes to accumulate information about spoilage domain of SSOs under actual conditions. The origin of the raw material, the initial handling and conditions in the processing factories including the time after catch before processing influenced the shelf-life. Improper icing and handling of raw material, leading to higher temperature of the fillets, especially influenced the development of *P. phosphoreum*, but growth of H_2S-producing bacteria was also accelerated at higher temperatures contributing to a shorter shelf-life of temperature abused cod fillets. Initial cooling of the traditionally processed fillets resulted in a lower average temperature of the fillets during storage and *Pseudomonas* spp. were dominating under these conditions resulting in less offensive odors and longer shelf-life. Under superchilling conditions the growth rate of all the bacterial groups was slower, however, higher levels of TVB-N were observed at sensory rejection for superchilled fillets stored under chilled conditions (>0 °C). The importance of the H_2S-producing bacteria in one batch of superchilled fillets was related to the initial contamination of the raw material in the processing while *P. phosphoreum* was dominating in superchilled fillets produced from another batch. The spoilage characteristics of superchilled as well as chilled and temperature abused fillets were mainly explained by high TVB-N content as well as metabolites that the CO sensor could detect, most likely short chain alcohols, aldehydes and esters. Classification of all the samples according to sensory quality (Torry score >7 and <7) using the counts of *P. phosphoreum, Pseudomonas* spp and H_2S-producing bacteria in combination with the CO sensor and TVB-N value gave 85% correct classification, indicating that these quality indicators do not adequately describe the quality changes of the samples. Further studies should include more sensitive detection of the microbial metabolites, including detection of ketones and other oxidatively or enzymically derived degradation compounds contributing to the sensory rejection of chilled and superchilled fillets. Superchilling with the new CBC technique and storage at 0.5 °C resulted in an overall sensory shelf life of 12.5 to 14 days. When combined with superchilled storage at -1.5 °C an extension in shelf-life to at least 15 days was achieved. Comparing the marketable shelf-life (Torry

score=7) of all the groups tested revealed that the CBC-process increased the shelf-life, by 2 days, while further storage of such fillets under superchilled conditions gave an additional 2 days extension.. Such gain in shelf-life is of high economical value since it would allow the distant transportation of fresh fillets by ship or truck, which is less costly compared to air freight.

Acknowledgments

The authors thank The Icelandic Centre for Research and the AVS research fund of the Ministry of Fisheries for partly financing the project. The staff at IFL is thanked for their valued contribution in chemical, microbial and sensory analysis of samples as well as the personnel at the participating companies Tangi, Tros and Skaginn for their support.

References

Anonymous 1995. Decision of the European Commission to fix TVB-N limits for certain categories of fishery products (95/149/EEC) of March 28, 1995.

Ashie IN, Smith JP, Simpson BK. 1996. Spoilage and shelf-life extension of fresh fish and shellfish. Crit Rev Food Sci Nutr 36(1-2):87-121.

Baranyi J, Roberts TA. 1994. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol 23: 277-94.

Castell CH, Greenough F, Dale J. 1959. The action of *Pseudomona*s on fish muscle: 3. Identification of organisms producing fruity and oniony odours. J Fish Res Board Can 16(1):13-9.

Chang KLB, Chang JJ, Shiau CY, Pan BS. 1998. Biochemical microbiological and sensory changes of sea bass (*Lateolabrax japonicus*) under partial freezing and refrigerated storage. J Agric Food Chem 46(2):682-6.

Dalgaard P, Gram L, Huss HH. 1993. Spoilage and shelf life of cod fillets packed in vacuum or modified atmospheres. Int J Food Microbiol 19:283-94.

Dalgaard P. 1995. Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int J Food Microbiol 26:319-33.

Dalgaard P, Mejlholm O, Huss HH. 1996. Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. J Appl Bacteriol 81:57-64.

Dalgaard P. 2000. Fresh and lightly preserved seafood. In: Man CMD, Jones AA, editors. Shelf life evaluation of foods. 2nd ed. Maryland, USA: Aspen Publishing Inc. p 110-39.

Debevere J, Boskou G. 1996. Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. Int J Food Microbiol 31:221-9.

Di Natale C, Olafsdottir G, Einarsson S, Mantin A, Martinelli E, Paolesse R, Falconi C, D'Amico A. 2001. Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets. Sens Act B77 572-8.

Esaiassen M., Nilsen H, Joensen S, Skjerdal T, Carlehog M, Eilertsen G, Gundersen B, Elvevoll E. 2004. Effects of catching methods on quality changes during storage of cod (*Gadus morhua*). Lebensm-Wiss Technol 37:643–8.

Fennema OR, Powrie WD, Marth EH. 1973. 4-6.2 Kinetics of enzymic reactions in the frozen state. In: Low-temperature preservation of foods and living matter. New York: Marcel Dekker INC. p 217-39.

Gram L, Huss HH. 1996. Microbiological spoilage of fish and fish products. Int J Food Microbiol 33:121-37.

Guldager HS, Bøknæs N, Österberg C, Nielsen J, Dalgaard P. 1998. Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2°C. J Food Prot 61:1129-36.

Herbert RA, Ellis JR, Shewan JM. 1975. Isolation and identifiaction of the volatile sulphides produced during chill-storage of North Sea cod (*Gadus morhua*). J Sci Food Agric 26:1187-94.

Herbert RA, Shewan JM. 1976. Roles played by bacterial and autolytic enzymes in the production of volatile sulphides in spoiling North Sea cod (*Gadus morhua*). J Sci Food Agric 27:89-94.

Huidobro A, Lopez-Caballero ME, Mendes R. 2002. Onboard processing of deepwater pink shrimp (*Parapenaeus longirostris*) with liquid ice: Effect on quality. Eur Food Res Techn 214:469-75.

Huss HH, Dalgaard P, Gram L. 1997. Microbiology of fish and fish products. In: Luten JB, Börresen T, Oehlenschlager J, editors. Seafood from producer to consumer; intergrated approach to quality. Amsterdam, The Netherlands: Elsevier. p 413-30.

Huss HH. 1995. Quality and quality changes in fresh fish. FAO Fisheries Technical Papers - T348. Rome: Food & Agriculture Organisation of the United Nations & Danish International Development Agency (FAO/DANIDA). 195 p.

Ingram M, Mackey BM. 1976. Inactivation by cold. In: Skinner FA, Hugo WB, editors. Inhibition and inactivation of vegetative microbes. London: Academic Press. p 111-151.

Ingram M. 1951. The effect of cold on microorganisms in relation to food. Prot Soc Appl Bacteriol 14:243-9.

ISO 1993. Sensory analysis - general guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors, 8586-1. Geneva, Switzerland: The International Organization for Standardization. p 1-10.

Jeyasekaran G, Ganesan P, Jeya Shakila R, Maheswari K, Sukumar D. 2004. Dry ice as a novel chilling medium along with water ice for short-term preservation of fish Emperor breams, lethrinus (*Lethrinus miniatus*). Innov Food Sci Emerg Technol 5:485–93.

Jørgensen BR, Gibson DM, Huss HH.1988. Microbiological quality and shelf life prediction of chilled fish. Int J Food Microbiol 6:295- 07.

Jørgensen BR, Huss HH. 1989. Growth and activtiy of *Shewanella putrefaciens* isolated from spoiling fish. Int J Food Microbiol 9:51-62.

Koutsoumanis K, Nychas GJE. 2000. Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. Int J Food Microbiol 60:171–84.

Lakshmanan R, Jeya SR, Jeyasekaran G. 2002. Survival of amine-forming bacteria during the ice storage of fish and shrimp. Food Microbiol 19:617-25.

Larsen E, Hyldig G, Dalgaard P, Bech AC, Österberg C. 2003. Consumers and experts responses to fresh cod fillets. In: Luten JB, Oehlenschläger J, Olafsdottir G, editors. Quality of Fish form Catch to Consumer. Wageningen, The Netherlands: Wageningen Academic Publishers 2003. p 345-60.

Lauzon HL. 2000. Shelf-life and bacteriological spoilage of American plaice (*Hippoglossoides platessoides*). In: Shahidi F, editor. Seafood in health and nutrition, transformation in fisheries and aquaculture: global perspectives. NF, Canada: A Science Tech. Book, St. John´s. p 327-54.

Lauzon HL, Stefánsson G, Jónsson GP, Sveinsdóttir K. 2002. Sensory quality and shelf life of MAP redfish (*Sebastes marinus*) fillets as affected by previous bulk storage of whole fish. J Aquat Food Prod Technol 11(3/4):251-73.

Lauzon HL. 2003. Notkun Malthus leiðnitækni til hraðvirkra örverumælinga. IFL project report 30-03, 30p (in Icelandic).

LeBlanc RJ, LeBlanc EL. 1992. The effect of superchilling with CO_2 snow on the quality of commercially processed cod (*Gadus morhua*) and winter flounder (*Pseudopleuronectes americanus*) fillets. In: Huss HH, Jakobsen M, Liston J, editors. Quality Assurance in the Fish Industry. Amsterdam, The Netherlands: Elsev Sci Pub B.V. p 115-23.

Lee CM, Toledo RT. 1984. Comparison of shelf life and quality of mullet stored at zero and subzero temperature. J Food Sci 49:317-22.

Lindsay RC, Josephson DB, Olafsdottir G. 1986. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In: Kramer DE, Liston J, editors. Proceedings of an International Symposium, University of Alaska Sea Grant Program, Anchorage, Alaska, USA. Amsterdam, The Netherlands: Elsev Sci Pub B.V. p 221-34.

Losada V, Piñeiro C, Barros-Velásques J, Aubourg SP. 2004. Effect of slurry ice on chemical changes related to quality loss during European hake (*Merluccius merluccius)* chilled storage. Eur Food Res Technol 219:27-31.

Magnussen OM, Nordtvedt TS, Torstveit AK. 1998. Use of partial freezing in the cold chain. In: IIR Proceeding Series “Refrigeration Science and Technolgy” 1998/6. Paris, France: Intl. Inst. of Refrigeration. p 363-70.

Magnússon H, Martinsdóttir E. 1995. Storage Quality of fresh and frozen-thawed fish in ice. J Food Sci 60:273-8.

Malle P, Poumeyrol M. 1989. A new chemical criterion for the quality control of fish: Trimetylamine / total volatile basic nitrogen (%). J Food Protect 52:419-23.

Martens H, Martens M. 1999. Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). Food Qual Pref 11:5-16.

Miller III A, Scanlan RA, Lee JS, Libbey, LM. 1973a. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas putrifaciens, Pseudomonas fluorescens,* and an *Achromobacter* species. Appl Microbiol 26 (1):18-21.

Miller III A, Scanlan RA, Lee JS, Libbey LM. 1973b. Identification of the volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas fragi.* Appl Microbiol 25 (6):952-5.

Miller III A, Scanlan RA, Lee JS, Libbey LM, Morgan ME. 1973c. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas perolens.* Appl Microbiol 25:257-61

Oehlenschläger J. 1997. Volatile amines as freshness/spoilage indicators. A literature review. In: Luten J, Börresen T, Oehlenschläger J, editors. Seafood from producer to consumer, integrated approach to quality. Amsterdam: Elsevier p 571-88.

Oehlenschläger J. 1998. Suitability of ammonia-N, dimethylamine-N, trimethylamine-N, trimethylamine oside-N and total volatile basic nitrogen as freshness indicators in seafoods. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K, editors. Methods to determine the freshness of fish in research and industry. Paris: International Institute of Refrigeration. p 92-9.

Olafsdottir G, Hognadottir A, Martinsdottir E, Jonsdottir H. 2000. Application of an electronic nose to predict total volatile bases in capelin (*Mallotus villosus*) for fishmeal production. J Agric Food Chem 48:2353-9.

Olafsdottir G, Martinsdottir E, Jonsson EH. 1997. Rapid gas sensor measurement to determine spoilage of capelin (*Mallotus villosus*). J Agric Food Chem 45:2654-9.

Olafsdottir G, Xiuchen L, Lauzon H, Jonsdottir R. 2002. Precision and application of electronic nose for freshness monitoring of whole redfish stored in ice and modified atmosphere bulk storage. J Aquat Food Prod Technol 11:229-49.

Olafsdottir G. 2003. Developing rapid olfaction arrays for determining fish quality. In: Tothill IE, editor. Rapid and on-line instrumentation for food quality assurance. Cambridge, England: Woodhead Publishing Ltd. p 339-60.

Olafsdottir G, Lauzon H, Martinsdottir E, Kristbergsson K. Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction. Forthcoming.

Olson JC, Nottingham PM. 1980. Temperature. In: ICMSF Editorial Committee (J.H. Silliker (chairman)). Microbial Ecology of Foods. Vol. I. ICMSF. New York: Academic Press. 1-37.

Riaz-Fatima R, Khan MA, Qadri RB. 1988. Shelf life of shrimp (*Penaeus merguiensis*) stored in ice (0°C) and partially frozen (-3°C). J Sci Food Agric 42:235-47.

Shaw DH, Botta JR. 1975. Preservation of inshore male capelin (*Mallotus villosus*) stored in refrigerated sea water. J Fish Res Board Can 32: 2047-53.

Shewan JM, 1962. The bacteriology of fresh and spoiling fish and some related chemical changes. In: Hawthorn J, Leitch JM, editors. Recent advances in food science 1:167-93.

Shewan JM, Macintosh RG, Tucker CG, Ehrenberg ASC. 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J Sci Food Agric 4:283-98.

Sivertsvik M, Rosnes JT, Kleiberg GH. 2003. Effect of modified atmosphere packaging and superchilled storage on the microbial and sensory quality of Atlantic salmon (*Salmo salar)* fillets. J Food Sci (4):1467-72.

Smith JSM, Hardy R, MacDonald I, Templeton J. 1980. The storage of herring (*Clupea harengus*) in ice, refrigerated sea water and at ambient temperature. Chemical and sensory assessment. J Sci Food Agric 31:375-85.

Tryggvadóttir SV, Olafsdottir G, Einarsson S. 2001. Multisensor for fish: Storage studies of cod in Reykjavik and Tromsö. Texture, electronic nose (FreshSense), FIGD analysis, RT-Freshmeter and sensory analysis. RF report 02-01 p 19-24.

Van Spreekens KJA. 1974. The suitability of Long & Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. Archiv fur Lebensmittelhygiene 25 (10):213-19.

Van Spreekens KJA, Toepoel L. 1981. Quality of fishery products in connection with the psychrophilic and psychrotrophic bacterial flora. In: Roberts TA, Hobbs G, Christian JHB, Skovgaard N, editors. Psychrotrophic microorganisms in spoilage and pathogenicity. London, UK: Academic Press. p 283-94.

Wold S. 1976. Pattern recognition by means of disjoint principal components models. Pattern recognition 8(3):127-39

Zeng QZ, Thorarinsdottir KA, Olafsdottir G. 2005. Quality changes of shrimp (*Pandalus borealis*) stored under different cooling conditions. J Food Sci Forthcoming.

Table 1- Overview of average storage temperature, shelf-life estimation and experimental data comparing initial (first sampling day) and final values measured at or near sensory rejection for all experimental groups of cod fillets stored in styrofoam boxes at different temperatures

	Initial values[a]			**Data at sensory rejection**								
	Batch 1	**Batch 2**	**Batch 3**	**Traditional process and temperature abuse**					**Superchilling process**			
	(A, B, C, D)	**(E, F, G)**	**(H, I)**	**A 0°C**	**B 0°C + T**	**D 0 °C + T**	**H 0 °C**	**I 0 °C + T**	**C 0 °C**	**E 0.5 °C**	**F -1.5°C**	**G -1.5/0.5°C**
Average temperature of fillets during storage				1.9 ± 0.9	2.0 ± 0.9	3.9 ± 1.9	0.4 ± 0.5	1.9 ± 2.3	-0.9 ± 0.6	-0.3 ± 0.5	-1.3 ± 0.03	-0.8 ± 0.7
Estimated shelf-life (days)[b]				**12**	**11.5**	**8**	**13.5**	**10.5**	**12.5**	**14**	**>15**	**15**
Estimated shelf-life (days)[c]				11	10.2	7	>15	11.5	11	12	15	15
Measurement day of data				*12*	*12*	*8*[d]	*14*	*11*	*12*	*15*	*15*	*15*
Sensory analysis (Torry score)	9.0 ± 0.2	8.6 ± 0.1	9.0 ± 0.2	5.4 ± 0.2	5.3 ± 0.5	4.0 ± 0.1[d]	5.6 ± 0.2	5.5 ± 0.1	5.9 ± 0.4	5.4 ± 0.4	5.7 ± 0.2	5.6 ± 0.5
TVC ($\log_{10}$ CFU/ g)	3.9 ± 0.0 (A,B) 4.8 ± 0.0 (C) 7.0 ± 0.1 (D)	5.0 ± 0.2	4.2 ± 0.1	**8.1 ± 0.1**	**8.0 ± 0.1**	**7.8 ± 0.1**	**8.0 ± 0.1**	**8.0 ± 0.1**	**7.9 ± 0.1**	**8.0 ± 0.1**	**8.0 ± 0.1**	**8.1 ± 0.5**
H_2S-producer counts	2.8 ± 0.1 (A,B) 3.0 ± 0.0 (C) 3.4 ± 0.5 (D)	2.3 ± 0.6	2.3 ± 0.7	7.0 ± 0.2	6.1 ± 0.1	6.2 ± 0.2	7.1 ± 0.0	6.6 ± 0.0	5.9 ± 0.4	7,4 ± 0.1	7,6 ± 0.2	7,5 ± 0.7
% H_2S-producers / TVC				**7.0%**	**1.4%**	**2.6%**	**12.9%**	**3.7%**	**1.0%**	**25.1%**	**36.3%**	**24.0%**
Pseudomonas counts	3.1 ± 0.3 (A,B) 3.5 ± 0.1 (C) 3.8 ± 0.0 (D)	3.4 ± 0.3	2.8 ± 0.1	6.6 ± 0.6	6.4 ± 0.4	4.4 ± 0.1	7.5 ± 0.3	7.4 ± 0.1	5.8 ± 0.5	6.4 ± 0.1	6.5 ± 0.2	6.5 ± 0.4
% *Pseudomonas* spp. / TVC				**4.9%**	**3.0%**	**0.04%**	**37.5%**	**25.4%**	**0.2%**	**2.5%**	**2,9%**	**2,4%**
P. phosphoreum counts	2.7 ± 0.4 (A,B) 3.1 ± 0.3 (C) 6.0 ± 0.3 (D)	2.2 ± 0.7	1.3 ± 0.5	7.2 ± 0.1	7.2 ± 0.1	7.1 ± 0.4	7.2 ± 0.1	7.4 ± 0.1	6.8 ± 0.2	6.8 ± 0.1	6.0 ± 0.1	6.7 ± 0.1
% *P. phosphoreum* / TVC				**12.6%**	**16.2%**	**12.7%**	**17.2%**	**24.0%**	**6.7%**	**6.3%**	**0.9%**	**3.8%**
TVB-N (mg N / 100 g)	12.2 ± 0.3 (A,B) 12.4 ± 1.2 (C) 18.0 ± 1.4 (D)	12.1 ± 0.5	12.4 ± 0.9	48.5 ± 0.9	47.5 ± 0.1	94 ± 15[d]	26.6 ± 0.6	33.6 ± 5.3	53.4 ± 0.4	55.8 ± 4.7	38.4 ± 5.0	38.7 ± 1.6
pH	6.70 ± 0.01 (A,B) 6.58 ± 0.06 (C) 6.55 ± 0.11 (D)	6.78 ± 0.13	6.90 ± 0.03	6.85 ± 0.09	6.73 ± 0.04	6.85 ± 0.05	6.86 ± 0.03	6.91 ± 0.08	7.00 ± 0.04	6.91 ± 0.01	6.92 ± 0.06	6.98 ± 0.02
CO sensor (nA)	37 ± 5 (A,B) 42 ± 2 (C) 232 ± 70 (D)	34 ± 20	14 ± 5	366 ± 72	439 ± 165	215 ± 65	98 ± 29	253 ± 22	158 ± 89	300 ± 156	136 ± 43	225 ± 108
NH_3 sensor (nA)	<10	<10	<10	<10	23 ± 6	34 ± 13	<10	33 ± 8	52 ± 13	<10	<10	<10
H_2S sensor (nA)	<20	<20	<20	<20	21 ± 24	20 ± 6	35 ± 8	22 ± 11	<20	20 ± 3	28 ± 33	<20

[a)] Initial values on first day of sampling (days after catch): Groups A, B, C (day 4), group D (day 6), goups E, F, G, H and I (day 2)

[b)] total shelf life, including days from catch, based on the sensory evaluation of cooked fish (Torry score = 5.5)

[c)] total shelf life, including days from catch, based on TVB-N = 35 mgN/g [d)] values for sensory and TVB-N data for group D from measurements on day 10

Table 2. - Maximum specific growth rate (h^{-1}) of different bacterial groups (*Pseudomonas* spp., *P. phosphoreum*, H_2S-producing bacteria) assessed by curve fitting using DMFit in cod fillets stored in styrofoam boxes under different temperature conditions

	Traditional process and temperature abuse					**Superchilling process**			
	A 0°C	**B 0°C + T**	**D 0 °C +**	**H 0 °C**	**I 0 °C +**	**C 0 °C**	**E 0.5 °C**	**F -1.5°C**	**G -1.5/0.5°C**
TVC	0.063	0.117 (28.4 h)[a]	0.028	0.034	0.114 (49.9 h)[a]	0.035	0.035	0.031 (48.9 h)[a]	0.024
Pseudomonas spp.	0.039	0.085 (38.8 h)[a]	0.040	0.039	0.089 (32.1 h)[a]	0.022	0.031	0.026 (29.5 h)[a]	0.024
S. putrefaciens	0.039	0.062	0.079	0.043	0.124 (59.5 h)[a]	0.030	0.043	0.034	0.042 (38.9 h)[a]
P. phosphoreum	0.377 (60.1 h)[a]	0.153 (44.2 h)[a]	0.035	0.055	0.442 (52.9 h)[a]	0.042	0.072 (75.2 h)[a]	0.059 (105.6 h)[a]	0.034

[a] estimated lag phase (h); [b] high initial counts

Table 3 - Correlation (r^2) and the error of prediction (root mean square error of prediction RMSEP) for different PLSR models based on the measured variables (X): chemical (TVB-N, pH), microbial (TVC, Pseud, Pp, H2S counts), electronic nose (CO, NH_3, H_2S, SO_2) to predict the sensory Torry scores (Y) of differently treated cod fillets.

