PROJECT REPORT
10 - 02

Rannsóknastofnun fiskiðnaðarins

JUNE 2002

MULTISENSOR FOR FISH

STORAGE STUDIES OF FROZEN HAKE IN MADRID AND FRESH COD IN HAMBURG

ELECTRONIC NOSE, TEXTURE AND SENSORY ANALYSIS

FAIR CT – 98 - 4076

THIRD INDIVIDUAL PROGRESS REPORT

DECEMBER 2000 - NOVEMBER 2001
DECEMBER 2001 - APRIL 2002
Extension period

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Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins

Icelandic Fisheries Laboratories Report Summary

Titill / Title	**Multisensor for Fish** - Storage studies of frozen hake in Madrid 2000 and fresh cod in Hamburg 2002 - Texture, electronic nose (FreshSense) and sensory analysis
Höfundar / Authors	*Guðrún Ólafsdóttir, Soffía Vala Tryggvadóttir og Rósa Jónsdóttir*
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Ágrip á íslensku:

Þessi skýrsla er þriðja áfangaskýrsla Rf í Evrópuverkefninu ***Þróun á margþátta skynjaratækni til að meta gæði fisks***. Markmið verkefnisins er að prófa og bera saman ýmsa tækni, sem getur nýst í fjölskynjara til að mæla ferskleika fisks. Sex Evrópulönd taka þátt í verkefninu en allir þátttakendurnir vinna að þróun nýrrar tækni til ferskleikamælinga. Niðurstöður áferðarmælinga, rafnefsmælinga og skynmats (QIM) frá tveimur vinnufundum eru kynntar og bornar saman við niðurstöður frá fyrri vinnufundum. Gerðar voru samtímis mælingar með ýmsum aðferðum í geymslutilraunum á frosnum lýsingi í Madrid 2000 og ferskum þorski í Hamborg 2002.
Niðurstöðum skynmats, áferðar og rafnefsmælinga ber vel saman þegar notaðar eru sömu aðstæður við sýnatöku og mælingar, þrátt fyrir mismunandi uppruna, árstíð og meðhöndlun. Ósamræmi í gögnum er aðallega vegna þess að tækin voru í þróun og sýnatökuaðstæður og mælingum var breytt lítilsháttar á tímabilinu til að auka næmni aðferðanna.

Niðurstöður áferðamælinga sýna að hægt er að greina breytingar sem verða á fyrstu 3-4 dögum geymslu sem tengjast dauðastirðnum, en litlar breytingar verða á áferð við frekari geymslu í ís. Áferðamælingar gætu nýst í fjölskynjara til að gefa upplýsingar um breytingar á ástandi fisks á fyrstu dögum geymslu þegar aðrar aðferðir hafa litla næmni. Áferðamælingar nýtast einnig til að greina hvort fiskur hafi verið frystur.

Samanburður á rafnefsgögnum fyrir þorsk frá þremur vinnufundunum sýndu að helstu breytingar sem verða á rokgjörnum efnum við geymslu í ís voru sambærilegar. Rafnefið getur greint þegar skemmdareinkenni koma í ljós í þorski geymdum í ís og hægt var að aðgreina sýni eftir skemmd. Rafnefið nýtist ekki til að nema breytingar sem verða við geymslu í frysti.

Í verkefninu gafst einstakt tækifæri til að mæla samtímis með mismunandi tækni sama fisk úr geymslutilraunum á þorski og lýsing.

Niðurstöðurnar nýtast til að gefa tækjaframleiðendum ráðleggingar varðandi frekari þróun á framleiðslutækjum til notkunar við gæðamat í fiskiðnaði. Notagildi gagna, byggð á mælingum með tækjum í spámódel til að ákvarða gæði, eru háð því að mælingar séu alltaf gerðar á sama hátt.

Betri skilgreining á skemmdareinkennum og breytingum sem verða við geymslu fæst þegar margar aðferðir eru notaðar til að fylgjast með breytingum á sýnum. Á þessu byggist hugmynd verkefnisins um fjölskynjara, að engin ein mæling getur sagt til um þær flóknu breytingar sem verða á fiski við geymslu í ís. Gerð verður grein fyrir samantekt á gagnaúrvinnslu fyrir verkefnið í heild af þátttakanda 6.

Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins

Icelandic Fisheries Laboratories Report Summary

Lykilorð á íslensku: *fiskur, gæði, ferskleiki, skemmd, áferð, rafnef, skynmat, spálíkön*

Summary in English:

This report is the third annual progress report of IFL for the EU project ***Multisensor for fish*** (CT98-4076). Six European countries are participating in the project focusing on the development of new measurement techniques and a multisensor instrument to evaluate fish freshness.

Data on texture and electronic nose measurements from two work-ins are presented and comparison of data from earlier work-ins. Simultaneous measurements were done during a storage study of frozen hake in Madrid 2000 and fresh cod in Hamburg 2002 using various instrumental techniques and sensory analysis using QIM.

The results show that sensory analysis, texture and electronic nose data is consistent between work-ins when the same sampling conditions are used, irrespective of origin, season and handling.

The inconsistency in the data is mainly attributed to differences in sampling, since the instruments used in the project have been developed and improvements and modifications made on them.

The texture results show that non destructive texture measurements on fresh fillets can detect distinctive changes in firmness in the first 3-4 days due to *rigor mortis*, but with extended storage time on ice, hardly any textural changes can be identified. Texture measurements can give information in a multisensor especially in the first days when other methods which are only sensitive to signs of spoilage do not show any response at all. Texture measurements can also detect whether the fish has been frozen.

Comparison of electronic nose data from three different work-ins on cod showed that the overall trend of the changes which occur during storage on ice are similar and the electronic nose appears to be able to detect when signs of spoilage appear. Samples stored on ice can be discriminated based on their level of spoilage. The electronic nose does not appear to be useful to detect changes which occur during frozen storage.

The opportunity to do experiments on important commercial fish species in various, different countries, measuring the same fish with the same instruments, is unique. The resulting data is useful to give directions and recommendations to manufactureres of instruments for further developments of the techniques and to evaluate the validity of the data which is used in models to predict quality. The benefits of using combined data from different experiments is dependent on that sampling conditions are always the same.

The characterisation of samples is improved when more techniques are used for the evaluation. Models based on more than one instrumental technique are likely to give better performance to predict quality. The multi-sensor approach is the aim of the project and data analysis using the combined techniques will be presented in the reports of partners 6 and 7.

English keywords: *fish, quality, freshness, spoilage, texture, electronic nose, QIM ,prediction*

MUSTEC
MUltiSensor TEChniques for monitoring the quality of fish

FAIR CT-98-4076

Freshness is the most important attribute of the quality of fish. Freshness is a complex concept but can be estimated as a combination of several sensory attributes such as appearance, smell, taste and texture. Sensory evaluation using a well-defined scheme such as the Quality Index Method (QIM) and a trained panel can give reliable quantitative evaluation of freshness. However, such panels are expensive, training is critical and the panel is not always accessible. Consequently, to satisfy the need for quality measurements in the fish industry, instrumental methods are needed.
The objective of the MUSTEC project is to enable rapid measurements of freshness. The approach is to use different techniques to mimic the human senses. Mathematical data fusion combines the outputs of multiple physical sensors. Calibrating with the QIM enables to construct an Artificial Quality Index (AQI).

The MUSTEC project uses the following techniques:

Texture: **Instruments compress the body of the fish to measure its firmness and elasticity. This varies before and during rigor mortis and for several days (months) of chilled (frozen) storage.**

Volatile compounds: **Odour is strongly associated with freshness. Electronic noses capture fish volatiles allowing recognition of fish freshness.**

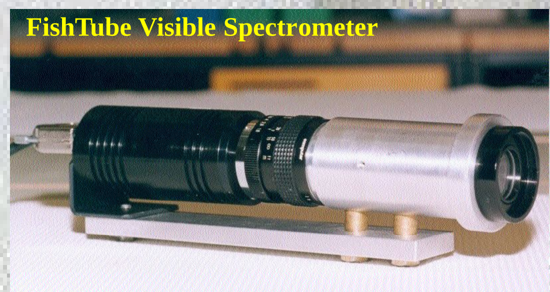
Electrical impedance: **This quantity depends on the disruption of fish muscle by autolytic spoilage and correlates very well with freshness of mechanically undamaged and unfrozen fish. There are commercial instruments on the market.**

Optical methods (colour, spectral and image analyses): **Colour and visible transmission spectra change with fish freshness. The appearance of the mucus on the skin and the coarseness of protein fibres on the cut surface of fish fillets, quantified by image analysis, also correlates with freshness.correlates with freshness.**

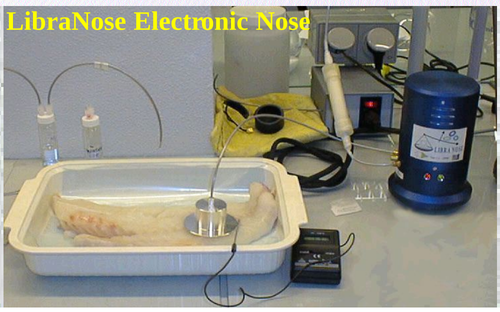

Texture analyser


Colorimeter


Electrical Impedance


FishTube Visible Spectrometer


Sensory Panel at work


LibraNose Electronic Nose


FreshSense Electronic Nose


Image Analysis

THE ARTIFICIAL QUALITY INDEX

The Artificial Quality Index (AQI) is a number characterising the freshness of fish by combining the outputs of physical sensors. The results of the MUSTEC project show that instrumental measurements can be calibrated to be as good as those of trained sensory panel. The calibration enables the valuable skill of the sensory panel to be transferred to the physical multi-sensing system.

This allows rapid and potentially inexpensive measurements of the freshness of fish. The added benefit of the system is that measurements could be made on-line and at locations not accessible to a sensory panel.

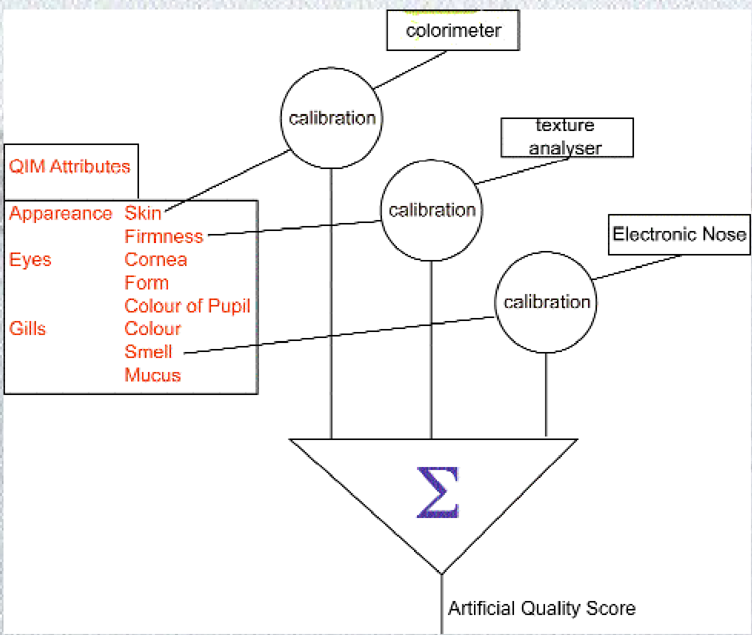


Figure 1: Construction of the Artificial Quality Index (AQI). After calibration with sensory data (the QIM score) the instrumental readings are combined into the AQI.

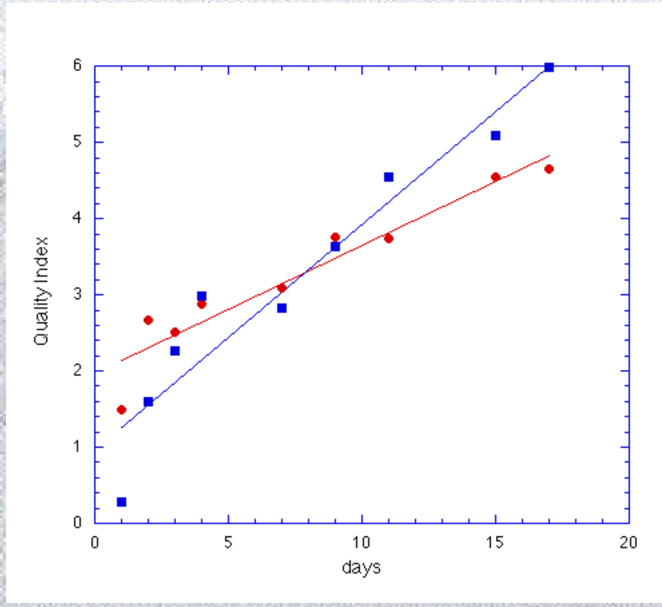


Figure 2. This shows the dependence of the QIM scores (data denoted by blue squares) and the AQI score (red dots) on the chilled storage time. For a given AQI score the storage time can be estimated with an uncertainty of ± 0.5 days. This is close to the reliability of the QIM.

