

**PROJECT REPORT**  
**02 - 01**



**Rannsóknastofnun**  
**fiskiðnaðarins**

**FEBRUARY 2001**

**MULTISENSOR FOR FISH**

- STORAGE STUDIES OF COD  
IN REYKJAVÍK AND TROMSÖ
- TEXTURE, ELECTRONIC NOSE,  
FIGD ANALYSIS, RT-FRESHMETER  
AND SENSORY ANALYSIS

**FAIR CT - 98 - 4076**

**SECOND PROGRESS REPORT**  
**DECEMBER 1999 - NOVEMBER 2000**

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<i>Titill / Title</i>	<b>Multisensor for Fish</b> Questionnaire on Quality Attributes and Control Methods Texture and Electronic Nose to Evaluate Fish Freshness		
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<i>Ágríp á íslensku:</i>	<p>Þessi skýrsla er önnur framvinduskýrsla Rf í Evrópuverkefninu <b>Þróun á margþátta skynjaratækni til að meta gæði fisks</b>. Sex Evrópulönd taka þátt í verkefninu en markmiðið er að þróa nýja mælitækni til ferskleikamælinga á fiski.</p> <p>Á tímabilinu voru tveir á vinnufundir. Fyrsti vinnufundurinn var hjá Rf, en sá seinni hjá Fiskeriforskning í Tromsø. Þátttakendur mættu allir með sín tæki og mældu með reglulegu millibili sýni af þorski sem geymdur var í ís í 17 daga. Í Tromsø var einnig mældur lax og uppþiðinn þorskur. Þátttakendurnir frá Rf stóðu fyrir áferðarmælingum, mælingum á rokgjörnum efnum með rafnefi (FreshSense) sem þróað er á Íslandi, mælingum á reikulum bösum (TVB/TMA) með nýrri aðferð (FIGD; flow injection gas diffusion), leiðnimælingum með íslenska RT ferskleikamælinum og einnig tóku þeir þátt í skynmati með gæðastuðulsaðferð (QIM).</p> <p>Niðurstöður skynmats með gæðastuðulsaðferðinni (<math>R^2=0.98</math>) og mælingar með RT ferskleikamælinum (<math>R^2&gt;0.90</math>) sýndu góða línulega fylgni við daga í ís og niðurstöður tilraunanna í Reykjavík og Tromsø voru sambærilegar. Niðurstöður áferðamælinga sýndu að hægt er að greina breytingar í fiskholdi fyrstu fjóra dagana í ís en eftir það er lítil sem engin breyting. Aðal áferðarbreytingarnar verða í sambandi við dauðastirðnun, hvort sem fiskurinn er að fara inn í dauðastirðnunina eða úr henni. Rafnefið og mælingar á TMA /TVB sýna mestu svörun í lok geymslutímans. Fjölpáttaúrvinnsla (höfuðþáttagreining) á rafnefsgögnum sýna að rafnefið getur nýst til að greina á milli daga í ís á geymslutímanum.</p> <p>Í verkefninu er unnið að þróun mælitækjanna og á árinu voru gerðar endurbætur á áferðarmælingum þannig að fiskvöðvinn skemmdist ekki og næmni FreshSense rafnefsins varð meiri.</p> <p>Úrvinnsla á niðurstöðum með fjölbreytuaðhvarfsgreiningu (PLS partial least squares regression) sýndi að með því að nota samhliða áferðarmælingar og TMA/TVB er hægt að spá fyrir um skynmat (<math>R^2=0.82</math>) og einnig er hægt að nota rafnefið til að spá fyrir um skynmat (<math>R^2=0.97</math>). Frekari þróun á mæliaðferðum og gagnaúrvinnslu er þörf áður en slík mælitækni nýtist saman í fjölskynjara til að meta ferskleika og gæði fisks.</p>		
<i>Lykilorð á íslensku:</i>	<i>fiskur, ferskleiki, skemmd, áferð, rafnef, RT ferskleikamælir, TMA/TVB, FIGD, skynmat QIM</i>		



### *Summary in English:*

This report is the second 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.

During the second year two work-ins took place. The first one was at IFL in Reykjavik and the second at Fiskforskning in Tromsø. All participants brought their own instruments and measured at regular intervals samples from a storage study of fresh cod stored in ice for 17 days. In Tromsø thawed cod and two batches of salmon stored in ice were also measured.

In this report are results from the two work-ins on texture analysis using Stable Micro Systems instrument and conductivity measurements using the Icelandic RT Freshmeter. In addition are results on measurements of volatile compounds using the electronic nose FreshSense developed in Iceland and measurements of TMA/TVB with a new method (FIGD; flow injection gas diffusion). Finally, results of sensory analysis using the Quality Index Method (QIM) are also included.

The sensory Quality Index scores (QI) and the RT Freshmeter measurements show a good linear trend ( $R^2=0.98$  and  $R^2>0.90$ , respectively) with storage time and the results of the two work-ins are very similar. The texture measurements show changes in the beginning of the storage time while the FreshSense measurements and the TMA/TVB analysis have higher signals at the end of the storage time. Principle component analysis (PCA) of the FreshSense data shows that it is possible to discriminate between samples of cod from different storage time.

The FreshSense instrument and the texture measurements are still under development. Improvements and modifications made during the year resulted in better sensitivity of the FreshSense measurements and the texture measurements improved and became less destructive.

Preliminary data analysis using PLS (partial least squares) indicates that it is possible to predict sensory QI scores for cod by using the combined data from texture and TMA/TVB ( $R^2=0.82$ ) or by using the FreshSense data ( $R^2=0.97$ ). Further development of the measurements techniques and data analysis are needed to verify the use of these techniques together in a multi-sensor instrument to evaluate fish freshness and quality.