	X	No of variables	Correlation r^2	RMSEP	N
All variables measured	sensors (4), microbes (4), TVB-N and pH	10	0.97	0.29	41
Microbial counts	TVC, Pseud, Pp, H_2S counts	4	0.96	0.32	40
Electronic nose	CO, NH_3, H_2S, SO_2	4	0.70	0.80	40
Classical	TVB-N, TVC, pH	3	0.92	0.45	41
E-nose+ TVB-N + SSO	CO, TVB, Pseud, H_2S counts, Pp	5	0.97	0.30	41

Fig. Captions

Figure 1- Influence of processes, initial average temperature (first day after packing) and average temperature of the fillets during storage on the shelf-life of experimental groups from different factories processed from different batches. Values next to experimental groups labels (A, B, C, D, E, F G, H and I) indicate the sensory shelf-life (days from catch). Temperature conditions of each group are shown in circles and abusive temperature treatments in triangles (RT = room temperature).

Figure 2 - a) Temperature profiles and b) sensory analysis (Torry score) of cod fillets from storage studies of traditionally processed and temperature abused experimental groups from batch 1 in factory I (A 0 °C (-■-); B 0 °C + post processing abuse (-□-); D 0 °C + pre- and post processing abuse (-x-) and experimental groups from batch 3 in factory II (H 0°C (-♦-); I 0 °C + abused (- ◊ -)). Lines indicate limit of sensory rejection (Torry score = 5.5) and marketable shelf-life (Torry score = 7.0).

Figure 3 - a) Temperature profiles and b) sensory analysis (Torry score) of cod fillets from superchilled experimental groups in factory I from batch 1 (C 0 °C new (--■--) and batch 2 (E 0.5 °C (-▲-); F -1.5 °C (-●-); G – 1.5 °C / 0.5 °C (-o-)). Lines indicate limit of sensory rejection (Torry score = 5.5) and marketable shelf-life (Torry score = 7.0).

Figure 4 - Counts of a) total viable psychrotrophic bacteria (TVC), b) *P. phosphoreum* (Pp), c) presumptive *Pseudomonas* spp. and d) H_2S-producing bacteria in traditionally processed and temperature abused experimental groups of cod fillets stored in styrofoam boxes: A 0 °C (-■-), B 0 °C + post processing abuse (-□-) and D 0 °C + pre- and post processing abuse (-x-) from batch 1. Groups H 0°C (-♦-) and I 0 °C + abused (- ◊ -) from batch 3.

Figure 5 - Counts of a) total psychrotrophic bacteria (TVC), b) *P. phosphoreum* (Pp) counts. c) presumptive *Pseudomonas* spp and d) H_2S-producing bacteria in superchilled experimental groups of cod fillets stored in styrofoam boxes under different conditions: C

0 °C new (--■--) from batch 1 and E 0.5 °C (-▲-), F -1.5 °C (-●-)and G – 1.5 °C / 0.5 °C (-o-) from batch 2.

Figure 6 - TVB-N values in a) traditionally processed and temperature abused experimental groups: A 0 °C (-■-), B 0 °C + post processing abuse (-□-), D 0 °C + pre- and post processing abuse (-x-), H 0°C (-♦-) and I 0 °C + abused (- ◊ -). b) superchilled experimental groups: C 0 °C (--■--), E 0.5 °C (-▲-), F -1.5 °C (-o-) and G – 1.5 °C / 0.5 °C (-●-). Dotted line indicates the limit of acceptability TVB-N = 35mg N/100g

Figure 7 - Electronic nose CO sensor response a) in traditionally processed and temperature abused experimental groups: A 0 °C (-■-), B 0 °C + post processing abuse (-□-) and D 0 °C + pre- and post processing abuse (-x-) from batch 1. H 0°C (-♦-) and I 0 °C a + abused (- ◊ -) from batch 3. b) in superchilled experimental groups: C 0 °C (--■--) from batch 1 and E 0.5 °C (-▲-), F -1.5 °C (-o-) and G – 1.5 °C / 0.5 °C (-●-) from batch 2.

Figure 8 - PLSR correlation loadings based on all the measured variables: TVC, pseudomonads, H_2S counts, *Photobacterium phosphoreum* (PP), TVB-N, pH, T_{acc} and electronic nose sensors (CO, NH_3, SO_2 and H_2S) as predictors (X) for the Torry scores as a response variable (Y) for the cod samples stored at different temperatures. The outer and the inner ellipses indicate 100% and 50% explained variance, respectively. Significant variables ($p<0.05$) are symbolised with small circles.

Figure 9 - Biplot for the principal components of a PLSR model ($r^2=0.94$; RMSEP=0.49) based on all the data (N=41) of cod fillets stored at different temperatures and 5 variables: *P. phosphoreum*, pseudomonads, H_2S-producers, TVB-N and the electronic nose CO sensor as predictors for the response variable (Torry score). Samples are labeled with letters indicating storage groups (A, B, C, D, E, F,G, H and I) and storage days. The arrow shows the spoilage trend of the samples with increasing storage time and the samples encircled (dotted line) in the middle were badly described by the variables.

Figures

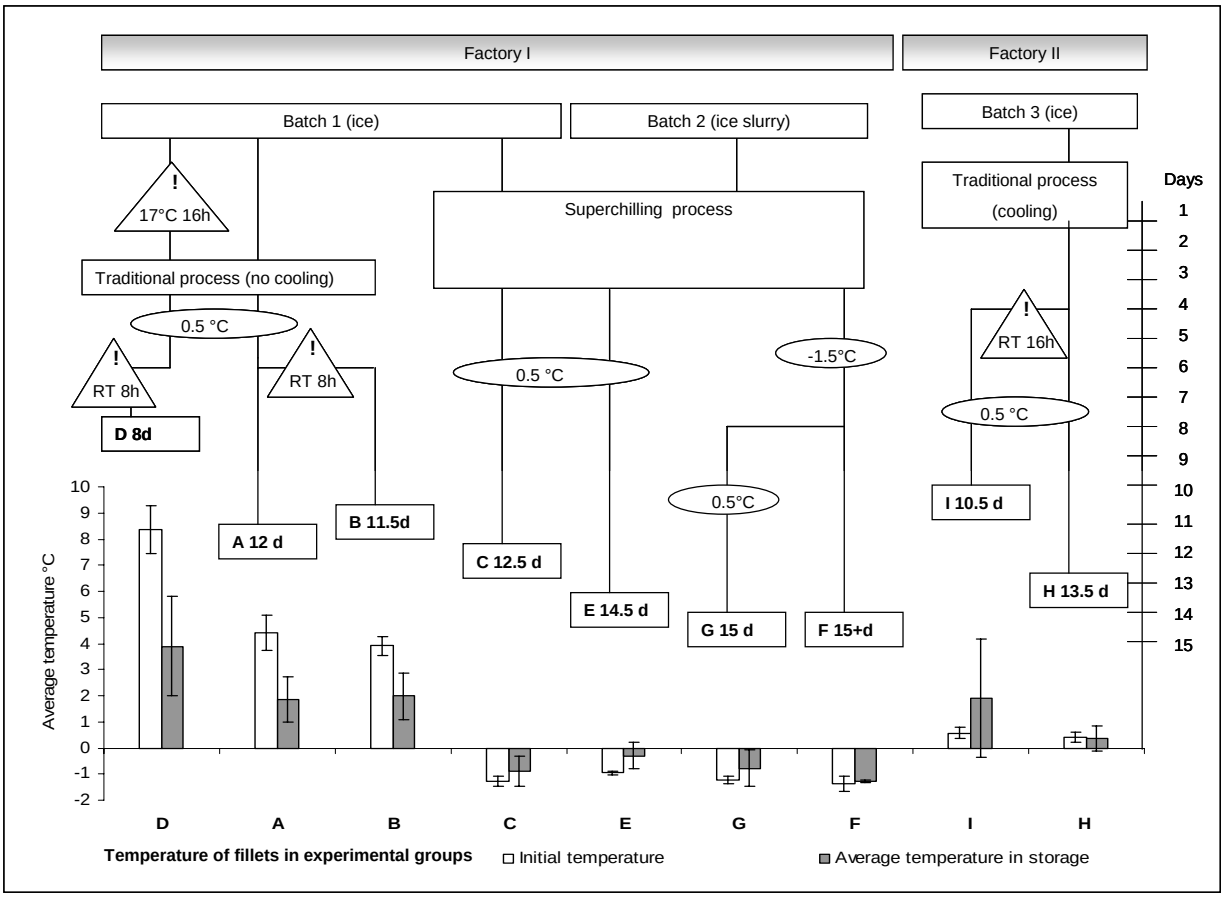


FIGURE 1.

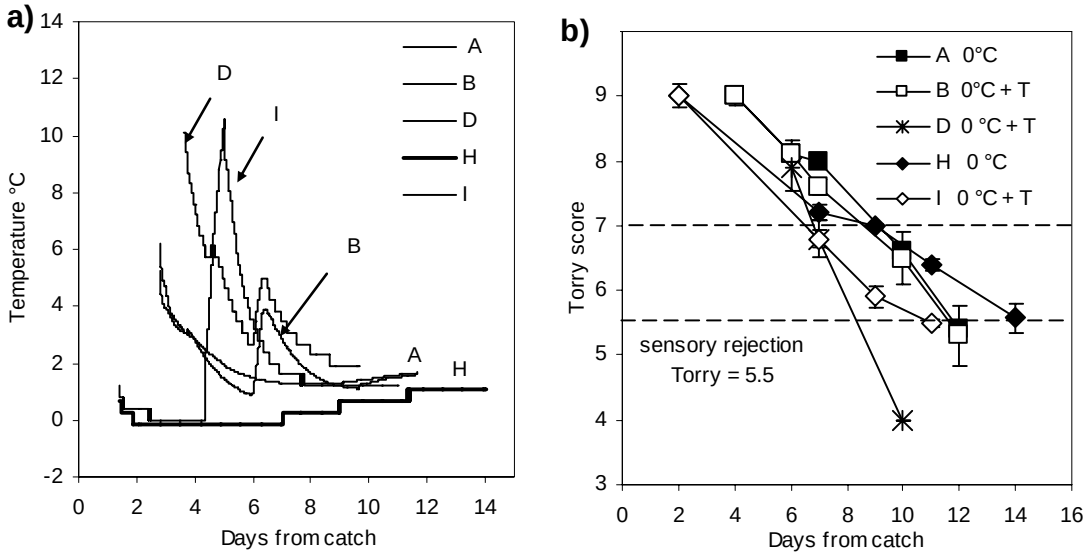


FIGURE 2.

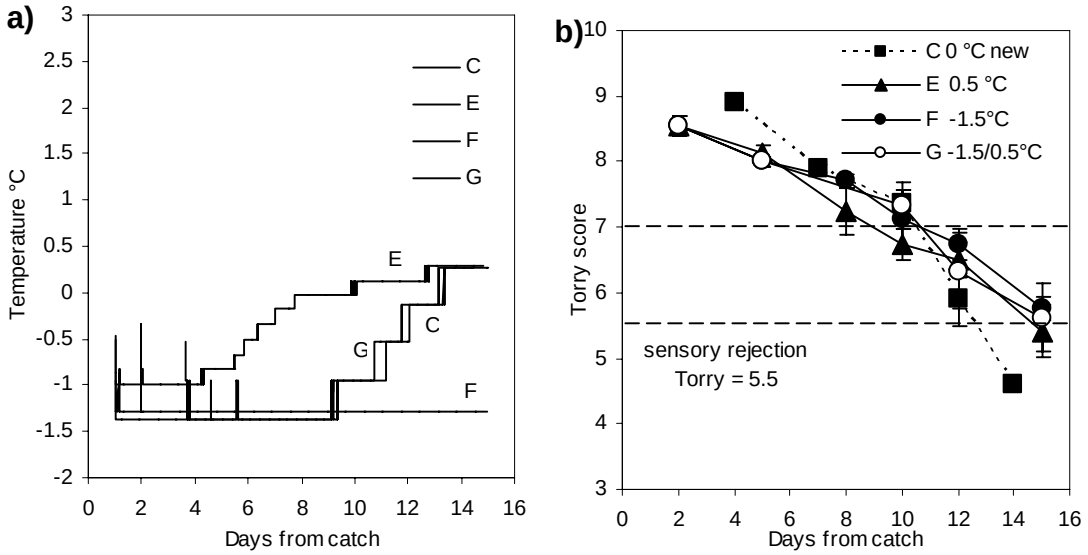


FIGURE 3.

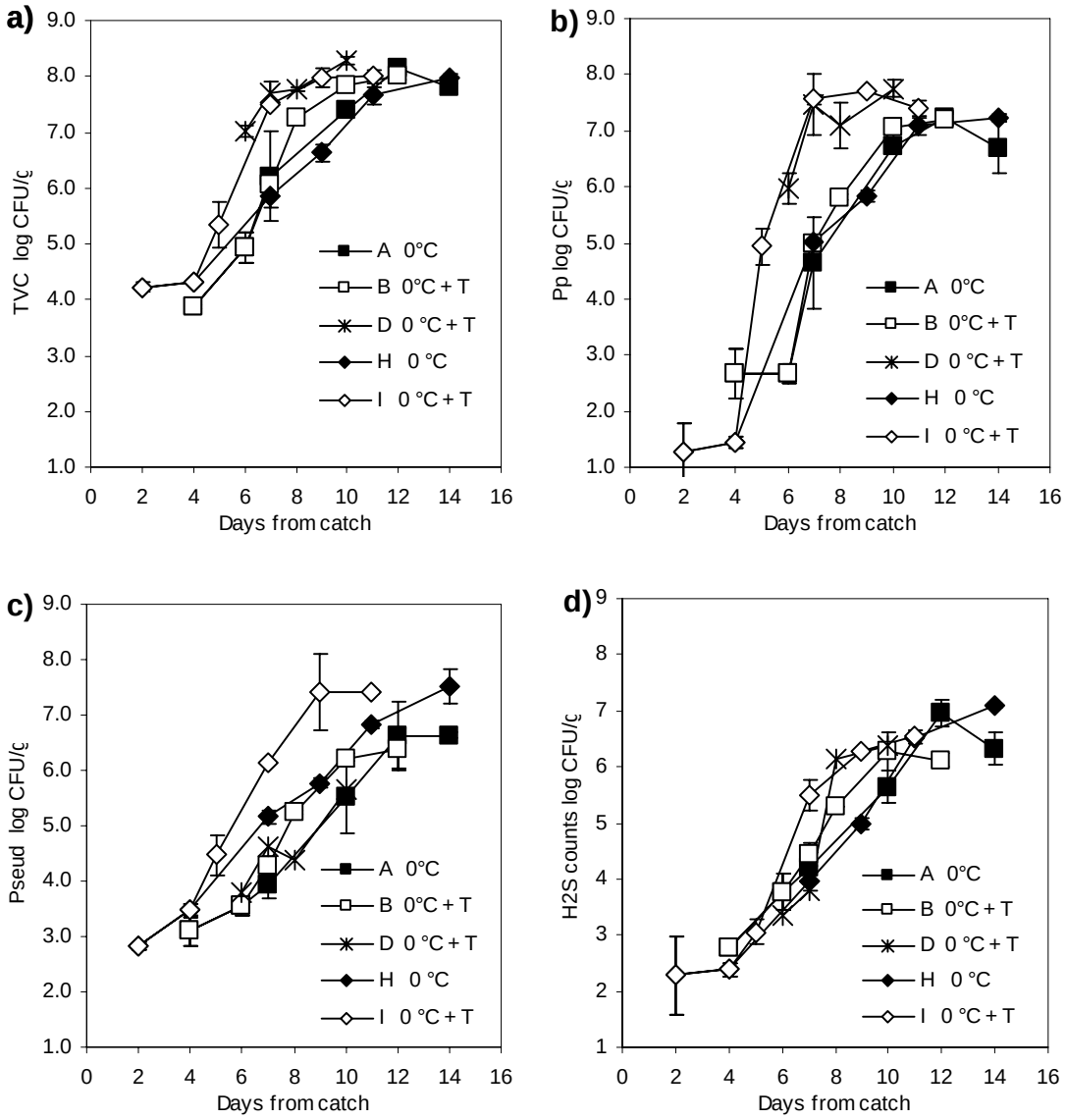

a)
TVC log CFU/ç
Days from catch
A 0°C
B 0°C + T
D 0 °C + T
H 0 °C
I 0 °C + T
b)
Pp log CFU/ç
Days from catch
A 0°C
B 0°C + T
D 0 °C + T
H 0 °C
I 0 °C + T
c)
Pseud log CFU/ç
Days from catch
A 0°C
B 0°C + T
D 0 °C + T
H 0 °C
I 0 °C + T
d)
H2S counts log CFU/ç
Days from catch
A 0°C
B 0°C + T
D 0 °C + T
H 0 °C
I 0 °C + T

FIGURE 4.