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FAIR- CT98-4076

Development of multi-sensor techniques for monitoring the quality of fish

Specific RTD and D Programme on Agricultural and Fisheries - FAIR (1994-1998)

Third individual progress report
December 2000 to November 2001 - 3rd year
December 2001 to April 2002 - Extension period

Multisensor for fish
Storage studies of frozen hake in Madrid 2000 and
fresh cod in Hamburg 2002
Texture, electronic nose (FreshSense) and sensory analysis

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Summary

This report is the third annual progress report of IFL for the EU project ***Multisensor for fish*** (CT98-4076). Six European countries are participating in the project focusing on the development of new measurement techniques and a multisensor instrument to evaluate fish freshness.

Data on texture and electronic nose measurements from two work-ins are presented and comparison of data from earlier work-ins. Simultaneous measurements were done during a storage study of frozen hake in Madrid 2000 and fresh cod in Hamburg 2002 using various instrumental techniques and sensory analysis using QIM.
The results show that sensory analysis, texture and electronic nose data is consistent between work-ins when the same sampling conditions are used, irrespective of origin, season and handling. The inconsistency in the data is mainly attributed to differences in sampling because the instruments have been under development in the project and improvements and modifications have been made.

The texture results show that non destructive texture measurements on fresh fillets can detect distinctive firmness changes due to *rigor mortis* the first 3-4 days but with extended storage in ice there are hardly any textural changes identified. Texture measurements can give information in a multisensor especially the first days when other methods that are only sensitive to spoilage signs do not show any response. Texture measurements can also detect whether fish has been frozen.

Comparison of electronic nose data from three different work-ins on cod showed that the overall trend of the changes occuring during storage in ice are similar and the electronic nose appears to be able to detect when spoilage signs appear. Samples stored in ice can be discriminated based on their spoilage level. The electronic nose is not to useful to detect changes during frozen storage.

The opportunity to do experiments on important commercial fish species in different countries with the same instruments is unique. The resulting data is useful to give directions and recommendations for further developments of the instrumental techniques and to evaluate the validity of the data to use in models to predict quality.
The benefit of using combined data from different experiments is dependent on that sampling conditions are always the same. The characterisation of samples is improved when more techniques are used for the evaluation. Models based on more than one instrumental technique are likely to give better performance to predict quality. The multi-sensor approach is the aim of the project and data analysis using the combined techniques will be presented in the reports of partners 6 and 7.

Key words: fish, quality, freshness, spoilage, texture, electronic nose, QIM ,prediction

Table of Contents

1. Objectives

The main objective of the project is to devise a multi-sensor method for rapid monitoring of fish quality. This involves the following sub-objectives:

- To ascertain the needs of the fish industry for quality determinations of fresh and frozen fish.
- To consult with industry and the regulatory authorities on the type of measuring instruments needed.
- To evaluate the ability of simultaneous measurements of several physical properties of fish to determine and monitor the quality of fish
- To contribute to the formulation of the multi-sensor device
- To disseminate the results of the project and pursue the exploitation of the instruments

The role of IFL in the project is to integrate electronic nose and texture methods into the multi-sensor frame. IFL has also studied other methodologies in the project such as the RT Freshmeter, a rapid technique for TMA and TVB analysis based on flow injection gas diffusion anlysis and the Quality Index Method.

2. Actions in the project

Table 1.Timetable of tasks for Partner 2 (Icelandic Fisheries Laboratory)

Sub-task	*1st year*	*2nd year*	*3rd year*
1.1	Identify critical points for quality measurements.		
1.2	Determine requirements of the industry for quality measurements		Determine scenarios for use of multi-sensor device
2.1	Prepare sensors		
2.2		Simultaneous measurements	Simultaneous measurements
2.3		Data analysis and fusion	Data analysis and fusion
3.1		Recommend exploitation route	Recommend exploitation route
3.2		Formulate industrial device	Formulate industrial device
4.1	Dissemination.	Dissemination	Dialogue with manufacturers

3. Planned Research Activities

Sub-task 1.1 Identifying at which Critical Points (CPs) along the fish processing / distribution chain measurements of fish quality are needed.

To fulfill Task 1.1 a flow chart is introduced in this report to give details of the processing and distribution chain and structure of the retail trade for the main fish species (cod) in Iceland (Figure 1).

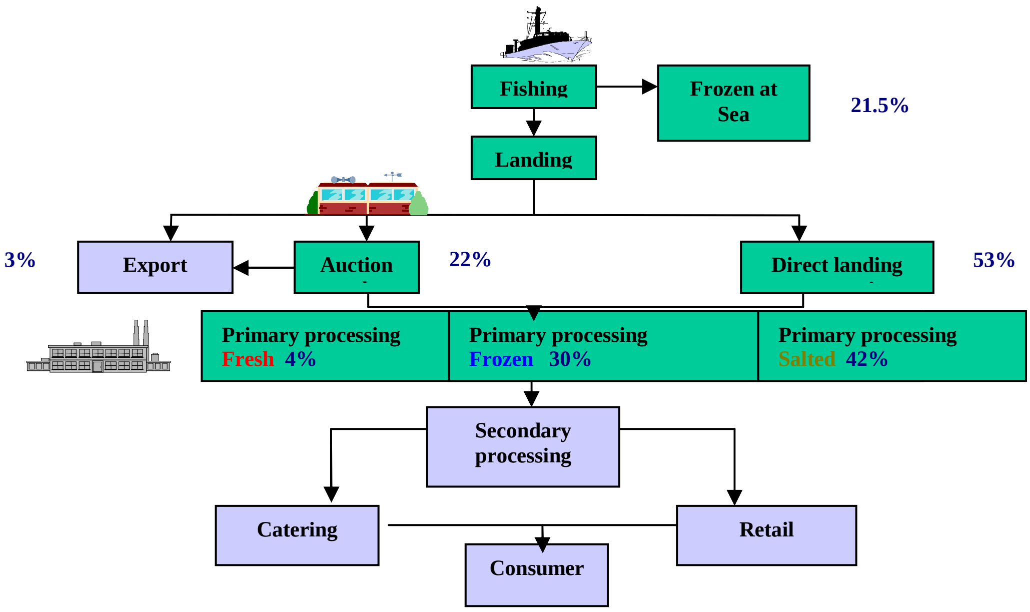

Figure 1. Distribution chain for cod in Iceland (From: Guðrún Ólafsdóttir, Brynhildur Benediktsdóttir, Friðrik Blomsterberg, 2000. Working Document on Fish Quality Labelling for Iceland - FAIR CT98 4174. RF Project Report 12-00)

The fish distribution chain is similar for the most important species and the role of the fish processing industry in Iceland is very important. The products are in most cases exported after primary processing, however in some cases secondary processing or packing is at source. In general, Iceland is exporting the raw material mainly after the primary processing step and the customers are not directly consumers but rather supermarket chains and secondary processing operations.

Sub-task 1.2 Determining current practices and requirements for measuring quality at the CPs.

Sensory evaluation to determine fish freshness is used in the fish industry in Iceland mainly in the processing sector. The most common scheme used in the evaluation of fillets is the Torry scheme for cooked fish. Some factories have their own sensory scheme to evaluate the freshness of raw fillets (Tryggvadóttir and Olafsdóttir, 2001). The Quality Index Method is being introduced in the fish industry and has received most attention in fish auctions (Martinsdóttir et al., 2002). The need for a standardized method to evaluate the freshness of fish has been expressed especially to facilitate the trade of fish via electronic commerce.

A survey conducted within the MUSTEC and FQLM projects in 1999 (Jörgensen *et al*., 2002; Tryggvadóttir and Olafsdóttir, 2001) gave information about the view of the different actors in the chain regarding the importance of various quality criteria of fish and the need for methods to evaluate these. According to the responses in the survey, there was a genereal concensus in Europe about the importance of documenting various criteria for quality and freshness of fish. 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. The single instruments to measure the individual properties were not considered important, but there was a concensus on the importance of the needs for rapid instrumental methods to measure the overall concepts freshness and quality.

The fish industry does not appear to have confidence in the single techniques to evaluate the complex concepts, freshness and quality. This may be one of the reasons, why the implementation of already existing techniques based on, for example, electrical properties (Fishtester, RT Freshmeter and Torry meter) to evaluate fish freshness has been slow in the industry. The reason for the reluctance to use instrumental methods for freshness and quality in Iceland may also be that the demand for fish is more than supplies and therefore, all fish is sold at a high price despite 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.

Sensory inspection of processed fish is used in the fish industry to find defects that have occurred during handling and processing (Oehlenschlager 1997). These defects are well described in the technical specifications for the products. Defects can be related to the condition of the fish flesh, appearance, which includes colour defects (bruises, bloodspots) and dehydration, workmanship defects such as improper packaging and cutting and trimming imperfections, scales, bones, foreign matters, skin and black membrane and the size of fillets. Evaluation of defects is widely used in control of processes and to grade fish for selling or buying purposes.

Parameters related to origin, handling and defects are considered very important in the quality systems in the fish processing industry in Iceland, but evaluation of the freshness of the raw material is not considered as important in the documentation according to a field study in one of the major fish processing companies in Iceland (Palacios, 2002).

The evaluation of the raw material is done at the moment of landing or in the reception area in the plant and information about species, catching area and catching day is recorded. The batches are evaluated by looking at the handling 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. No evaluation of freshness is done at this stage. For the evaluation of fillets, samples are taken randomly after trimming and checked for defects in appearance (bones, parasites, bloodspots, bruises, black membrane). For freshness determination of fillets, color and smell are evaluated on a scale from 2 to 5 for redfish, but the Torry score for cooked fish is used for cod as required by their customers.

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 that evaluation. The evaluation of the whole fish by sensory evaluation using the QIM method is reliable, but an instrumental multi-sensor device would facilitate the evaluation.

The possible scenarios for use of multi-sensor device for evaluation of species like cod in Iceland are the following:

Evaluation of whole fish:

- at the point of landing
- in fish auctions
- in the reception area of fish processing factories

Evaluation of fish fillets:

- in fish processing (e.g. after trimming the fillets)
- at secondary processing before further processing
- in retail before labelling for sale

Task 2 Simultaneous evaluation of physical methods for monitoring the quality of fish

The aim of the work-ins was to generate sufficient data to evaluate the effectiveness of the physical methods in measuring the quality of fish at different stages of storage and processing. The individual reports of the participants give details about the different methods.

Sub-task 2.2 Simultaneous application of physical methods

During the third year of the project the main activity was data analysis from the simultaneous measurements of frozen hake and cod in a work-in in Madrid in November 2000. In the extension period of the project an additional work-in took place in Hamburg in February 2002 measuring cod samples of different freshness. This study was done to be able to validate the data collected in the previous trials. The data analysis of the measurements collected in November 2000 in the Madrid work-in is included in this report and the results from the Hamburg work-in in February 2002. All participants came together in both work-ins to determine simultaneously the quality and freshness of the same fish by several physical techniques (optical -colour, NIR and imaging, gas sensors, texture and electrical).

Sub-task 2.3 Data analysis and fusion

Analysis of the overall results from Task 2.2 using statistical analysis and data fusion will be carried out by partner 6. Data from the texture analysis and electronic nose measurements of Partner 2 is evaluated separately in this report and compared to the QIM.

Task 3 Collaboration with the industry to pursue commercial exploitation of the multi-sensor instrument

Sub-task 3.1 Recommendation of exploitation route for physical methods

The outcome of sub-task 2.3 will give ideas about the best combination of the various sensors to fulfill the need for various quality measurements in the fish processing industry.

Sub-task 3.2 Formulate industrial device for fish freshness determination

The company Bodvaki (Hlidarsmári 14, Kópavogur, Icleand) has cooperated with IFL on the development of the electronic nose FreshSense during the last eight years. In 1990 the University of Iceland and IFL started a joint project on the development of a gas sensor instrument that was designed to monitor the freshness of fish. Tinoxide sensors were used in the beginning, but since 1993 electrochemical sensors have been used in the instrument. The research has been partly funded by the Icelandic Research Council and has been ongoing since 1990. The electronic nose is a new technique that has gained a lot of attention. Various sensor technologies are being utilized in these instruments but to date very few of these instruments have been implemented in the food industry.

The electronic nose FreshSense is based on commercial electrochemical gas sensors and a static sampling system. The aim of the research has been to provide the fish industry with a low cost, easy to use instrument that can rapidly and accurately assess the freshness or quality stage of fish products. The sensors are sensitive to low molecular weight volatile compounds that typically develop during microbial spoilage of fish such as amines (ammonia and TMA), sulfur compounds (hydrogen sulfide, methyl mercaptan and dimethyl disulfide) and alcohols (ethanol, propanol, butanol). These microbial metabolites have been suggested as useful indicators of spoilage and measurements using the electronic nose have shown that the results are comparable to traditional measurements to evaluate spoilage such as TVB (total volatile bases) and sensory analysis.