*English keywords:* **fish, freshness, spoilage, texture, electronic nose, sensory analysis, RT Freshmeter, FIGD, TMA/TVB**

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)

Second progress report - Dec.1999 to Nov. 2000

Multisensor for fish

Storage studies of cod in Reykjavik and Tromsø

Texture, electronic nose (FreshSense), FIGD analysis, RT-Freshmeter and  
sensory analysis

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# 1 Objectives

- To ascertain the requirements of the fish processing industry
- To integrate electronic nose and texture methods into the multi-sensor frame
- To contribute to the formulation of the multi-sensor device
- To disseminate and commercialise the results of the project

The objectives are the same as in the beginning of the project. To fulfill the first objective a questionnaire was sent to all parts of the fish sector to ascertain the requirements of the fish industry for a rapid instrument to monitor the freshness and quality of fish. The results of the questionnaire indicate that all sectors of the fish chain agree that such measurement techniques are needed (Tryggvadottir and Olafsdóttir, 2000).

Continuing efforts have been made to integrate electronic nose and texture measurements into the multi-sensor frame by collecting data from simultaneous measurements of different techniques during storage studies of fish.

# 2 Actions in the project

Actions by tasks are summarised in the following table. During the second year of the project tasks 2.2 simultaneous measurements and task 2.3 formulation of a practical multi-sensor instrument were performed during two work-ins in Reykjavik in Nov. 1999 and in Tromsø in May 2000. Moreover tasks 3.1 and 3.2 about collaboration with fish industry and manufactureres of instruments are currently being explored.

## Timetable of tasks for Partner 2 (Icelandic Fisheries Laboratory)

<i>Sub-task</i>	<i>1st year</i>	<i>2nd year</i>	<i>3rd year</i>
1.1	Identify critical points for quality measurements.		
	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

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

The aim of the work-ins is to generate sufficient data for evaluating the effectiveness of the physical methods of measuring the quality of fish at different stages of storage and processing.

##### **Sub-task 2.2 Simultaneous application of physical methods**

Two work-ins were planned for the second year of the project. All participants came together 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 each measurement technique of Partner 2 is evaluated separately in this report. Moreover, some preliminary data fusion is done on the combined data from the Icelandic techniques i.e. electronic nose, texture analysis, TMA/TVB and RT meter using PCA (principal component analysis). The ability of individual instrumental techniques or combination of the techniques to predict sensory score was also evaluated by PLS (partial least squares) using the Unscrambler<sup>®</sup> software (CAMO A/S).

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

##### **Sub-task 3.1**

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**

The company Bodvaki (Ártorg 1, Saudarkrokur, Iceland) has expressed an interest to exploit the results of the instrumental development and produce a commercial instrument to monitor quality of fish. Bodvaki is owned by TM Software which is the biggest information technology company in Iceland specializing in IT solutions for the fish and food industry.

Currently the electronic nose FreshSense and the FIGD instrument are under development and both instruments are being used in various research projects at IFL to monitor freshness and onset of spoilage of fish during storage. The research projects are in close collaboration with the fish industry.

Further developments of the electronic nose instrument to improve the sensitivity and to make it more user friendly in industry applications have been done in collaboration with the Icelandic company Bodvaki.



**Figure 1. Work-in in Reykjavik in November 1999. Dr Paul Nesvadba informs Geir Arngrímsson the manager of Bodvaki about the function of the handheld texture meter developed in Aberdeen.**

#### 4 Research activities during the second reporting period carried out by partner 2

The participants from the Icelandic Fisheries Laboratories measured texture, volatile compounds and conductivity 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 (Artorg 1, Saudarkrokur; Iceland) and Icelandic Fisheries Laboratories.
- Volatile bases: TMA and TVB-N measured with a new technique. *Instrument:* FIGD (Flow Injection / Gas Diffusion). A prototype developed in the EU project FAIR CT97 3253
- Electrical measurement. *Instrument:* RT-Freshmeter (Rafagnataekni, Reykjavik, Iceland). Commercial instrument developed in Iceland, but it is not in production any more.
- Sensory evaluation using QIM (Quality Index Method).



## 4.1 Work-ins in Reykjavik 1999 and in Tromsö 2000

Two work-ins were carried out during the second year 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 cod and also a few samples of salmon stored in ice. The first work-in was hosted by the Icelandic Fisheries Laboratories in Reykjavik in November 1999 and the second work-in was at the Fiskeriforskning in Tromsö in May 2000.



**Figure 2. MUSTEC participants performing sensory evaluation using the QIM during the work-in in Reykjavik.**

## 4.2 Materials and Methods

*Reykjavik, November 12 - 20, 1999*

Simultaneous measurements were done on fresh cod which was stored in ice for 17 days. Three batches of fish (A, B,C) were obtained from small boats from Reykjanes, from a fishing ground in Faxaflói, southwest of Iceland. The first batch was caught on Monday 1.Nov (A). The second batch was caught on Monday 8.Nov.(B) and the last batch was caught on Tuesday 16.Nov. (C).

The fish was caught with longline, gutted, iced and brought to IFL the following day (Day 1). The fish was stored iced in boxes at 0-2°C until analysed on days 1,2,3,4, 7,9,11, 15 and 17. For each storage time 5 fish were measured as whole fish and 8 fishes as fillets (see sampling plan in Appendix 1).