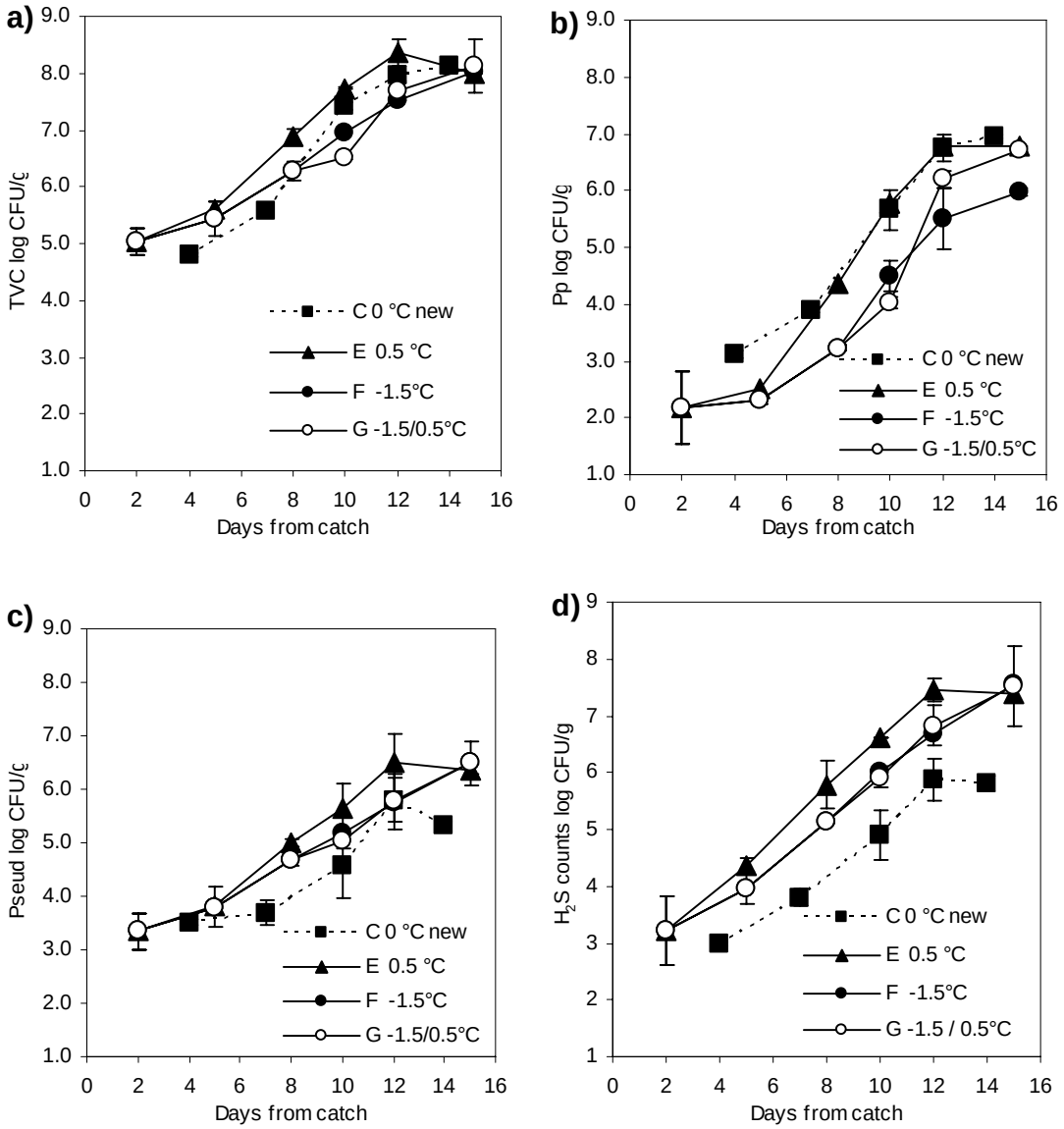

a)
TVC log CFU/g
Days from catch
C 0 °C new
E 0.5 °C
F -1.5°C
G -1.5/0.5°C
b)
Pp log CFU/g
Days from catch
C 0 °C new
E 0.5 °C
F -1.5°C
G -1.5/0.5°C
c)
Pseud log CFU/g
Days from catch
C 0 °C new
E 0.5 °C
F -1.5°C
G -1.5/0.5°C
d)
H2S counts log CFU/g
Days from catch
C 0 °C new
E 0.5 °C
F -1.5°C
G -1.5 / 0.5°C

FIGURE 5.

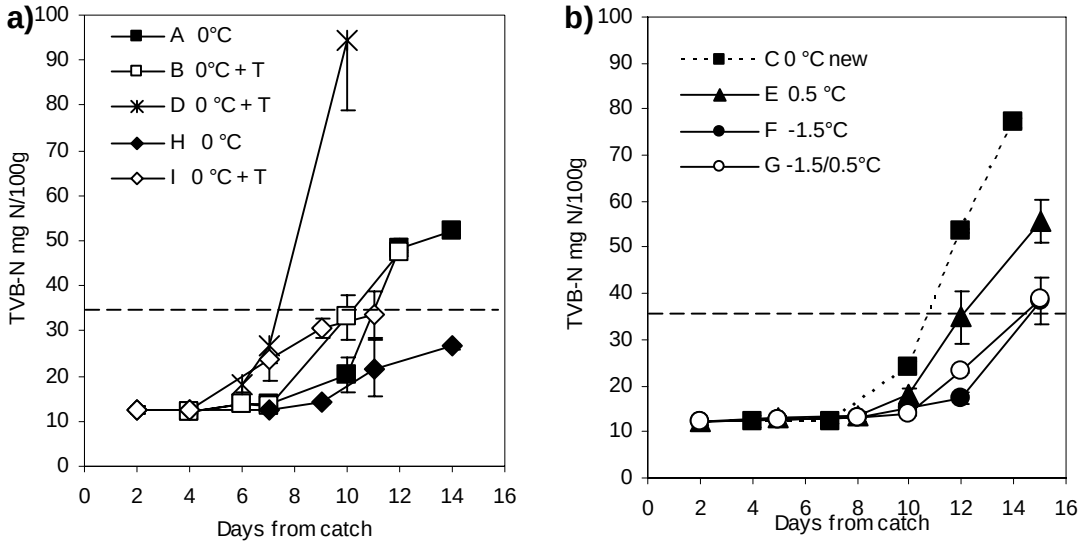

a)
A 0°C
B 0°C + T
D 0 °C + T
H 0 °C
I 0 °C + T
TVB-N mg N/100g
Days from catch
b)
C 0 °C new
E 0.5 °C
F -1.5°C
G -1.5/0.5°C
TVB-N mg N/100g
Days from catch

FIGURE 6.

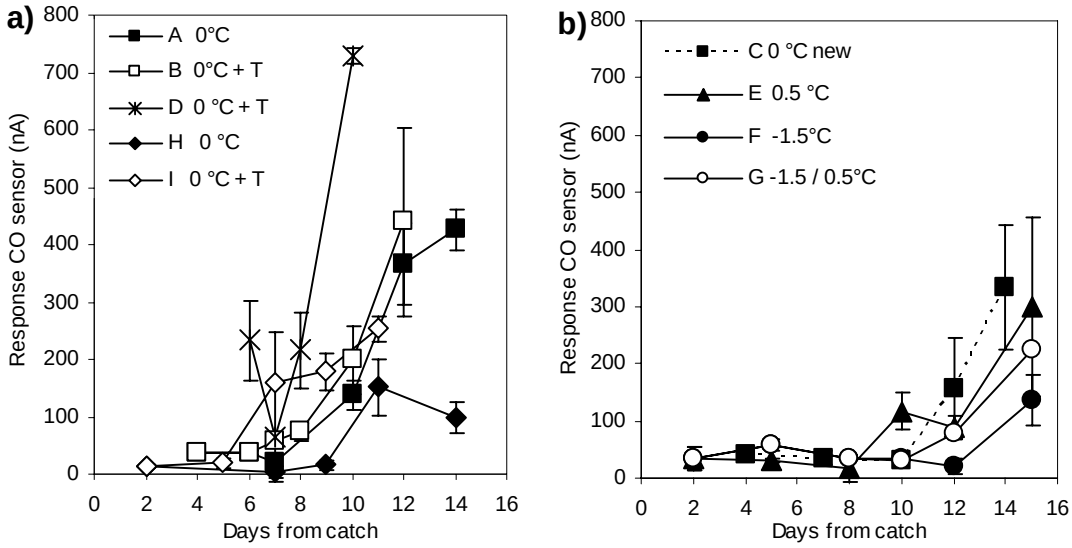

a)
A 0°C
B 0°C + T
D 0 °C + T
H 0 °C
I 0 °C + T
Response CO sensor (nA)
Days from catch
b)
C 0 °C new
E 0.5 °C
F -1.5°C
G -1.5 / 0.5°C
Response CO sensor (nA)
Days from catch

FIGURE 7.

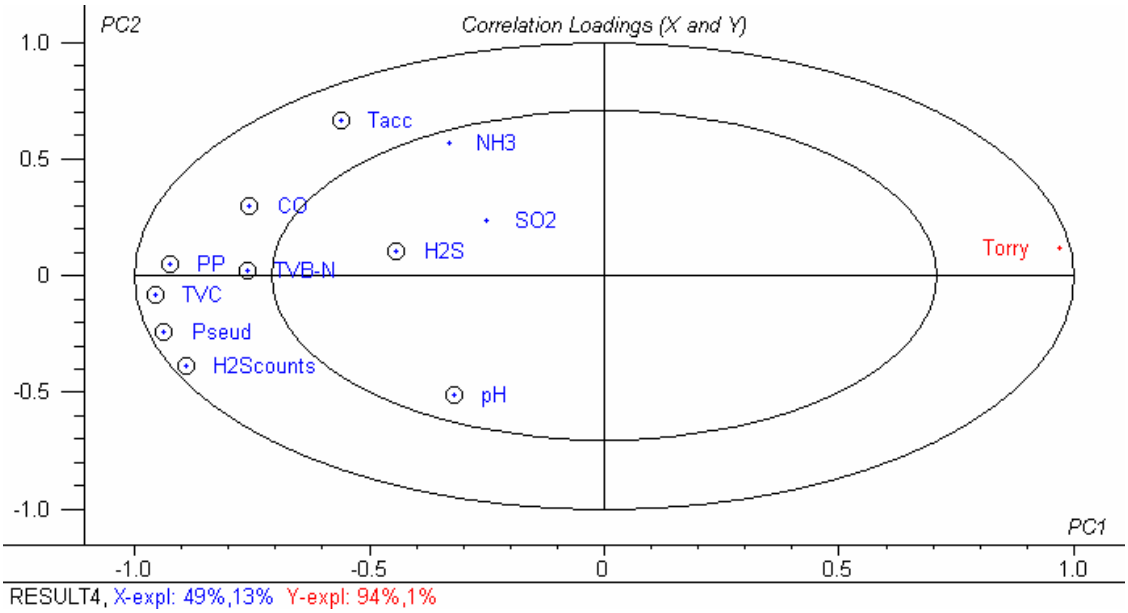


FIGURE 8.

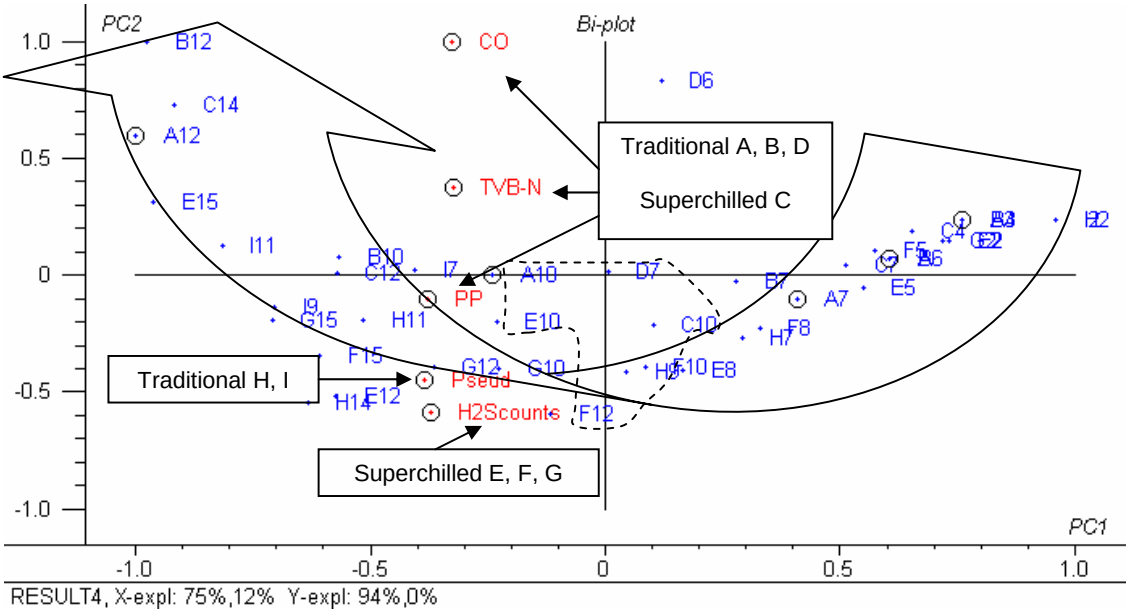


FIGURE 9.

PAPER VI

Characterization of Volatile Compounds in Chilled Cod (*Gadus morhua)* Fillets by Gas Chromatography and Rapid Detection of Quality Indicators by an Electronic Nose

Guðrún Ólafsdóttir, Rósa Jónsdóttir, Hélène L. Lauzon, Joop B. Luten and Kristberg Kristbergsson

VI

Submitted for publication

Characterization of Volatile Compounds in Chilled Cod *(Gadus morhua*) Fillets by Gas Chromatography and Rapid Detection of Quality Indicators by an Electronic Nose

Gudrun Olafsdottir[*Ψ], *Rosa Jonsdottir*[Ψ], *Hélène L. Lauzon*[Ψ], *Joop Luten*[δ] *and Kristberg Kristbergsson*[γ]

[Ψ]Icelandic Fisheries Laboratories, Skulagata 4, 101 Reykjavík, Iceland

[δ] Norwegian Institute of Fisheries and Aquaculture Research, Muninbakken 9-13, 9291 Tromsö, Norway

[γ c]Department of Food Science, University of Iceland, Hjardarhagi 2 - 6, 107 Reykjavik, Iceland

TITLE RUNNING HEAD: Volatile compounds as quality indicators for cod fillets

[*] To whom correspondence should be addressed. E-mail: gudrun@rf.is. Tel.: +354 5308600, Fax: +354 5308601

ABSTRACT

Volatile compounds in cod fillets packed in styrofoam boxes were analyzed during chilled storage (0.5 °C) by GC-MS and GC-O to screen potential quality indicators present in concentrations high enough for detection by an electronic nose. On day 12 when the fillets were rejected by sensory analysis, ketones, mainly 3-hydroxy-2-butanone, were detected in the highest level (33 %), followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%) and a low level of esters (<1%). The electronic nose's CO sensor showed increasing response with storage time coinciding with the production of ethanol and 2-methyl-1-propanol that were produced early in the storage, followed by the production of 3-methyl-1-butanol, 3-methyl-butanal, 2,3-butandiol and ethyl acetate. Lipid derived aldehydes, like hexanal and decanal were detected in similar levels throughout the storage time and contributed to the overall sweet odors of cod fillets in combination with other carbonyls (3-hydroxy-2-butanone, acetaldehyde, 2-butanone, 3-pentanone and 6-methyl-5-heptene-2-one).

KEYWORDS: Volatile compounds; quality indicators; gas chromatography; electronic nose; cod fillets

INTRODUCTION

The use of electronic nose based on different sensor technologies has been suggested for the rapid detection of quality related volatile compounds for various food products (*1-3*) including monitoring the quality and spoilage processes in fish (*4-12*). Gas sensors that are commonly used in electronic noses are non-selective towards individual compounds, but show sensitivity towards certain classes of compounds. This property induces their potential for monitoring quality and the onset of spoilage associated with varying levels of different classes of volatile compounds produced in fish during storage (*13-14*). The progression of characteristic odors in fish during chilled storage caused by microbial growth is well documented (*15-17*) and has been associated with the formation of volatile compounds produced by the main spoilage organisms (*18-21*). Both single compounds and a combination of compounds representing the different changes occurring during storage have been suggested as indicators for freshness and spoilage (*22-31*).

Alcohols, aldehydes, ketones, amines and sulfur compounds have been identified in different seafood products during chilled storage and related to the growth of specific spoilage organisms (SSOs) (*32-34*). However, because of the interaction of the microorganisms (*35-36*) and the complexity of the dynamic spoilage changes the levels of the volatile compounds may vary and they are often not detected until the products are overtly spoiled. Storage temperature, packaging and the inherent composition of available nutrients in the fish influence the growth and spoilage potential of the dominating SSO. The SSO in chilled fish are mainly Gram-negative, psychrotrophic bacteria like *Pseudomonas* spp. and *Shewanella* spp. (*37*). Pseudomonads typically cause sweet, malty, fruity, and onion like odors contributed by alcohols, carbonyls, esters and sulfur compounds (*18,19*), while *S. putrefaciens* can produce more potent odors related to high levels of sulfur compounds and fishy odors because of reduction of TMAO to TMA (*38,21*). *Photobacterium phosphoreum*, is also of interest as SSO in chilled fish and has been identified as an active TMA producer in iced cod, and in cod fillets (*39-40*) and more recently in modified atmosphere packed fish (*41-43*). Oxidative processes occurring during storage of fish will also result in the accumulation of both saturated and unsaturated aldehydes that contribute to the development of rancid cold store flavors (*44-45*).

The information on the identity and quantity of volatile compounds present in the headspace during storage of fish is essential when selecting sensors in an array for quality monitoring of fish (*14*). It should be clearly stated that in many cases, the most abundant volatiles may have minimal odor significance. However, they may be indicative for the degradation processes occurring in the products like the production of metabolites by the SSOs. Therefore, the potential ability of the electronic nose to monitor quality is not necessarily directly related to detecting the most influential aroma active compounds contributing to off odors, since these may be present in too low concentrations.

The aim of the study was to screen the most abundant volatile compounds produced by SSOs that could be used as quality indicators for chilled fillets. This study was done in parallel to extensive storage studies on cod fillets (*46*) where the SSOs were monitored and the sensory shelf-life was determined. Herein are the results from gas chromatography analysis of cod fillets stored in Styrofoam boxes under chilled conditions (0.5 °C) and comparison was made with electronic nose analysis, TVB-N and pH measurements. The results obtained will be useful to characterize the spoilage potential of the SSO and to guide the future development of the electronic nose technique based on selecting sensors that are sensitive to the indicator compounds identified in chilled cod fillets.

MATERIALS AND METHODS

The fish was processed by a conventional process three days after catch as described earlier (*46*). The process includes mechanical filleting, deskinning and packing of fillets in styrofoam (EPS, expanded polystyrene) boxes (160 x 400 x 263 mm) lined with a plastic bag. The fillets were stored at 0.5 °C until analyzed. Gas chromatography analysis (GC-MS and GC-O), electronic nose, TVB-N and pH measurements were performed on days 4, 7, 10, 12 and 14 after catch

GC-MS measurements. The headspace from approximately 500 g of fish (1-2 fillets) in a glass container (2.3L, Ø 17 cm) was collected by an air pump sampling (ALPIN-2, Air sampler, METEK) by sweeping volatiles from the surface of the fish. Aqueous heptanoic acid ethyl ester solution (10 µL/L) was used as an external standard, using an amount of 1 mL in a 25 mL beaker (Ø 3.5 cm) located in the glass sampling container.

Quantification of volatiles PAR (peak area ratio) was based on comparison of peak area to the peak area of the external standard. Sampling time was 2 hours at room temperature (RT) (20 to 22 °C) using a flow rate of approximately 100 mL/min. Duplicate headspace samples were collected on 250 mg Tenax 60/80 (Alltech, IL) in stainless steel tubes (Perkin-Elmer, Buchinghamshire, UK). Volatiles were thermally desorbed (ATD 400, Perkin-Elmer, Buchinghamshire, UK) from the sampling tubes prior to separation on a DB-5ms column (30 m × 0.25 mm i.d. × 0.25 µm, J&W Scientific, Folsom, CA, USA) using a GC-MS (HP G1800C GCD, Hewlett-Packard, Palo Alto, CA, USA). Helium was used as a carrier gas and 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 injector temperature was 250°C and the detector temperature was 280°C. The mass detector ion range was 35-300 m/z.

GC-O measurements. Samples were prepared by weighing 100 ± 2 g of fish fillets and 100 ± 5 g of saturated aqueous solution of NaCl into a 250 mL and blended manually in a round bottom flask. Saturated NaCl solution (200 ± 5 g) was prepared as a reference sample. Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 1 mL of 10 µL/L aqueous solution of the standard to the 200 g fish and $NaCl_{sat}$ solution to evaluate the extraction based on the FID response. The sample was purged at RT with nitrogen at about 100 mL/min for 2.5 hours. Volatiles were collected on 150 mg Tenax in a Pasteur pipette. Each sample was prepared in duplicate. Volatiles were extracted from the Tenax traps with 1 mL diethyl ether. The sample was concentrated by passing nitrogen over the solution leaving a small amount of sample (20-30 µL) and 1µL was then injected splitless onto the column. 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 ionization detector (FID) and an ODO-1 olfactory detector outlet (SGE International Pty. Ltd, Australia). Nitrogen bubbled through water to add moisture was used to drive the sample to the sniffer port. Two trained panelist with former experience in describing seafood odor sniffed the effluent. Description of each odor and its duration in time was recorded and the intensity evaluated using a category scale with a description of intensity of odor at each score (*47-48*); 0 none; 0.5 thresholds or just detectable; 1

slight; 2 little; 3 moderate; 4 strong and 5 very strong. GC-O analysis were done on days 4, 10 and 17.

Identification of volatiles was done by matching retention indices (RI) of ethyl esters and mass spectra of samples with authentic standards (Sigma-Aldrich Chemical Co. St. Louis, MO, USA). Tentative identifications were based on the MS library data in the HP GCD ChemStation software (Hewlett Packard Co, 1997) and manually checked against literature sources and the database Flavornet (*49*).

Electronic nose measurements The electronic nose instrument (FreshSense, Bodvaki-Maritech, Kópavogur, Iceland) is based on four electrochemical gas sensors: CO, H_2S, SO_2 (Dräger, Luebeck, Germany); NH_3 (City Technology, Portsmouth, Britain). The measurements were performed at room temperature as described earlier (*9*). Approximately 500g of fish fillets were weighted and tempered for 30 minutes in the sampling container (2.3 L, Ø 17 cm). Measurement time was 5 minutes and temperature of fillets reached 8 to 12°C before measurements started. All measurements were done in duplicate.