Currently the electronic nose FreshSense is used in various research projcets at IFL to monitor freshness and onset of spoilage of fish during storage. The FreshSense instrument has also been used in a Nordic project to monitor the ripening process of cod roe. IFL is now working with the fish processing company Tros Ltd. in Sandgerdi, Iceland. Tros produces fresh fillets that are mainly sold to USA and UK by airfreight. The storage life of fresh fillets is short and different seasons, catching techniques and handling influence the spoilage rate (Olafsdóttir *et al.,* 2002). To verify the freshness of the products it is important for the commercial partners to have access to objective and reliable measurements of freshness quality. There is an interest in the fish industry to implement this new technique for freshness evaluation of fish. However, more research is needed in cooperation with the fish industry where actual extrinsic parameters such as icing techniques and temperature fluctuations of the raw material during storage are studied to evaluate the performance of the electronic nose to predict the freshness and quality of fish and fish products.

The company Bodvaki was interested in exploiting the results of the instrumental development of the FreshSense in the MUSTEC project. The aim was to produce a commercial instrument to monitor quality of fish. However, because of recent changes within the company these plans have been postponed and the focus is only cooperating in research projects. Bodvaki is now owned by Maritech in Iceland, that is one the biggest information technology company specializing in IT solutions for the fish industry. Maritech has also collaborated with IFL in a CRAFT project on the development of a software for QIM evaluation which is now a commercial product.

The experience gained in the MUSTEC project is very valuable for future developments of the FreshSense instrument. The opportunity to do experiments on important commercial fish species in different countries with the same instruments is unique. The resulting data is useful to give directions and recommendations for further developments of the instrumental techniques and to evaluate the validity of the data to use in models to predict quality. This has implications for manufacturers of instruments that may want to supply their instruments with a model to predict the quality of fish. They need to know if a single model based on data from different experiments has acceptable performance or if a model has to be developed for each experiment or application. It would be practical if a single model could be developed for each species irrespective of origin, different stocks and seasons. Catching techniques and handling will influence the spoilage, but these can always be controlled. The approach taken for the development of the QIM has been to develop a single model for each species although small seasonal variations are observed. The model is only valid when the fish is caught and handled according to best practices (Martinsdottir *et al*., 2001).

The evaluation and development of sampling techniques in the project also clearly indicate that well controlled sampling conditions are of utmost importance to ensure the performance of the FreshSense instrument.

Task 4.1 Dissemination

Dissemination from the project was done jointly at a conference held in collaboration with the EU project Fish Quality Labelling and Monitoring (FQLM) in Firenze in Italy on April 15-17. The conference attracted more than 100 participants and was an excellent forum to disseminate the results with a mixed audience from researh and industry. The proceedings from the meeting will published in a book.

A leaflet was prepared with information about the project and distributed at the Firenze meeting (Appendix 2).

4. Research activities during the third reporting period carried out by partner 2

The participants from the Icelandic Fisheries Laboratories measured texture and volatile compounds using the following instruments and took part in sensory evaluation using the QIM:

- Texture measurement. *Instrument:* Stable Micro Systems texture analyser, model TA.XT2i (Stable Micro Systems Ltd, England)

- Volatiles. *Instrument*: FreshSense an electronic nose. A prototype developed by Bodvaki (Hlidarsmari 14 , Kopavogur; Iceland) and Icelandic Fisheries Laboratories.

- Sensory evaluation using QIM (Quality Index Method).

4.1 Work-ins Madrid 2000 and Hamburg 2002

Two work-ins were carried out in the last reporting period of the project. All participants came together to determine simultaneously the quality and freshness of fish from the same batch in a storage study of frozen hake and cod. The work-ins were hosted by the Instituto del Frio in Madrid November 2000 and by the Federal Research Centre for Fisheries in Hamburg in February 2002.

4.2 Materials and Methods

Madrid, November, 2000

Hake (*Merluccius capensis* and *Merluccius paradoxus*) were obtained from Pescanova-Chapela S.A., Redondela, Spain. The fish was caught off the South West African coasts (area of Namibia). The hake was stored frozen (-20 ºC) for 2, 4, 8, 10, 12 and 18 months (frozen and thawed immediately). Two additional tests were included in the Madrid work-in: (i) Chilled storage experiment for nine days in ice for the hake frozen for 4 month and (ii) Measurement of thawed cod from work-in in Tromsö which had been kept frozen for 8 months.

Hamburg, February, 2002

Cod (*Gadus morhua* L.) originated from the Baltic sea. Three batches of fish were stored in ice for periods from 0 to 9 days. The batch of fish that was stored for 7 and 9 days in ice was caught in nets and was kept alive for 3-5 days prior to slaughtering. One batch of pre-rigor fish was analysed within 3.5 hours from slaughtering and part of that batch was stored for one day in ice and analysed as 1 days old sample. The third batch was bought at the market and was 1 or 2 days old *(see Appendix2)*

4.2.1 Sensory analysis

Quality Index Method (QIM) for whole cod (Bremner, 1985; Martinsdottir, 1995) was performed in the Reykjavik and Tromsö work-ins by all the Mustec participants, about 15 people. The QIM scheme for frozen hake used in the Madrid work-in was under development.

4.2.2 Texture measurements

The texture measurements applied during the work-ins were the puncture test (firmness test) and the creep test using the Stable Micro Systems texture analyser, model TA.XT2i .

The puncture test consists of measuring the force required to push a plunger into a food sample, which is thus subjected to a combination of compression and shearing in proportion to the area of the cross-section of the plunger (Barroso *et al*. 1998). The plunger was set to go to a certain % of the height of the fillet (thickness). The penetration distance was non-destructive to the fillet. The creep test is mainly useful for the characterization of viscoelastic materials. A constant shear stress is applied and the resulting strain determined as a function of time.

The creep test values were given as creeping distances which is the difference in distances (d1-d2). D1 is the distance after a 100g force had been applied for 30 sec and d2 is the distance of permanent deformation which was measured after 30 sec of relaxation. These give the viscoelastic nature of the product. For example, a product that flows will have a greater difference in distance between these two points than a very elastic (less creeping) product.

Probes and calibrations:

Puncture test (firmness test)

- Ebonite cylinder probe, 10 mm in diameter (P/10)
- Spherical stainless (P0,5s)
- Pre test speed 2,0 mm/s; speed in sample 0,8 mm/s
- Strain (distance) 40%, for flesh side (Madrid and Hamburg)

Creep test

- Ebonite cylinder probe, 10 mm in diameter (P/10)
- Pre test speed 2,0 mm/s; speed in sample 0,8 mm/s
- 100g force applied for 30 sec and allowed to recover for another 30 sec (Tromsö).

4.2.2.1 Sample preparation for texture analysis

Madrid work-in

In the Madrid work-in the main target was to see if texture measurements could detect changes in hake during frozen storage. The thawed fish was filleted few hours before the measurement and the right fillets were measured on the flesh side. The

puncture test was done with two different methods regarding probes and placement of the mesurement. Ten millimeters in diameter cylinder probe with a flat contact area was depressed into each fillet four times and the reported value is the average of the four measurements. The first contact was done about 3 cm from the top (neck cut) of the fillet and again about 6 cm from the top, parallel measurements were done in each location about 2-3 cm apart. The other puncture test was done with a spherical probe (10mm in diameter) that was pressed in the middle of the fillet at three locations. First measurement was about 5-6 cm from the neck cut and then about 6-8 cm apart along the fillet. The average of the 3 measurements of each fillets was given as the firmness value for each individual.
The creep test included four measurements along the fillet starting about 2-3 cm from the neck-cut and proceeding down along the fillet about 6 cm apart. The creeping distances were calculated and the average of the four distances was given as a result for each fillet. The creep test was used on the frozen-thawed cod that was transfered from Tromsö as it had been used on that batch 5 months earlier in Tromsö.

Hamburg work-in

The puncture test (firmeness) was applied with a 10 mm ebonyte cylinder probe.
On the first day of the work-in measurement were to be done on cod stored for 7 days in ice but there was a malfunction in the Texture Analyser so the texture could not be measured on the fillets for that day.

4.2.3 Electronic nose measurements

Electronic nose measurements were performed using a gas sensor instrument called "FreshSense", developed by the IFL and Bodvaki-Maritech (Hlidarsmari 14, Kopavogur, Iceland). The instrument is based on electrochemical gas sensors (Dräger, Germany: CO, H_2S, and SO_2; City Technology, Britain: NH_3A7AM). The measurement technique and modifications of the instrument were described in the last annual report (Tryggvadóttir and Olafsdóttir, 2001).
Figure 2 shows the electronic nose FreshSense. A small sampling container (2,3L) is used and a dynamic sampling system using a pump to ensure the transport of the headspace from the sampling container into a small measurement chamber. The headspace is circulated between the sampling container and the measurement chamber

and no extra air is introduced into the system. A PC with a Labview measurement and data analysis software is used for data acquisition and analysis. Measurements are taken every 10 seconds for 5-10 minutes. The reported value (current) is the average of last three measurements of the 10 minutes measurement cycle minus the initial value (the average of 6 measurements before the measurement starts).

Figure 2. The electronic nose FreshSense with the electrochemical gas sensors (CO, H_2S, SO_2, NH_3) developed by Bodvaki and Icelandic Fisheries Laboratories.

4.2.3.1 Preparation and measurements of fish samples

The fish was filleted and the skin removed. Each fillet was measured separately. The samples were placed in the glass container and temperature of the samples was measured before the container was closed.

Table 2. Weight, temperature of fillets and size of the sampling container in the three work-ins

Fillets	**No samples**	**Average weight (g)**	stdev	**Temp°C**	stdev	**Sampling container**
Reykjavík	8	623,7	170,0	8,8	1,5	5,2L
Tromsö	5	571,4	177,9	8,9	1,2	2,3L
Hamburg	6	176,5	92,5	6,7	2,7	2,3L
Madrid	5	not measured		6,6	2,3	2,3L

Table 3. Weight, temperature of heads and size of the sampling container in the three work-ins

Heads	**No**		**Average weight (g)**	min	max	stdev	**Temp°C**	stdev	**Sampling container**
Hamburg	6	(1 head)	344,8	170	580	104,1	6,5	1,3	2,3L
Reykjavík	3	(2 heads)	1629,6	1100	2390	392,8	7,0	1,8	5,2L

Tables 2 and 3 show the number of replicate samples analysed, the average weight and temperature of the samples, and the size of the sampling container used during sampling in all the work-ins.

An overview of this is given here because a comparison of all the data from the Reykjavik, Tromsö and Hamburg will be done in this report.

4.2.4 Data analysis

Microsoft Excel 97 was used to calculate means and standard deviations for all multiple measurements and to generate graphs. Systat® 7.0 for Windows® was used for statistical calculations and multivariate analysis was performed by the Unscrambler® 7.5 software package (CAMO A/S). Principal component analysis (PCA) was performed on the electronic nose and QIM data from the Reykajvik, Tromsö and Hamburg work-ins to study the main variance in the data on cod. The main purpose was to study the ability of the electronic nose to discriminate between days of storage or spoilage level. PLS (partial least squares regression) was used to evaluate the possibility to predict QI scores from the electronic nose. In all PCA runs two principal components and full cross validation were used. All the data was standardized to equal variance prior to PCA.

4.3 Results and Discussion

The results of the texture measurements, electronic nose, and sensory analysis (QIM) of frozen/thawed hake and fresh cod from the work-ins in Madrid and Hamburg, respectively, are reported herein. Moreover, these results are compared to the earlier results from the Reykjavik and Tromsö work-ins

4.3.1 Sensory analysis

The results of the QIM analysis are shown for cod in Figure 3 to illustrate that the results were similar in all the work-ins. The highest slope in the Hamburg series may indicate more rapid spoilage, but since samples were not analysed after day 9 in Hamburg as was done in the other series this can not be confirmed. The pre-rigor sample in the Hamburg series had higher scores than anticipated because the QIM scheme does not take into account pre-rigor samples and should have had lower

scores. The best linear fit reported by Martinsdóttir et al. 2001 is the following:
y = 1,02 x days in ice + 1.08, R^2= 0.965.

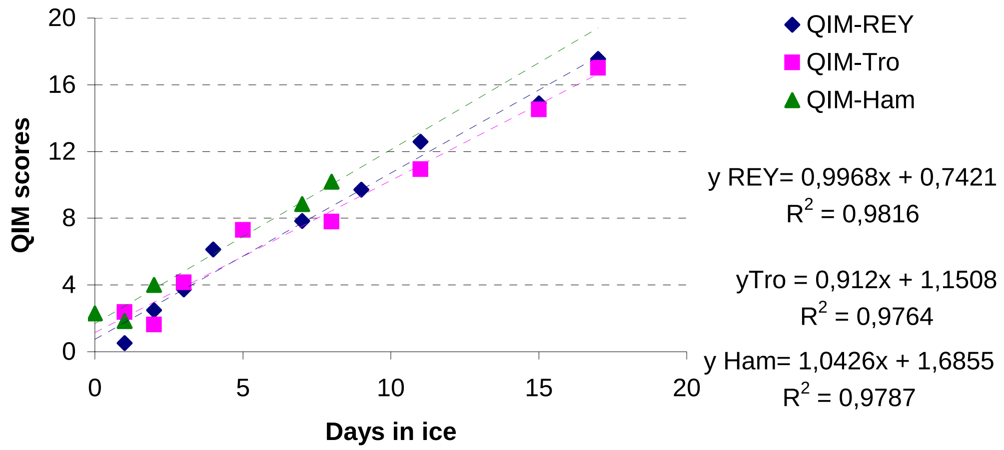

Figure 3. Results of averages and the best linear fit of QIM measurements of cod vs. days in ice in the Reykjavik, Tromsö and Hamburg work-ins.

