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Electronic Nose to Monitor the Freshness of Red Fish (*Sebastes marinus*) Stored in Ice and Modified Atmosphere Packaging (MAP)

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ABSTRACT

An electronic nose called FreshSense was used as a rapid technique to monitor the freshness of red fish stored in ice and modified atmosphere packaging (MAP). Standard compounds were measured to study the characteristic response of the FreshSense sensors. Volatile compounds produced during storage of red fish were monitored and the results were analysed by multivariate analysis methods. The sensors showed good selectivity, sensitivity and repeatability to standard compounds that are representative of spoilage compounds in fish. The FreshSense could discriminate between standard compounds and their mixtures and was also able to discriminate between fresh samples and spoiled samples of red fish. The CO sensor increases earlier than the other sensors and is most likely responding to short chain alcohols and carbonyls formed during the storage of red fish. The NH_3 and H_2S sensors are sensitive to amines and sulphur compounds respectively formed in high concentrations at the end of the storage period. A slower spoilage rate was observed in MAP storage than in iced. The electronic nose measurements are generally in agreement with the results of sensory evaluation, but give detailed information about the spoilage pattern and the composition of the headspace of red fish.

Key words: Electronic nose; Red fish (*Sebastes marinus*); Freshness / Spoilage

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1. INTRODUCTION

Odour is one of the most important indicators of fish freshness. Traditionally, analysis of odour has been performed either by sensory panel or by gas chromatography which are time-consuming and costly. The electronic nose has proven to be a rapid, non-destructive technique for measuring volatile compounds which exhibit spoilage odours in fish (Ólafsdóttir et al. 1997a).
The advent of electronic noses has opened a variety of applications in the food industry, environmental management and medical diagnoses (Keller et al. 1996). Research focusing on fish has also been done, such as evaluation of fish freshness and monitoring of odour (Di Natale et al. 1998a, Ghosh et al. 1998).
Red fish (*Sebastes marinus*) is a commercially important marine fish species sold on markets. As the demand for high quality fresh red fish has increased in recent years, it is economically important to extend the shelf life of fresh red fish. Many studies have shown that cold storage and modified atmosphere packaging (MAP) of fish can meet this requirement (Dalgaard 1995, Huss 1995), however no work has been done to evaluate the quality of red fish stored in MAP using electronic nose.
The aims of conducting this project were to achieve a good understanding of the principal function of electronic noses, and to become familiar with the methodology involved in using electronic nose to evaluate the quality of seafood.
An electronic nose namely FreshSense developed in Iceland was used to assess the freshness of red fish (*Sebastes marinus*) stored in ice and MAP. The FreshSense is based on a closed semi-dynamic sampling system, an array of electrochemical gas sensors and a personal computer for data acquisition recognising and processing.
The project was divided into the following three parts:
1) A literature review report.
2) Standard compounds representing the main classes of spoilage volatile compounds were measured to: a) determine the sensitivity of the electronic nose, b) evaluate the instrument's ability to discriminate between compounds and mixtures, and c) determine the repeatability of the measurements.
3) Measurement of volatile compounds produced in red fish during storage in ice and MAP were done using the FreshSense instrument. This work was part of an EU funded demonstration project conducted at the Icelandic Fisheries Laboratories. The results of electronic nose measurements were analysed by principal component analysis (PCA) and partial least square regression (PLS-R) methods and compared with the results of sensory evaluation. The ability of the FreshSense to monitor the freshness of red fish stored in ice and MAP was evaluated.

2. LITERATURE REVIEW

2.1 Spoilage process of fish and the influence of packaging techniques

The initial quality loss of fresh fish is due to autolytic changes mainly related to the break down of nucleotides, while spoilage is due primarily to bacterial action (Huss 1995). The shelf life of fresh fish depends mainly on storage temperature and the atmosphere around the fish (Gram and Huss 1996).
Temperature changes have a great impact on microbiological growth and activity. Many bacteria are unable to grow at temperatures below 10°C (Huss 1995). When fish stored in ice aerobically, *Pseudomonas sp.* and *S. putrefaciens* have been identified as specific spoilage bacteria. These bacteria also played a dominant role in fish spoilage at higher temperature (i.e. 20°C), but other spoilage organisms particularly *Vibrionaceae* developed as well (Gram et al. 1987). The rate of spoilage is usually doubled or tripled for every 10°C increase in temperature of fresh fish

(http://www.distam.unimi.it). Taoukis et al. (1999) found that the shelf life of whole boque stored at 0°C and 10°C are 7 days and 2 days respectively.
MAP changes the microbial flora and spoilage patterns of fresh fish stored in ice (Dalgaard *et al.* 1993), and thus has the ability to extend the shelf life of the fish (Dhananjaya and Stroud 1994, Dalgaard 1995). As mentioned above, *Pseudomonas sp*., which produce aldehydes, ketones and esters and *S. putrefaciens*, the H_2S and TMA-producing bacteria, are responsible for the spoilage of fish stored under aerobic condition in ice (Lindsay et al. 1986). In contrast, CO_2-packaging has a specific inhibitory effect on *S. putrefaciens* and *Pseudomonas sp*. TMA-producing bacteria *P. phosphoreum* was identified as specific spoilage organism in CO_2-packaged fish (Dalgaard 1995). The spoilage pattern for vacuum packaged and iced fish is characterised by both *S. putrefaciens* and *P. phosphoreum*, so H_2S and TMA dominate the flavour of vacuum packaged fish (Jorgensen et al. 1988).
Many reports have documented the effects of packaging on the shelf life of meat, poultry and seafood (Dalgaard et al. 1993, Church 1998). Huss (1995) pointed out that vacuum and CO_2 packaging extend the shelf life of meat products by several weeks or months. In contrast, the shelf life of fresh fish is not affected by vacuum packaging and a small increase in shelf life depending on fish species can be obtained by CO_2 packaging. Dalgaard (1995) indicated that the shelf life for CO_2 packaged cod fish is 20 days, whereas the shelf life of iced cod is about 13-14 days. *Lindsay et al.* (1986) demonstrated that whitefish held in ice aerobically was putrid after 14 days, but the carbon dioxide packaged fish still had an acceptable aroma. Dhananjaya and Stroud (1994) also found a useful extended shelf life of haddock and herring packaged in MAP.

2.2 Methods of fish odour evaluation

During storage of fish the odour changes from fresh through flat, sweet and stale and ends as spoilage or putrid odour (Ólafsdóttir and Fleurence 1998). Research has shown that during each phase of storage different volatile compounds are present and characterise the odour. Fresh fish odour is mainly contributed by compounds that are oxidatively derived from long chain polyunsaturated fatty acids such as eicosapentaenoic acid 20:5ω3 (Josephson et al. 1984). These compounds have low odour thresholds and are present at low concentrations (ppb). Compounds that contribute to microbial spoilage odours of fish are well known. TMA, ethanol and hydrogen sulphide that result from microbial degradation of amino and fatty acids exhibit odour such as fishy, stale, rotten and putrid and are present in high concentrations (ppm) in the fish during storage (Gram and Huss 1996).
To date, sensory analysis is still the key technique in odour analysis. Quality Index Method (QIM) is frequently used for freshness evaluation of whole raw fish (Luten and Martinsdóttir 1997). QIM is based on significant sensory attributes using many weighted parameters and a score system from 0 to 3 demerit points. The scores for all characteristics of the fish are added to give an overall sensory score, giving zero score for very fresh fish and increasing scores as the fish deteriorates (Hylidig and Nielsen 1997). However, sensory analysis often provides mainly qualitative information. Panellists are subject to fatigue and low threshold concentrations of stale odour compounds may not be perceived (Josephson et al. 1986).
Gas chromatography has proven to be useful when measuring volatile compounds that are present in very low concentration such as those characterising fresh odour (Josephson et al.1986, Ólafsdóttir et al. 1997b). Some chemical measurements could also be classified as odour measurements when they measure one specific compound or a class of compounds that are important indicators of volatile substances. Examples are TVN (total volatile nitrogen) and TMA (trimethylamine) measurements (Ólafsdóttir et al. 1997c). Unfortunately, these methods are laboratory techniques that are complicated, destructive and time consuming, and sometimes give no information about the quality changes during the early storage of fish (Ólafsson et al. 1992, Rehbein et al. 1994).

In recent years, electronic noses have been introduced as an alternative rapid technique to supplement or replace traditional odour evaluation technique in food industries (Bartlett et al. 1997). An electronic nose is used to "sniff" the headspace of a product in a closed sampling system by transferring the headspace of the product to the sensor array. Low molecular weight volatile compounds that contribute to the spoilage odour and are present in high concentration in the headspace of fish can be analysed using an electronic nose (Egashira et al.1994, Ólafsdóttir and Fleurence 1998).

2.3 Development of electronic noses and applications

2.3.1 Electronic nose measurements

An electronic nose is an instrument which is comprised of an array of chemical sensors and matched with a suitable data processing method, capable of measuring and recognising volatile compounds which contribute to odours (Haugen and Kvaal 1998). The concept of electronic nose dates to 1982 and the first commercial instrument was introduced to the market in 1993 (Hurst 1999). Many types of chemical sensors are now available for use in electronic nose instruments. The response of a sensor is usually measured as the change of some physical parameters, e.g. conductivity or current. The responses of all sensors form a response pattern that can be learned by a computer (Holmberg et al. 1996).