TVB-N and pH measurements. Total volatile basic nitrogen content (TVB-N) was measured by the steam distillation method described by Malle and Poumeyrol (*50*). The pH was measured in 5 grams of mince moistened with 5 mL of deionized water. The pH meter was calibrated using buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

Data analysis. Statistical analysis was carried out with the Number Cruncher Statistical Software (NCSS) 2000, using ANOVA. In case of statistical significance, the Duncan's multiple range was performed. An effect was considered significant at the 5% level.

RESULTS AND DISCUSSION

Volatile compounds in cod fillets analyzed by GC – potential quality indicators

Volatile compounds detected in the highest level by GC-MS and dominating SSO: TMA, 2-methyl-1-propanol (isobutanol) and 3-hydroxy-butanone (acetoin) were detected in the highest amount in cod fillets and their levels increased with storage time (Table 1).

However, dynamic changes in their level were noticed, which could be related to the growth of the spoilage bacteria. An initial decline of the pH (Fig. 1) could be explained by the *post mortem* changes in chilled fish which are initially dominated by autolytic activity, including degradation of nucleotides, accumulation of hypoxhanthin, lowering of pH and endogenous enzyme activity (*51*). Proliferation of the microflora follows these changes and development of microbial metaboliltes contributing to spoilage changes as seen by increased pH value coinciding with the detection of trimethylamine (TMA) and higher TVB-N values on day 12 (Fig. 1). TMA has been suggested as a spoilage indicator since its level increases at later stages of storage (*29*), while DMA (dimethylamine) which forms enzymically very early after harvest of fish has been suggested as a freshness indicator along with its precursor TMAO (trimethylamine oxide) (*24*).

In the parallel study on sensory and microbiological changes of packed cod fillets (*46*) the shelf-life was determined as 12 days by sensory analysis using the Torry scheme (*16*). TMA and acetoin which were present in the highest concentration on day 12 (Table 1) have earlier been associated with dominating *P. phosphoreum* growth (*40*). Increasing levels of acetoin were detected on day 7 coinciding with a rapid *P. phosphoreum* growth reaching counts of log 7.2 CFU/g at sensory rejection. *P. phosphoreum* was identified as the main SSO in the cod fillets based on its high counts throughout the storage time and was found in the highest levels (12.6 %) at the end of shelf-life, while *Pseudomonas* spp. and H_2S-producing bacteria represented 4.9 % (log 6.6 CFU/g) and 7% (log 7 CFU/g), respectively, of the total microflora at sensory rejection. (*46*).

The variation in GC analysis for duplicate samples was high for the very volatile compounds like TMA and ethanol (Table 1), most likely because of their high breakthrough volume on the Tenax and also the fact that in some cases compounds with RI<173 coeluted and made both identification and quantification difficult. This was the case for ethanol, TMA and dimethyl sulfide as well as 2-methyl-1-propanol which was also identified as pentane in the MS library database (Table 1).

Characteristic odor of cod fillets: The overall odor of the fillets was observed as a mild and sweet odor that became sour, fruity and stale during storage and much less potent odors developed on the fillets than are generally observed on whole fish. Characteristic odor development during storage of the fillets could be explained by the odor description and odor scores for the individual compounds analyzed by GC-O (Table

1). The odor impact of the different compounds was also evaluated based on their amount in the samples measured by GC-MS and the reported odor threshold (OU (Odor Unit) = PAR / odor threshold x 1000) to determine the potential contribution of the individual compounds at sensory rejection on day 12. The GC-O scores were generally low but increased with storage time in agreement with the increased level of the compounds measured by GC-MS. The values and ranges of the GC-O odor scores detected on days 4, 10 and 17 are shown in Table 1. The most potent odor described as spicy, flowery, sweet, onion and mushroom-like, was detected at RI 457 in all samples during storage, with increasing odor scores with time of 3 (moderate) to 4.8 (very strong). This complex odor is most likely contributed by coeluting compounds in low concentrations which were not detected by GC-MS. Aldehydes appeared to influence the characteristic odors of the fresh fillets throughout the whole storage time and decanal had the highest odor impact as evaluated by OU (1200-24000), but 3-methyl-butanal and heptanal, probably in combination with methional and 2-heptenal that are known to coelute at the same retention time (*12*), received the highest odor scores of the aldehydes by GC-O (Table 1). TMA is a potent odorant with a characteristic fishy, dried fish, ammonia-like odor as detected by GC-O and received high odor scores. The odors of esters and sulfur compounds were detected by GC-O at advanced spoilage on day 17.

The calculated odor unit values for the volatiles detected by GC-MS on day 12 when the end of shelf-life was determined by sensory analysis are shown in Table 1. The reported odor threshold of TMA varies but values as low as 0.3 ppb have been reported (*52*) (Table 1). The calculated OU based on the different odor thresholds showed that TMA had by far the highest odor impact on day 12 with two to three orders of magnitude higher OU than the straight chain aldehydes (nonanal and decanal) that appeared to have a high odor impact as well, based on their odor threshold values. The high levels of acetoin suggested that this compound could contribute to the onset of spoilage odors in cod fillets (Table 1). The odor of acetoin has been described as butter- cream like (*49*), but a mild, sweet-sour like odor was detected by GC-O (Table 1). However, acetoin appeared to have less odor impact (OU=119) than the aldehydes (decanal and nonanal) because of its higher odor threshold (800 ppb), even though it was present in much higher levels. Aldehydes have generally low odor thresholds (~ 0.1 to 4.5 ppb) and therefore their odor impact was greater than the alcohols (odor thresholds range 0.4 to 270 ppm)

and the ketones (odor thresholds range 0.05 to 70 ppm) although their overall levels were less (Table 1). It appears that even though the alcohols like 2-methyl-1-propanol and aldehydes like decanal were detected in lower levels on day 12 than on day 10 ($p<0.05$), the presence of the more potent odorants like TMA on day 12 overpowered the odor and contributed to the sensory rejection of the fillets. Ethyl acetate was first detected on day 12 and probably also influenced the overall spoilage odor development based on its OU value (0.1-120) in combination with the other compounds detected on day 12. Additionally, the change in the pH value on day 12 may have influenced the overall odor perception and synergistic effects may have occurred. For example, TMA has been noted for intensifying fishiness by a synergistic action with certain volatile unsaturated aldehydes derived from autoxidation of polyunsaturated fatty acids (*53*). The possible influence of other compounds present in lower levels like the unsaturated autoxidatively derived aldehydes should not be overlooked, but the sampling techniques used in our study were not sensitive enough to allow detection of these compounds. The main aim was to detect the compounds present in the highest concentration in the headspace for evaluation of potential quality indicators for electronic nose detection.

Alcohols, carbonyls, esters and acids. The dynamic changes in the levels of the most abundant alcohols, esters, acids and carbonyls are demonstrated in Figs. 2 and 3. Ethanol was detected in high levels initially, followed by an increase in 2-methyl-1-propanol, 3-methyl-1-butanol, 2,3-butandiol, 3-methyl-butanal, and ethyl acetate (Fig. 2). The early detection of the ethanol and 2-methyl-1-propanol is of interest to monitor the initial changes related to the loss of freshness before the obvious spoilage signs appear. However, their levels did not increase continuously with time.

The initial production of ethanol in spoilage of fish has been related to the utilization of carbohydrate sources, while the formation of branched-chain alcohols and aldehydes like 2-methyl-1-propanol, 3-methylbutanol and 3-methyl-butanal probably originate from degradation of valine and leucine, respectively (*28*). The branched chain aldehyde, 3-methyl butanal, is characterized by a malty and caramel like odors (*54*), which was perceived as a sweet, caramel and fish fillet like odor by GC-O (Table 1). The corresponding alcohol 3-methyl-1-butanol, and 2-methyl-1-propanol exhibit alcoholic and fruity odours. 3-methyl -1-butanol was first detected on day 10 and significant continuous increases ($p<0.05$) were seen with time. Levels of 2-methyl-l-propanol were

much higher but as discussed above its level fluctuated with time. The flavor thresholds of the alcohols are high compared with the carbonyls and their odors were not detected by GC-O and therefore they probably did not contribute much to the overall odor of the fillets as seen by the low OU value on day 12 (Table 1). On the basis of odor evaluation 3-methyl-butanal in combination with acetaldehyde, methional, 1,5-octadiene-3-one, 2,6-nonadienal and 2,4-decadienal were determined as character impact odorants of boiled cod and the malty flavour of 3-methyl butanal was suggested to be mainly responsible for the malty off flavor defect of boiled cod by Milo and Grosch (*55*).

The formation of acetic acid and a decline in the ethanol level were observed on day 10 followed by the detection of ethyl acetate at the end of the shelf-life on day 12 and a sickenly sweet odor detected by GC-O on day 17 and identified as ethyl butanoate. The formation of esters suggested the activity of *Pseudomonas fragi* (*18*). At the end of the shelf-life on day 12 the counts of *Pseudomonas spp*. reached log 6.6 CFU/g (data from the parallel study) (*46*) and increasing level of ethyl acetate was seen on day 14 and much higher levels on day 17 at overt spoilage (Table 1). Esters have low odor thresholds and are known to contribute to sweet and fruity spoilage odors at advanced stages of spoilage. Similarly, ethanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 3-hydroxy-2-butanone, ethyl acetate and butanoic acid ethyl ester were the most abundant volatiles in the headspace of haddock stored in ice associated with the growth of *Pseudomonas* spp. in an earlier study on packed haddock fillets stored under chilled conditions (*56*). *Pseudomonas* species have also been found responsible for the formation of volatile sulfides, alcohols (3-methyl-1-butanol, 1-penten-3-ol) and ketones (2-butanone) contributing to the stale and putrid off odors in fish because of amino acid and lipid degradation (*18, 19*).

Much higher levels of 3-methyl-1-butanol, 2,3-butandiol, 2-methyl-1-propanol, TMA, ethyl acetate and acetoin were detected on days 14 and 17 compared to day 12 when the end of sensory shelf-life was reached (Table 1). The late development of the spoilage indicators is in agreement with studies on spoilage indicators for cultured and wild sea bream stored in ice for 23 days (*31*). TMA, 3-methyl-1-butanol, 1-penten3-ol, piperidine, methanethiol, dimethyl disulfide, dimethyl trisulfide, and acetic acid were suggested as spoilage indicators and the increase in the levels of most of the compounds were detected

around day 10 of storage, however, methyl mercaptan and dimethyl trisulfide appeared to accumulate later when the products were already spoiled.

Ketones: The formation of microbially derived acetoin was characteristic for the spoilage of chilled cod fillets as discussed above. Levels of acetoin increased earlier than TMA and therefore, it is more useful to monitor the loss of freshness as an early indicator of spoilage. Acetoin can be formed from carbohydrate sources via pyruvate and diacetyl, however the mechanism of its formation in fish is not well known but acetoin and diacetyl have been suggested as early indicators of spoilage in beef (*57*). The concentration of acetoin was much higher than the other lipid derived ketones detected like 2-butanone and 3-pentanone and carotenoid derived 6-methyl-5-heptene-2-one (Fig 3) that were present in cod fillets throughout storage but no obvious increase occurred until at the end of shelf-life and during continued storage (Fig. 3). Ketones can influence the overall odor because of their typical odors and their low odor thresholds (Table 1). Butanone has a butterscotch odor and pentanone a sweet fruity odor (*58*), but a sweet, caramel like odor was detected by GC-O for 3-pentanone (Table 1).

Aldehydes: The straight chain lipid derived aldehydes, hexanal, heptanal, nonanal, decanal and undecanal were detected in the cod fillets throughout the storage time. Their levels did not appear to increase until after the end of shelf life was reached (Table 1). Therefore, they did not appear to be useful as indicators of spoilage during chilled storage of the cod fillets, but aldehydes have been suggested as indicators of spoilage in fatty species (*45*). These compounds exhibit green, fatty, soapy, tallowy odors and hexanal is characterized by green odor (58). The aldehydes most likely contributed to the overall mild and sweet odors of the fillets since their odor threshold is low and therefore they are likely to have high odor impact as seen by the high odor unit for these compounds (Table 1). Based on the GC-O it is suggested that the lipid derived ketones in combination with 3-methyl-butanal and aldehydes like hexanal, nonanal and decanal, contributed to the characteristic sweet, caramel, and flowery odor of the cod fillets. Oxidation of fatty acids contributes to the rancid odors of fish with the formation of aldehydes like hexanal, 2,7-heptadienal, and 2,4,7-decadienal (*44, 59*). The unsaturated aldehydes were not detected by GC-MS in this study using the surface stripping sampling of the volatiles.

Odor of fresh cod fillets is typically characterized by a very mild and pleasant, marine like odor and aroma active compounds contributing to the fresh fish odors are present in

very low concentrations (*14*). Species specific odors of fresh fish are contributed by long chain alcohols and carbonyl compounds like 1,5-octadien-3-ol and 2,6-nonadienal, respectively, that are derived from polyunsaturated fatty acids (*60*). These compounds are more pronounced on the skin than in the muscle and were not detected in the fillets using the sampling conditions herein. However, an earthy and potato-like odor was detected by GC-O at the same retention time as heptanal which is most likely caused by low levels of methional and 4-heptenal eluting at the same retention time (*12*). These compounds were identified earlier as the most potent odorants in boiled cod (*55*).

Electronic nose and GC-MS analysis of the main classes of compounds in cod fillets during storage

Quality indicators: The sum of the peak area ratio (PAR) for all the volatile compounds detected in cod fillets by GC-MS showed that the total amount of volatiles increased with storage and alcohols, ketones and TMA (amines) were present in the highest amount (Table 2). On day 4 the alcohols were the most abundant of the volatiles (29%), mainly contributed by ethanol. Again on day 7 the alcohols were still the most abundant volatiles (45%) and 2-methyl-1-propanol was detected in the highest level. The ketones (36%) increased considerably on day 10 with the development of acetoin and acid (2%) was first detected on day 10. At sensory rejection on day 12 the ketones were detected in the highest level (33 %) followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%) and low level of esters (ethyl acetate) (<1%). Esters and acids were not detected during early storage and sulfur compounds were only detected in low levels (Table 2) as expected since these compounds are typically produced at advanced spoilage of seafood (*18-20, 27, 14, 32*). TMA was the most abundant volatile compound at advanced spoilage on day 17 and alcohols and ketones were also in high levels but in addition the level of esters had increased considerably comprising 8% of the total PAR (Table 1).

Miscellaneous classes of compounds: Although alcohols, aldehydes, ketones, acids, sulfur compounds, esters and acids are primarily of interest as spoilage indicators of fish, other classes of compounds were also present in the headspace, which may have an impact when measuring the total headspace with electronic noses. The concentration of the straight chain alkanes (nonane, decane and undecane) appeared to be similar throughout storage. Additionally, numerous branched chain alkanes were identified by

the MS library database, but their RIs (retention indices) were not confirmed and therefore, they were classified with the group "unknown" which represented compounds that remained unidentified. The alkanes will not influence the responses of the electrochemical sensors of the electronic nose and are not considered of interest as quality indicators since they are not aroma active.

Compounds classified as "others" appeared to increase with storage time. Among these compounds were some odorous compounds like piperidine which was tentatively identified. Piperidine has been reported earlier in low levels in fish and has been associated with 1,5-diaminopentane (cadeverine) (*61*). Piperidine was earlier suggested as quality indicator of sea bream (*31*). The terpene derivative limonene was also classified with "other" compounds but did not show an increasing trend with time. The origin of limonene in fish has been related to the diet derived from plant sources (*31*). Limonene has low odor threshold and a fresh lemon odor was detected by GC-O analysis, suggesting that it may have an impact on the overall odor of fish fillets (Table 1).

The aromatics detected were mainly benzene derivatives. Styrene and chloroform were most abundant and were found to increase with storage. Styrene was also identified in wild and cultured sea bream packed in polystyrene boxes during chilled storage (*31*). The odor of styrene is described as kerosene like and has been associated with off odors in surimi based products related to the growth of yeasts (*62*). However, it is speculated that both styrene and chloroform could be originating from the Styrofoam boxes, however this was not confirmed. Tainting from the packaging may be of concern when monitoring spoilage changes with an electronic nose, if the sensors are sensitive to the respective compounds. Therefore, it is very important to analyze the total headspace by gas chromatography and understand the origin of the suggested quality indicating compounds prior to the selection of suitable sensors for monitoring the relevant spoilage changes.

Electronic nose measurements: The results of the electronic nose measurements showed that the response of the CO sensor was much higher than the other sensors' responses (H_2S, SO_2 and NH_3) and significant increase ($p<0.05$) was first observed between days 7 and 10, and thereafter a continuous increase occurred (Fig. 4). Earlier studies have shown that the main classes of spoilage indicator compounds present in the headspace of fish can be estimated based on the individual sensor responses (*63, 9*).

Selected standard compounds representing the main classes of compounds causing spoilage odors showed that the CO sensor is sensitive to alcohols, aldehydes and esters. The NH_3 sensor is sensitive to amines and the H_2S and SO_2 sensors can detect sulfur compounds. The comparison of the electronic nose measurements and the GC analysis has some drawbacks related to the different sampling techniques used. Therefore, the results are only a semiquantitative approach to screen the major changes in the composition of the volatiles and the electronic nose responses can be partly explained by comparison to the compounds identified in the highest concentration by the GC-MS.

Based on the electronic nose responses it appears that only the CO sensor was useful to monitor the changes in volatiles during storage. The increasing PAR for alcohols, aldehydes and esters (Table 2) could explain the increasing CO sensor response with time based on its sensitivity towards these classes of compounds. It is of interest that much higher responses were observed for the CO sensor in an earlier study of haddock fillets stored under identical conditions as the cod fillets at the end of shelf-life. This can be explained by higher levels of alcohols and esters produced by the SSO in the haddock fillets (*56*). Although *P. phosphoreum* was also identified as the main SSO in the haddock fillets, high counts of *Pseudomonas* spp were observed at sensory rejection. The pseudomonads are not able to reduce TMAO to TMA (*64*) and therefore, they may have contributed to the higher levels of alcohols because of their metabolism and need for hydrogen acceptors, hence favouring the production of alcohols. Therefore, the dynamic spoilage changes and development of volatiles are dependent on the combination of the dominating SSO.

The detection of the high level of acetoin produced in cod fillets was not achieved by the electronic nose since none of the sensors is sensitive to ketones. The ketones, mainly acetoin, increased continuously with storage (Fig. 3) and appeared to be promising indicators for cod fillets packed in styrofoam boxes. Improvements should be made to include selective sensors in the electronic nose for the detection of ketones.

The NH_3 sensor's response increased first on day 14 although not significantly because of the high standard deviation. The high standard deviations for replicate samples for both the electronic nose and GC analysis, was partly caused by the influence of the temperature on the very volatile compounds during sampling (*56, 65*). Although high levels of TMA were detected by GC–MS on day 12 in agreement with high TVB-N

level (48.5 mgN/100g), the NH_3 sensor was not sensitive enough to detect this. Higher responses for the NH_3 sensor were observed in earlier studies for both shrimp and capelin but in these products TVB-N levels were higher than in cod fillets (*66-67, 63, 8, 68*).

The low response of the H_2S and SO_2 sensors suggested that the H_2S-producing bacteria were not important in the development of spoilage odors in packed cod fillets in agreement with the GC-MS analysis. Dimethyl sulfide was detected on day 4 suggesting that this compounds was most likely not associated with microbial spoilage but rather reflected the feeding conditions as has been suggested by others (*69*). Sulfur compounds like hydrogen sulfide and methylmercaptan, which are produced by microbial degradation of fish constituents (*70-71*) were not detected, but GC-O analysis on day 17 indicated that dimethyl trisulfide was present in the spoiled fillets (Table 2). Sulfur compounds contribute to potent spoilage odors because of their low odor thresholds (Table 2). Earlier studies using the FreshSense electronic nose have shown increasing response of the H_2S and SO_2 sensors at advanced spoilage of whole capelin and redfish (*66, 8-9*), but response to fillets of cod and haddock is generally low (*56, 72*).