4.3.2 Texture measurements

4.3.2.1 Results of texture measurements - Madrid, November 2000

The main objective of the texture measurement in the Madrid work-in was to find out if it is possible to detect with non destructive measurements the changes in fish muscles that occur with prolonged frozen storage.

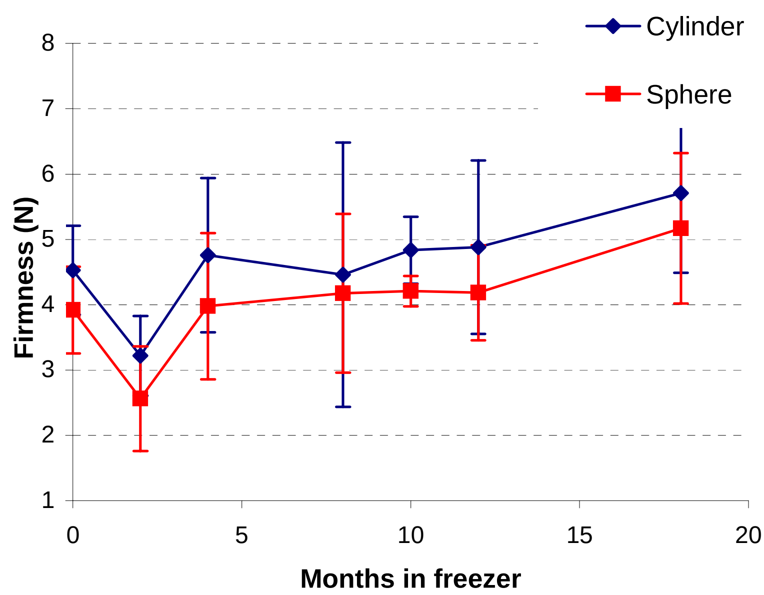

Figure 4. Texture result from puncture test on frozen thawed hake using two different probes and measuring areas on the fillets.

Figure 4 shows the results from the non destructive puncture test. Using the spherical probe and measuring along the fillet rather than using the cylinder probe and measure close to neck area lowers the average values about half a Newton. The pattern of the two graphs is almost identical which implies that the measuring place and type of probe do not make much difference in the texture measurement. The low values at month two in the freezer is unexpected. Figure 4 shows decreasing firmness values at month 2 and then slight continuous increase until month 18. Apparently, very small texture changes take place between months 4 and 12 in the freezer. This could be interpreted as a stable storage period regarding texture. It has to be considered that the standard deviation in the texture measurements is very large due to individual differences and the measurable changes are very small.

Figure 5 shows the results from the punctue test (firmness test) using two different probes on hake fillets after storage in a freezer for four months. Following the four months freezer storage the fish was thawed and stored in ice for 9 days. As seen in Figure 4 there are no clear detectable changes in texture during storage in ice after thawing. It can be seen from the standard deviation both in Figures 4 and 5 that the individual differences are large. The individual texture differences between the fillets could even be detected by touching the fillets by hand.

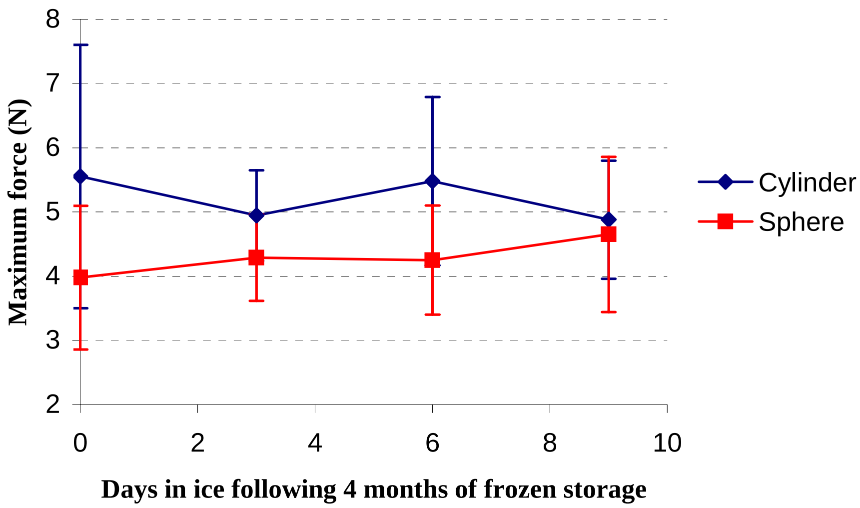

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Figure 5. Results from texture measurements on frozen /thawed hake during storage in ice for nine days. Measured with two different probes and measurement areas.

The individual differences in the texture measurements have in fact been a problem in the project. It is not surprising that there is a texture difference between individual fishes. A batch can be defined as one fishing haul which can be composed of fishes of different ages, different upbringing, from different environment and in different nutritional status which are all factors that may have an impact on texture

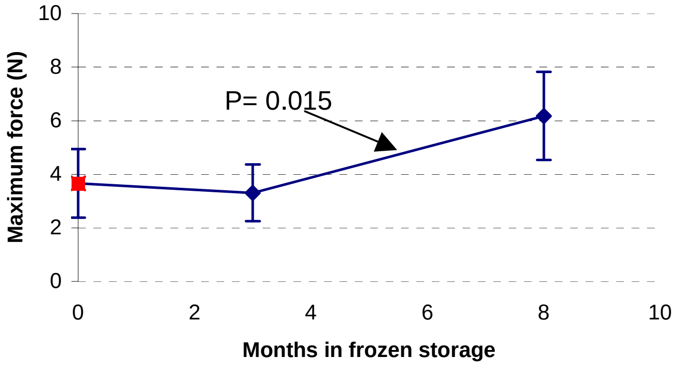

Figure 6. Puncture test (firmness test). Result from frozen thawed cod from two work-ins in Tromsö and Madrid. The red square is a firmness value from fresh Tromsö cod (3 days in ice) for comparison.

The results from the texture measurements on fillets of thawed cod that had been in frozen storage for 3 and 8 months are shown in Figures 6 and 7 for the puncture test and creep test, respectively.

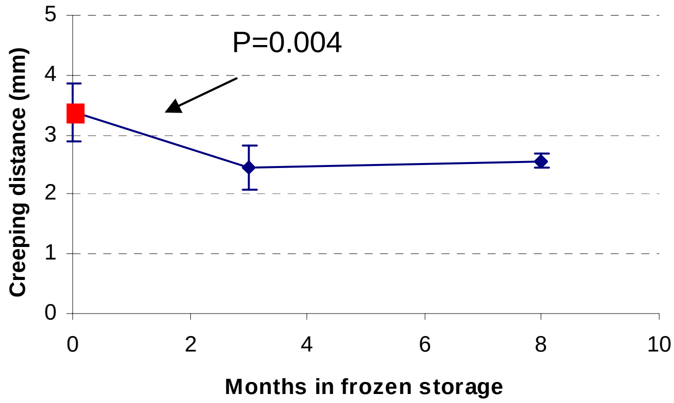

Figure 7. Creep test. Result from frozen thawed cod from the work-ins in Tromsö and Madrid. The red square is a firmness value from fresh Tromsö cod (3days in ice) for comparison.

The puncture test does not show texture changes between the fresh cod and cod frozen for 3 months, but a significant difference ($P \leq 0.05$) in increased hardness (toughness) from 3 months to 8 months of frozen storage. On the other hand the creep test shows significant difference ($P \leq 0.05$) in decreased elasticity from fresh cod to frozen for 3 months. After that there is hardly any viscoelastic change from 3 months to 8 months storage. These results indicate that the puncture test can detect length of frozen storage and the creep test detects the physical change that occurs from fresh to frozen stage, which could be helpful to determine whether fish had been frozen or not.

4.3.2.2 Results of texture measurements - Hamburg February 2002

Four batches of fresh cod fillets were measured in the Hamburg work-in. The extra work-in was planned to be able to validate the earlier measurements of fresh cod from the work-ins in Reykjavik and Tromsö. The sample from day 7 is missing because the instrument was not functioning. The results appear to be similar as before, i.e. decrease in texture values the first few days (Figure 8).

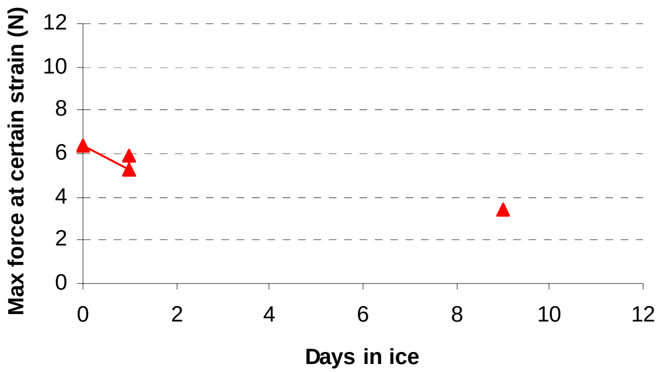

Figure 87. Results from the texture (puncture test) measurement of fresh cod in the Hamburg work-in.

4.3.2.3 Results from the puncture test from the three work-ins in Reykjavik, Tromsö and Hamburg.

In Figure 9 the texture measurements of fresh cod from all three work-ins are shown together. The overall patterns appear to be quite similar, but the values from the Reykjavik work-in are higher. This can be explained because the strain is 55% in Reykjavik, but 40% in the Tromsö and Hamborg work-ins. The Hamburg values coincide with the Tromsö values except for the pre-rigor/rigor values on day 0, which is not surprising since the exact rigor status of the fish is not known. The measurements themselves appear to be consistent in the Tromsö and Hamburg work-

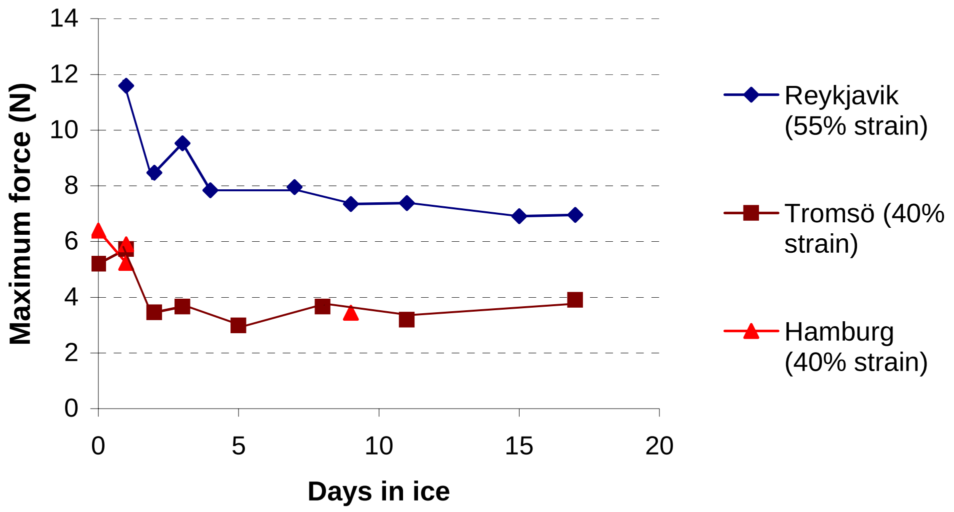

Figure 9. Texture measurements (puncture test) on cod fillets stored in ice for up to 17 days in the three work-ins.

ins, but the undestructive analysis method is not sensitive enough to detect softening of the muscle (if there is any) from day 3 to day 17 in ice storage.

The finger test of the QIM when humans push with the finger on the whole fish might be cruder than the non destructive 40% strain that the analyser is programmed for, and softening of texture is observed during ice storage. It is also possible that the filleting process like cutting into the myotomes and loosening up the muscle may destroy the ability for the texture analyser to detect small softening changes that occur during storage in ice. The different size of the fish does not influence the measurements. The average weight of the fish was considerably lower in Hamburg than Tromsö, 1.1 kg vs 3.2 kg.

Figure 10 is shown here to illustrate that sensory changes are measurable in cooked fillets during storage in ice when evaluated by Quantitative Descriptive Analysis (QDA). It is interesting to see how the fish gets gradually dryer and tougher with storage. This data is from an experiment of fresh and thawed fillets stored in a cooler (Martinsdottir *et al*., 2001b). For comparison the texture values (firmness) from the Tromsö work-in on fresh cod are put into the figure. In Tromsö the fish was stored in ice as whole fish and filleted just before the measurements but in the experiment of Martinsdóttir *et al*. (2002) the fillets were wrapped in plastic and stored in a cooler.