Sampling is a critical step in electronic nose measurements. The goal of sampling is to collect the volatile compounds that represent the real condition of an analytical problem and to provide adequate concentration/amount of compounds to the sensors for detection. Static headspace sampling methods are commonly used in measuring low molecular weight volatile compounds with low boiling points such as hydrogen sulphide, dimethysulphide and amine (Ólafsdóttir et al. 1997d). They are simple and low-cost because the headspace is not transported to the measuring chamber and not diluted (Hurst 1999). More efficient dynamic headspace methods are necessary for collecting and concentrating less-volatile compounds such as those contributing to 'fresh fish' and 'oxidised' odours (Ólafsdóttir et al. 1997d).

Data analysis is an important issue in electronic nose measurements. Its role is to determine the relation between sensor output patterns and the properties of the samples being analysed (Di Natale et al. 1998a). Artificial neural networks (ANNs) and chemometric analysis such as principal component analysis (PCA) and partial least squares regression (PLS-R) are frequently used. ANNs that are based on a non-linear approach are powerful pattern-recognition techniques. Many ANN configurations and training algorithms have been used in electronic noses including back propagation-trained feed-forward networks and self-organising maps (SOMs). One of the main problems of an ANN approach is that the training typically becomes more difficult, and the class prediction less than satisfactory when the data sets become smaller (Singh et al.1996, Haugen and Kvall 1998). As a linear approach, 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. PLS which is based on known probability of variable distribution, is used as a prediction model. 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 sensor carries to the total information of the data set (Esbensen et al. 1996).

If an electronic nose is to operate for a long period of time, drift must be considered and instrument must be calibrated (Holmberg et al. 1996). As the details of drift are in general not known, no drift-compensating model can be made for chemical sensors. Standard compounds are usually used for calibration of the system (Ólafsdóttir et al. 1997c). A great deal of research work has been done to improve the selectivity, sensitivity and reproducibility of the gas sensors and the hardware and software in electronic noses (Haugen and Kvaal 1998).

2.3.2 Development of chemical sensors

The most frequently used sensors in electronic noses are metal oxide semiconductor sensors (MOS), conducting polymer sensors (CP), quartz microbalance (QMB), surface acoustic wave sensors (SAW), metal oxide field effect transistors (MOSFET) and electrochemical sensors (Bartlett et al. 1997, Haugen and Kvaal 1998).
MOS sensors are made from a metal-oxide film (e.g. tin oxide). The odorant molecules undergo a reaction on the film surface producing a conductivity change in the sensor. Heater within the sensors aids in the reaction process. The advantages of MOS sensors include low cost, longevity and electronic simplicity. The disadvantages are the necessity to operate at high temperatures (200-500°C), limited selectivity, high power requirements and modest sensitivity (Haugen and Kvaal 1998, Mielle 1996).
MOSFET sensors consist of a doped semiconductor and an insulator (oxide) covered by a catalytic metal. The output signal is based on a change of potential in the sensor due to electrical polarisation when molecules react on the catalytic surface. The sensors operate at temperatures between 100-200°C. The selectivity and sensitivity of this type of sensors is dependent on temperature and choice of metal (Haugen and Kvaal 1998).
CP sensor is a semi conducting polymer film coated to adsorb specific species of molecules. When the odorant molecules interact with the coating, the conductivity of the sensor changes. CP sensors have wide selectivity, high sensitivity, stability, and operate at ambient temperature. The drawback of CP sensors is a strong sensitivity to humidity (Mielle 1996).
QMB sensors are piezoelectric quartz crystals coated with selective coatings that adsorb molecular species. The adsorbed molecules increase the mass of the sensor changing its resonance frequency. By measuring this shift, the concentration of the odorant can be derived. The advantages of QMB sensors include high selectivity and sensitivity, stability over wide temperature ranges, low response to humidity, and good reproducibility. The disadvantage is the complexity in the interface electronics. SAW sensors consist of two pairs of finger structure electrodes fabricated onto a piezoelectric substrate with a sensing layer between them. The functions of SAWs are similar with that of QMB, but the former operate at much higher frequencies (50-100MHz) than the latter (5-30MHz) (Haugen and Kvaal 1998).
Electrochemical sensors, which consist of several electrodes and an electrolyte are not sensitive to the humidity (Hurst 1999). The gas molecules are either oxidised or reduced at the working electrode, while the opposite reaction takes place at the counter electrode. The reaction between the electrodes generates a voltage between the electrodes, which is measured as the output signal. This type of sensor is very sensitive to short chain alcohols that are produced during the spoilage of fish (Ólafsdóttir and Fleurence 1998).

2.3.3 Applications of electronic noses

The advent of the electronic nose has opened a variety of applications and new possibilities in many fields where the presence of odours is the relevant phenomenon (Mielle 1996, Keller et al. 1996, Di Natale et al.1998a).
The biggest market for the electronic nose is the food industry including quality monitoring or grading of food, beverage and fruits, inspection of food packaging materials, etc. Aishima (1991) used an array of six MOS sensors and a headspace concentrator to measure the aroma of eight different types of whisky, wine and beers and succeeded in classifying them by cluster analysis. Similarly, good classification of different coffee samples or blueberries could be obtained by using MOS sensors and dynamic sampling technique (Singh et al.1996). Chattonet and Dubourdieu (1999) pointed out that MOS sensors were capable of monitoring the quality and toasting homogeneity of oak wood used to make barrels for ageing wines and spirits. Jonsson et al.(1997) used an array of MOSFET sensors, a MOS sensor, and an IR-absorption based CO_2 sensor to classify oats as good, mouldy, weakly musty or strongly musty by pumping the headspace into the

test chamber. He could also determine the percentage of mouldy grains in barley and rye using the same sampling method. Di Natale et al. (1998b) showed good correlation between the results of QMB sensors under dynamic sampling conditions and the results of sensorial analysis for tomato paste and UNT milk.
Research focusing on fish has also been done using electronic noses for freshness monitoring and odour evaluation. Ólafsson et al. (1992) used an array of MOS sensors to monitor the freshness of haddock and cod stored in ice. The sensors exhibited enough sensitivity to monitor the changes in the headspace while the fish was deteriorating, however the static headspace sampling technique had to be refined. Schweizer-Berberich et al. (1994) used an array of eight amperometric sensors to monitor changes in odour of cold stored trout. The responses of the sensors correlated with storage time, but the sensors were not sensitive enough to detect long chain alcohols and carbonyls in the first period of deterioration. Hurst (1999) demonstrated that an array of twelve CP sensors could correlate the odour of salmon fillets with storage times when the moisture of the samples was the same. By pumping the headspace of cod fillets and using an array of metalloporhyrins-coated QMB sensors, small compounds such as alcohol, sulphur compounds, and trimethylamine in fish were detected successfully (Di Natale et al.1998a).
An electronic nose with electrochemical gas sensors has been developed in recent years for rapid detection of volatile compounds in seafood. Ólafsdóttir et al.(1997a, 1997b) used this kind of instrument and a static headspace sampling technique to monitor the volatile compounds such as alcohols, amines, and sulphur compounds in the headspace of capelin during spoilage. The instrument showed good sensitivity, selectivity and reproducibility, and correlated also well with classical TVB-N measurements. Another positive attribute was that the electronic nose did not respond to water. Similar results were obtained when measuring herring, whole or peeled shrimp and fresh roe (Ólafsdóttir et al.1997c, 2000, Högnadóttir 1999).
Electronic noses are found to be effective tools in environmental management (Keller et al. 1996). Applications include that analysis of fuel mixtures, detection of oil leaks, testing ground water for odours, and identification of household odours. Potential applications include identification of toxic wastes, air quality monitoring, and monitoring factory emissions (Mielle 1996).
Electronic noses have also been used in medical diagnosis. An electronic nose can examine odours from the body (e.g., breath, wounds, body fluids, etc.) and identify possible problems (Keller et al. 1996).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Standard compounds

The main classes of volatile compounds which characterise the spoilage odour of fish i.e. alcohols, aldehydes, amines and sulphur compounds were tested to estimate the sensitivity, selectivity and repeatability of the electronic nose. Ethanol and acetaldehyde were selected from alcohols and aldehydes respectively. Trimethylamine (TMA) represented amines and dimethyldisulfide (DMDS) was selected from the sulphur group.
Standard ethanol(1-100ppm), TMA(10-300ppm), DMDS(0.5-5ppm) and acetaldehyde (1-100ppm) in aqueous solutions were prepared in various concentrations. Mixtures of these standards in concentrations strong enough to show a significant response were also measured three times each. In addition, several concentrations of DMDS (5-50ppm) in oil (paraffinum liquidum) solutions were measured to compare the characteristic response of the electronic nose to DMDS in water.