CONCLUSION

Analysis of volatile compounds during storage of packed cod fillets by gas chromatography showed that acetoin and TMA were produced in the highest and increasing amounts coinciding with the growth of *P. phosphoreum* which was identified as the dominating SSO in the parallel study. The response of the electronic nose's CO sensor was explained by the increasing level of alcohols during storage like ethanol, 2-methyl-1-propanol and 3-methyl-1-butanol in addition to the presence of aldehydes and the formation of esters at the end of the shelf-life. At sensory rejection of the packed cod fillets on day 12, the ketones were detected in the highest level (33 %) followed by amines (TMA) (29%), alcohols (15%), acids (4%), aldehydes (3%) and a low level of esters (ethyl acetate) (<1%). It is suggested that selective sensors for the detection of these classes of compounds could be used for monitoring spoilage changes in different fish products because similar volatile compounds emerge in the products. The CO sensor is useful for detecting incipient spoilage since its levels increased significantly between days 7 and 10. None of the sensors in the electronic nose was sensitive to ketones and

acids and therefore selective sensors for these components would be useful to monitor spoilage of cod fillets in addition to a more sensitive sensor for the detection of TMA. Increased sensitivity of existing methods is required for monitoring of quality deterioration and the early detection of microbially produced compounds. Low levels of sulfur compounds in the cod fillets suggested that *S. putrefaciens* was not very important in the spoilage of chilled cod fillets stored in styrofoam boxes.

However, because of the complexity of the spoilage processes caused by the diversity of the microflora and their different spoilage potential, it is likely that fixed values to determine the end of shelf-life or the quality of fish fillets based on electronic nose responses or amount of the main classes of compounds will have to be developed for each product and the respective storage conditions.

ABBREVIATIONS USED

GC-MS, gas chromatography-mass spectrometry; GC-O, gas chromatography-olfactometry; IFL, Icelandic Fisheries Laboratories; ATD, automated thermal desorber; RI, retention index; TMA, trimethyl amine; TVB-N, total volatile basic nitrogen; OU, odor unit; PAR, peak area ratio; SSO, specific spoilage organisms.

ACKNOWLEDGMENT

The authors thank The Icelandic Centre for Research for partly financing the project. The staff of IFL is thanked for their valued contribution in chemical, microbial and sensory analysis of samples.

REFERENCES

(1) Schaller, E.; Bosse, J.O.; Escher, F. 'Electronic noses' and their application to food. *Lebensm.-Wiss. Technol.* **1998**, *31*, 305-316.

(2) Gardner, J.W., Bartlett, P.N. Electronic noses. Principles and applications, Oxford University Press: Oxford, England, 1999.

(3) Haugen, J.E. Electronic noses in food analysis. In *Headspace Analysis of Food and Flavors: Theory and Practice*; Rouseff, R.L., Cadwallader, K.R., Eds.; Kluwer Academic / Plenum Publishers: New York, 2001; pp. 43-57.

(4) Ólafsson, R.; Martinsdóttir, E.; Ólafsdóttir, G.; Sigfússon, Þ.I.; Gardner, J.W. Monitoring of fish freshness using tin oxide sensors. In *Sensors and Sensory Systems for an Electronic Nose*; Gardner, J.W., Bartlett, P.N., Eds.; Kluwer Academic Publ.: Amsterdam, The Netherlands, 1992; pp. 257-272.

(5) Schweizer-Berberich, M.; Vahinger, S.; Göpel, W. Characterization of food freshness with sensor array, *Sens. Act.* **1994**, *B, 18-19*, 282-290.

(6) Di Natale, C.; Brunink, J.A.J.; Bungaro, F.; Davide, F.; d´Amico, A.; Paolesse, R.; Boschi, T.; Faccio, M.; Ferri, G. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. *Meas. Sci. Technol.* **1996**, *7*, 1103-1114.

(7) Di Natale, C.; Olafsdottir, G.; Einarsson, S.; Mantini, A.; Martinelli, E.; Paolesse, R.; Falconi, C.; D'Amico, A. Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets. *Sens. Act.* **2001**, *B77,* 572-578.

(8) Olafsdottir, G.; Högnadottir, A.; Martinsdottir, E.; Jonsdottir, H. Application of an electronic nose to predict total volatile bases in capelin (*Mallotus villosus*) for fishmeal production. *J. Agric. Food Chem*. **2000**, *48*, 2353-2359.

(9) Olafsdottir, G.; Xiuchen, L.; Lauzon, H.; Jonsdottir, R. Precision and application of electronic nose for freshness monitoring of whole redfish stored in ice and modified atmosphere bulk storage. *J. Aquat. Food Prod. Technol*. **2002**, *11*, 229-249.

(10) Zhao, C.Z.; Pan, Y.Z.; Ma, L.Z.; Tang, Z.N.; Zhao, G.L.; Wang, L.D. Assay of fish freshness using trimethylamine vapor probe based on a sensitive membrane on piezoelectric quartz crystal. *Sens. Act.* **2002**, *B81,* 218-222.

(11) Haugen, J.E.; Undeland, I. Lipid oxidation in herring fillets (*Clupea harengus*) during ice storage measured by a commercial hybrid gas-sensor array system. *J. Agric. Food Chem*. **2003,** *51*, 752-759.

(12) Jonsdottir, R.; Olafsdottir, G.; Martinsdottir, E.; Stefansson, G. Flavor Characterization of Ripened Cod Roe by Gas Chromatography, Sensory Analysis and Electronic Nose. *J. Agric. Food Chem*. **2004,** *52,* 6250-6256.

(13) Lindsay, R.C. Fish flavors. *Food Rev. Int.* **1990,** *6*, 437-455.

(14) Olafsdottir, G.; Fleurence, J. Evaluation of fish freshness using volatile compounds - Classification of volatile compounds in fish. In *Methods to Determine the Freshness of Fish in Research and Industry*; Olafsdottir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdottir, E., Heia, K., Eds.; International Institute of Refrigeration: Paris, France, 1998; pp. 55-69.

(15) Elliot, R.P. Evaluation of surface pH as a freshness index for fish fillets. *Food Research*. **1947**, *12 (2)*, 87-98.

(16) Shewan, J.M.; Macintosh, R.G.; Tucker, C.G.; Ehrenberg, A.S.C. The development of a numeric scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. *J. Sci. Food Agric.* **1953**. *4*, 283-298.

(17) Castell, C.H.; Greenough, M.F. The action of *Pseudomonas* on fish muscle: 1. Organisms responsible for odour produced during incipient spoilage of chilled fish muscle. *J. Fish. Res. Board Can*. **1957**, *12*, 617-625.

(18) Miller III, A.; Scanlan, R.A.; Lee, J.S.; Libbey, L.M. Identification of the Volatile Compounds Produced in Sterile Fish Muscle (*Sebastes melanops*) by *Pseudomonas fragi. Appl. Microbiol.* **1973**, *25*, 952-955.

(19) Miller III, A.; Scanlan, R.A.; Lee, J.S.; Libbey, L.M. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas putrifaciens, Pseudomonas fluorescens,* and an *Achromobacter* species. *Appl. Microbiol.* **1973**, *26*, 18-21.

(20) Miller III, A.; Scanlan, R.A.; Lee, J.S.; Libbey, L.M.; Morgan, M.E. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas perolens. Appl. Microbiol*. **1973**, *25*, 257-261.

(21) Herbert, R.A.; Shewan, J.M. Roles played by bacterial and autolytic enzymes in the production of volatile sulphides in spoiling North Sea cod (*Gadus morhua*). *J. Sci. Food Agric*. **1976**, *27*, 89-94.

(22) Lerke, P.; Huck, R.W. Objective determination of canned tuna quality: Identification of ethanol as a potentially useful index. *J. Food Sci*. **1977**, *42*, 755-758.

(23) Human, J.; Khayat, A. Quality Evaluation of Raw Tuna by Gas Chromatography and Sensory Methods. *J. Food Sci*. **1981**, *46*, 868-873.

(24) Hebard, C.E.; Flick, G.J.; Martin, R.E. Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In *Chemistry and Biochemistry of Marine Food Products*; Martin, R.E., Ed.; AVI Publishing Co., Inc.: Westport, Connecticut, 1982; pp. 149–304.

(25) Kelleher, S.D.; Zall, R.R. Ethanol accumulation in muscle tissue as a chemical indicator of fish spoilage. *J. Food Biochem*. **1983**, *7*, 87-91.

(26) Ahmed, A.; Matches, J.R. Alcohol production by fish spoilage bacteria. *J. Food Prot*. **1983,** *46*, 1055-1059.

(27) Josephson, D.B.; Lindsay, R.C.; Olafsdottir, G. Measurement of Volatile Aroma Constituents as a Means for Following Sensory Deterioration of Fresh Fish and Fishery Products. In *Seafood Quality Determination*, *Proceedings of an International Symposium*, University of Alaska Sea Grant Program, Anchorage, Alaska, U.S.A Kramer D.E.; Liston J. Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1986; pp. 27-47.

(28) Lindsay, R.C.; Josephson, D.B.; Olafsdottir, G. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In *Seafood Quality Determination, Proceedings of an International Symposium*, University of Alaska Sea Grant Program, Anchorage, Alaska, U.S.A. Kramer D.E.; Liston J. Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1986; pp. 221-234.

(29) Oehlenschläger, J. Evaluation of some well established and some underrated indices for the determination of freshness and/or spoilage of ice stored wet fish. In *Quality Assurance in the Fish Industry*; Huss, H.H., Jakobsen, M., Liston, J.; Eds.; Elsevier Science Publisher: Amsterdam, The Netherlands, 1992; pp 339-350.

(30) Jørgensen, L.V.; Dalgaard, P.; Huss, H.H. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *J. Agric. Food Chem*. **2000**, *48*, 2448-2453.

(31) Alasalvar, C.; Taylor, K.D.A.; Shahidi, F. Comparison of volatiles of cultured and wild sea bream (*Sparus aurata*) during storage in ice by dynamic headspace analysis gas chromatography mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 2616-2622.

(32) Chinivasagam, H.N.; Bremner, H.A.; Wood, A.F.; Nottingham, S.M. Volatile components associated with bacterial spoilage of tropical prawns. *Int. J. Food Microbiol.* **1998**, *42*, 45-55.

(33) Joffraud, J.J.; Leroi, F.; Roy, C.; Berdague, J.L. Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. *Int. J. Food Microbiol*. **2001**, *66 (3)*, 175-184.

(34) Jørgensen, L.V.; Huss, H.H.; Dalgaard, P. Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar)* analyzed by GC-MS and multivariate regression. *J. Agric. Food Chem*. **2001,** *49*, 2376-2381.

(35) Gram, L.; Melchiorsen, J. Interaction between fish spoilage bacteria *Pseudomonas sp.* and *S. putrefaciens* in fish extracts and on fish tissue. *J. Appl. Bacteriol*. **1996**, *80*, 589-595.

(36) Gram, L.; Ravn, L.; Rasch, M.; Bruhn, J.B.; Christensen, A.B.; Givskov, M. Food spoilage – interactions between food spoilage bacteria. *Int. J. Food Microbiol.* **2002**, *78,* 79-97.

(37) Huss, H.H.; Dalgaard, P.; Gram, L. Microbiology of fish and fish products. In *Seafood from producer to consumer; intergrated approach to quality*; Luten, J.B., Börresen, T., Oehlenschlager, J., Eds.; Elsevier: Amsterdam, The Netherlands, 1997; pp. 413-430.

(38) Shewan, J.M. The bacteriology of fresh and spoiling fish and some related chemical changes. In *Recent Advances in Food Science;* Hawthorn, J., Leitch, J.M., Eds.; Butterworths: London, 1962; 1, 167-193.

(39) Van Spreekens, K.J.A. The suitability of Long & Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. *Arch. Lebensmitt.* **1974**, *25 (10)*, 213-219.

(40) Van Spreekens, K.J.A.; Toepoel, L. Quality of fishery products in connection with the psychrophilic and psychrotrophic bacterial flora. In *Psychrotrophic microorganisms in spoilage and pathogenicity*; Roberts, T.A., Hobbs, G., Christian, J.H.B., Skovgaard, N., Eds.; Academic Press: London, UK, 1981; pp. 283-294.

(41) Dalgaard, P.; Gram, L.; Huss, H.H. Spoilage and shelf life of cod fillets packed in vacuum or modified atmospheres. *Int. J. Food Microbiol*. **1993**, *19*, 283-294.

(42) Dalgaard, P.; Mejlholm, O.; Christiansen, T.J.; Huss, H.H. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. *Lett. Appl. Microbiol*. **1997,** *24*, 373-378.

(43) Dalgaard, P. Qualitative and quantitative characterization of spoilage bacteria from packed fish. *Int. J. Food Microbiol.* **1995**, *26*, 319-333.

(44) McGill, A.S.; Hardy, R.; Burt, J.R. Hept-*cis*-4-enal and its contribution to the off-flavour in cold stored cod. *J. Sci. Food Agric.* **1974**, *25*, 1477-1489.

(45) Aro, T.; Tahvonen, R.; Koskinen, L.; Kallio, H. Volatile compounds of Baltic herring analysed by dynamic headspace sampling-gas chromatography-mass spectrometry. *Eur. Food Res. Technol*. **2003**, *216*, 483-488.

(46) Olafsdottir, G.; Lauzon, H.L.; Martinsdóttir, E.; Oehlenschläger, J.; Kristbergsson, K. Shelf-life extension of superchilled cod (*Gadus morhua*) fillets and influence of temperature fluctuations on microbial growth and metabolites. In press.

(47) ISO 1985. Sensory analysis – methodology - genereral guidance. ISO 6658. Geneva, Switzerland: The International Organization for Standardization. 14 p.

(48) ISO 1987. Sensory analysis – methodology – evaluation of food products by methods using scales. ISO 5124. Geneva, Switzerland: The International Organization for Standardization. 7 p.

(49) Acree T.;Arn, H. http://www.flavornet.org/flavornet.html; Flavornet, Cornell University, 1997.

(50) Malle, P.; Poumeyrol, M. A New Chemical Criterion for the Quality Control of Fish: Trimethylamine/Total Volatile Basic Nitrogen (%). *J. Food Prot*. **1989,** *52*(*6)*, 419-423.

(51) Huss, H.H. Quality and quality changes in fresh fish. FAO Fisheries Technical Papers - T348. Rome: Food & Agriculture Organisation of the United Nations & Danish International Development Agency (FAO/DANIDA). 1995; 195 p.

(52) Fazzalari, F.A., Ed. Compilation of Odor and Taste Threshold Data, ASTM Data Series DS 48A, 1978, Philadelphia, PA.

(53) Karahadian, C., Lindsay, R.C. Evaluation of compounds contributing characterizing fishy flavors in fish oils. *J. Am. Oil Chem. Soc.* **1989**, *66*, 953-960.

(54) Sheldon, R.M.; Lindsay, R.C.; Libbey, L.M.; Morgan, M.E. Chemical nature of malty flavour and aroma produced by *streptococcus lactis*. *Appl. Microbiol.* **1971**, *22*, 263-266.

(55) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366-2371.

(56) Olafsdottir G. Developing rapid olfaction arrays for determining fish quality. In *Rapid and On-Line Instrumentation for Food Quality Assurance*; Tothill, I.E., Ed.; Woodhead Publishing Ltd: Cambridge, England, 2003; pp. 339-360

(57) Dainty, R.H.; Edwards, R.A.; Hibbard, C.M. Time course of volatile compound formation during refrigerated storage of naturally contaminated beef in air. *J. Appl. Bacteriol.* **1985,** *59*, 303-309.

(58) Curioni, P.M.G.; Bosset, J.O. Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int. Dairy J.* **2002**, *12*, 959-984.

(59) Hardy, R.; McGill, A.S.; Gunstone, F.D. Lipid and autoxidative changes in cold stored cod (*Gadus morhua*). *J. Sci. Food Agric.* **1979**, *30*, 999-1006.

(60) Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. Variations in the occurrences of enzymically derived volatile aroma compounds in salt- and freshwater fish. *J. Agric. Food Chem*. **1984**, *32*, 1344-1347.

(61) Kawai T. Fish flavor. *Crit. Rev. Food Sci. Nutr*. **1996,** *36, 3*, 257-298.

(62) Koide, K.; Yanagisawa, I.; Ozawa, A.; Satake, M.; Fujita, T. Studies on the kerosene-like off-flavour producing yeast in surimi-based products. *Bull. Jap. Soc. Sci. Fish*. **1992,** *58*, 1925-1930.

(63) Olafsdottir, G.; Högnadottir, A.; Martinsdottir, E. Application of gas sensors to evaluate freshness and spoilage of various seafoods. In *Methods to Determine the Freshness of Fish in Research and Industry*; Olafsdottir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdottir, E., Heia, K., Eds.; International Institute of Refrigeration: Paris, France, 1998; pp 100-109.

(64) Castell, C.H.; Greenough, F.; Dale, J. The action of *Pseudomona*s on fish muscle: 3. Identification of organisms producing fruity and oniony odours. *J. Fish. Res. Board Can.* **1959**, *16*, 13-19.

(65) Mielle, P.; Marqui, F. An alternative way to improve the sensitivity of electronic olfactometers. *Sensors and Actuators* **1999**, *B-Chemical 58(1-3)*, 526-535.

(66) Olafsdottir, G.; Martinsdottir, E.; Jonsson, E.H. Rapid gas sensor measurements to predict the freshness of capelin (*Mallotus villosus*). *J. Agric. Food Chem.* **1997**, *45*, 2654-2659.

(67) Olafsdottir, G.; Martinsdóttir, E.; Oehlenschläger, J.; Dalgaard, P.; Jensen, B.; Undeland, I.; Mackie, I.M.; Henehan, G.; Nielsen, J.; Nilsen, H. Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Technol.* **1997**, *8*, 258- 265.

(68) Zeng, Q.Z.; Thorarinsdottir, K.A.; Olafsdottir, G. Quality changes of shrimp (*Pandalus borealis*) stored under different cooling conditions. In press *J. Food Sci*. 2005, accepted for publication.

(69) Ackman, R.G.; Hingley, J. Dimethyl sulfide as an odor component in Nova Scotia fall mackerel. *J. Fish. Res. Board Can.* **1972**, *29*, 1085-1088.

(70) Herbert, R.A.; Ellis, J.R.; Shewan, J.M. Isolation and identifiaction of the volatile sulphides produced during chill-storage of North Sea cod (*Gadus morhua*). *J. Sci. Food Agric.* **1975**, *26*, 1187-1194.

(71) Kamiya, A.; Ose, Y. Study of the odorous compounds produced by putrefaction of foods. V. Fatty acids, sulphur compounds and amines. *J. Chromat*. **1984**, *292*, 383-391.

(72) Tryggvadóttir, S.V.; Olafsdottir, G.; Einarsson, S. Multisensor for fish: Storage studies of cod in Reykjavik and Tromsö. Texture, electronic nose (FreshSense), FIGD analysis, RT-Freshmeter and sensory analysis. RF report 02-01. 2001; pp 19-24.

(73) Buttery, R.G.; Guadagni, D.G.; Ling, L.C.; Seifert, R.M.; Lipton, W. Additional volatile components of cabbage, broccoli and cauliflower. *J. Agric. Food Chem.* **1976**, *24*, 829-832.

(74) Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem*. **1988**, *36*, 1006-1009.

(75) Sheldon, R.M.; Lindsay, R.C.; Libbey, L.M.; Morgan, M.E. Chemical nature of malty flavour and aroma produced by *streptococcus lactis*. *Appl. Microbiol.* **1971**, *22*, 263-266.

(76) Whitfield, F.B.; Tindale, C.R. The role of microbial metabolites in food off-flavours. *Food Technol. Australia*, **1984**, *36*, 204-213.

(77) Buttery, R.G.; Teranishi, R.; Ling, L.C.; Turnbaugh, J.G. Quantitative and sensory studies on tomato paste volatiles. *J. Agric. Food Chem.* **1990**, *38*, 336-340.

FIGURE CAPTIONS

Figure 1. Changes in pH (--Δ--), development of TMA (-♦-) measured by GC-MS (PAR: peak area ratio) and TVB-N (mgN/100g) (-■-) in cod fillets packed in styrofoam boxes during storage at 0.5 °C. Vertical line indicates the end of shelf-life determined by sensory analysis.

Figure 2. PAR (peak area ratio) for alcohols (ethanol (-♦-), 3-methyl-1-butanol (-■-), 2,3-butandiol (-▲-) and isobutanol (-Δ-) detected in highest concentration and ethyl acetate (-x-)-in cod fillets packed in styrofoam boxes during storage at 0.5 °C. Vertical line indicates the end of shelf-life determined by sensory analysis.

Figure 3. PAR (peak area ratio) for ketones (6-methyl-5-hepten-2-one (-■-), 2-butanone (-●-), 3-pentanone (-▲-), acetoin (-Δ-)) and acetic acid (-x-) in cod fillets packed in styrofoam boxes during storage at 0.5 °C. Vertical line indicates the end of shelf-life determined by sensory analysis.

Figure 4. Response of the electronic nose sensors (CO, H_2S, NH_3 and SO_2) towards cod fillets during storage in styrofoam boxes at 0.5 °C on days 4, 7, 10, 12, and 14 after catch.