*Tromsø, May 23 - 30, 2000*

Simultaneous measurements were done on fresh cod in the Tromsø work-in experiment. The cod was caught in the Norwegian Sea, at fishing grounds outside Tromsø two months prior to the work-in. The fish was kept alive in a net pen in an aquaculture station situated half an hour drive from the institute. In preparation for the experiment the fish was slaughtered once a week to make up the storage times 0, 1, 3, 5, 8, 11, 14, and 17 days in ice (see sampling plan in Appendix 1). For the texture analyses an extra batch of cod, which had been stored in ice for 2 days, was measured. Two batches of salmon, 1 day and 13 days in ice, were measured with all methods except texture. The salmon was not measured for texture due to malfunction of the texture analyser at the time of the measurement. One batch of frozen thawed cod was measured. The cod was frozen in rigor at -40 °C (1 day) and then stored at -30 °C for 47 days and then thawed in air for two days before it was measured. For each storage time 5 fish were measured.

#### **4.2.1 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 aim was to have the penetration distance 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 ( $d_1-d_2$ ).  $d_1$  is the distance after a 100g force had been applied for 30 sec and  $d_2$  is the distance of permanent deformation which was measured after 30 sec of relaxation.



**Figure 3. Stable Micro Systems texture analyser, model TA.XT2i**

### *Probe and calibrations*

#### Puncture test (firmness test)

- Ebonite cylinder probe, 10 mm in diameter (P/10)
- Pre test speed 2,0 mm/s; speed in sample 0,8 mm/s
- Strain (distance) 55%, for flesh side (Reykjavik)
- Strain (distance) 40%, for flesh side (Tromsö)
- Strain (distance) 30% for skin side (Reykjavik)

#### 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).

#### *4.2.1.1 Sample preparation for the texture analysis*

##### *Reykjavik*

The left unskinned fillets were used in the texture measurement. Each fillet was both measured on the flesh side and on the skin side. The test done was the puncture test with 55% strain on the flesh side and 30% strain for the skin side. The flesh side of the fillet was laid down flat on the texture analyser and the probe was penetrated into the fillet four times. First 3 cm from the neck-cut and again about 6 cm from the neck-cut. Parallel penetration was done from both location about 3-4 cm apart. The values for the four measurements were averaged and given as one firmness (hardness) value for each fillet. The measurements on the skin side were four penetrations along the fillet starting about 2-3 cm from the neck-cut, down along the fillet 8-10 cm apart. Again the average of the four measurements were given as one firmness (hardness) value for each fillet. The creep test was not used in the Reykjavik work-in

##### *Tromsö*

As in the Reykjavik work-in the left unskinned fillets were used for the texture measurement in Tromsö. This time the fillet was only measured on the flesh side. The procedure for the puncture test on the flesh side of the fillet was the same as on the flesh side in Reykjavik except that the strain (%) was decreased to 40% as it had been observed that the 55% penetration was sometimes a little destructive. The creep test included four measurements along the fillet starting about 2-3 cm from the neck-cut, down along the fillet 8-10 cm apart. The creeping distance was calculated and the average of the four distances was given as a result for each fillet.

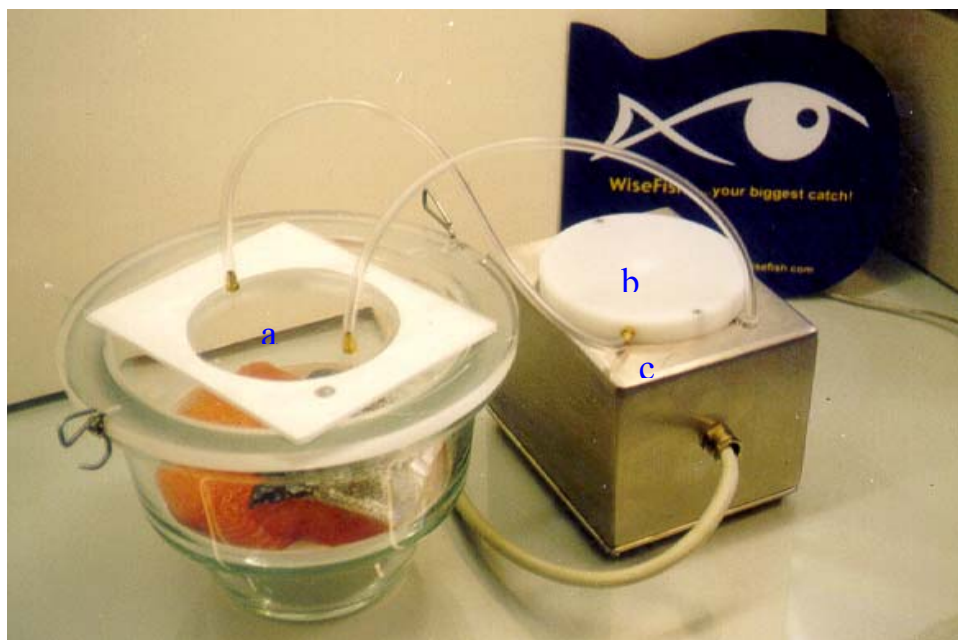
#### **4.2.2 Electronic nose measurements**

Electronic nose measurements were performed using a gas sensor instrument called "FreshSense", developed by the Icelandic Fisheries Laboratories and Bodvaki-Element Sensor Systems (Artorg 1, 550 Saudarkrokur, Iceland). The instrument is based on electrochemical gas sensors (Dräger, Germany: CO, H<sub>2</sub>S, and SO<sub>2</sub>; City Technology, Britain: NH<sub>3</sub>A7AM). The measurement technique, described earlier by Tryggvadóttir and Olafsdóttir (2000), was used in Reykjavik, but major modifications were done before the Tromsö work-in to improve the sensitivity of the measurements.