3.1.2 Red fish

Fresh whole red fish (*Sebastes marinus*) was bought in the market two days after being caught, and stored in ice immediately to keep the temperature inside the fish at 0°C. Then a sample of 160 red fish was stored in ice in 8 boxes (20 fish/box). The boxes were put into a container and sealed. A mixture of gases ($60\%CO_2+40\%N_2$) was injected into the container and the modified atmosphere (MAP) storage environment for the fish was built up. Another sample of 100 red fish was put directly into ice and stored in cold storage at 0-2°C. Both samples were stored for 22 days.
Table 1 shows the overall schedule of the storage experiment. On day 5, 33 samples were taken from iced storage to be further stored in MAP for 9 and 15 days and analysed later as ice5/MAP9 and ice5/MAP15. Meanwhile the same number of samples were taken from MAP and stored in ice for additional 9, 14 and 17 days and labelled as MAP5/ice9, MAP5/ice14 and MAP5/ice17. Other 33 samples were taken from MAP on day 14 and further stored in ice for 8 days and measured as MAP14/ice8. On day 19, 33 samples were taken from MAP to be stored further in ice for 3 days and analysed as MAP19/ice3.
Samples were analysed on days 0, 5, 14, 19, 20 and 22 by sensory evaluation, microbial and chemical analysis and by the electronic nose. The same samples were analysed by sensory evaluation and the electronic nose. The results of the electronic nose measurements were compared with the results of sensory evaluation (QI scores).
As the day 0 samples were missing for electronic nose measurement, we selected 10 red fish samples from another catch and measured instead as ice 0 and MAP 0.

Table 1: Schedule of red fish storage experiment

Sampling dates	**Storage condition**
16. Nov. (day 0)	Ice0, MAP0
21. Nov. (day 5)	Ice5, MAP5
30. Nov. (day14)	Ice14, MAP14, Map5/Ice9, Ice5/MAP9
5. Dec. (day19)	MAP5/Ice14, MAP14/Ice5
6. Dec. (day20)	Ice20, MAP20, Ice5/MAP15
8. Dec. (day22)	MAP5/Ice17, MAP14/Ice8, MAP19/Ice3

3.2 Methods

3.2.1 Electronic nose measurement

Measurements were performed using the FreshSense developed by the Icelandic Fisheries Laboratories (IFL) and Bodvaki — Element Sensor Systems (Figure 1).

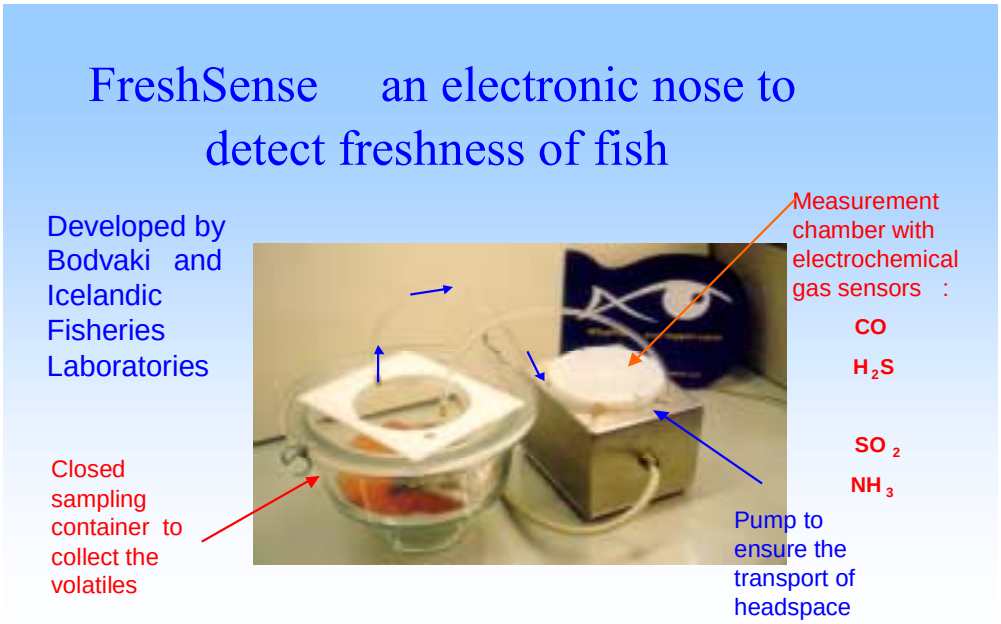


Figure 1: The electronic nose (FreshSense) used to measure red fish.

The FreshSense is based on a semi-dynamic headspace system that was reported earlier by Ólafsdóttir et al. (1997a) but slightly modified. The instrument consists of a glass container (2.3 l) closed with a plastic lid, a stainless steel sensor box (dimension: 16 × 12 × 10 cm) which can either be fastened directly to the lid or used in a separate measurement chamber. The sensor box contains four electrochemical gas sensors: CO, H_2S, SO_2 (Dräger Germany) and NH_3 (City Technology, Portsmouth, Britain) and a temperature sensor (PT100). A pump in the box is used to transfer volatiles from the headspace (see Figure 1). An A/D converter and a microprocessor to read the measurements and send them to a PC computer that runs the Labview measurement program are also in the box.
The 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
one minute measurements minus the initial value that is the average of 6 values before the measurement starts.
Standard compounds measurement: 25 ml aliquots of four to five concentrations of each standard or their mixtures were pipetted into a Petri dish (diameter 8.8 cm), and placed into the sampling container and closed with the plastic lid. Measurements were performed in triplicate.
Red fish measurement: The red fish samples taken from ice storage (0-2°C) were allowed to reach 5-7°C before measuring. Three whole fishes 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.

3.2.2 Sensory analysis

Sensory analysis was performed according to the Quality Index Method (QIM) by 11 trained panellists at the Icelandic Fisheries Laboratory. The QIM scheme for red fish as described by Martinsdóttir (1995) was used.

3.3 Data analysis

Microsoft Excel 97 was used as the main method for data analysis in this study. The data was also analysed by multivariate methods.
The Unscramber (CAMO A/S), a multivariate analysis program (Esbensen et al. 1996), was used to conduct principal component analysis (PCA) to: a) study the main tendencies of the data set being

measured, b) discriminate between standard compounds and their mixtures, and c) discriminate between fresh and spoiled red fish samples stored in ice and MAP. In all PCA runs two principal components and full cross validation were used and the data was standardised with 1/*Sdev* (Esbensen et al. 1996)

Partial least square regression (PLS-R) was conducted to evaluate the ability of the electronic nose to predict Quality Index (QI) scores. Data from all electronic nose measurements was used and red fish samples comprised of various storage conditions were analysed by PLS-R.

4. RESULTS

4.1 Characteristic response of the FreshSense sensors to standard compounds

4.1.1 Sensitivity

The sensitivity of the FreshSense sensors was tested for different classes of compounds. Figure 2 shows the responses of the sensors to various concentrations of ethanol, TMA, DMDS and acetaldehyde in aqueous solutions. Each solution was measured three times.

As expected, the sensors show different responses to the volatile compounds tested. The CO sensor is the only sensor responding to ethanol, while TMA is only detected by the NH_3 sensor. The SO_2 sensor is only sensitive to DMDS. The results also show that both the CO and H_2S sensors respond to acetaldehyde and all the sensors respond to DMDS.

There is a strong linear relationship between the responses of the sensors and the concentrations (Figure 2). The sensitivity (nA/ppm) was obtained by calculating the slope of the regression line. The results show that the sensitivity of the FreshSense sensors to each compound is different (Table 2). The CO sensor is more sensitive to acetaldehyde than to ethanol. All the sensors are more sensitive to DMDS aqueous solution than any other compounds and DMDS in oil. DMDS is not soluble in water and is therefore present in much higher concentrations above the aqueous phase than the oil phase.