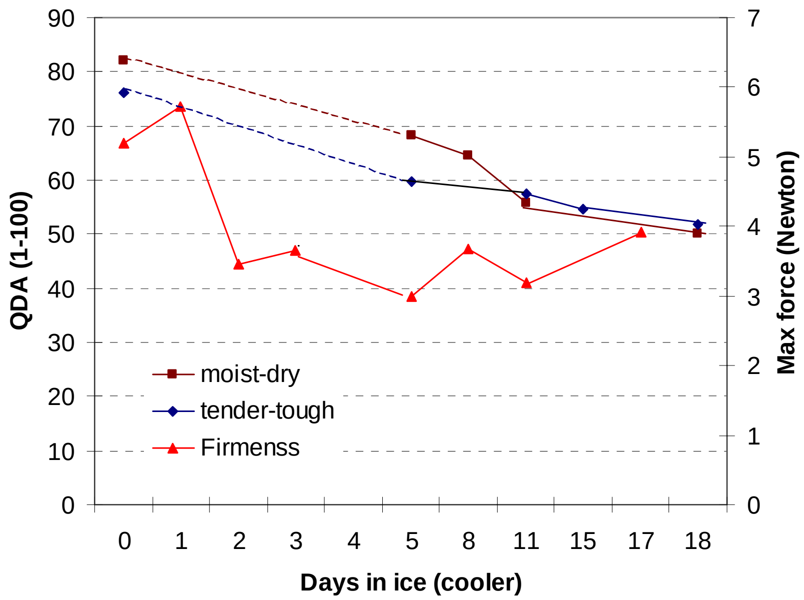

Figure 10. Results from modified QDA (quantitative descriptive analysis) on fresh cod fillets store at 0°C for 18 days (Martinsdottir 2001b). Texture (firmness) measured instrumentally (results from Tromsö work-in)

4.3.2.4 Conclusion Texture Measurements

- The results show that that texture measurements on fresh fillets can detect distinctive firmness changes due to onset and resolution of *rigor mortis* the first 3-4 days but with extended storage in ice there are hardly any textural changes identified.
- When the same test and setting are used in the non destructive texture measurements on fresh cod fillets the results are quite consistent. The values from the Hamburg work-in coincide with the values from the Tromsö work-in and the data is consistent for these two work-ins, although cod batches are from different fishing grounds (Baltic Sea vs Norwegian Sea) and caught at different seasons (winter vs spring). It has to be considered that the standard deviation in the texture measurements is very large due to individual differences.
- The texture measurements worked quite well with frozen thawed fish. The results of frozen and thawed hake (fillets) show decreasing firmness after two months of frozen storage compared to fresh hake but from two months in frozen storage and onward there is a gradual increase in hardness as

frozen storage prolongs. Texture measurements give no detectable changes in texture of hake fillets during storage in ice after thawing.

- For frozen cod the puncture test gives no information whether cod is fresh (3 days in ice) or has been in frozen storage for 3 months but gives significant difference in increased firmness when it has been frozen for 8 months. Creep test shows significant difference in elasticity of fresh and frozen cod, but very little difference is seen in elasticity during frozen storage for 8 months.

4.3.3 Electronic nose measurements

Measurements with most of the instrumental techniques in the project including the elecronic nose were done on fillets, but the QIM evaluation was done on the whole fish. Spoilage signs on the whole fish are more noticable than the changes that can be detected on the fillets by sensory evaluation. In the fish industry the color and the smell are the most important sensory attributes for raw fillets. The odor of the fresh fillet is very little or neutral. Odors like sweet, fruity, salted fish or stock fish, cheesy and foul are detected when the fillets become spoiled and at overt spoilage the odor is TMA and ammonia-like, sour and putrid - like.

The compounds contributing to these odors are not necessarily detected by the electronic nose. Only the compounds that are very volatile and are present in the highest concentrations in the headspace can be detected. The concentration of a compound present in the headspace above an aqueous sample, is related to the vapour pressure and to the liquid phase concentration of the compound. This means that compounds with high vapour pressures are abundant in the headspace and the headspace changes dynamically with the variation of temperature. The amount of compounds reaching the sensors is also dependent on the surface area of the sample and the ratio of sample to headspace in the sampling container (Olafsdóttir *et al.*, 2002a). Sampling has to be carefully designed because sampling conditions can influence the composition of volatile compounds in the headspace and alternatively influence which compounds are detected by the sensors. In the work-ins the sampling conditions were not exactly the same. Efforts have been made to compensate for that and corrections have been done to take into account differences in sample size, sampling containers and baseline value of the sensors. This facilitates comparison of data in this report.

4.3.3.1 Results of electronic nose measurements - Madrid work-in Nov. 2000.

Figures 11 and 12 show the results of the electronic nose measurements of the frozen (thawed) hake samples from the Madrid work-in. The responses are very low (< 100nA) towards the pre-frozen / thawed fillets and only the CO sensor shows some response during frozen storage. No changes are observed in the thawed hake during storage in ice. This was expected because it is known that microbial activity is lower in chilled fish after frozen storage compared to fresh fillets (Magnússon and Martinsdóttir, 1995). The electronic nose is in particular sensitive to very volatile compounds that are typically produced during microbial spoilage and these do not appear to be present in the thawed fillets. This is in agreement with the results of Magnusson and Martinsdóttir (1995) who found that very low levels of TMA were present in thawed cod fillets at the point of sensory rejection. The spoilage flora appears to be different resulting in the formation of different or very low concentrations of volatile compounds that are not detected by the electronic nose.

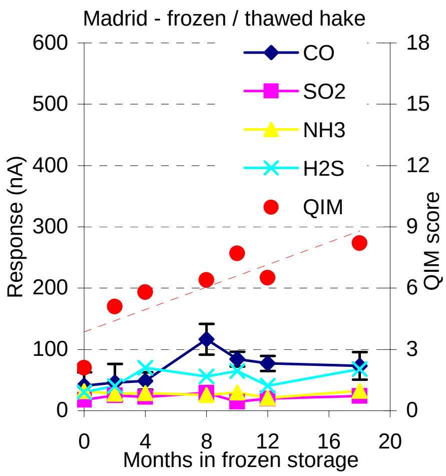

Figure 11. Electronic nose measurements of frozen hake from Madrid work-in 2000.

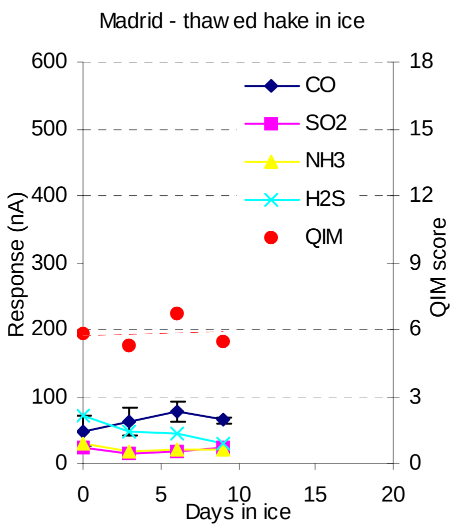

Figure 12. Electronic nose measurements of frozen /thawed hake stored in ice from Madrid work-in 2000

The odor of the fillets was neutral and no signs of ammonia-like or sulfurous-like odors were noticed. The scores for the QIM were also low and did not increase during storage in ice. The QIM scheme for frozen hake was under development during the work-in and apparently the attributes selected are not giving information about the changes occuring during storage in ice as seen by the results in Figure 12. Further developments of the QIM scheme for hake are needed.

4.3.3.2 Results of electronic nose measurements - Hamburg work-in Feb. 2002.

The results of the electronic nose measurements from the Hamburg work-in are shown in Figures 13 and 14 for the fillets and heads, respectivley. The responses of the sensors were low as was expected for the fresh fish fillets (days 0,1, and 1.5) but slight increases were noticed for the CO sensor on day 7, but somewhat less on day 9. When comparing this to the earlier work-ins in Reykjavik and Tromsö (Figures 15 and 17, respectively) the Hamburg values are slightly lower which may be explained by the very small size of the samples in Hamburg (see Table 2).

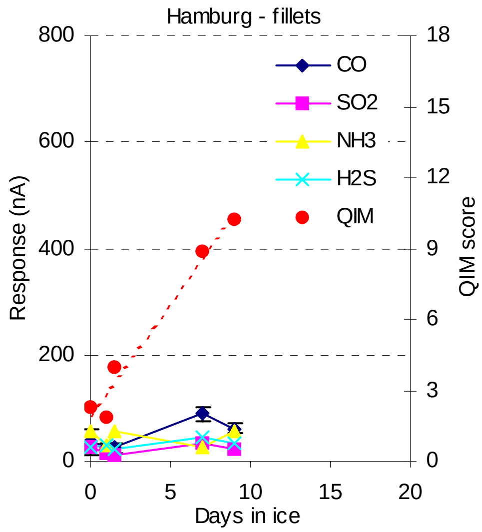

Figure 13. Electronic nose measurements of fillets and QIM score of cod stored in ice for 9 days in Hamburg work-in 2002

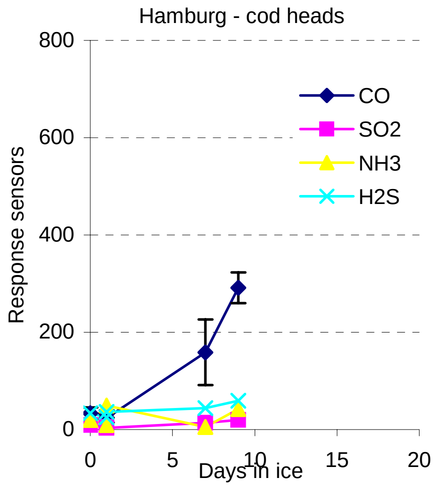

Figure 14. Electronic nose measurements of heads of cod stored in ice for 9 days in Hamburg work-in 2002

The responses are lower for the fillets than the heads as was also noticed in former experiments in the Reykjavik work-in (Figures 15 and 16). Measurements on heads were done in Reykjavik and Hamburg and the values appear to be similar in the two work-ins (on day 9 the CO sensor response is approx. 300nA). The higher responses of the sensors to the volatiles of the cod heads imply that the FreshSense electronic nose may have better abilities to monitor changes of the whole fish during storage of in ice rather than the fillets.

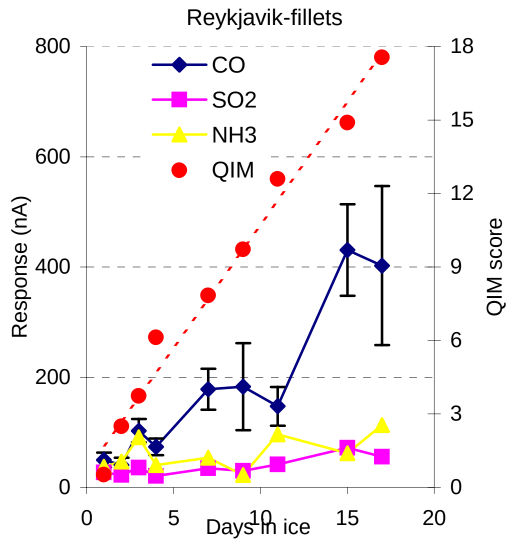

Figure 15. Electronic nose measurements of fillets and QIM score of cod stored in ice for 17 days in Reykjavík work-in 1999

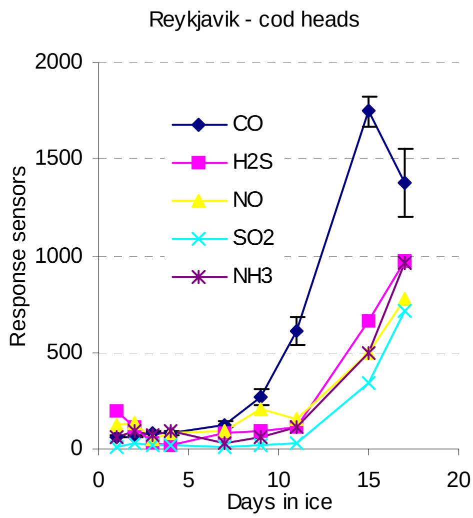

Figure 16. Electronic nose measurements of cod heads stored in ice for 17 days in Reykjavík work-in 1999.

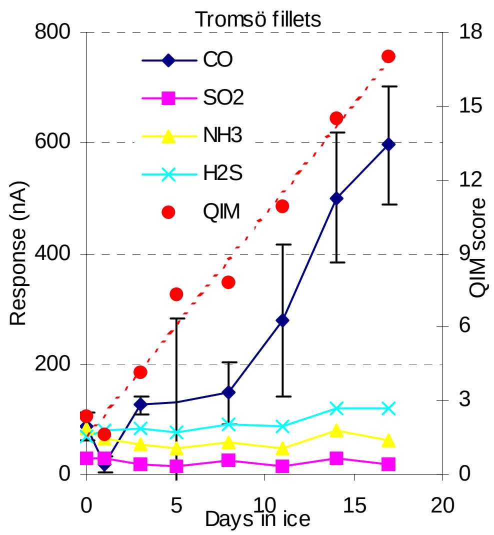

Figure 17. Electronic nose measurements and QIM of cod stored in ice from Tromsö work-in 2000

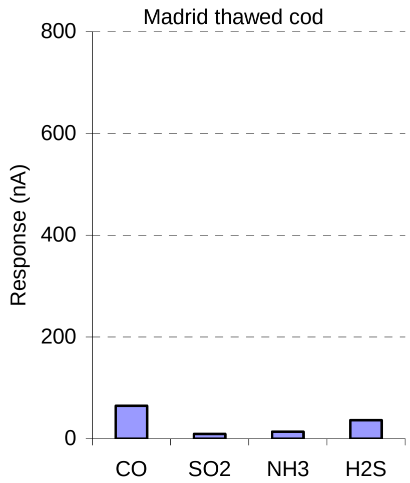

Figure 18. Electronic nose measurements from Madrid 2000 of thawed cod from Tromsö

The electronic nose measurement of the thawed sample of cod from Tromsö in Madrid is shown in Fig 18. The low responses of the sensors to a pre-frozen sample is in agreement with the result of the pre-frozen hake samples in the Madrid work-in. The FreshSense nose is not well suited to detect changes in pre-frozen fish.