*Modifications of the FreshSense.* A smaller sampling container (2,3L) is used in the new version (instead of 5.2L) to increase the concentration of volatiles in the headspace. A dynamic sampling system has been added to the instrument instead of using a static headspace sampling analysing directly the headspace of fish stored in the closed glass container (see Figure 4). The headspace from the sampling container is transported with a pump 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. Moreover, a new Labview measurement and data analysis software has been installed. Measurements are taken every 10 seconds for 10 minutes. The reported value (current) is the average of last three measurements of the 10 minutes measurement cycle

#### *4.2.2.1 Preparation and measurements of fish samples*

The fish was filleted and the skin removed. Each fillet was measured separately (300-500g). In Reykjavik additional measurements were done on the heads. Two heads were measured simultaneously (approximately 1500g). The samples were placed in the glass container and temperature of the samples was measured before the container was closed.



**Figure 4.** The new version of the electronic nose FreshSense with the electrochemical gas sensors (CO, H<sub>2</sub>S, SO<sub>2</sub>, NH<sub>3</sub>) developed by Bodvaki and Icelandic Fisheries Laboratories. A pump ensures the transport of headspace from the closed glass sampling container (a) to the measurement chamber (b).

#### *4.2.2.2 Measurements of standard compounds*

Measurements of standard compounds are done routinely at IFL to monitor the performance of the instrument. Measurements using different concentrations of aqueous ethanol solutions (50, 100, 200 ppm) were selected in this report as an example to demonstrate the reproducibility of the measurements from May 2000 and November 2000. The value reported is the average of the last three measurements minus the average of 6 values (1 minute) before the measurement starts.

### 4.2.3 TMA and TVB-N measured with FIGD (Flow Injection / Gas Diffusion).

#### 4.2.3.1 Determination of TVB and TMA using the Flow Injection/ Gas Diffusion (FIGD)

The FIGD analysis was done according to Capillas & Horner, 1999 and Capillas *et al.* (2000). Six standards of ammonia (in the case of the TVB determination) or TMA (in the case of the TMA determination) in the range of 0-200 micromoles per litre (approximately equivalent to 0-3 mg TVB or 0-10 mg TMA per 100g fish flesh) were prepared by taking appropriate dilutions of a 0,5 mM stock solution of ammonium chloride or trimethylamine crystalline hydrochloride in 7,5% trichloroacetic acid (TCA) solution. 100 microliter quantities of these standards (and samples) were injected into the FIGD manifold (a Rheodyne 5020 low-pressure injection valve supplied by Anachem, Luton with a 100-microliter sample loop), which was then closed.

#### Preparation of the sample:

One part of minced fish muscle was blended with two parts 7,5% TCA solution and filtered through Whatman No. 1 paper.

#### Determination of TVB:

The flow of 1,0 M NaOH (1 ml/min) from the peristaltic pump (Ismatec, 4-channel with variable flow rate) carries the injected liquid (standard or sample) through the mixing coil alkalising it and releasing its contained nitrogen in the form of ammonia gas. On flowing through the gas diffusion cell (a laboratory-built gas diffusion cell with channel dimensions 240mm x 1,5mm x 0,2 mm with a microporous, chemically inert and acid resistant PTFE membrane RS No. 8003525) this released ammonia passes through the gas permeable membrane into a 0,3 g/L (pH 6,5) solution of bromothymol blue (BTB) indicator flowing from the peristaltic pump. The colour change caused in the indicator produces a proportionate response from the detector; a photometer incorporating a red light-emitting diode connected to a Perkin-Elmer 56 recorder.

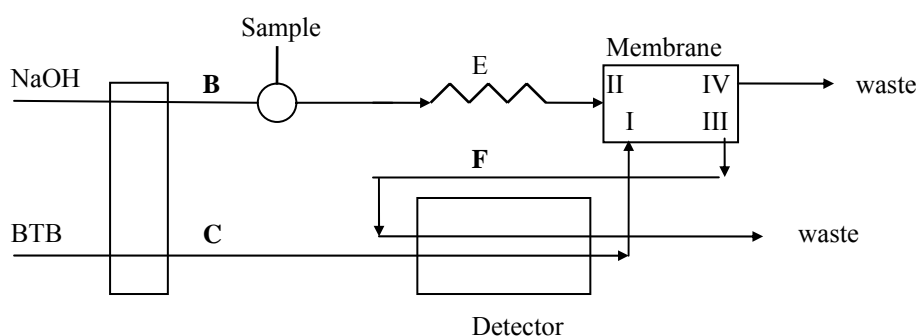
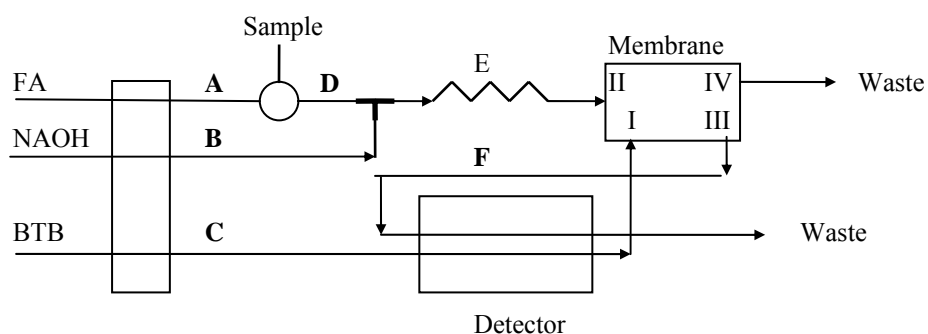


Figure 5. A schematic diagram of the Flow Injection/Gas Diffusion apparatus used for the determination of TVB-N.

#### Determination of TMA:

The third feed through the peristaltic pump of 20% formaldehyde solution (the carrier solution) met the 0,1 ml of injected sample, from which sequesters all the non-TMA volatile based, prior to alkalisation. This third feed is disconnected for the TVB determinations.