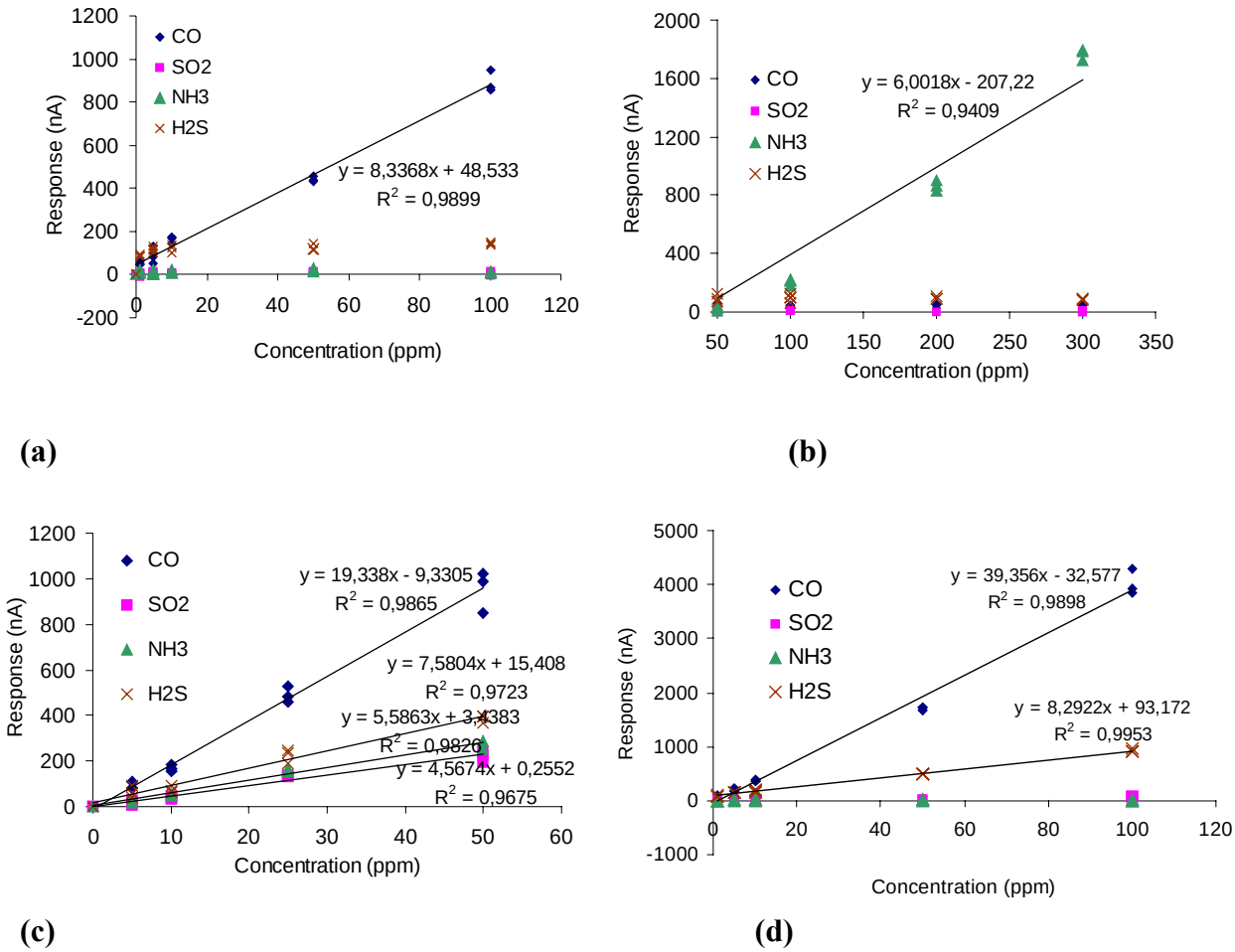


Figure 2: Responses of the FreshSense sensors to the aqueous solutions of ethanol, TMA, DMDS and acetaldehyde at different concentrations: (a) ethanol (1, 5, 10, 50, 100 ppm); (b) TMA (50, 100, 200, 300 ppm); (c) DMDS (5, 10, 25, 50 ppm); (d) acetaldehyde (1, 5, 10, 50, 100 ppm).

Table 2: Sensitivity (nA/ppm) of the FreshSense sensors obtained from three replicate measurements of each concentration between 1 and 300ppm.

	Sensitivity of the sensors (nA/ppm)			
Compounds	**CO**	**H_2S**	**SO_2**	**NH_3**
ethanol	8.3	0	0	0
acetaldehyde	39.3	8.3	0	0
TMA	0	0	0	6.0
DMDS	164.5	96.2	42.0	52.6
DMDS(in oil)	19.3	7.7	5.6	4.6

4.1.2 Repeatability

Generally, the precision of the electronic nose is studied by measuring the repeatability and reproducibility. 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. As the time for this study was limited, the measurement of reproducibility was not conducted.

The repeatability of the measurements was evaluated by calculating the coefficient of variability CV = (standard deviation / mean)*100 when measuring each concentration of the four standard compounds in triplicate. Figure 3 shows the response of the CO sensor to three different aqueous solutions of ethanol during repeated measurement. The results relevant to the repeatability of the sensors are shown in Table 3.

The repeatability is good (Figure 3 and Table 3). The curves generally overlap for each concentration of ethanol (Figure 3) and the CV in most situations is less than 6% except for the DMDS solutions which has slightly higher CV (Table 3), possibly because of the insolubility of DMDS in water. Although the solubility of DMDS in oil is relatively high, it still has high CV for the H_2S sensor for repeated measurements of low concentration of DMDS in oil. The main reason for this is that it is difficult to get homogenous samples because of the thickness of the oil.

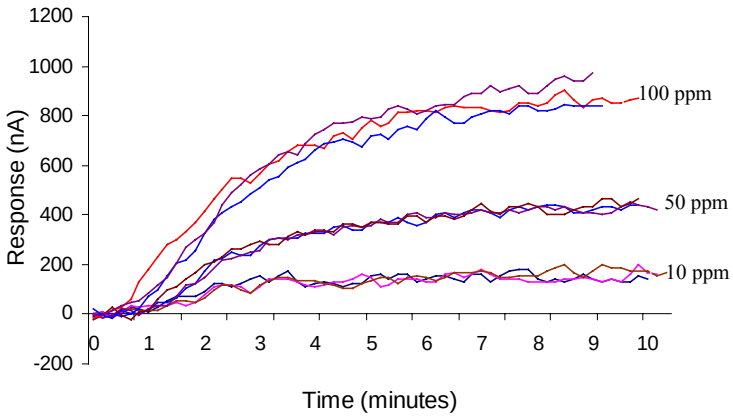


Figure 3: Response of the CO sensor to three different concentrations (10, 50, 100 ppm) of aqueous ethanol solutions during repeated measurement.

Table 3: Repeatability of the FreshSense sensors to standard compounds.

Compounds	Concentration (ppm)	Sensor	Average response (nA)	SD	CV%	n
Ethanol	10	CO	491.7	16.0	3.3	3
	50	CO	1325.8	14.1	1.1	3
	100	CO	2672.0	48.7	1.8	3
Acetaldehyde	10	CO	363.9	16.7	4.6	3
	50	CO	1703.3	21.8	1.3	3
	100	CO	4015.9	239.8	5.9	3
TMA	100	NH_3	217.0	5.9	2.8	3
	200	NH_3	867.3	38.5	4.4	3
	300	NH_3	1767.0	40.5	2.3	3
DMDS	0.5	H_2S	168.0	11.2	6.6	3
(in water)	1	H_2S	211.7	26.5	12.5	3
	5	H_2S	574.2	13.6	2.4	3
DMDS	5	H_2S	63.3	26.7	42.1	3
(in oil)	10	H_2S	74.6	13.8	18.4	3
	50	H_2S	387.3	16.4	4.2	3

4.1.3 Selectivity

Figures 4 and 5 show the responses of the FreshSense sensors to aqueous solutions of standard compounds, their mixtures as well as water. The results show that when a sensor responds to both standards in a mixture, its response to the mixture is higher than to either one alone. For example, the responses of the CO sensor to ethanol and acetaldehyde in the mixture of ethanol + TMA + acetaldehyde (E + T + A) aqueous solution were nearly the sum of the response of both the compounds measured individually (Figure 5). The responses of the CO sensor to ethanol and DMDS in the mixture of ethanol + TMA + DMDS (E + T + D) in aqueous solution are also additive (Figure 4). The NH_3, SO_2 and H_2S sensors also showed good selectivity to their specific class of compounds. The NH_3 sensor gave similar response values to TMA both by itself and in the mixture of E + T+ A or E + T + D. Only DMDS influenced the response of the SO_2 sensor in the mixture of E + T + D. None of the sensors were sensitive to water.

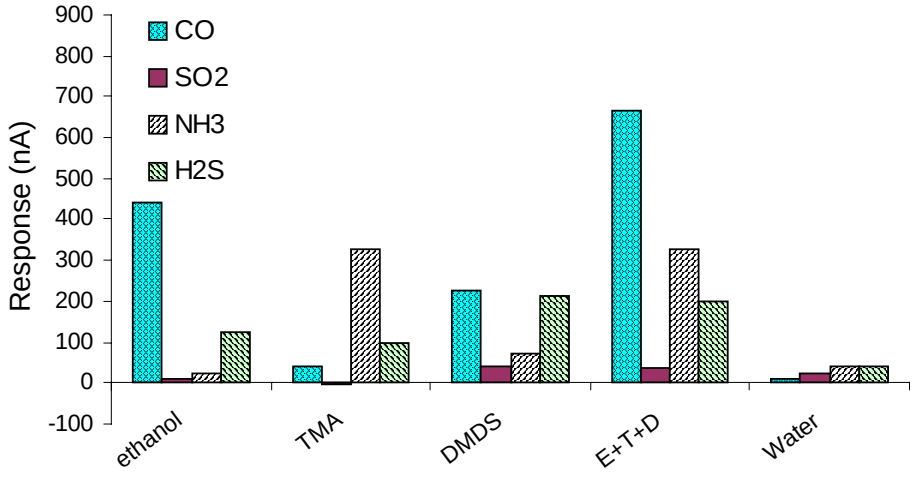


Figure 4: Selectivity of the FreshSense sensors to aqueous solutions of ethanol (50ppm), TMA (100ppm), DMDS (1ppm), and E+T+D (50+100+1ppm).