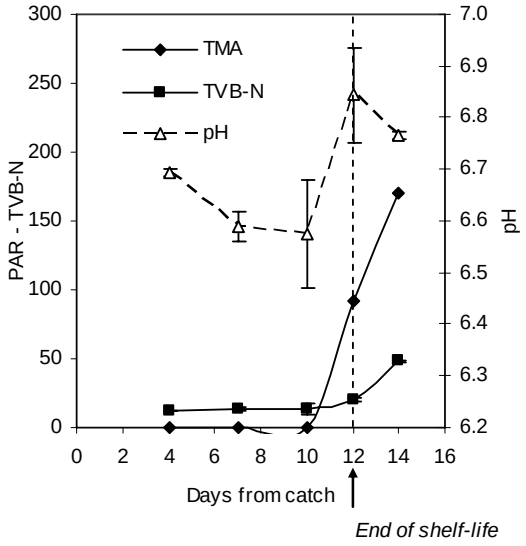


Figure 1

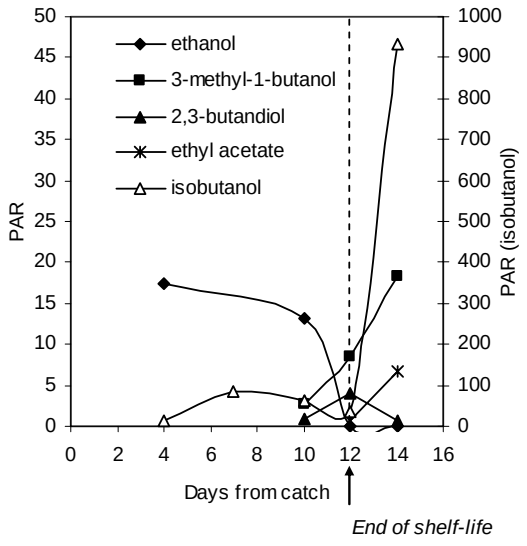


Figure 2

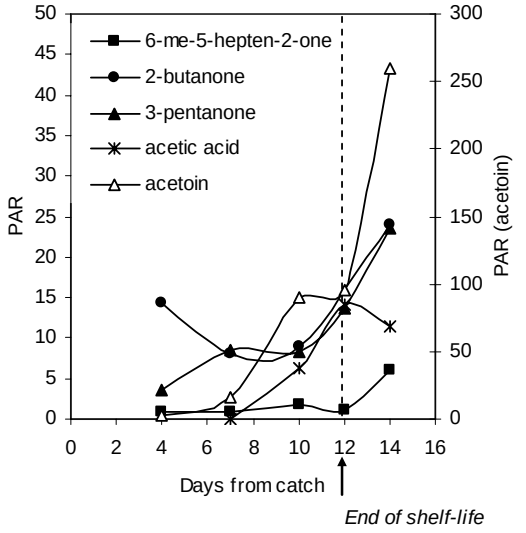


Figure 3.

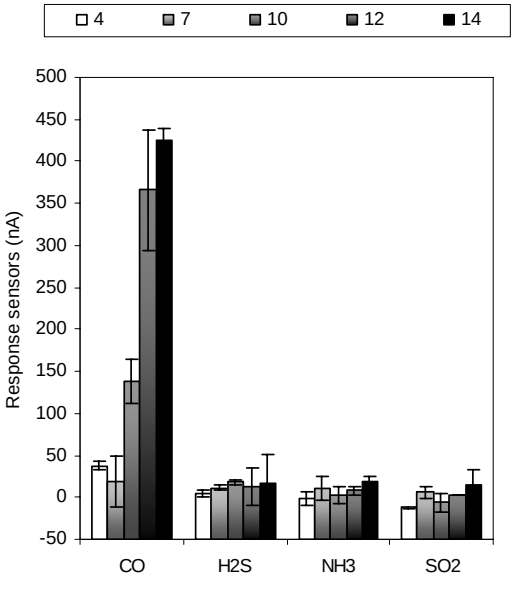


Figure 4.

Table 1. Volatile compounds associated with spoilage in cod fillets during storage in styrofoam boxes at 0.5 °C for 17 days. PAR (peak area ratio) of compounds analyzed by GC-MS, odor descriptions and odor scores based on GC-O analysis and OU (odor unit) value of potent odorants at sensory rejection on day 12.

Compounds	RI[a]	PAR[b]						Odor description[c]	Odor score[d]	OU[e]	Odor threshold[f]	ID means[g]
		Day 4	Day 7	Day 10	Day 12	Day 14	Day 17			Day 12	in water	
Alcohols												
ethanol	<173	17.4 ± 20.7	-	13.1 ± 3.8	-	-	-	-			100000 ppb [1]	MS, RI
2-methyl -1-propanol/ pentane[h]	<173	14.0 ± 1.4	85.4 ± 36.5	64.4 ± 13.2	35.0 ± 2.8	934.6 ± 1096.8	451.9	-		5	7000 ppb [1]	MS, RI
1-penten-3-ol	245	2.3	0.4	0.8 ± 0.1	1.2 ± 0.0	2.5 ± 1.7	-	-		3	400 ppb [1]	MS, RI
3-methyl-1-butanol	300	-	-	2.7 ± 2.4	8.5 ± 1.0	18.3 ± 6.2	31.6 ± 5.6	-		28-34	250-300 ppb [2]	MS, RI
2-methyl-1-butanol	306	-	-	-	-	5.3 ± 2.7	-	-				MS, RI
2,3-butandiol	359	-	-	0.9 ± 1.2	4.0 ± 4.4	0.7	21.3 ± 30.1	-				MS, RI
2-ethyl-1-hexanol	633	-	2.0 ± 2.1	2.3 ± 1.0	1.8 ± 0.2	1.4	2.5 ± 1.1	-			270000 ppb [1]	MS, RI
Aldehydes												
acetaldehyde	<173	2.8 ± 0.8	1.4 ± 0.4	0.9 ± 0.6	0.8 ± 0.5	5.8 ± 4.4	7.5 ± 6.4	-		7-160	15-120 ppb [4]	MS, RI
3-methyl-butanal	227	-	-	1.7	1.3 ± 0.2	2.6	1.2	sweet, caramel, fish fillet	1.5 - 3.0	22	60 ppb [5]	MS, RI, GC-O
hexanal	378	1.9	2.1 ± 2.0	3.2 ± 1.2	2.0 ± 0.1	8.7 ± 6.2	-	-		444	4.5ppb [6]	MS, RI
heptanal	501	-	2.4	1.7 ± 0.8	0.7 ± 0.2	2.9 ± 2.2	3.5 ± 1.8	earthy, boiled potato	2.0 - 3.0	233	3 ppb [1]	MS, RI, GC-O
octanal	606	-	-	1.4 ± 0.6		-	-	-			0.7 ppb [1]	MS, RI
nonanal	710	-	4.3 ± 3.7	6.3 ± 1.1	3.7 ± 0.6	10.2 ± 4.4	-	-		3700	1 ppb [1]	MS, RI
decanal	807	2.3 ± 0.2	2.5 ± 1.9	3.5 ± 0.4	2.4 ± 0.3	8.0 ± 4.2	14.9 ± 5.7	fresh, floral	1.5	1200 - 24000	0.1-2 ppb [1]	MS, RI, GC-O
undecanal	910	-	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	-	2.0 ± 1.1	sweet, candy	1.5	100	4 ppb [1]	MS, RI, GC-O
Ketones												
2-butanone	191	14.3 ± 10.2	8.1 ± 1.1	9.0	-	24.0 ± 13.9	-	-			50000 ppb [1]	MS, RI
3-pentanone	255	3.7 ± 3.2	8.5 ± 5.2	8.2 ± 3.9	13.6 ± 6.2	23.5 ± 17.7	34.6 ± 18.0	sweet, caramel	1.5 - 2.0	0.2	70000 ppb [1]	MS, RI, GC-O
3-hydroxy-2-butanone	273	2.6 ± 1.3	16.8 ± 20.8	90.3 ± 51.6	95.3 ± 5.6	259.2 ± 177.5	341.8 ± 129.3	sweet, sour	1.5 - 2.0	119	800 ppb [7]	MS, RI, GC-O
6-methyl-5-hepten-2-one	588	0.8 ± 0.2	0.9 ± 0.2	1.9 ± 0.4	1.0 ± 0.1	6.1 ± 4.1	5.4 ± 1.8	spicy, flowery	1.5	20	50 ppb [2]	MS, RI, GC-O
Acids												
acetic acid	191	-	-	6.3 ± 4.2	14.2 ± 13.0	11.4 ± 4.3	15.5	-		0.4	34200ppb[3]	MS, RI
Amines												
TMA	<173	-	-	-	91.6 ± 28.5	922.2 ± 1064.2	1721.4 ± 47.6	TMA-like, dried fish	3.0	91600-305333	0.3-1 ppb [1]	MS, RI, GC-O
Esters												
ethyl acetate	200	-	-	-	0.6	6.6 ± 1.4	258.9 ± 330.0	-		0.1-120	5-5000ppb [1]	MS, RI
ethyl butanoate	377	-	-	-	-	-	13.9 ± 2.2	sickenly sweet, vomit	2.3			MS, RI, GC-O
Sulfur compounds												
dimethyl sulfide	173	4.5	-	-	-	-	-	-			900 ppb [6]	MS, RI
dimethyl disulfide	312	-	-	-	-	-	1.2	onion like	1.5 - 2.5		12 ppb [2]	MS, RI, GC-O
dimethyl trisulfide	564	-	-	-	-	-	-	rotten, sulfur, cabbage	2.5		0.01ppb [2]	GC-O
Unknown	457	-	-	-	-	-	-	spicy, fish, flowery, sweet, onion, mushroom	3.0 -4.8			GC-O

[a]RI: calculated retention index on DB-5ms capillary column; [b]PAR: peak area ratio, average of duplicate analysis ± standard deviation when detected in both samples or " - " if not detected; [c]odor evaluated by GC-O; [d]GC-O odor scores (average value of two panelists) giving the range of scores on days 4, 10 and 17; [e]OU: odor unit calculated by dividing the PAR by the odor threshold value x 1000; [f]odor thresholds from literature: 1. Fazzalari, 1978 (52); 2. Buttery et al., 1976 (73); 3. Kawai 1996 (61); 4. Buttery et al., 1988 (74); 5. Sheldon et al., 1971 (75); 6. Whitfield and Tindale, 1984 (76); 7. Buttery et al., 1990 (77); [g]identification on the basis of MS database,retention index and odor evaluation; [h]coeluting peaks.

Table 2. PAR of main classes of compounds identified by GC-MS in cod fillets packed in styrofoam boxes during storage at 0.5 °C for 17 days

	PAR [a]					
Class	Day 4	Day 7	Day 10	Day 12	Day 14	Day 17
Quality indicators						
Alcohols	34 (29)	88 (45)	84 (28)	51 (15)	963 (39)	507 (15)
Aldehydes	7 (6)	13 (6)	19 (6)	11 (3)	38 (2)	27 (1)
Ketones	21 (18)	34 (18)	109 (36)	110 (33)	313 (13)	382 (11)
Acids	-	-	6 (2)	14 (4)	11 (<1)	15 (<1)
Amines	-	-	-	92 (27)	922 (37)	1721 (51)
Esters	-	-	-	1 (<1)	7 (<1)	273 (8)
Sulfur compounds	5 (4)	-	-	-	-	1 (<1)
Miscellaneous						
Alkanes	3 (3)	4 (2)	6 (2)	3 (1)	4 (<1)	3 (<1)
Aromatics	17 (15)	39 (20)	40 (13)	22 (7)	80 (3)	76 (2)
Other	5 (4)	5 (3)	3 (1)	12 (4)	97 (4)	351 (10)
Unknown	26 (22)	10 (5)	36 (12)	17 (5)	28 (1)	44 (1)
Total volatiles	118	194	306	335	2464	3402

[a] PAR (peak area ratio) and percentage of total volatiles each day shown in parenthesis

PAPER VII

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

Guðrún Ólafsdóttir, Eric Chanie, Frank Westad, Rósa Jónsdóttir, Claudia R. Thalmann, Sandrine Bazzo, Saïd Labreche, Pauline Marcq, Frank Lundby, John-Erik Haugen,

VII

Accepted for publication in J Food Sci.

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

Gudrun Olafsdottir [a], Eric Chanie[b], Frank Westad[c], Rosa Jonsdottir[a], Claudia R. Thalmann[d,e], Sandrine Bazzo[b], Said Labreche[b], Pauline Marcq[b], Frank Lundby[c], John Erik Haugen[c¶]

[a]Icelandic Fisheries Laboratories, Skulagata 4, 101 Reykjavik, Iceland
[b]Alpha-Mos 20 Av Didier DAURAT 31400, Toulouse, France
[c]MATFORSK AS, Osloveien 1, N-1430 Ås, Norway
[d]ttz Bremerhaven, Fischkai 1, D-27572 Bremerhaven, Germany
[e]SENSIENT Flavors GmbH - Regulatory Affairs / Technical Information
Beim Struckenberge 1-9, D-28239 Bremen, Germany

Contact information for Corresponding Author:
Gudrun Olafsdottir
Icelandic Fisheries Laboratories, Skulagata 4, 101 Reykjavik, Iceland
Fax: +354 530 8601, Phone: +354 530 8600, gudrun@rf.is

ABSTRACT:

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

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

Introduction

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

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

Quality monitoring of smoked salmon in the industry is often based on sensory evaluation of appearance, texture, smell and taste. Desirable attributes from smoking diminish during storage and the characteristic deterioration takes over, including softening of the fish flesh, fading colors and unpleasant odors and flavors. Different schemes have been used in the various studies on cold smoked salmon and sensory descriptors for spoilage have been suggested. Sensory descriptors like sweet/sour, bitter, fecal, ammonia and cabbage were used by Hansen and others (1998) to describe the spoilage of vacuum packed cold smoked salmon stored at 5°C. The main

spoilage characteristics described by Leroi and others (1998) in cold smoked vacuum packed salmon stored for 5 weeks at 8 °C were a pasty texture and pungent, acid, rancid and sour flavors and odors. Cardinal and others (2004) identified different quality classes based on sensory characteristics of cold smoked salmon from supermarkets in the European market. Color, intensity of smoke related odors, amine odors and salty perception were the main sensory characteristics discriminating between quality classes.

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

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

Materials and Methods

Preparation of samples

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

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

Sampling

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

Microbial and chemical analyses were done in the participating laboratories in the different countries on each day of sampling. Samples for electronic nose measurements and sensory analysis were vacuum packed, frozen (-24 °C) and

transported in a Styrofoam box by courier service at the end of the experiment to AlphaMOS and to IFL, respectively.

Chemical analysis

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 using an extraction apparatus 2050 Soxtec Avanti Automatic System (AOCS 1998). Salt content was measured by extracting the soluble chloride from the sample with water containing nitric acid. The chloride content of the solution was titrated with silver nitrate and the end point determined potentiometrically (AOAC 1995)

Microbial analysis

The microbial analysis included total psychrotrophic counts (TVC) (total viable counts) using modified Long & Hammer's medium (LH) (Van Spreekens 1974) and incubation at 15 °C. Analysis of lactic acid bacteria (LAB) counts was done using NAP (Nitrite Actidione-Polymyxin) medium slightly modified (Davidson and Cronin 1973).

Sensory analysis

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

/ flavor, appearance, and texture. Odor and flavor attributes were: smoked salmon odor/flavor, metallic odor/flavor, sweet/sour fruity odor/flavor, rancid odor/flavor, off- odor/flavor. Taste attributes included: salt and bitter taste. Appearance attributes evaluated were: fat secretion, translucent, hue, color intensity and three texture attributes: elasticity, oiliness, juiciness. Further training of the panel and testing of the scheme was done in a pre-trial, using samples that were stored for 14, 28, 35 and 42 days at 5 °C.

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

Electronic nose

The GEMINI electronic nose (Alpha M.O.S, Toulouse, France) equipped with 6 metal oxide semiconductors (MOS) sensors (PA/2, P10/1, P40/2, P40/1, LY2/G, LY2/LG) was used in the project. A prototype sampling unit developed by OPTOTEK (Slovenia) was connected to the sensor unit GEMINI. The sampling unit has a 10 ml sample loop, a heated inlet tube (55°C) and a pump (flow rate 200 ml/min). The sampling was performed by inserting the inlet tube into a bell shaped unit (10 cm 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 inert property. Sampling temperature (headspace generation temperature) was 5 °C and loading time of 7 s was used.

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

Data analysis

Sensory analysis of smoked salmon was performed using the software Fizz (France). Statistical analysis was done on the sensory data using Number Cruncher Statistical

Software (NCSS 2000 and Pass Trial, Kaysville, Utah). 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 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. In this context prediction means cross-validated predictions, as there were no new independent sets of samples present for prediction. However, cross-validation is more conservative than just numerical fit of all samples. The quality criteria established to discriminate good samples from bad samples were based on overall results of the analysis in this study, taking into account commercial critical limits for total viable counts (TVC), results of earlier studies of cold smoked salmon and sensory scores of selected attributes in this study.

Results and Discussion

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

The data from the FishNose sensors for all the 96 samples were analyzed by Principal Component Analysis (PCA) (Fig 1). The two first dimensions described 99 % of the total variance in the data set. The first PC explaining 94 % of the variation appears to be describing the spoilage level of the samples. Most of the samples located to the right were stored samples from producer A and a few from producer D with high bacterial numbers and high scores in sensory odor attributes (sweet/sour and off odors). All the samples on the left had low microbial counts and low spoilage odor scores indicating that the majority of the samples in the experiment were of good microbial and sensory quality. The second PC explaining 5 % of the variance in the data appears to be discriminating the different smokehouses: The samples were clustered together, however samples from the same producers appear to be closer together indicating differences in the headspace of the samples from the different smokehouses.

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

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

cold smoked salmon indicating the importance of spoilage organism in product shelf-life since characteristic spoilage off-odors were only found in samples of cold smoked salmon with high bacterial loads (Hansen and others 1996).

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

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

Chemical analysis

The correlation of the chemical parameters and the gas sensors were low. Therefore, they were not selected to calibrate the FishNose responses. However, variation in salt content can influence the microbial growth and may explain the proliferation of spoilage. An overview of the chemical composition (fat, water and salt) of the cold

smoked salmon samples from the different producers is shown in Table 2. Considerable variation in fat and water content of samples was found even within samples from the same batch. The A samples had the highest fat content and samples from C had the lowest fat content. This variation in fat content is expected and reflects both the age/size and feeding condition of the salmon.

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

Microbial analysis - TVC

The limits for the end of shelflife in the industry are often set at 10^6 cfu/g. However, this limit has been questioned and not in agreement with the results of sensory panelists who estimated that samples with counts of 3 x 10^6 cfu/g had not exceeded the limit of shelf life (Leroi and others 1998). 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 (Hansen and others 1995, 1998). Estimation of shelf life of smoked salmon products based on storage days is not practical, because the various handling, smoking processes and the different storage conditions influence the spoilage processes and the shelf life of the products (Hansen and others, 1995; 1996; Dondero and others, 2004). Accordingly, the shelf life of smoked salmon products varies considerably from about 2 weeks to 2 months depending mostly on the temperature during storage.

Storage at 5 °C: The initial TVC numbers for the day 0 samples in the storage studies varied from 1.5 to almost 4 in log cfu/g (Fig 4). The increase in TVC numbers with storage time for samples stored at 5°C for 28 days is obvious for all the samples except from smokehouse D. The D samples had very low counts (< 10^3 cfu/g) throughout the study which may be explained by the high salt content (mean = 5.3%) of those samples (see Table 2). Slow spoilage rate was also observed for samples from smokehouse B and at the end of the study the TVC values did not exceed 10^6 cfu/g. Samples from smokehouse B were packaged as whole fillets, but not as slices.

This may have influenced the spoilage rate for the B samples. Other researchers have reported longer shelf life for fillets (32 - 49 days) than for slices (21 - 36 days) of the same product evaluated by a sensory panel (Hansen and others 1998). The initial high counts of the A samples probably reflected the hygienic conditions in the smokehouse. Additionally, the A samples had the highest microbial counts throughout the study which may also be explained by the handling, salting, the smoking process and the packaging. In particular it is of interest that the smoking time was very short for the A samples (Table 1).