**Figure 6.** A schematic diagram of the Flow Injection/Gas Diffusion apparatus used for the determination of TMA-N.



**Figure 7.** The new version of the Icelandic FIGD (IFL-FIGD)

#### 4.2.3.2 Determination of TVB by steam distillation of TCA extract

WEFTA Codex Method (Vyncke *et al.* 1987). One hundred grams of fish were mixed with 200 ml of trichloroacetic acid (TCA). Twenty-five ml of the TCA extract, 6 ml of NaOH (or enough to make the pH of the solution 11) was transferred into a Kjeldahl flask. The ammonia of the solution was liberated by steam distillation (on Gerhardt distillator) into a receiver beaker containing 20 ml of 3% boric acid and a few drops of mixed indicator. The distillation was carried on until 100 ml of distillate had been collected. The titration end point was a colour change from green to grey at pH 5.

#### 4.2.3.3 Determination of TMA by conventional method

Determination of TMA was done by the Dyer method, modified by Tozawa (1971).



### 4.2.3 RT-Freshmeter ; electrical measurement.

The RT-Freshmeter is an Icelandic version of the idea of measuring electrical properties to determine fish freshness. Due to enzymatic autolytic activity that takes place in post-mortem fish the cell membrane will disrupt and the fish get gradually spoiled. The intracellular electrically conductive electrolyte will leak into the intercellular space and the electrical resistance [R] and the capacitance [C] in the tissue will decrease (Oehlenschläger and Nesbvadba, 1997). It has been shown (Jason and Richards, 1975) that combination of C and R give good correlation with fish freshness sensory scores. The RT-Freshmeter Type RT-SE, that was developed and produced by Rafagnataekni - Electronics Ltd, Sidumula 1, Reykjavik, Iceland, makes frequent measurements while being drawn across the side of the fish and integrates these readings to an average. Five whole cods were measured with the RT meter at all the different storage days. The procedure was the same for the Reykjavik and the Tromsø work-in.



Figure 8. The Icelandic RT-Freshmeter

### 4.2.4 pH measurements

pH was measured at room temperature, with an Orion Ag/AgCl combination electrode (TRIODE TM pH electrode) connected to an Orion model 290A pH meter. The pH of fish mince was determined at 20-22°C by mixing 20 g sample with 80 ml of distilled water on a magnetic stirrer and measuring the pH in the slurry after 5-10 min equilibration.

### 4.2.5 Sensory analysis

Quality Index Method (QIM) for whole cod (Bremner, 1985; Martinsdottir, 1995) was performed in the two work-ins by all the Mustec participants, about 15 people.

#### 4.2.6 Data analysis

Microsoft Excel 97 was used to calculate means and standard deviations for all multiple measurements and to generate graphs. Multivariate analysis was performed by the Unscrambler<sup>®</sup> 7.5 software package (CAMO A/S). Principal component analysis (PCA) was performed on all data from the Reykjavik and Tromsø experiments to study the main variance in the data set. The main purpose was to study the ability of the instrumental techniques (texture, electronic nose and FIGD) 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 instrumental techniques. 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

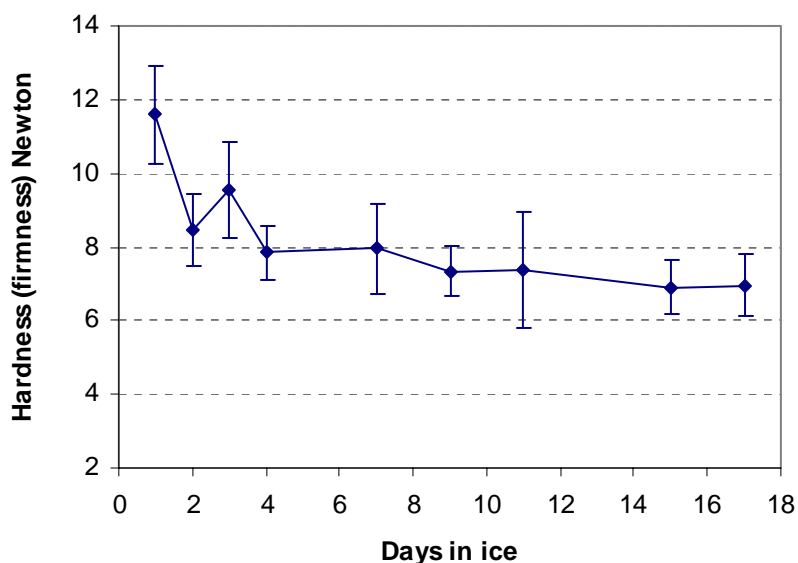
Following are the results of the texture measurements, electronic nose, FIGD analysis, RT-Freshmeter and sensory analysis (QIM) from the work-ins in Reykjavik and Tromsø.

#### 4.3.1 Texture measurements

##### 4.3.1.1 Results of texture measurements from the work-in in Reykjavik in Nov. 1999.

##### *Puncture test measured as force (N)*

The puncture measurements (Figures 9 and 10) show pronounced changes in firmness during the first four days of storage. After day four and throughout the storage period the firmness values change very little, but a decreasing trend in hardness is observed.



**Figure 9.** Puncture test (55% strain), a texture measurement on the *flesh side* of cod fillets. The texture value for each storage day is an average of five measured fishes.



As expected the measurements on the skin side with 30% strain show lower values than the 55% strain measurement on the flesh side otherwise the two measurements give similar patterns. The values for the skin side do not show as well defined decrease in firmness for the first few days as they do for the flesh side. The skin might give the measurement more resistance i.e. more elasticity than when penetrated into the flesh. The values for the skin side give also larger standard deviation, which could indicate less reliable measurement.