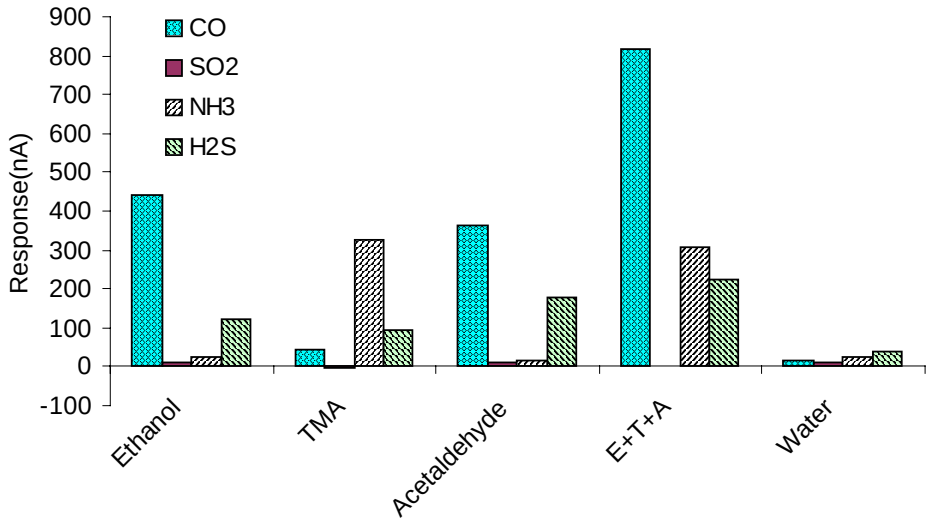


Figure 5: Selectivity of the FreshSense sensors to aqueous solutions of ethanol (50ppm), TMA (100ppm), acetaldehyde (10ppm), and E+T+A (50+100+10 ppm).

4.1.4 Discrimination study

It is necessary to understand the ability of the FreshSense sensors to discriminate both similar and dissimilar classes of chemicals. A PCA was performed from data of repeated measurements of aqueous solutions of ethanol, TMA, DMDS and acetaldehyde and their mixtures. Figure 6 shows the PCA scores of all the samples. The discrimination among the four compounds and their mixtures is good since all replicates of samples form groups on the PCA plot and are separated from each other (Figure 6).

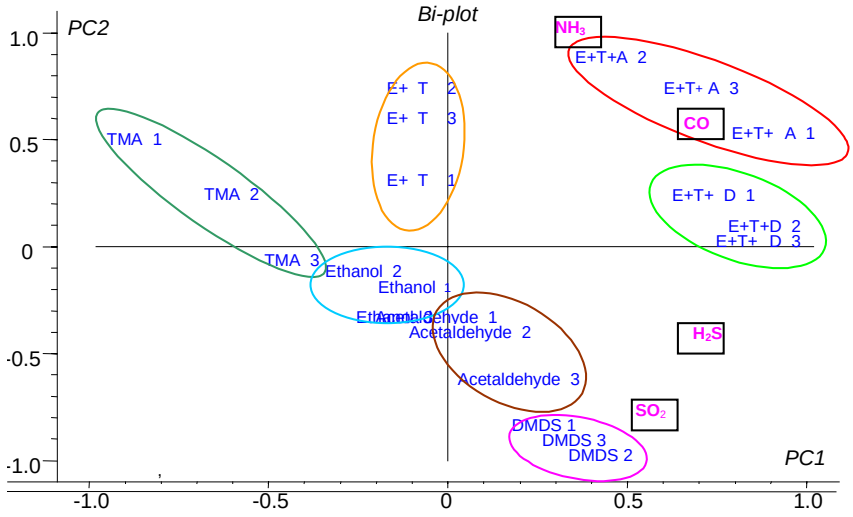


Figure 6: PCA plot of triplicate measurements of standard compounds and their mixtures. Sample scores are labelled with names: ethanol, TMA , acetaldehyde, DMDS, E+T, E+T+A and E+T+D. Loadings of sensors(CO, NH_3, SO_2 and H_2S) are shown in boxes. PC1 and PC2 explain 46% and 33% respectively of the variance in the data.

Sensor loadings were also superimposed on the scores plot to determine which sensors were more sensitive to particular compounds. Those sensors are the most sensitive to the compounds that are closest to them on the scores/loading plot (bi-plot) (Figure 6). It can be seen that the four sensors gave different loadings to the samples, and was near the samples that they were most sensitive (Figure 6). This indicates that the sensors have good selectivity to the standard compounds being measured. The NH_3 sensor had highest loading on PC2 and was closer to E+T+A than to E+T and TMA (Figure 6), since the NH_3 sensor responds to both TMA and acetaldehyde in E+T+A. The SO_2 sensor had the lowest loading on PC2 and was close to DMDS that it responds only. The CO sensor had the high loading on PC1 and was most sensitive to E + T + A and E + T+ D, because both ethanol and acetaldehyde in E+T+A and ethanol and DMDS in E+T+D were responded to by the CO sensor. Similar result was obtained as the CO sensor, the H_2S sensor also had high loading on PC1 and was near acetaldehyde and E + T + D that are sensitive to it.

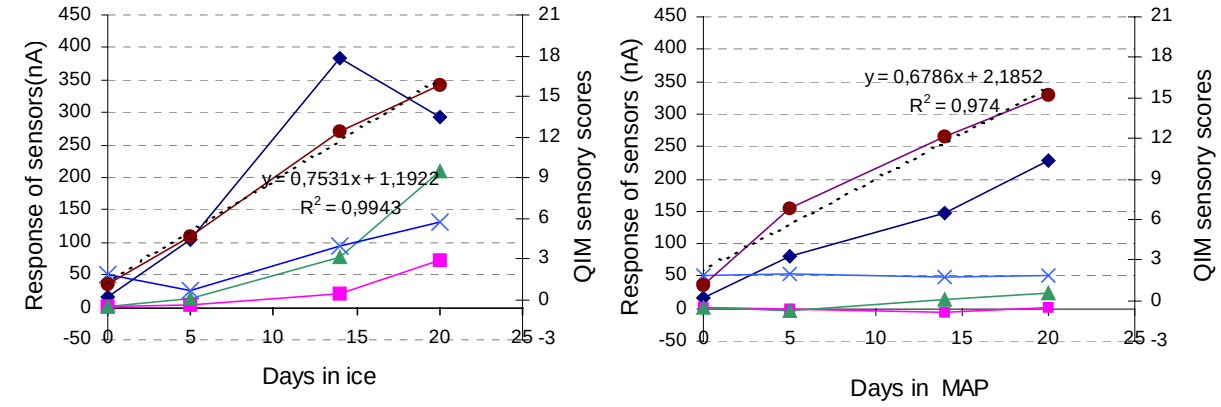
4.2 Red fish storage experiment

4.2.1 Red fish stored in ice

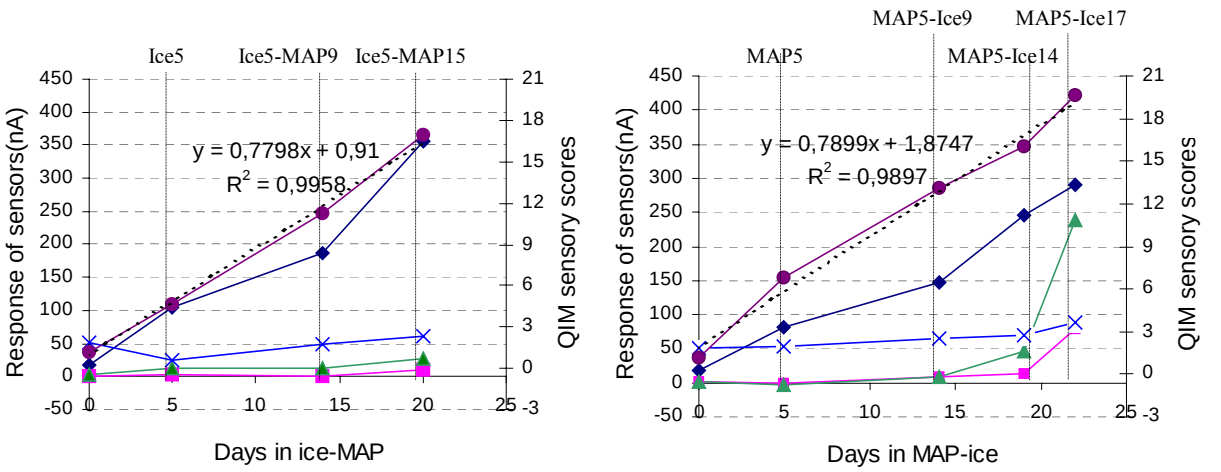
Figure 7a shows the responses of the FreshSense sensors and the QI scores of red fish stored in ice. The CO sensor has the highest response and the response increases from the beginning but appears to decline after 14 days of storage. The responses of the NH_3 and SO_2 sensors start to increase after 5 days of storage and increase markedly after day 14. During the first 5 days of storage, the response of the H_2S sensor decreased a little, but increased linearly after day 5 and reached 130 nA on the last day.
The QIM scores show a linear increase with time in ice stored fish throughout the storage time (Figure 7a), but the sensors do not correlate linearly with the storage time.