4.3.2.3. Comparison of Reykjavik, Tromsö and Hamburg electronic nose data of cod

Correction of data: The values have been corrected to compensate for differences in the sampling. This is necessary to facilitate the comparison between the work-ins. First of all the values were all corrected taking into account the baseline value, which changes with time, because of the drift in the sensors (corrected value = final value-average initial value for all the samples in each work-in). A correction was also done on the Reykjavik data to compensate for the different size of container used for sampling in that experiment (corrected value=value x 5.2L/2.3L). The weight of the heads varied considerably (two heads were used in Reykjavik, but one per sample in Hamburg) and a correction has been made to compensate for the difference in sample weight of the heads in the two work-ins (corrected value = value x average weight/weight of sample). It is assumed here that the sample weight is directly related to the exposed surface area. In fact it is important that the samples always have the same surface area, but this is difficult to achieve when samples are irregular in shape as the heads are. In this case it would be better to use larger samples (more heads and bigger containers) so that the error in the surface area would be less. A practical approach for analysis of whole fish is to use fish boxes equipped with a lid and extract samples from the headspace into the electronic nose.

An alternative way to overcome these variation is to use a small sampling probe (10ml) like the LibraNose (Partner 5) is supplied with, that fits onto the surface of the sample and works quite well for fillets.

No effort has been done to correct for the different size of the fillets. The performance of the instrument would improve if the sample size was always the same. For future development of the sampling system of the instrument it is necessary to make sure that the surface area of the exposed sample is always the same. In the work-ins this was not possible because we decided to measure individual fish. The size of the fish varied considerably between work-ins and the size of the cod in the Hamburg work-in was by far the smallest (see Tables 2 and 3).

Comparison of sensor responses to QIM: The responses of the sensors were generally low to the fish fillets and only the CO sensor appeared to be responding to the fillets. To further study the trend in the responses of the sensors it is of interest to plot the values of the sensors v.s. the QIM score for all the samples from the three work-ins. Values lower than 100nA for the sensor are not considered significant and Figure 19 shows that only the CO sensor has significant responses. The H_2S sensor responses are scattered with no obvious trend. The SO_2 and NH_3 sensors appeared to show some trend of increasing responses with increasing QIM score but again the responses are very low.

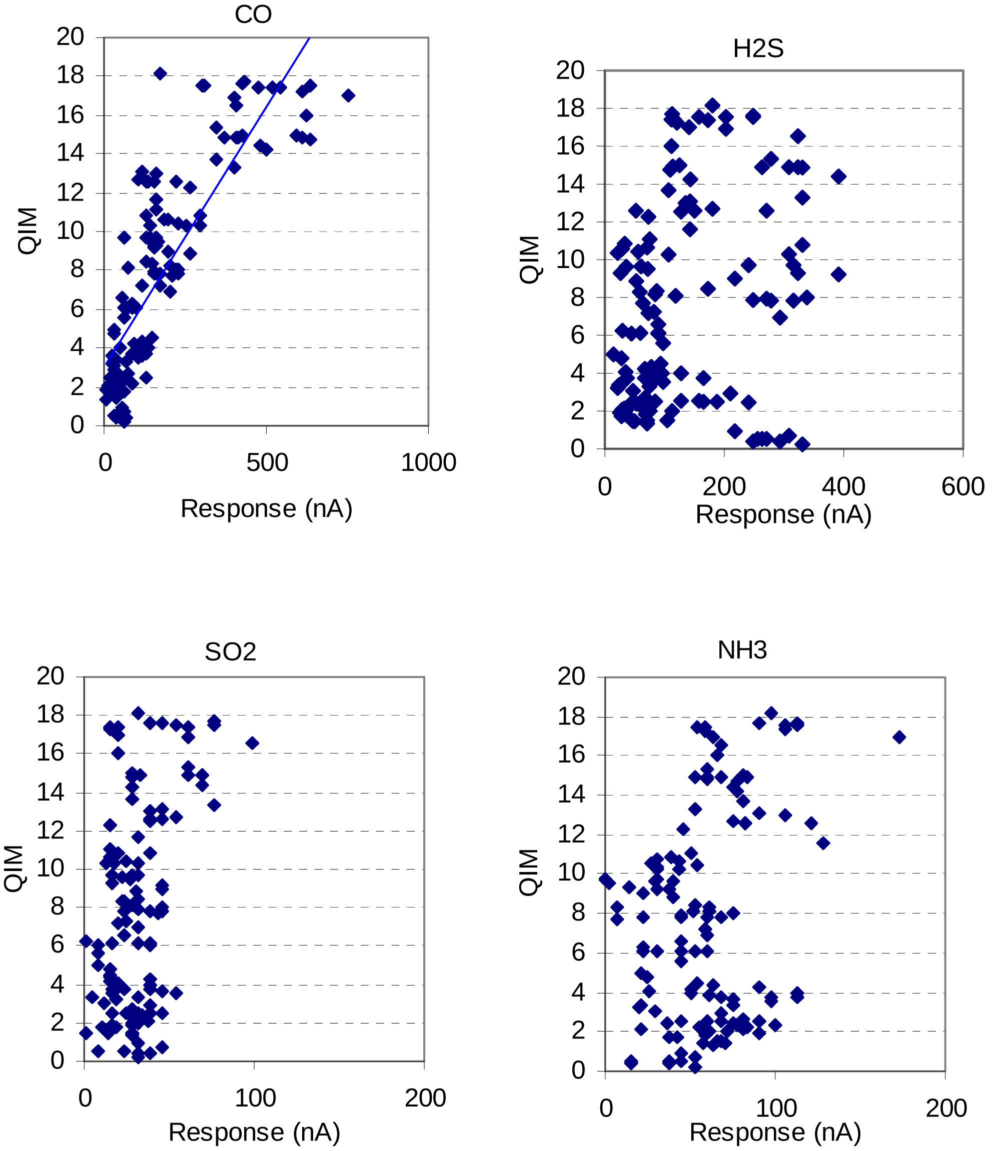

Figure 19 Values of the different sensor responses (CO, H_2S, SO_2, NH_3) v.s. the QIM score for all the samples from the three work-ins

Principle Component Analysis: By using PCA more information can be extracted from the electronic nose data. PCA of the data on cod from the three work-ins Reykjavik, Tromsö and Hamburg was done to study the overall trend and to see if samples could be discriminated based on their spoilage level, expressed as days of storage. Grouping of the data according to storage days is observed on the PCA plot in Figure 20. However, samples of same storage days from the different work-ins do not overlap and the Reykjavik data is positioned on the upper half of the plot because of lower values of the CO sensor. PC1 explains 47% of the variation of the data and PC2 explaines 22%. The CO sensor and the QIM score have similar loadings on the plot and appear to be highly correlated as was also seen in Figure 19.

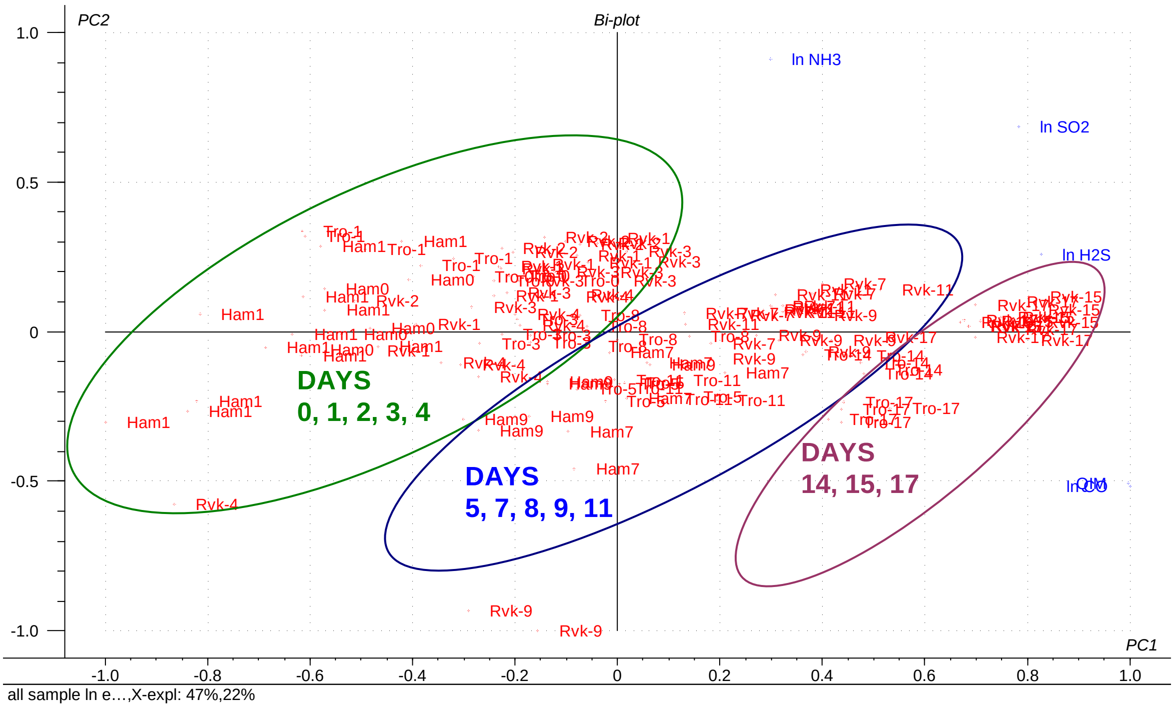

Figure 20. Principle component analysis (PCA) of all samples from the Reykjavik, Tromsö and Hamburg work-ins and the electronic nose variables (ln of the values of the CO, SO_2, H_2S and NH_3 sensors)

Figure 21 showing a PCA plot of the averages of the sensor responses illustrates this more clearly and the patterns appears to be slightly different in the three work-ins. This could be explained because of different cod stocks, difference in initial handling and different seasons, that can contribute to difference in spoilage rate. These factors are beyond control and reflect the actual situation in the industry where handling, season and different origin are the facts of life.

However, other factors directly related to the sampling conditions are more likely to be influencing the variation. First of all the modifications done on the FreshSense instrument between the Reykjavik and Tromsö work-in resulted in more sensitivity of the instrument. Smaller samping container was used and a pump was installed to circulate the headspace. The simple corrections done to compensate for the diffferent size of the containers were not adequate. Other factors are also very important such as; slight variation in sensor because of differences in the sensors responses with time (i.e. drift); difference in the weight of the samples; variation in the ambient temperatures and consequently a slight variation in the temperature of the sample. This was very difficult to control during the work-ins because the same samples had to be used for different measurement techniques. Control of all these factors is very important to ensure consistency of the measurements. Standardisation of sampling parameters would improve the precision of the FreshSense instrument.