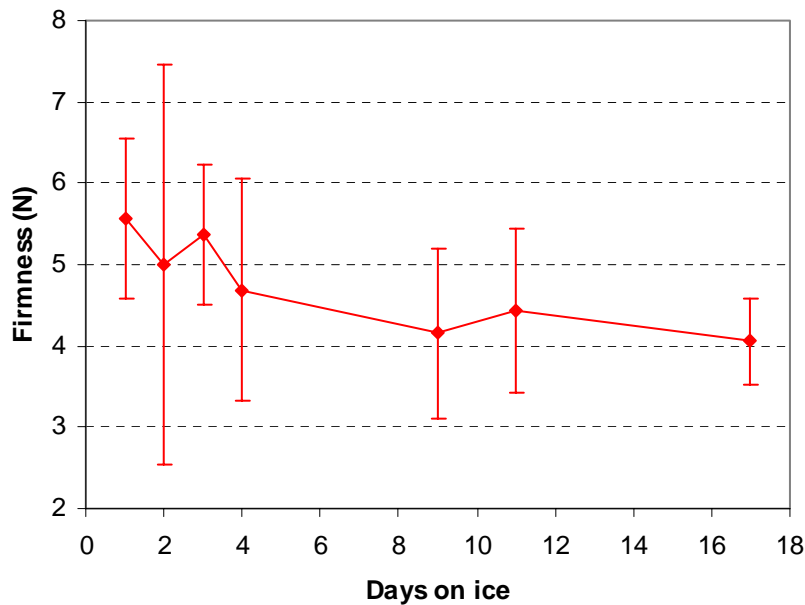


Figure 10. Puncture test (30% strain), a texture measurement on the skin side of cod fillets. The texture value for each storage day is an average of five measured fishes.

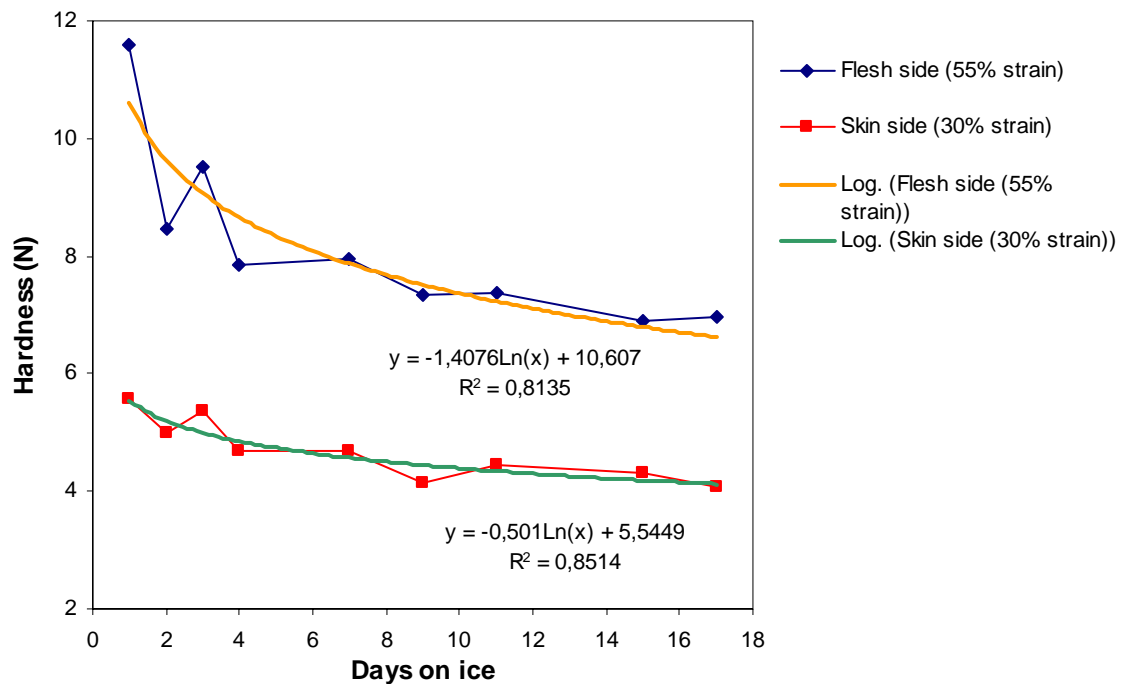


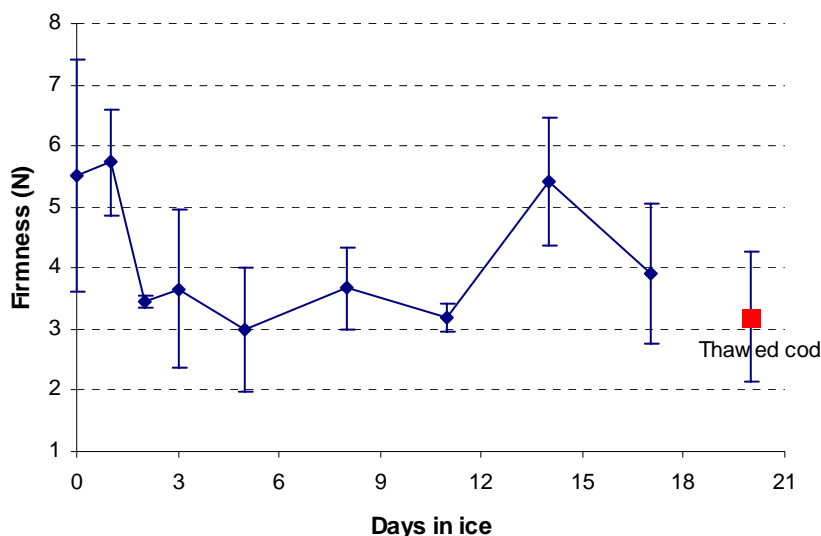
Figure 11. Non-destructive measurements using a puncture test on cod fillets. A logarithmic trendline with its equation and R<sup>2</sup> factor are shown for both the time periods

In Figure 11 the data from the puncture measurement is shown together and a logarithmic trendline is shown for both the flesh side and the skin side. The fluctuation in the measurements at the beginning of the storage influence the values for  $R^2$  (0,81 and 0,85 for flesh and skin, respectively), especially for the measurement on the flesh side.