4.2.2 Red fish stored in MAP

The overall trend of the sensors' response and QI scores of red fish stored in MAP is shown in Figure 7b.
The CO sensor shows a similar response trend as in the ice stored fish during the first 14 days of storage, but the response is much lower in MAP than in ice. The response of the CO sensor after day 14 continued to increase. The increase in response of the NH_3 sensor occurred after 5 days of storage but the values were much lower compared to those of iced fish. For example, the responses of the NH_3 sensor to ice and MAP stored fish on day 20 are 210 nA and 24 nA respectively (Figure 7a and b). The responses of the H_2S and SO_2 sensors show no change during the 20 days of storage in MAP (Figure 7b).



(a) **(b)**



(c) **(d)**

Figure 7: Electronic nose (FreshSense) measurements and QIM scores of red fish stored for 22 days in: (a) Ice; (b) MAP; (c) Ice-MAP; (d) MAP-Ice (◆CO, ■ SO_2, ▲ NH_3, ✕ H_2S, ● QIM, ----- QIM Linear), different storage conditions are label on top in (c) and (d).

QI scores show linear relationship with storage time for fish stored in MAP and in ice. However the spoilage rate is slightly slower in MAP stored fish, as can be seen by the slope of the lines (0.68 and 0.75 respectively) (Figures 7a and b). Under this storage condition (Figure 7b), the CO sensor has

the best linear correlation to storage time (R^2= 0.986). The NH_3 sensor also shows good linear correlation to storage time (R^2 = 0.844).

4.2.3 Red fish stored in ice-MAP

One group of samples was stored in ice only for the first 5 days, and then stored in MAP and sampled after 9 and 15 days. The results of electronic nose measurements and QI scores for this storage condition are shown in Figure 7c.
Although the CO sensor showed increasing response during storage, the response on day 14 was lower (154 nA) than that of fish stored continuously in ice (384 nA) (Figures 7a and c). On the other hand, the response of the CO sensor on day 20 was higher (356 nA) than for fish stored continuously in MAP (227 nA) (Figures 7b and c). The NH_3, SO_2 and H_2S sensors showed low responses.
Under this storage condition (Figure 7c), the QI scores also increased linearly with storage time. The CO and NH_3 sensors show good linear correlation with storage time (R^2 =0.951 and 0.854 respectively).

4.2.4 Red fish stored in MAP-ice

Figure 7d shows the results of the electronic nose measurements and QI scores of red fish stored in MAP for 5 days and then stored further in ice.
The results also show that the response tendency of the sensors depends on the specific storage conditions. The response pattern of the sensors was similar to ice stored fish after the fish was stored in ice, but significant increase in responses showed a lag phase compared with iced fish (Fig. 7a and d). The response of the CO sensor increased quickly after day 14 and slow down after day 19. Considerable increase in response of the NH_3 and SO_2 sensors occurred after day 20. The response of the CO sensor under MAP-ice condition was lower than ice stored fish, but was higher than fish stored continuously in MAP after day 14 (Figure 7a and b).
QI scores in Figure 7d also show significant linear correlation with storage time, and the spoilage rate appears to be slightly higher than for other storage conditions. The CO sensor shows good linear correlation with storage time (R^2 =0.974).

4.2.5 PCA and PLS—R analysis

Figure 8 shows PCA plot of all the data from electronic nose measurements and QI scores of red fish stored under various conditions and the loading of the sensors.

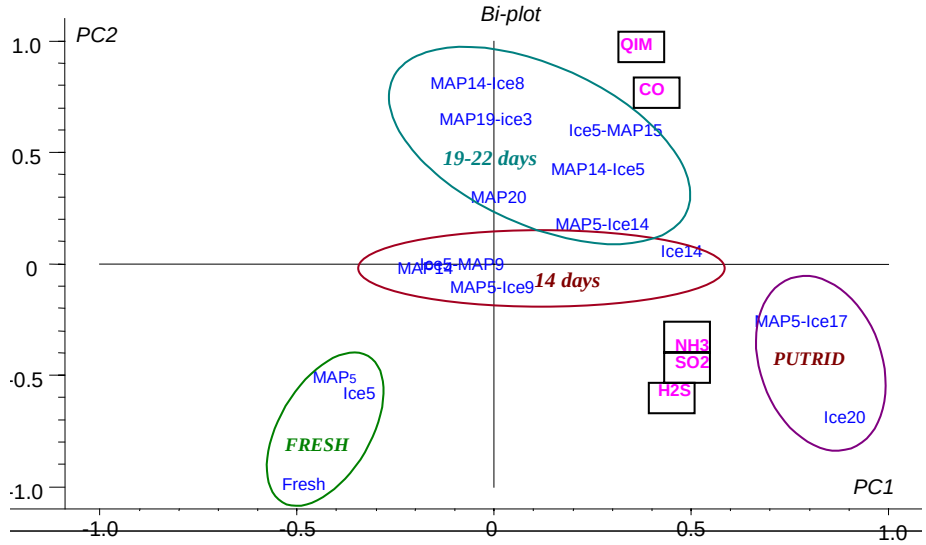


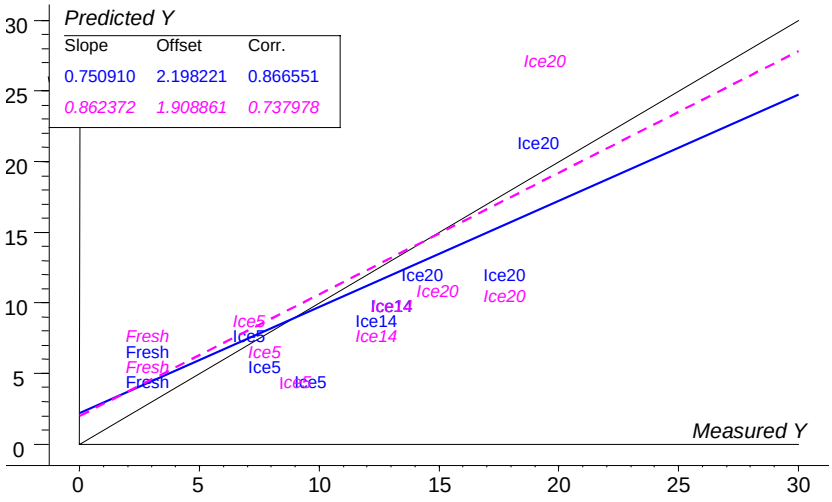
Figure 8: PCA bi-plot of the FreshSense measurements and QIM data from experiment on red fish stored in ice, MAP, ice-MAP and MAP-ice. Sample scores are labelled with the storage condition. Loadings of sensors(CO, NH_3, SO_2 and H_2S) and QIM are shown in boxes. PC1 and PC2 explain 66% and 24% respectively of the variance in the data.

Figure 8 shows that the first principle component (PC1) x-axis explains 66% of the variance in the data set. It can also be seen that PC1 represents the spoilage of the samples. As storage time increases from left to right along PC1, the odour of the red fish 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 are low. As storage time increases, the onset of spoilage of most the samples was related to the response of the CO sensor especially the samples ice5-MAP15, ice14 and MAP14-ice5. Some samples such as ice20 and MAP5-ice17 were represented by the other three sensors which characterise putrid odour.

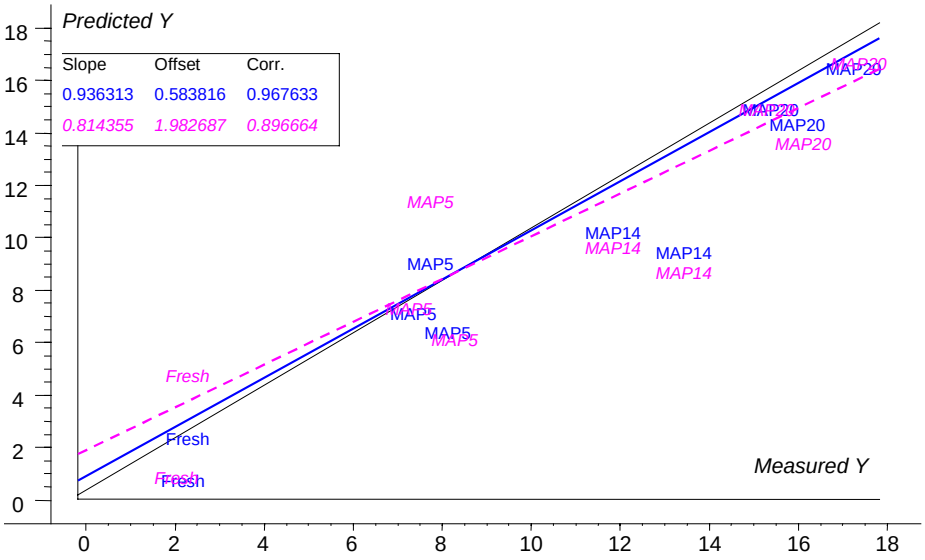
Similar to the CO sensor, QIM shows high loading on PC2 and close to the CO sensor (Figure 8). This demonstrates that the CO sensor gives similar information as QI scores. The fresh samples are far from QIM because they have low QI scores. On the other hand, the putrid samples are also far from QIM, therefore additional information about the onset of putridity of the sample 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 MAP are similar, but 9 days later the ice 14 and MAP 14 samples are very different (Figure 8) indicating that MAP is efficient in slowing down the spoilage rate. Sample ice 20 showed the highest production of putrid volatile compounds.