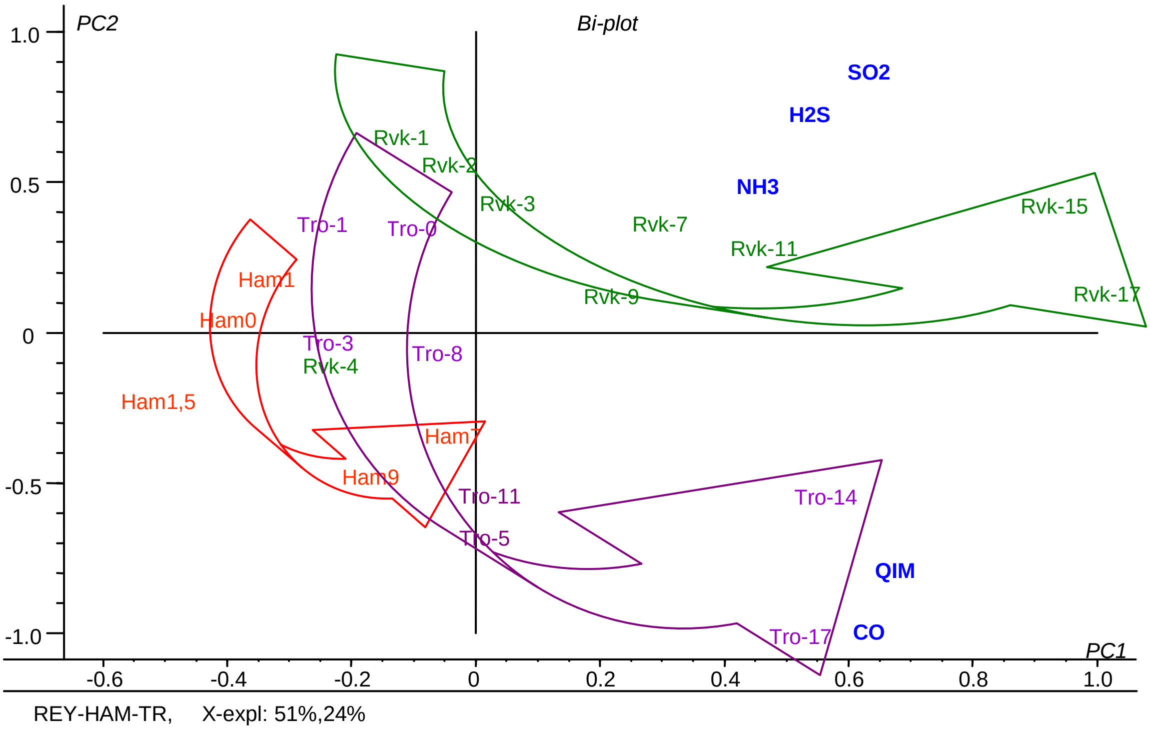

Figure 21. Principle component analysis (PCA) of averages of all samples from the Reykjavik, Tromsö and Hamburg work-ins and the electronic nose variables (CO, SO_2, H_2S and NH_3 sensors)

Partial Least Squares Regression: When analysing samples of unknown origin with the electronic nose it is essential to have a reliable model to be able to predict the quality or the QIM score. Instrument manufactures may want to supply their customers with models to predict the quality of fish based on the electronic nose measurements. Therefore they need to know, if a single model based on data from

different experiments has acceptable performance or if a model has to be developed for each experiment or application. Di Natale *et al* 2001 showed that a PLS-DA on the combined electronic nose data (FreshSense and LibraNose) from Reykjavik showed a good performance to predict storage days but some errors were evidenced mainly beacuse of the effect of different batches with slightly different spoilage rates that were used in Reykjvik. In this analysis it was very clear that information on storage days of fish does not give an absolute indication of freshness quality because initial handling of the fish influences the spoilage rate. Therefore, it was suggested to predict the QIM values as a measure of freshness or quality rather than storage days in ice. Analysis of the Reykjavik data applying outer product technique for PLS regression of the FreshSense and Libranose joint data gave better performance to predict QIM smell attributes, than the individual noses (Olafsdóttir *et al.,* 2002a). This is because characterization of the samples is improved when the data is fused.
In an earlier report (Tryggvadottir *et al*., 2001) a PLS model based on only the Tromsö electronic nose data showed good performance to predict the QIM score (Corr = 0.97 and 0.89 for calibration and validation, respectively, and RMSEP=2.61).

The aim of the Hamburg work-in was primarily to collect more data on cod stored in ice to be able to study the performance of models based on the Reykjavik and Tromsö work-ins. Figure 22 shows a PLS model based on the corrected Reykjavik, Tromsö and Hamburg electronic nose data to predict the sensory score. The error is higher (RMSEP=3,08) than for models based on only Reykjavik or only Tromsö data. This is expected because of the inconsistency in the data mainly between the Reykjavik and Tromsö work-ins as was illustrated by the PCA. The variation is because of lower CO responses in Reykjavík resulting from differences in sampling.
Another way to validate the model based on Reykjavík and Tromsö is to use data from Hamburg as a test set (n=141; $n_{\text{test samples}}$=29). This validation had a correlation of 0.85 for the prediction and 0.82 for the calibration and RMSEP = 2.83. This is a good performance, however, it should be mentioned that samples from Hamburg did not represent the distribution of the model samples, since samples with QIM values above 12 were not present (no spoiled samples).

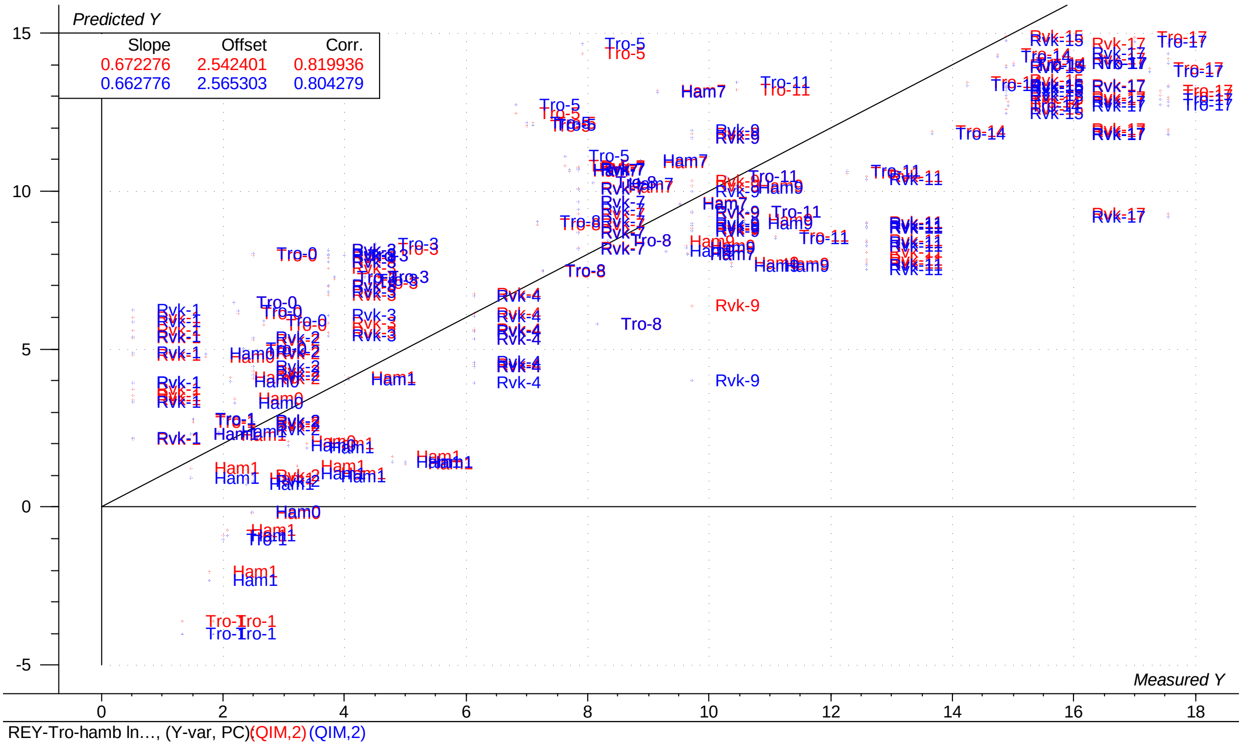

Figure 22. PLS regressin of electronic nose (ln of CO, H2S, SO2, NH3) as X variables and QIM scores as Y variables using data from Reykjavik, Tromsö and Hamburg work-ins, full cross validation and 2 significant factors (RMSP 3.08).

A third attempt was done to use the data from all the experiments and a model based on the Reykjavik and Tromsö data to predict the Hamburg QIM scores. Figure 23 shows the PLS model with a correlation of 0.82 for the prediction and 0.79 for the calibration and RMSEP = 3.28.

Table 4 shows the averages of the predicted QIM scores and the measured QIM values for each sample from different days. The predicted values for days 0, 1, and 1,5 are underestimated, as can be expected because the electronic nose values for the Hamburg work-in were very low. A good QIM prediction for day 7 is promising but the standard deviation is large compared to the standard deviation of the measured QIM.

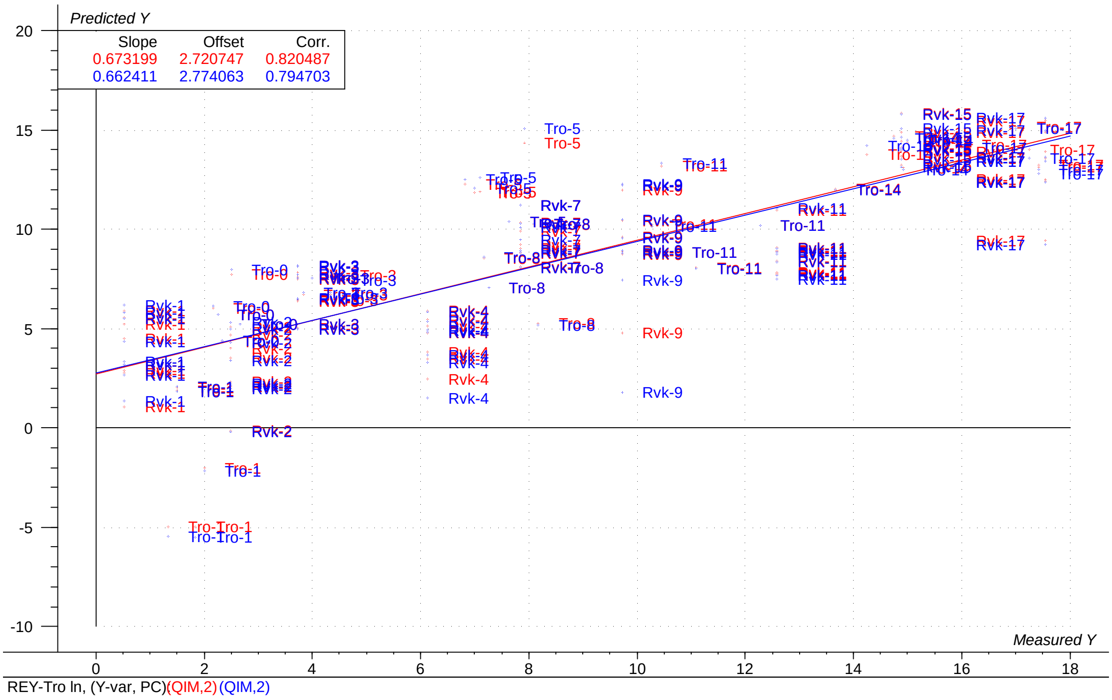

Figure 23. PLS regression of electronic nose (ln of CO, H2S, SO2, NH3) as X variable and QIM as Y variable using data from Reykjavik, and Tromsö work-ins (RMSP 3.28).

Table 4 Averages of the predicted QIM scores using the PLS model in Figure 23 and the measured QIM scores for the Hamburg work-in.

	Average predicted QIM	stdev predicted QIM	Average measured QIM	stdev measured QIM
0	1,67	2,09	2,32	0,45
1	0,35	1,31	1,85	0,36
1,5	-0,95	1,62	4,00	0,75
7	8,87	2,06	8,86	0,75
9	7,75	1,16	10,19	0,60

The QIM evaluation done on the whole fish is based on evaluation of 10 different attributes while the electronic nose measurements are done on the fillets using 4 different sensors. The CO sensors responses are the highest, but the low values for the other sensors are also contributing to the model.

The PLS analysis has shown that models based on the combined electronic nose data from the three work-ins have inferiour performances than models made with only one data set from a single work-in. This is mainly because sampling conditions changed between work-ins and underlines the importance of standardizing sampling and selecting representable samples for the models. A model based on Tromsö and Hamburg data may give better performance. Further analysis of the data will be done in a paper that will be published in a journal.

Models based on more than one instrumental technique are likely to give better performance to predict QIM. The multi-sensor approach is the aim of the project and data analysis using the combined techniques will be presented in the report of partners 6 and 7.

4.3.3.3 Conclusions Electronic Nose Measurements

Evaluation of iced fish - whole fish and fillets

- The responses of the sensors of the FreshSense nose were generally low to the cod fish fillets and only the CO sensor appeared to be responding to the fillets. The CO sensor and the QIM score had similar loadings on the PCA plot and appear to be highly correlated.
- The response of the electronic nose to cod heads was higher than to the fillets and all the sensors responded giving more information about the freshness quality of the samples. The higher responses of all the sensors to the volatiles of the cod heads imply that the FreshSense electronic nose may have better abilities to monitor changes of whole fish during storage of in ice rather than the fillets.

Evaluation of frozen fish - thawed fillets

- The FreshSense nose responses were very low towards thawed hake fillets and thawed cod. The electronic nose does not appear to be useful to detect changes during frozen storage. The responses of the nose to thawed hake fillets stored in ice were also very low indicating different spoilage pattern than in fresh fillets stored in ice.

Prediction of fish freshness quality (QIM scores) by FreshSense electronic nose

- Evaluation of the performane (PCA) of the electronic nose to detect changes in fish fillets during storage from three different experiments (work-ins) showed that fish samples stored in ice can be discriminated based on their spoilage level. The electronic nose appears to be able to detect when spoilage signs appear and can discriminate between fresh samples (1-4 days in ice) intermediate samples (5-11 days in ice) and spoiled samples stored (14-17 days in ice) level of ice stored cod. The use of an e-nose to complement the sensory odor evaluation of fillets in the fish processing is suggested.
- PLS prediction of QIM scores using models based on electronic nose and QIM

data from three experiments (work-ins) showed that QIM scores can be predicted with models having correlation >0.80 for the prediction and RMSEP =2.68-3,28 (QIM score) which corresponds to an error of 2.5-3 days. This error appears to be quite large, however, it can be argued that the models are based on inhomogeneous sample set, that is data from experiments measuring fish from different origin, different seasons, using different fishing gear and handling. The inconsistency in the data is mostly attributed to different sampling conditions. Earlier reports showed that models from one work-in showed better performance to predict the QIM score by the electronic nose than models based on the three different work-ins.