#### 4.3.1.2 Results of texture measurements from the work-in in Tromsø in May 2000.

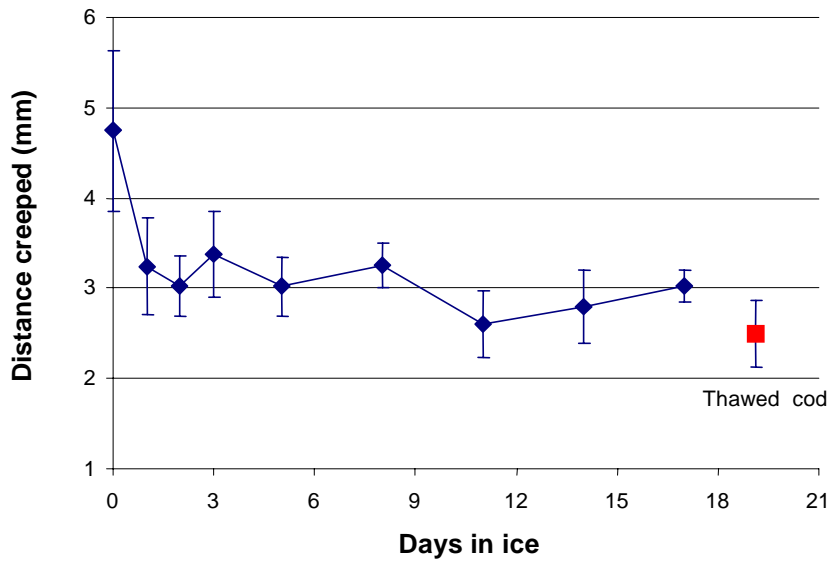
##### *Puncture test (firmness test) measured as force (N)*

The values from the nondestructive puncture measurement (40% strain) from the Tromsø work-in is shown in Figure 12. The result is very similar to the puncture test result from the Reykjavik work-in i.e. apparent changes in firmness during the first few days which level off during extended storage. However, the high value for the measurement on day 14 is unexpected. The Tromsø, cod that was measured after ice storage of 14 and 17 days, came from the same batch which was slaughtered on the 12<sup>th</sup> of May 2000. This batch might have been handled differently during the slaughtering, gutting and icing process. The average value for thawed cod is separately added into Figures 12 and 13 for comparison. The texture for the frozen thawed cod show similar values as the lowest values for fresh the cod.



**Figure 12** Non-destructive texture measurement (Puncture test, 40% strain) on the flesh side of cod fillets. The texture value for each storage day is an average of five measured fish. The value for one batch of frozen thawed cod is added into the figure for comparison (independent of days in ice).

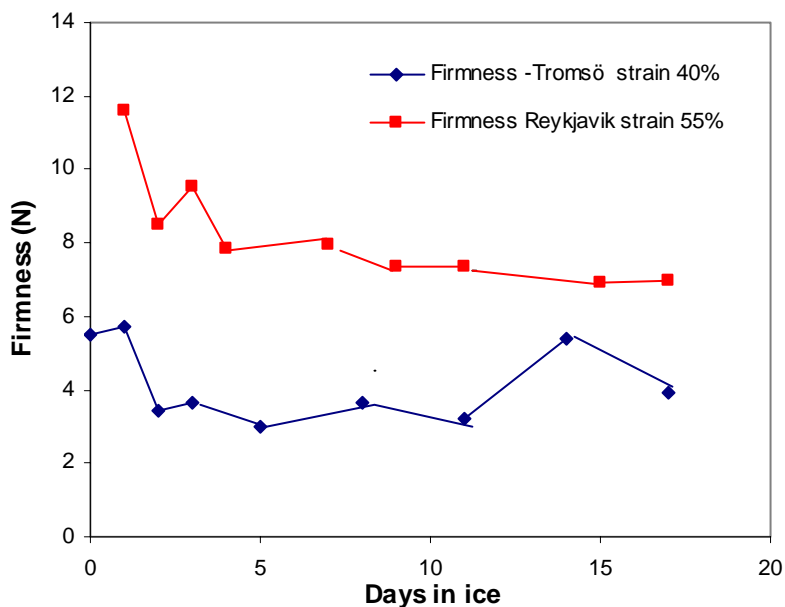
Figure 13 shows the values for the creep test. Although a different test is used, the measurement outcome is very similar to the puncture test i.e. apparent change in texture for the first few days in ice and little change after that. The creep test gives the indication of the viscoelastic nature of the product. A product that flows will have a greater difference in distance than a very elastic (less creeping) product. Therefore from this it can be interpreted that the fish muscle flows when it is in rigor and it gets more elastic as the storage in ice continues.



**Figure 13.** Creep test measurements on cod fillets stored for 17 days in ice. Value from creep test for one batch of frozen thawed cod is added into the figure for comparison (independent of days in ice).

**4.3.1.3 Combined texture results from the Reykjavik and Tromsö work-ins**

When comparing the puncture test (firmness test) values from the two work-ins (Figure 14) it can be seen that the pattern is very similar. The strain used in the Reykjavik work-in was 55% but 40% in the Tromsö work-in and this explains the difference in force values. The overall trend is a decreasing firmness during the first four days which levels off during extended storage. As mentioned before the high value on day 14 in the Tromsö work-in is unexpected and can not be explained.



**Figure 14.** Firmness (puncture) measurement on fresh cod measured at two work-ins in Reykjavik Nov1999 and in Tromsö May 2000.