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

Process samples: The TVC in samples from the process (labeled: a,b,c…h) varied from 10^2 to almost 10^7 cfu/g. This variation is explained by the different age of the samples. Two processors (A and C) provided fresh samples from the process, but the others (B and D) provided both fresh and stored samples. One batch from D had been kept for 10 days at 2 °C before delivery to the laboratory and another batch from B was selected from old stored products (15-22 months in freezer), to obtain samples of bad quality reflecting frozen storage conditions

Initially it was decided that a quality criterion corresponding to a 10^6 cfu/g for total viable count should be applied to discriminate between accepted “good” and rejected “bad” samples in this study, since this is the general microbiological safety guideline applied for food quality. Only 6 samples (6 %) of the total sample set from different production batches were of “bad” quality based on the TVC criterion 10^6 cfu/g. These were samples A1 and A2 stored for 28 days and two samples from the process. It should be mentioned that according to the specification of the A products the indicated shelflife was only 2 weeks. Since the majority of the samples were not spoiled based on the TVC criteria of 10^6cfu/g at the end of the study (Fig 4), it was decided to use lower limits (10^5cfu/g) and aim at discriminating between good samples and samples that are just starting to show spoilage signs but still of

acceptable quality. Fifteen samples exceeded values above 10^5 cfu/g (16 %) and 33 samples (35 %) exceeded values above 10^4 cfu/g.

Microbial analysis - LAB

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

In some samples the counts of LAB exceeded the TVC value. It is expected that the LAB will grow on the modified 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.

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

Sensory analysis

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

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

Statistical evaluation of the data using 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 ($p < 0.05$) were found in odor and flavor attributes and color intensity for some sample groups (data not shown). Since flavor was not evaluated in all the samples the odor scores will be used for the quality criteria and data from the odor evaluation is shown herein (Fig 6 - 9).

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

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

The scores for the initial samples (0 days) from C and D and samples from day 4 from B for the sample groups stored at 10 °C had already high scores for the sweet/sour, rancid and off odor spoilage attributes in agreement with higher microbial counts for

these samples. Part of the samples from the process from D had high scores for the spoilage attributes that can be explained because they had been stored for 10 days before the delivery to the laboratory.

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

FishNose measurements

The sensor responses of the FishNose prototype sensor system showed a similar pattern for the 96 samples analyzed, suggesting covariation in their responses. Figure 10 gives an example of one the sensors PA/2 showing a pattern which is very similar to the microbial analysis of TVC (Fig 4) and the spoilage related sensory attributes (sweet/sour, odor off and rancid odor) (Fig 6, 7 and 8). High sensor readings corresponded to high TVC numbers for two of the producers (A and D) in agreement to higher sensory scores for spoilage odors in those samples.

Studies in the project on the responses of the FishNose to varying concentrations of standard 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 (Olafsdottir and others 2005). Therefore, it is likely that the gas sensors are mainly detecting changes in the very volatile compounds present in the headspace of the samples mainly representing microbial metabolism and oxidatively derived compounds.

Classification modeling

Global models: Different classification models were investigated for prediction of samples of different quality. Using single numeric criteria of separate reference parameters like TVC numbers or single sensory quality related parameters like sweet/sour odor, rancid odor or off-odor on all the combined measurement data gave in general low classification rates. It should be emphasized that the establisment of quality critera in this study is aimed at detecting samples that are showing initial spoilage signs, but not necessarily of unacceptable quality, because the sample set did not contain many samples that were spoiled. Therefore, the sensors sensitivity is challenged because the concentration of spoilage volatiles may be low and as a result the classification rates may be poorer than otherwise expected if the samples were indeed spoiled.

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

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

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

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

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

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

The samples that showed the highest correlation with TVC numbers were the A samples, except for the 28 days old samples, where an unexpected decrease was observed in the sensor response signal (Fig 10). 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 D samples from the process, but higher correlation of sensor responses with the TVC values, suggests that other bacteria than LAB may have contributed to the TVC counts like *Enterobacteriacea*. This indicates that these samples were not handled properly and perhaps poor hygienic conditions in the factory. In a prestudy done in the project *Enterobacteriacea* counts were indeed high in samples from producer D (unpublished data).

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

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

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

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

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

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

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

Combined criteria: The combined criteria improved the overall predictions slightly for the A samples but not for the D samples where 8 samples were expected 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 (C and B), because of a lack of structural correlation of variables and sample groups. Moreover, a robust prediction was achieved with the single criteria for those samples.

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

Conclusions

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

The majority of the samples in this study were of "good" quality and most of the samples defined as "bad" were of marginal quality, since the criteria for the individual quality attributes were set lower than the commercial rejection criteria. Only a few of the samples would have been judged unacceptable based on commercial quality criteria. The practical implication of this study for the smoked salmon producer is that

the FishNose system was able to discriminate samples of marginal quality from the good samples. This allows more effective quality control of samples in the production and could also be useful in retail and distribution. Rapid quality grading of samples is possible before the samples are actually spoiled. Additionally, the FishNose gas sensors appeared to group the samples according to the fish processors indicating that there were differences in the composition of the headspace because of the different smoking and handling processes.

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

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

References

AOAC. 1995. 976.18 Determination of salt (chlorine as sodium chloride) in seafood potentiometric method. Sec. 35.1.19. In: Cunniff P, editor. Official Methods of Analysis of AOAC Int. Gaithersburg, Md: AOAC Int. 8 p

AOCS. 1998. Official method BA 3-38 and application note Tecator no. AN 301. Firestone D, editor. Official Methods and Recommended Practices of the American Oil Chemists' Society. 5th ed. Champaign, IL, USA: American Oil Chemists Society. 4 p.

Becker B, Schillinger U, Holzapfel WH. 2002. Microbiological quality and listeria contamination of vacuum packaged smoked salmon. Archiev fur Lebensmittelhygiene , 53 (1): 4-7.

Bugueno G, Escriche I, Martinez-Navarrete N, Camacho MD, Chiralt A. 2003. Influence of storage conditions on some physical and chemical properties of smoked salmon (*Salmo salar*) processed by vacuum impregnation techniques. Food Chem (1): 85-0.

Cardinal M, Gunnlaugsdottir H, Bjoernevik M, Ouisse A, Vallet JL, Leroi F. 2004. Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. Food Res Int 37(2): 181-93.

Cardinal M; Knockaert C; Torrissen O, Sigurgisladottir S; Morkore T, Thomassen M; Vallet JL. 2001. Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). Food Res Int 34(6): 537-50.

Davidson AP, Cronin F. 1973. Medium for the selective enumeration of lactic acid bacteria in foods. Appl Microbiol 26 (3): 439-0.

Di Natale C. 2003. Data Fusion in MUSTEC: Towards the Definition of an Artificial Quality Index.. In: Luten JB, Oehlenschlager J, Olafsdottir G, editors. Quality of Fish from Catch to Consumer: Labeling, Monitoring and Traceability. Wageningen, The Netherlands: Academic Publishers. p 273-0.

Di Natale C, Brunink JAJ, Bungaro F, Davide F, D´Amico A, Paolesse R, Boschi T, Faccio M, Ferri G. 1996. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. Meas Sci Technol 7: 1103-14.

Di Natale C, Olafsdottir G, Einarsson S, Mantini A, Martinelli E, Paolesse R, Falconi C, D'Amico A. 2001. Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets. Sens Act B77: 572-8.

Dondero M, Cisternas F, Carvajal L, Simpson R. 2004. Changes in quality of vacuum-packed cold-smoked salmon (*Salmo Salar*) as a function of storage temperature. Food Chem 87(4): 543-0.

Du WX, Kim J, Huang TS, Marshall MR, Wei CI. 2001. Microbiological, sensory, and electronic nose evaluations of yellowfin tuna under various storage conditions. J Food Prot 64 (12): 2027- 36.

Du WX, Lin CM, Huang TS, Kim J, Marshall MR, Wei CL. 2002. Potential application of the electronic nose for quality assessment of salmon fillets under various storage conditions. J Food Sci 67 (1): 307-13.

Egashira M, Shimizu Y, Takao Y. 1990. Trimethylamine sensor based on semiconductive metal-oxides for detection of fish freshness. Sens Act B Chem 1 (1-6): 108-12 .

Gonzalez-Rodriguez MN, Sanz JJ, Santos JA, Otero A, Garcia-Lopez ML. 2002. Numbers and types of microorganisms in vacuum-packed cold-smoked freshwater fish at the retail level. Int J Food Microbiol 77 (1-2): 161-8

Gram L, Huss HH. 1996. Microbiological spoilage of fish and fish products. Int J Food Microbiol 33:121-37.

Hansen LT, Gill T, Huss H. 1995. Effects of salt and storage temperature on chemical, microbiological and sensory changes in cold-smoked salmon. Food Res Int 28(2): 123-30.

Hansen LT, Gill T, Rontved SD, Huss HH. 1996. Importance of autolysis and microbiological activity on quality of cold-smoked salmon. Food Res Int 29(2): 181-8

Hansen LT, Rontved SD, Huss HH. 1998. Microbiological quality and shelf life of cold-smoked salmon from three different processing plants. Food Microbiol 15(2): 137- 50.

Haugen JE, Chanie E, Westad F, Jonsdottir R, Bazzo S, Labreche S, Marcq P, Lundby F, Olafsdottir G. Rapid control of smoked Atlantic salmon quality by electronic nose: correlation with classical evaluation methods. Forthcoming.

Haugen E, Undeland I. 2003. Lipid oxidation in herring fillets (*Clupea harengus*) during ice storage measured by a commercial hybrid gas-sensor array system, J Agric Food Chem 51(3): 752-9

ISO 1983. 6496. Determination of moisture and other volatile matter content. Geneva, Switzerland: The International Organization for Standardization. 7 p.

ISO 1993. Sensory analysis - general guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors, 8586-1. Geneva, Switzerland: The International Organization for Standardization. p 1-10.

ISO. (1994). 11035 - Sensory analysis - identification and selection of descriptors for establishing a sensory profile by a multidimensional approach. Geneva, Switzerland: The International Organization for Standardization.

Joffraud JJ; Leroi F; Roy C; Berdagué JL. 2001. Characterisation of volatile compounds produced by bacteria isolated from spoilage flora of cold-smoked salmon. Int J Food Microbiol 66, 175-84.

Jørgensen, LV; Huss HH; Dalgaard P. 2001. Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar)* analyzed by GC-MS and multivariate regression. J Agric Food Chem 49: 2376-81.

Leroi F, Joffraud JJ, Chevalier F, Cardinal M. 1998. Study of the microbial ecology of cold –smoked salmon during storage at 8°C. Int J Food Microbiol 39: 111-21

Leroi F; Joffraud JJ; Chevalier F; Cardinal, M. 2001. Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. J Appl Microbiol 90: 578-87.

Luzuriaga DA, Balaban MO. 1999a. Evaluation of Odor Decomposition in Raw and Cooked Shrimp: Correlation of Electronic Nose Readings, Odor Sensory Evaluation and Ammonia Levels. In: Hurst WJ, editor. Electronic nose and sensor array based system, Design and Applications. Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. USA: Technomic Publication Company, Inc. p 177-84.

Luzuriaga DA, Balaban MO. 1999b. Electronic nose odor evaluation of salmon fillets stored at different temperature In: Hurst, WJ editor. Electronic nose and sensor array based system, Design and Applications. Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. USA, Technomic Publication Company, Inc. p.162-9.

Newman DJ, Luzuriaga DA, Balaban MO. 1999. Odor and Microbiological Evaluation of Raw Tuna: Correlation of Sensory and Electronic Nose Data. In: Hurst WJ, editor. Electronic nose and sensor

array based system, Design and Applications. Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. USA: Technomic Publication Company Inc. p 170-6.

Ohashi E, Takao Y, Fujita T, Shimizu Y and Egashira M. 1991. Semiconductive trimethylamine gas sensor for detecting fish freshness. J Food Sci 56(5):1275-8.

Olafsdottir G, Chanie E, Westad F, Jonsdottir R, Bazzo S, Labreche S, Marcq P, Lundby F, Haugen JE. 2005. Rapid Control of Smoked Atlantic Salmon Quality by Electronic Nose: Correlation with Classical Evaluation Methods. In: Marco S, Montoliu I, editors. Proceedings of the 11th International Symposium on Olfaction and Electronic Nose, ISOEN2005, Electronic Department, Physics Faculty, Barcelona University, Barcelona, Spain. p 110-4.

Olafsdottir G, Hognadottir A, Martinsdottir E, and Jonsdottir H. 2000. Application of an electronic nose to predict total volatile bases in capelin (*Mallotus villosus*) for fishmeal production. J Agric Food Chem 48:2353-9.

Olafsdottir G, Hognadottir A, Martinsdottir E. 1998. Application of gas sensors to evaluate freshness and spoilage of various seafoods. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdottir E, Heia K, editors. Methods to Determine the Freshness of Fish in Research and Industry. Paris: International Institute of Refrigeration p 100-9.

Olafsdottir G; Fleurence J. 1998. Evaluation of fish freshness using volatile compounds - Classification of volatile compounds in fish. In: Olafsdottir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdottir E, Heia K, editors. Methods to Determine the Freshness of Fish in Research and Industry. Paris: International Institute of Refrigeration p. 55-69.

Olafsdottir G, Martinsdottir E, Jonsson EH. 1997. Rapid gas sensor measurement to determine spoilage of capelin (*Mallotus villosus*). J Agric Food Chem 45:2654-9.

Olafsdottir G, Xiuchen L, Lauzon H, Jonsdottir R. 2002. Precision and application of electronic nose for freshness monitoring of whole redfish stored in ice and modified atmosphere bulk storage. J Aquat Food Prod Technol 11:229-49.

Olafsson R, Martinsdóttir E, Olafsdottir G, Sigfússon ÞI, Gardner JW. 1992. Monitoring of fish freshness using tin oxide sensors. In: Gardner JW, Bartlett PN, editors. Sensors and Sensory Systems for an Electronic Nose. The Netherlands: Kluwer Academic Publ. 257-272.

Bleibaum RN, Stone H, Tan T, Labreche S, Saint-Martin E, Isz S. 2002.Comparison of Sensory and Consumer Results with Electronic Nose and Tongue Sensors for Apple Juices, Food Quality Pref, 13: 409-22.

Schweizer- Berberich PM; Vaihinger S, Göpel W. 1994. Characterisation of food freshness with sensor arrays. Sens Act, B18(19): 282-0.

Stohr V, Joffraud JJ, Cardinal M, Leroi F. 2001. Spoilage potential and sensory profile associated with bacteria isolated from cold-smoked salmon. Food Res Int 34 (9): 797-6

Stone H, Sidel JL. 1985. Sensory evaluation practices. Orlando FL: Academic Press, Inc. p 311.

Van Spreekens KJA. 1974. The suitability of Long & Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. Archiv fur Lebensmittelhygiene. 25 (10): 213-9.

Zhao CZ, Pan YZ, Ma LZ, Tang ZN, Zhao GL, Wang LD. 2002. Assay of fish freshness using trimethylamine vapor probe based on a sensitive membrane on piezoelectric quartz crystal, Sens Actu B-CHEM 81 (2-3): 218-22

Acknowledgments

The research work presented herein was a part of the EU CRAFT project FishNose "Development of an electronic nose system for the automated quality control of smoked fish" (QLK1-CT-2002-71304) sponsored by the European Commission.

Table 1 - Smoking and storage conditions of samples from the different smokehouses, number of samples from each smokehouse and treatments.

Conditions		Smokehouse			
		A	B	C	D
Smoking	Temp. (°C)	27	16-22	22	28
	Time (hours)	0.5	14-18	5	6-12
	Humidity (%)	40	50-60	50-60	50
Storage	Packaging	MAP/VAC	VAC	VAC	VAC
	Temp. (°C)	5	5 / 10	5 / 10	5 / 10
Storage study	Batch 1 (n=64)	16	14	16	16
Process	Batch 2 (n=16)	4	4	4	4
Process	Batch 3 (n=18)	4	4	4	6
Total	n=96	24	22	24	24

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
A	5.8 – 16.6	10.5	56.1 – 65.7	61.4	2.9 – 5.7	3.8
B	5.8 – 12.9	9.0	60.2 – 66.2	63.3	2.1 – 6.6	4.7
C	3.5 – 11.7	7.0	62.9 – 68.9	65.5	2.3 – 4.0	3.1
D	4.7 – 11.4	8.5	57.3 – 63.8	61.2	4.3 – 7.0	5.3

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

Good/accepted samples:	Bad/rejected samples:
TVC < 5	TVC > 5
LAB < 4	LAB >4
Off odor < 20	Off odor > 20
Rancid odor < 10	Rancid odor > 10
Sweet/sour odor < 20	Sweet/sour odor > 20

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

		Expected number of samples	% correct prediction	% wrong prediction
n = 96 4 criteria	Good / accepted	65	92 (n = 60)	8 (n = 5)
	Bad / rejected	31	35 (n = 11)	65 (n = 20)
n = 96 5 criteria	Good / accepted	58	71 (n = 41)	29 (n = 17)
	Bad / rejected	38	37 (n = 14)	63 (n = 24)

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

		Sensors					
	Attributes	PA/2	P10/1	P40/2	P40/1	LY2/G	LY2/LG
A	Sweet/ sour	0.77	0.62	0.77	0.63	0.78	0.74
	Rancid odor	0.43	0.41	0.43	0.41	0.44	0.37
	Off-odor	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
D	Sweet/ sour	0.87	0.72	0.88	0.72	0.91	0.89
	Rancid odor	0.74	0.73	0.74	0.72	0.7	0.68
	Off-odor	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
C	Sweet/ sour	-0.52	-0.48	-0.23	-0.48	-0.22	-0.41
	Rancid odor	-0.47	-0.49	-0.27	-0.49	-0.25	-0.40
	Off-odor	-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
B	Sweet/ sour	-0.16	-0.29	-0.17	-0.26	-0.09	-0.16
	Rancid odor	0.37	0.22	0.35	0.22	0.41	0.42
	Off-odor	-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

Table 6 - PLSR classification results of local models based on gas sensor and selected quality criteria for samples from each smokehouse (A. B. C. D)

Quality criteria		A (n=24)			D (n=16)			C (n=16)			B (n=13)		
		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	Good	15	73	27	13	92	8	7	100	0	8	63	38
	Bad	9	100	0	3	33	67	9	100	0	5	100	0
LAB	Good	15	80	20	15	100	0	14	100	0	8	75	25
	Bad	9	89	11	1	0	100	2	0	100	5	100	0
Off odor	Good	17	94	6	12	100	0	15	100	0	13	100	0
	Bad	7	86	14	4	100	0	1	0	100	0		
Sweet /	Good	16	100	0	11	100	0	14	86	14	13	100	0
sour	Bad	8	88	13	5	100	0	2	100	0	0		
Rancid	Good	21	100	0	13	92	8	15	100	0	13	100	0
	Bad	3	0	100	3	67	33	1	0	100	0		
Combine d	Good	14	79	21	8	0	100						
	Bad	10	90	10	8	100	0						

List of Figures

Figure 8 - Rancid odor scores for samples stored at 5°C and 10°C for 28 and 10 days, respectively, and 8 samples from the process from the different smokehouses (A1, A2, B, C and D). Two replicate samples were analyzed each day. Line represents the quality criteria set at 10.

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

Figure 10 - Sensor response distribution for samples measured with the FishNose PA/2 sensor for samples stored at 5°C and 10°C for 28 and 10 days, respectively, and 8 samples from the process from the different smokehouses (A1, A2, B, C and D). Two replicate samples were analyzed each day.