4.3 Conclusions

Electronic nose: Comparison of electronic nose data from three different work-ins on cod showed that the overall trend of the changes occuring during storage in ice are similar and samples can be discriminated based on their spoilage level. The electronic nose does not appear to be useful to detect changes during frozen storage. Variations in the data may be because of slight differences in sampling conditions such as size of samples and volume of sampling containers. Possible variation may also be because of inherent differences in the samples because of origin, season and handling that may influence the spoilage pattern and development of volatile compounds that the electronic nose can detect.

More samples need to be measured to improve the performance of models based on electronic nose data. Consistent sampling conditions for the electronic nose will improve the performance of the models. Future development of the electronic nose need to ensure that variations in measurements will not be caused be obvious controllable factors in sampling such as sample size and temperature.

Texture analysis: The texture results show that that non destructive texture measurements on fresh fillets can detect distinctive firmness changes due to *rigor mortis* the first 3-4 days but with extended storage in ice there are hardly any textural changes identified. This result was consistant for all the three work-ins.

The texture measurements worked quite well with frozen thawed fish. For frozen cod the textural changes after 3 months in a freezer could be detected also it could be detected whether fish had been frozen before chilling.
Texture measurements can give information in a multisensor especially the first days when other methods that are only sensitive to spoilage signs do not show any response. The texture measurements look quite promising to detect whether fish had been frozen before chilling and whether it had been for a long time in a frozen storage.

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Oehlenschläger, J. (1997*).* Suitability of ammonia-N, dimethylamine-N, trimethylamine-N, trimethylamine oxide-N and total volatile basic nitrogen as freshness indicators in seafoods. In: *Methods to determine the freshness of fish. In research and industry* (edited by G. Olafsdottir *et al*.). Pp. 92-99. Nantes conference, November 12-14. Paris: International Institute of Refrigeration.

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5. Other activities during the reporting time

- 6th project meeting and the third work-in was in Madrid, Spain (Task 2.2.) on November 12th-21st, 2000. The data analysis and the results of the experiment are included in this report.
- 7th project meeting was in Aberdeen on June 23rd-26th 2001.
- 8th project meeting was in Copenhagen on November 19th-20th 2001
- 9th project meeting and the fourth work-in was in Hamburg in February 2002.
- 10th project meeting and final meeting of the MUSTEC project was held in Firenze in April 2002.
- Joint MUSTEC-FQLM conference: Final International CA-FQLM Conference "*Quality of Fish in the Supply Chain: Labelling, Monitoring and Traceability*" 15-17 April, Firenze, Italy. Congress centre Convitto della Calza, Piazza della Calza.

6. Significant difficulties or delays experienced during the reporting period

The progress of the project has been according to the timetable of the project and no unexpected difficulties or delays have been during the third year. The project was extended for four months and an additional work-in was scheduled.

7. Dissemination of results

All participants contributed to a presentation made by Jörg Oehlenschläger (Federal Research Center for Fisheries, Germany) in Vancouver 2001:

- Jörg Oehlenschläger 2001. Development of multi-sensor techniques for monitoring the quality of fish - MUSTEC. Technological developments in Processing & Products. 4th WORLD FISH INSPECTION & QUALITY CONTROL CONGRESS. Vancouver, Canada, October 24-26, 2001

The MUSTEC participated in the final CA-FQLM Conference in Firenze in April 2002. The proceedings will be published in a book. Ólafsdóttir,G., Di Natale C. and Macagnano A. 2002. Measurements of quality of fish by electronic noses. Final International CA-FQLMConference"*Quality of Fish in the Supply Chain: Labelling, Monitoring and Traceability*"15-17 April, Firenze, Italy.

- Careche, M., Tryggvadottir, S.V., Herrero, A., Schubring, R., Nesvadba, P. Instrumental methods for measureing texture. Final International CA-FQLMConference"*Quality of Fish in the Supply Chain: Labelling, Monitoring and Traceability*"15-17 April, Firenze, Italy.

A presentation at WEFTA 2002 of the results from the haddock experiments that were done in Iceland during the first year of the MUSTEC project. The paper will be published in a journal.

- Ólafsdóttir G., Tryggvadóttir S. V., Einarsson, S. and Lauzon,. H.L. 2002. Prediction of sensory quality of haddock fillets using various instrumental techniques. 32nd WEFTA meeting, May 13th-15th, 2002, Ireland.

Paper on the overall results of the questionnaire was sent to the journal Food Quality and Preferences in 2001. It was not considered suitable for that journal and instead if will be published in the proceeding of the Firenze meeting:

- Bo M. Jørgensen, Guðrún Ólafsdóttir, Soffía V. Tryggvadóttir, Jörg Oehlenschläger, Mercedes Careche, Karsten Heia, Maria L. Nunes, Bianca M. Poli, Corrado Di Natale, Begoña Pérez-Villarreal, Håvard Ballo, Joop Luten, Anita Smelt, Wesley Denton, Paul Nesvadba, Peter Bossier, Tapani Hattula, Göran Åkesson, 2002 A study of the attitudes of the European fish sector towards quality control and labelling. Proceedings of the CA-FQLMConference"*Quality of Fish in the Supply Chain: Labelling, Monitoring and Traceability*"15-17 April, Firenze, Italy

The paper presented by Corrado Di Natale has appeared in the journal Sensors and Actuators

- Corrado Di Natale, Gudrun Olafsdottir, Sigurdur Einarsson, Alessandro Mantini, Eugenio

Martinelli, Roberto Paolesse, Christian Falconi, Arnaldo D'Amico, 2000. Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets. *Sensors and Actuators B: special issue: Proc. of the 8th IMCS* 8th International Meeting on Chemical Sensors, Bazel Switzerland 2-5 July, 2000, Elsevier.

Papers in preparation:

- Ólafsdóttir G., Tryggvadóttir S. V., Einarsson, S. and Lauzon,. H.L. 2002. Prediction of sensory quality of haddock fillets using various instrumental techniques.
- Paper on the overall results of the MUSTEC project and the questionnaire to be published in Trends in Food Science and Technology
- Papers on the overall results of the FreshSense electronic nose, texture and QIM on cod from the three work-ins.

8. Appendix 1

Work-in schedules

1. Sampling plan for Madrid, November, 2000
2. Sampling plan for Hamburg, February, 2002

Work-in schedule in Madrid Nov 2000

Following measurements were done:

QIM: The QIM analysis was done by the MUSTEC participants
Torrymeter, Fishtester: Jörg
Visible/NIR: Karsten and Margarethe.
Colour/Image: Reinhard and Michael
Electronic Nose: Gudrun, Corrado and
TVN -TMA / FIGD (Flow-Injection-Gas-Diffusion) **:** Sigurdur, IFL
Texture: Soffia , Mercedes, Ana, Paul, Bert, Uwe and Reinhard

Fish batches:

Experimental plan

Date	Primary code	Fish	Conditions Temperature	Months frozen	Thawing (days)	Days on ice
Tu. 14th	A	Hake	-20°C	12	3	0
Tu. 14th	B	Hake	-20°C	4	3	3
We. 15th	C	Hake	-20°C	8	3	0
Th. 16th	D	Hake	-20°C	0	-	0
Th. 16th	E	Hake	-20°C	4	3	0
Fr. 17th	F	Hake	-20°C	10	3	0
Fr. 17th	G	Hake	-20°C	4	3	6
Mo. 20th	H	Hake	-20°C	4	3	9
Mo. 20th	I	Hake	-20°C	2	3	0
Tu. 21st	J	Hake	-20°C	18	3	0
Tu. 21st	K	Cod (NO)	-30°C/-40°C	7-8	3 (?)	0

Code A-J each consisted of 15 fish.
Five (01-05) were used as whole fish for QIM, FT and colour measurements.
Five (06-10) were used as whole fish for texture measurements.
Five (f01-f05) were filleted.

Code K consisted of 10 fish (as in Tromsø).
Five (01-05) were used for QIM and other measurements on whole fish.
Five (f01-f05) were filleted.

Experimental plan MUSTEC Work-in, Hamburg, February 2002

The fish:

Batch	**Comment**	Days in ice
Cod 1 (label A) 7days	Fish from the Baltic sea. Caught in net. Kept alive in the net for 3 to 5 days. Killed and gutted at the same time. Stored for 7 days on ice.	7
Cod 2 (label B)	Fish bought fresh from the market earlier in the morning. Estimated "days on ice" is 1 (could be 2).	1
Cod 3 (label C)	Just caught (3.5 hours old). I.e. "days on ice" = 0	0
Cod 4 (label D)	same as Cod 1	9
Cod 5 (label E)	The fish from batch 3. I.e. "days on ice" = 1	1

9. Appendix 2

Dissemination

1. Ólafsdóttir,G., Di Natale C. and Macagnano A. 2002. Measurements of quality of fish by electronic noses. Final International CA-FQLMConference"*Quality of Fish in the Supply Chain: Labelling, Monitoring and Traceability*"15-17 April, Firenze, Italy.

2. Ólafsdóttir G., Tryggvadóttir S. V., Einarsson, S. and Lauzon,. H.L. 2002. Prediction of sensory quality of haddock fillets using various instrumental techniques. in preparation

3. Careche, M., Tryggvadottir, S.V., Herrero, A., Schubring, R., Nesvadba, P. Instrumental methods for measureing texture. Final International CA-FQLMConference"*Quality of Fish in the Supply Chain: Labelling, Monitoring and Traceability*"15-17 April, Firenze, Italy.

4. MUSTEC Leaflet

Measurements of quality of fish by electronic noses

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Odor is an important attribute to evaluate the freshness of food. Electronic noses can give objective information about the freshness quality of fish by detecting volatile compounds produced during storage. The importance of selecting appropriate sampling conditions for electronic nose will be discussed. The results of measurements of cod stored in ice using two electronic noses will be compared to sensory analysis using the Quality Index Method (QIM). The two electronic noses *LibraNose* and *FreshSense* are based on different sampling procedures and sensor technologies. LibraNose is based on an array of eight thickness shear mode resonators coated with metalloporphyrins. FreshSense is based on four electrochemical sensors: CO, H_2S, SO_2, and NH_3. The data of the two instruments have been integrated using various conventional chemometrics and neural networks techniques. In particular the comparison of the odor evaluation scores of the QIM and the output of the sensors of the different noses will be illustrated.

Key words: electronic nose, cod, freshness, Quality Index Method, Artificial Quality Index

WESTERN EUROPEAN FISH TECHNOLOGISTS´ ASSOCIATION
32nd WEFTA meeting, May 13th-15th, 2002, Ireland
(not to be quoted without prior reference to the authors)

Prediction of sensory quality of haddock fillets using various instrumental techniques

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The value of exported fresh haddock fillets is about 25% of the total value of haddock products in Iceland. The fillets are mainly sold to USA and UK by airfreight and these products are about 17-18% of the total catch. The storage life of fresh fillets is short and different seasons, catching techniques and handling influence the spoilage rate. To verify the freshness of the products it is important for the commercial partners to have access to objective and reliable measurements of freshness quality.

Storage studies were done on haddock from two different catching seasons. Fish was stored whole in ice and fillets at 0-2°C. The changes of various properties of the fillets were monitored for 15 - 18 days using traditional methods (sensory analysis, TMA/TVN and microbial counts) and novel instrumental techniques (electronic nose, conductivity measurements, FIGD (flow injection gas diffusion) to measure TMA and TVN and texture analyser).

The aim of the storage studies was to test the ability of the different instrumental techniques to predict the sensory freshness score of haddock. The results show that the spoilage rate is different in the storage studies of whole fish from the two seasons and the fillets spoil most rapidly. Information about days in ice and microbial counts does not give reliable information about the sensory quality. Partial least squares regression models (PLS) based on data from instrumental measurements show that these could be used to predict the freshness sensory score of haddock.

Key words: fish freshness, electronic nose, texture analysis, flow injection gas diffusion, RT Freshmeter, microbial counts, sensory analysis.

Instrumental methods for measuring texture

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The objective was to evaluate the capabilities of commercial texture analysers (TAXT2*i* SMS Stable Microsystems) and hand-held devices (both commercial and new prototypes) for assessing the quality of fish in terms of their firmness and/or elasticity that could serve, together with other physical instruments, to construct a multi-sensor device. The instrumental data were compared and calibrated against the texture attributes of QIM in fish stored in ice and frozen/thawed fish with known history. The high degree of correlation between a combination of coefficients extracted from compression-relaxation curves and the firmness sensory score from QIM suggests that textural devices could be used in conjunction with other instruments (colour and electronic noses) in the multi-sensor system. This enables to construct the Artificial Quality Index as explained in a separate paper in these proceedings. The hand-held texture meters developed and evaluated in this project showed a potential to be included in portable and inexpensive multi-sensor instruments.

Key words: Fish, multi-sensor, firmness, elasticity, quality.