PLS-R analysis was done to predict the QI scores using electronic nose measurements. Results show that models made with all data from all of the storage conditions were not satisfactory (not shown here), but the models from data of only ice and only MAP storage gave satisfactory prediction of QI scores (Figure 9). It can be seen that the calibration (prediction) models have high correlation (0.87 and 0.96 respectively) under ice and MAP storage conditions, and also have reasonable validation in both storage conditions. The correlations of the validation models are 0.74 and 0.90 respectively (Figure 9). According to the values of the Root Mean Square Error (RMSEP) of the calibration (prediction) models (4.72 and 2.29 respectively), the results of future predictions

for QI scores can then be presented as: Predicted values ± 4.27 for ice stored fish and Predicted values ± 2.29 for MAP stored fish.



(a)



(b)

Figure 9: PLS-R results of the FreshSense data to predict QI scores for fish stored in: (a) ice and (b) MAP. X-axis and Y-axis are the measured and predicted QI scores, respectively. — Calibration (prediction), ---- Validation.

5. DISCUSSION

5.1 Standard compound experiment

Measurements of standard compounds in aqueous solutions show that the electrochemical gas sensors have different selectivity, sensitivity, and repeatability and can discriminate between four standard compounds that are representative of spoilage compounds in fish (TMA, ethanol and acetaldehyde, DMDS) and their mixtures. This is in agreement with previous work at IFL (Högnadottir 1999).
The intensity of the responses of the sensors is highly correlated with the vapour pressure and the concentration of individual compounds in the headspace. The higher the concentration of the standard, the higher the responses of sensors, and response curves (response vs. concentrations) are linear for the various standard compounds measured (Figure 2). The sensitivity of the CO sensor to acetaldehyde is higher than to ethanol at same concentrations. This is because acetaldehyde has lower boiling point than ethanol and thus has higher vapour pressure in the headspace. By comparing the responses of the sensors to DMDS in water and oil (Table 2), it is clear that water solubility is another factor affecting the response of sensor. The more solubility in water, the less will evaporate from the aqueous solution.
The lowest concentration the sensors can measure depends on the nature of the compounds and the respective sensors (Figure 2). The CO sensor responds to 1 ppm ethanol or acetaldehyde solutions and most sensors respond to 0.5ppm DMDS solutions. The concentration of TMA must be 100 ppm for the NH_3 sensor to respond. The results obtained here are complementary to previous work done at the IFL (Högnadottir 1999).
None of the sensors in the FreshSense are sensitive to humidity (Figures 4 and 5) which is in agreement with previous research and publications (Ólafsdóttir et al. 1997c, Högnadottir 1999). This insensitivity to humidity is an advantage when comparing with other type of sensors responsive to water vapour, such as conducting polymer sensors and metal oxides semiconductor sensors (Haugen and Kvaal 1998). The FreshSense can then be used accurately to measure samples of high water content.
The results have proven that the sensors show good repeatability under these experimental conditions, as the CV in repeated measurements of the same sample is less than 6% in most situations except for the DMDS solution. However the evaluation of long-term drift and calibration procedures of the sensors were not conducted due to the time limits.
All sensors responded to DMDS, which is important since the sensors are generally selective only toward certain groups of compounds. DMDS could then be used in a single measurement to ascertain that all the sensors are working. However the reproducibility (Högnadottir 1999) and the repeatability obtained here of the sensors to DMDS in water and oil vary enormously, so it is not practical for calibration purposes unless another suitable solvent for DMDS is found.
During the experiments we also observed that the FreshSense is sensitive to the environment especially the CO sensor, and it takes a long time for the sensors to recover after responding to contaminated environment or high concentrations of standard compounds. Moreover, the temperature of the samples also affects the volatility of compounds in the sample and hence influences the response of the sensors (see Appendix).

5.2 Red fish storage experiment

The CO sensor showed the highest response of the sensors for all the samples stored under various conditions. This is in agreement with the results of Lindsay et al. (1986) that ethanol is produced in air, vacuum or carbon dioxide packaged fish.
During the first 5 days of storage, the responses of all the sensors were low and showed no big difference in response patterns for red fish stored in various conditions. Red fish was characterised as fresh during this period, and was similarly described as freshly caught fish by sensory evaluation.

The response patterns of the sensors changed with storage conditions after day 5. According to Figure 7a and b there is some difference in sensors' responses between iced and MAP stored fish during 5-14 days. All the sensors showed higher responses to iced fish than MAP stored fish, but the headspace of red fish stored in both conditions was still composed mainly of alcohol compounds during this period. A significant difference in response patterns in ice and MAP stored fish occurred after day 14. A considerable increase in the responses of the NH_3, SO_2 and H_2S sensors was noticed in ice stored fish during this period, but no increase in responses of those sensors was detected in MAP stored fish. The overall responses of all the sensors in MAP stored fish were much lower than ice stored fish. The changes in response patterns of the sensors can be explained by the growth of micro-organisms and the production of microbial metabolites reported earlier (Lindsay 1986, Dalgaard 1995).

Lower concentration of volatiles was observed at the end of storage in ice-MAP condition (Figure 7c) compared with MAP-ice condition (Figure 7d). A possible explanation to this is that the microbial flora in ice is much more active in producing volatile spoilage compounds and MAP has been shown to inhibit the growth of some spoilage bacteria. Therefore the MAP-ice samples appeared more spoiled at the end of the storage period (Dalgaard 1995). But changing storage method during storage time has no advantage compared with ice or MAP storage conditions.

According to sensory evaluation, rancidity and bitterness descriptors appeared around day 14 and the end of shelf life was 19 days for ice stored fish, while MAP stored fish had not reached the end of shelf life on day 20. It appeared that the increase in responses of the NH_3, SO_2 and H_2S sensors were at the same time as the end of shelf life was reached for ice stored fish, and no compounds contributing to putrid odour were developed for MAP stored fish at the end of experiment (22 days).

When data from electronic nose measurements for all storage conditions was analysed by PCA, a clear tendency of the changes in freshness of red fish with storage time were obtained (Figure 8), and the influences of all the sensors to the odour patterns of red fish were also known. Based on the results of the electronic nose measurements, the spoilage patterns are believed to differ more than QIM indicates.

In order to predict the QI scores using electronic nose data for red fish stored in ice and MAP, two PLS-R models had to be made to obtain satisfactory results. This means that different spoilage patterns existed in iced and MAP stored fish, and thus different QIM schemes may be needed for MAP stored fish.

QI scores correlated linearly with storage time, but showed no big difference between different storage conditions. The FreshSense sensors showed different response patterns and response intensities for red fish stored in different storage conditions.

6. CONCLUSIONS

The FreshSense sensors showed good selectivity, sensitivity and repeatability when measuring standard compounds (TMA, ethanol, acetaldehyde and DMDS) that are representative of spoilage compounds in fish and the sensors could discriminate between those compounds and their mixtures. The results indicate that the FreshSense sensors could be used efficiently to measure volatile compounds that contribute to the spoilage odour in fish.

The FreshSense sensors have the ability to monitor freshness and the onset of spoilage of red fish stored under various conditions. The CO sensor appeared to increase earlier than the other sensors and was most likely responding to short chain alcohols (i.e. ethanol) and aldehyde that form during storage. The responses of the NH_3 and SO_2 sensors increased at later stages of storage. These sensors are sensitive to amines and sulphur compounds respectively, that typically form in high concentrations at the end of the storage life.

The freshness of red fish depends on storage time and storage conditions. Slower spoilage rate reflected by lower intensities of sensors' response was observed in MAP stored red fish compared with other storage conditions (ice, ice-MAP and MAP-ice).
The FreshSense measurements are generally in agreement with the results of sensory evaluation (QI scores). The FreshSense sensors give detailed information about the spoilage pattern and the composition of the headspace of red fish.
Different QIM schemes should be created for MAP stored fish.
Further analysis of the data of the electronic nose and comparison with microbial analysis may give more information about different spoilage patterns in red fish stored in ice and MAP.
The electronic nose measurements show that careful control of the environment and monitoring of the temperature of the sample are needed during measurement. It is also important that the samples measured have the same surface area.
The FreshSense is promising for application in the food industry, where rapid measurements and no sample preparation are required, and where chemicals and laboratory facilities are not available.

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APPENDIX I: RESPONSES OF THE FRESHSENSE SENSORS TO AQUEOUS SOLUTIONS OF STANDARD COMPOUNDS.