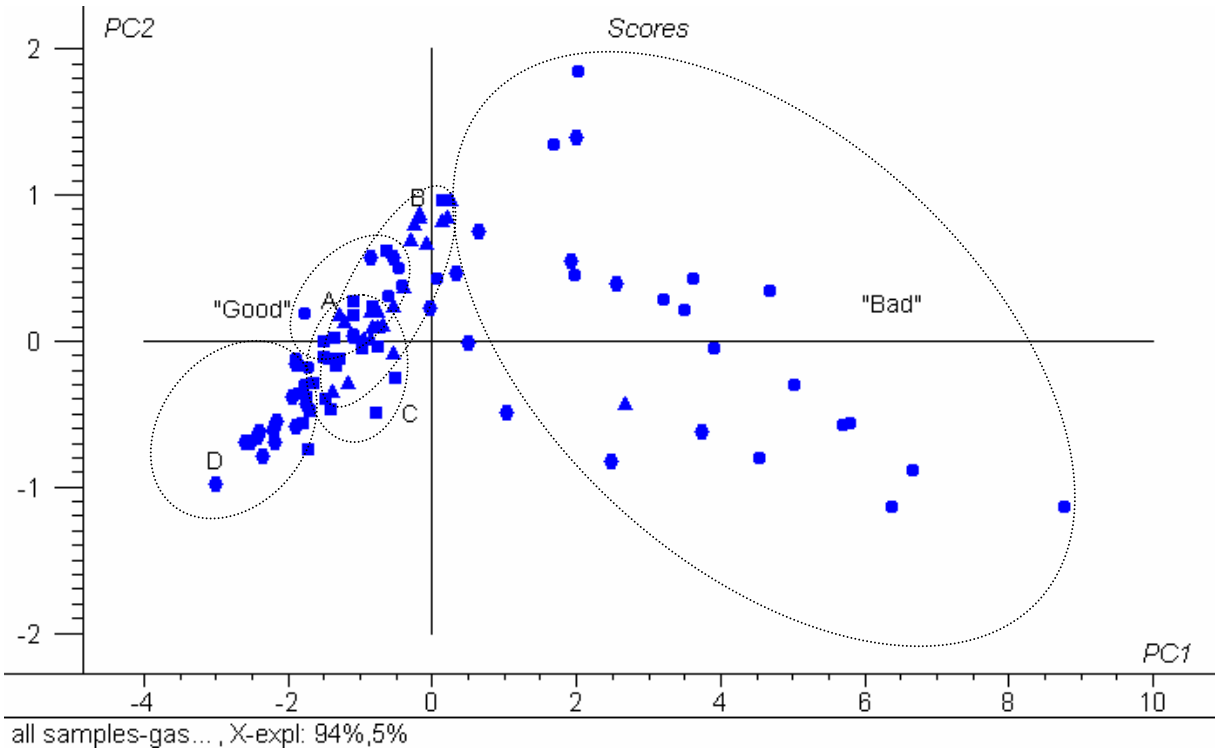

PC2
Scores
2
1
0
-1
-2
"Good"
"Bad"
A
B
C
D
PC1
-4
-2
0
2
4
6
8
10
all samples-gas... , X-expl: 94%,5%

Figure 1.

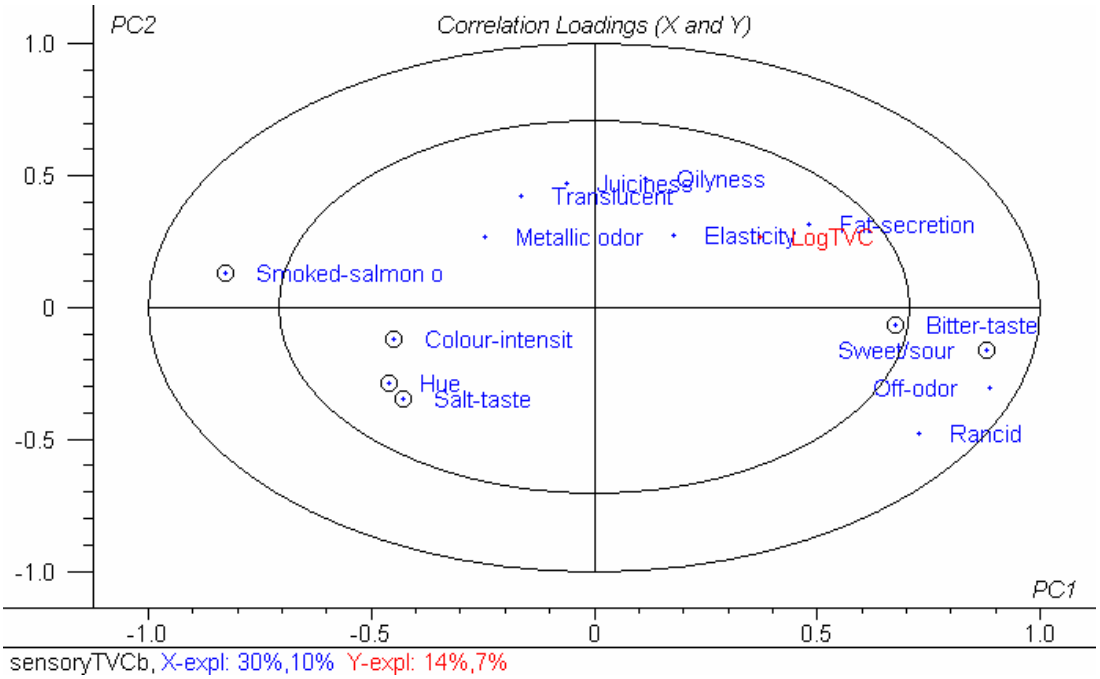


Figure 2.

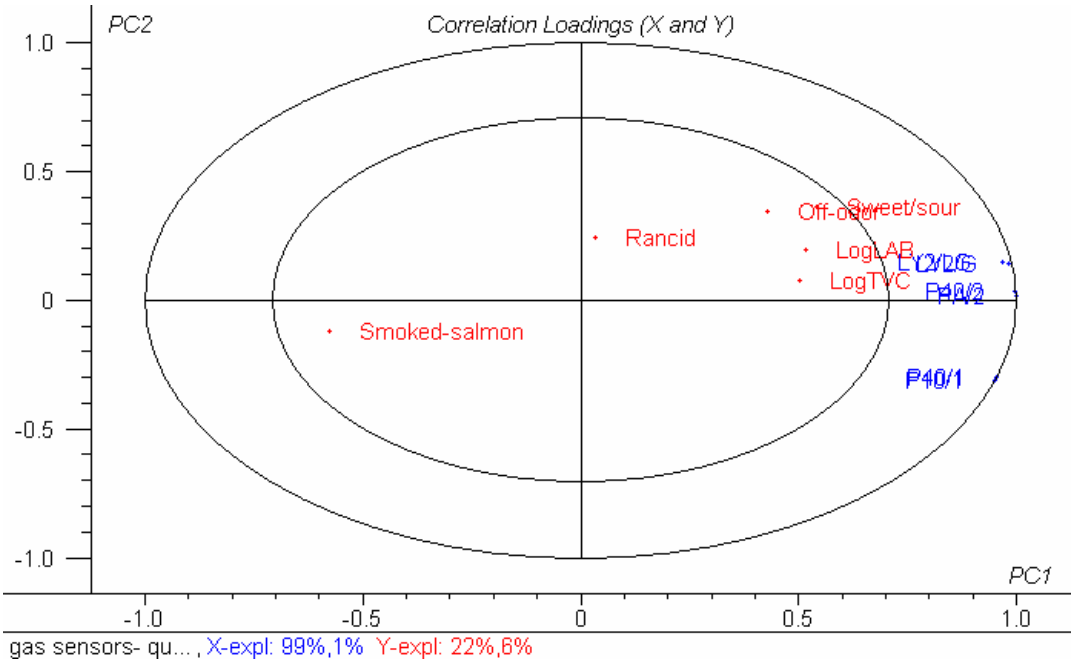


Figure 3

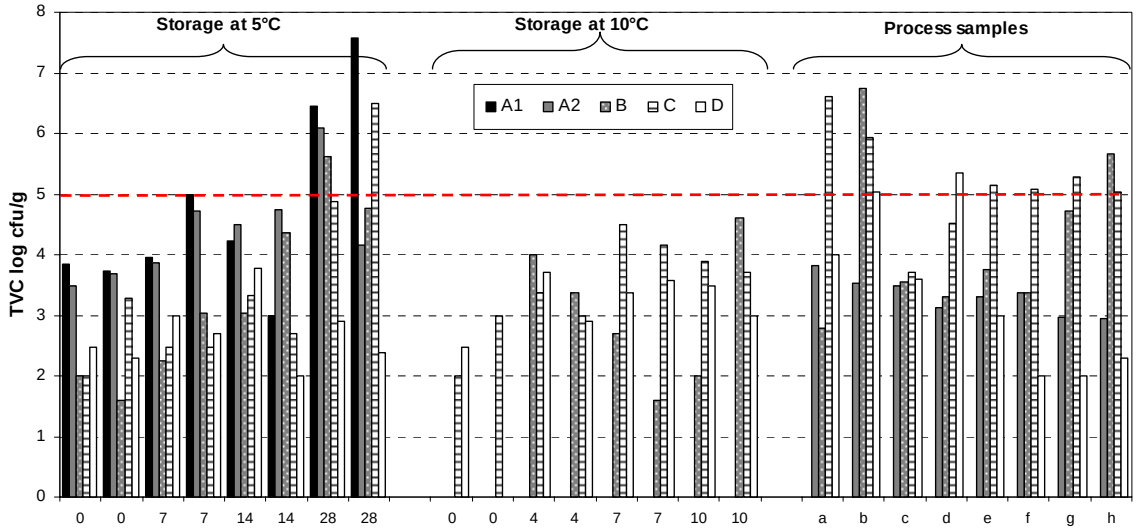


Figure 4

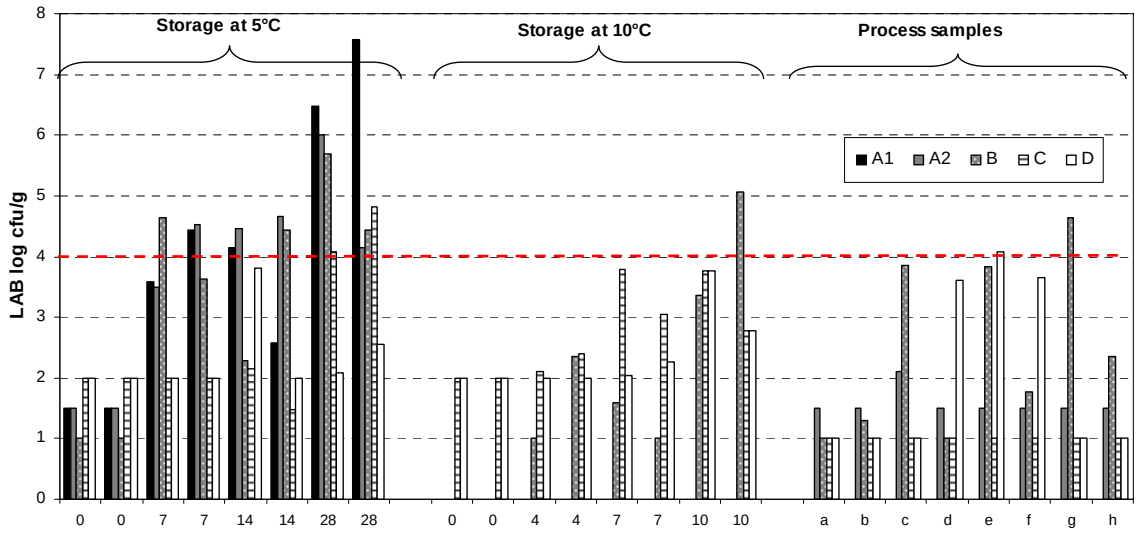


Figure 5

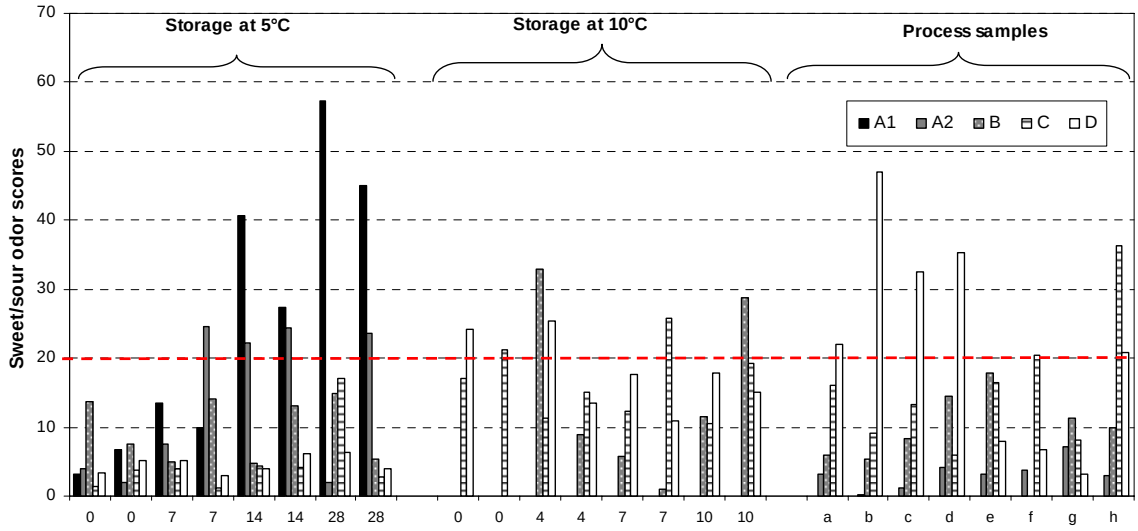


Figure 6

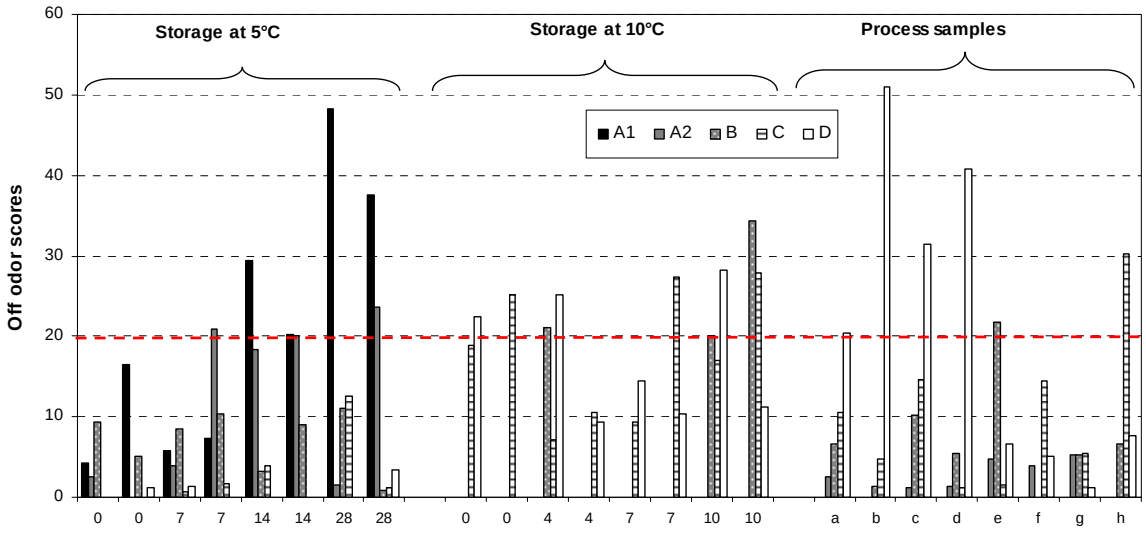


Figure 7

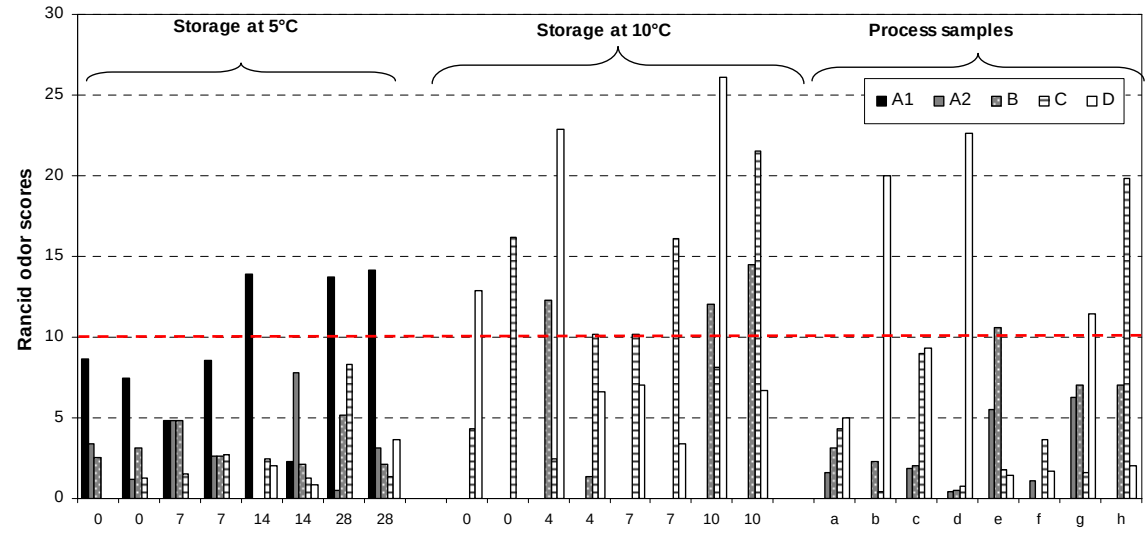


Figure 8

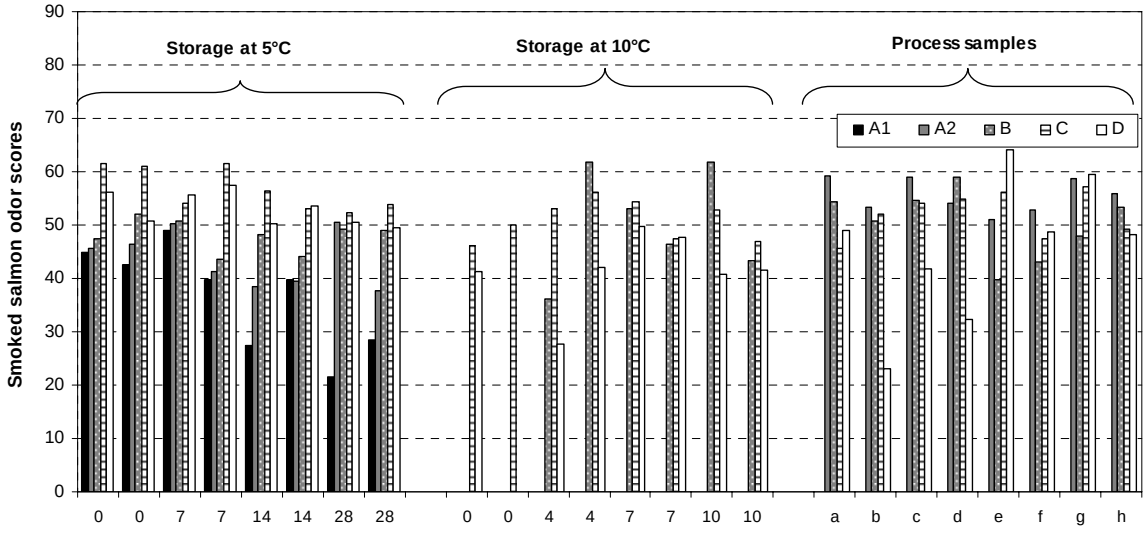


Figure 9

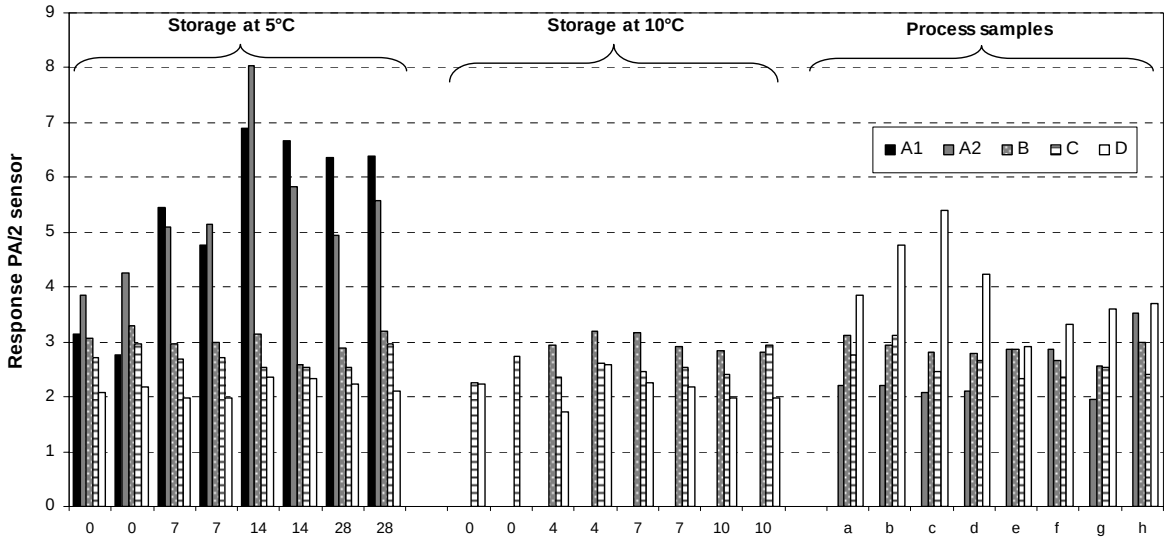

Storage at 5°C
Storage at 10°C
Process samples
A1
A2
B
C
D
Response PA/2 sensor
0
1
2
3
4
5
6
7
8
9
0 0 7 7 14 14 28 28
0 0 4 4 7 7 10 10
a b c d e f g h

Figure 10