Standard	Concentrations (ppm)	Sensors			
		CO	H_2S	NH_3	SO_2
Ethanol	1	50.9	0	9.0	81.4
Ethanol	1	64.4	-6.8	20.3	92.7
Ethanol	1	47.5	6.8	9.0	90.4
Ethanol	5	128.9	-2.3	9.0	28.9
Ethanol	5	84.8	15.8	2.3	122.1
Ethanol	5	101.7	0	22.6	115.3
Ethanol	10	169.5	9.0	11.3	124.3
Ethanol	10	176.3	4.5	11.3	101.7
Ethanol	10	145.8	4.5	18.1	135.6
Ethanol	50	437.4	9.0	24.9	142.4
Ethanol	50	430.6	9.0	29.4	110.8
Ethanol	50	457.8	13.6	15.8	119.8
Ethanol	100	871.5	-4.5	13.6	151.5
Ethanol	100	854.5	13.6	6.8	135.6
Ethanol	100	946.1	4.5	9.0	142.4
TAM	10	44.1	9.0	6.8	90.4
TAM	10	57.6	2.3	24.9	70.1
TAM	10	20.4	6.8	27.1	85.9
TAM	50	17.0	11.3	27.1	70.1
TAM	50	61.0	2.3	29.4	124.3
TAM	50	17.0	4.5	43.0	88.2
TAM	100	47.5	-15.8	214.8	65.6
TAM	100	44.1	-4.5	223.8	101.7
TAM	100	30.5	9.0	212.5	124.3
TAM	200	27.1	-4.5	827.4	85.9
TAM	200	40.7	2.3	870.3	106.3
TAM	200	57.7	-9.0	904.2	90.4
TAM	300	-3.4	0	1720.3	85.9
TAM	300	30.5	-2.3	1788.1	79.1
TAM	300	44.1	4.5	1792.6	90.4
DMDS	5	78.0	27.1	40.7	56.5
DMDS	5	81.4	6.8	38.4	40.7
DMDS	5	108.5	24.9	15.8	92.7
DMDS	10	186.5	45.2	45.2	90.4
DMDS	10	152.9	36.2	49.7	65.6
DMDS	10	166.2	31.7	56.5	67.8
DMDS	25	525.6	133.4	156.0	232.8
DMDS	25	457.8	133.4	156.0	248.7
DMDS	25	484.9	135.6	165.0	189.9
DMDS	50	851.1	196.7	255.4	395.6
DMDS	50	1020.7	239.6	289.4	397.9
DMDS	50	986.7	226.1	284.8	368.5
Acetaldehyde	1	111.9	11.3	-11.3	81.4
Acetaldehyde	1	54.3	20.4	4.5	67.8
Acetaldehyde	1	78.0	4.5	-6.8	108.5
Acetaldehyde	5	189.9	0	22.6	156.0
Acetaldehyde	5	240.8	9.1	-2.3	146.9
Acetaldehyde	5	186.5	-6.8	13.6	160.5
Acetaldehyde	10	383.2	2.3	20.3	165.0
Acetaldehyde	10	356.0	6.8	22.6	180.8
Acetaldehyde	10	352.7	18.1	2.3	192.2
Acetaldehyde	50	1678.5	27.1	15.8	490.5
Acetaldehyde	50	1712.4	29.4	-4.5	506.5
Acetaldehyde	50	1719.2	29.4	31.7	488.3
Acetaldehyde	100	3916.4	58.8	22.6	972.0
Acetaldehyde	100	3841.8	67.8	-11.3	906.5
Acetaldehyde	100	4289.4	58.8	2.3	904.2

APPENDIX II: RESPONSES OF THE FRESHSENSE SENSORS TO THE MIXTURES OF STANDARD AQUEOUS SOLUTIONS

Standard	Concentrations (ppm)	Response of sensors (nA)			
		CO	H_2S	NH_3	SO_2
Ethanol +TMA	10-50	152.6	6.8	18.1	85.9
Ethanol +TMA	10-50	101.7	2.3	67.8	76.7
Ethanol +TMA	10-50	125.5	9.0	43.0	156.0
Ethanol +TMA	50-100	400.1	0	300.7	142.4
Ethanol +TMA	50-100	434.0	11.3	366.2	92.7
Ethanol +TMA	50-100	413.7	4.5	316.5	117.6
Ethanol +TMA	100-200	718.9	15.8	863.5	101.7
Ethanol +TMA	100-200	742.6	27.1	958.5	108.5
Ethanol +TMA	100-200	739.2	15.8	1003.7	106.3
Ethanol+TMA+acetaldehyde	50+100+10	769.7	4.5	309.7	253.2
Ethanol+TMA+acetaldehyde	50+100+10	847.7	15.8	321.0	223.8
Ethanol+TMA+acetaldehyde	50+100+10	847.7	-2.3	300.7	221.5
Ethanol+TMA+DMDS	50+100+1	654.4	36.2	348.1	176.3
Ethanol+TMA+DMDS	50+100+1	681.6	33.9	321.0	214.8
Ethanol+TMA+DMDS	50+100+1	657.8	38.4	316.5	203.5

APPENDIX III: RESPONSES OF THE FRESHSENSE SENSORS TO ETHANOL AQUEOUS SOLUTIONS AT DIFFERENT TEMPERATURES

Standard	Temperature (°C)	Concentrations (ppm)	Response of sensors (nA)			
			CO	H_2S	NH_3	SO_2
Ethanol	4	50	247.5	13.6	4.5	54.3
Ethanol	4	50	271.3	9.0	-13.6	18.1
Ethanol	4	50	244.1	9.0	6.8	49.7
Ethanol	13	50	312.0	29.4	-13.6	94.9
Ethanol	13	50	322.1	9.0	6.8	49.7
Ethanol	13	50	291.6	-11.3	2.3	56.5
Ethanol	21	50	328.9	4.5	9.0	85.9
Ethanol	21	50	383.2	-4.5	0	135.6
Ethanol	21	50	373.0	6.8	2.3	74.6

APPENDIX IV: RESPONSES OF THE FRESHSENSE SENSORS TO RED FISH STORED IN ICE AND MAP

Samples	Weight (g)	Response of sensors (nA)			
		CO	H_2S	NH_3	SO_2
Fresh 124	561.0	10.2	-6.8	-9.1	67.8
Fresh 735	566.0	27.1	13.6	9.0	65.6
Fresh 644	568.0	13.6	-4.5	6.8	20.3
Ice5 741	415.9	54.3	6.8	11.3	20.3
Ice5 789	686.7	203.5	2.3	18.1	49.7
Ice5 258	738.0	54.3	2.3	9.1	56.5
MAP5 357	952.9	88.2	9.0	-4.5	54.3
MAP5 951	661.5	101.7	4.5	-4.5	54.3
MAP5 620	676.8	54.3	-15.8	2.3	54.3
Ice14 489	475.7	335.7	13.6	49.7	108.5
Ice14 156	366.6	447.6	29.4	126.6	106.3
Ice14 756	386.9	369.6	18.1	54.3	67.8
MAP14 557	550.2	115.3	-6.8	2.3	54.3
MAP14 807	696.1	210.3	2.3	22.6	63.3
MAP14 320	419.7	118.7	-9.0	13.8	31.7
MAP5-Ice9 287	617.9	217.0	18.1	11.3	65.6
MAP5-Ice9 480	406.3	149.2	-6.8	11.3	52.0
MAP5-Ice9 609	469.7	78.0	13.6	0	74.6
Ice5-MAP9 085	607.3	156.0	0	6.8	43.0
Ice5-MAP9 914	541.3	315.4	13.6	27.1	63.3
Ice5-MAP9 101	718.9	91.6	-9.0	6.8	43.0
MAP5-Ice14 442	528.8	200.1	29.4	67.8	76.9
MAP5-Ice14 207	576.3	257.7	2.3	43.0	63.3
MAP5-Ice14 710	401.8	281.4	11.3	24.9	70.1
MAP14-Ice5 654	604.6	328.9	15.8	9.1	52.0
MAP14-Ice5 573	481.5	213.6	15.8	4.5	76.9
MAP14-Ice5 195	797.3	420.5	9.1	24.9	67.8
Ice20 127	565.1	183.1	61.0	131.1	110.8
Ice20 437	631.7	393.3	124.3	393.3	185.4
Ice20 070	695.3	301.8	31.7	108.5	97.2
MAP20 590	583.7	328.9	2.3	20.4	56.5
MAP20 677	512.9	237.4	0	27.1	49.7
MAP20 201	385.7	115.3	4.5	24.9	45.2
Ice5-MAP15 603	494.8	437.4	6.8	27.1	88.2
Ice5-MAP15 882	399.7	440.8	18.1	22.6	45.2
Ice5-MAP15 739	418.1	189.9	6.8	29.4	52.0
MAP5-Ice17 050	434.4	278.1	49.7	196.7	65.6
MAP5-Ice17 498	479.0	325.5	110.8	201.2	117.6
MAP5-Ice17 331	474.0	264.5	74.6	316.5	79.1
MAP14-Ice8 873	510.5	203.5	-2.3	24.9	33.9
MAP14-Ice8 057	497.2	318.7	-2.3	13.6	4.5
MAP14-Ice8 949	425.5	257.7	-15.8	29.4	20.4
MAP19-ice3 795	381.7	223.8	6.8	20.4	33.9
MAP19-ice3 211	406.4	220.4	-4.5	9.1	9.0
MAP19-ice3 294	469.4	186.5	-4.5	2.3	49.7