### 4.3.2 Electronic nose measurements

Modifications were done after the Reykjavik work-in to improve the sensitivity of the FreshSense instrument. A smaller sampling container (2.3L instead of 5.2L) was used in Tromsö to increase the sample/headspace ratio and thus increase the concentration of volatiles in the headspace. Higher signals of the sensors were observed in the Tromsö experiment and the instrument was more able to discriminate samples from different storage days.

#### 4.3.2.1 Reproducibility of FreshSense measurements -Results of standard compounds measurements

The reproducibility of the FreshSense measurements is monitored routinely by measuring standard compounds. In Table 1 are results of repeated measurements of aqueous solutions of ethanol to demonstrate the repeatability and reproducibility of the measurements. The results show that the response of the the CO sensor is higher and RSD% lower in May 2000 than in November. This indicates more sensitive and precise measurements. For direct comparison with the fish measurements it would be more reliable to measure lower concentrations of ethanol solutions that would give similar intensities of sensor responses as the fish does during the first days of storage (i.e.100 - 300 nA). Further studies need to be done with lower concentrations to determine the limit of detection for these measurements.

**Table 1. Repeatability and reproducibility of CO sensor responses to different concentrations of aqueous ethanol solutions**

	Conc (ppm)	CO response (nA)			Average	stdv	RSD (%)
22-Nov-99	50	470	533	537	513	38	7,3
old instrument	100	1003	1043	1040	1029	22	2,2
$y = 7,4x + 201,5$ $R^2 = 0,9818$	200	1593	1677	1687	1652	52	3,1
06-May-00	50	580	587	597	588	8	1,4
new version	100	993	1010	1010	1004	10	1,0
$y = 8,52x + 158$ $R^2 = 0,9999$	200	1843	1820	1927	1863	56	3,0

**Table 2. Repeatability of CO sensor measurements of cod samples during the storage studies in Reykjavik and Tromsö (average, standard deviation and relative standard deviation)**

Days	Reykjavik			November 1999 (n=8)			Tromsö May 2000 (n=5)			
	CO heads	stdev	RSD%	CO fillets	stdev	RSD%	Days	CO fillets	stdev	RSD%
1	96,7	6,7	6,9	85,0	5,9	7,0	0	303,8	25,6	8,4
2	88,9	3,8	4,3	80,8	5,8	7,2	1	236,0	13,9	5,9
3	113,3	10,7	9,4	108,3	9,4	8,7	3	341,8	15,2	4,5
4	96,7	5,8	6,0	95,4	6,7	7,0	5	663,3	152,1	22,9
7	131,1	15,0	11,5	141,7	16,4	11,6	8	364,2	56,6	15,5
9	158,9	45,4	28,6	143,8	34,9	24,3	11	495,1	137,4	27,8
11	336,7	75,4	22,4	127,9	15,6	12,2	14	717,5	118,4	16,5
15	617,8	76,2	12,3	253,3	36,7	14,5	17	813,1	107,1	13,2
17	611,1	175,3	28,7	240,8	63,7	26,5				

Table 2 shows the relative standard deviation of the fish measurements. The %RSD appears to increase with increased sensor response or days of storage and %RSD is higher for fish measurements than the standard ethanol solution. Here the effect of small variation in sample weight and also slight differences in the temperature of the individual fish samples may influence the precision of the measurements. Temperature of the samples was measured before they were put into the sampling containers and the temperature varied from 5 to 10°C. It was difficult to control the temperature of the fillets during the work-ins because the samples were used for more than one technique and the temperature of the samples increases rapidly when kept at room temperature. Earlier results have shown that temperature does effect the responses of the sensors and careful monitoring of temperature is needed during the measurement (Tryggvadóttir and Olafsdóttir, 2000). The ideal situation is to have a temperature controlled sampling system, but this is costly and is not considered a feasible alternative for a low cost instrument. Therefore, for meaningful comparison of samples they must have the same temperature during the measurements. Further studies are needed to verify these effects and more careful control of sampling conditions may be needed to get better repeatability. Further statistical analysis of the data are needed to determine if differences between storage days are significant.

#### 4.3.2.2 *Results of FreshSense electronic nose measurement in Reykjavik and Tromsø work-ins*

The aim of the storage studies was to investigate the possibility to use the electronic nose measurements to detect freshness and onset of spoilage of cod. Measurements were done on both fillets and heads in Reykjavik, but in Tromsø only the fillets were measured. Similar overall trend is observed in both experiments and the response of the CO sensor to the headspace of both fillets and heads increases with storage time, The intensity of the CO sensor responses is lower for the fillets in the Reykjavik experiment (Figure 15 (note the CO sensor is on the secondary y-axis)) than in Tromsø. The reason for this is the smaller sampling container used in Tromsø. The responses of the NH<sub>3</sub> and SO<sub>2</sub> sensors start to increase after 11 days of storage for the heads (Figure 16) but their responses are very low for the fillets in both experiments. This is in agreement with earlier measurements of haddock stored in ice (Tryggvadottir and Olafsdóttir, 2000).

The CO sensor has the highest response in all cases and starts to increase during early storage but appears to level off after 15 days of storage in the Reykjavik experiment for both fillets and heads. This was also noticed for haddock in earlier experiments (Tryggvadottir and Olafsdóttir, 2000) and is most likely related to the availability of substrate for production of microbial metabolites (Lindsay *et al.* 1986).

In the Tromsø experiment the CO sensor showed a very high response on day 5 (Figure 17). This is unexpected and it is believed that some type of contamination must have occurred. The CO sensor is known to be very sensitive to poisoning effects from the environment. It is not clear if chemical contamination in the lab was influencing the response, but use of solvents or detergents close to the instrument is known to poison the sensor. Therefore, the data from day 5 has been omitted in the PCA data analysis.

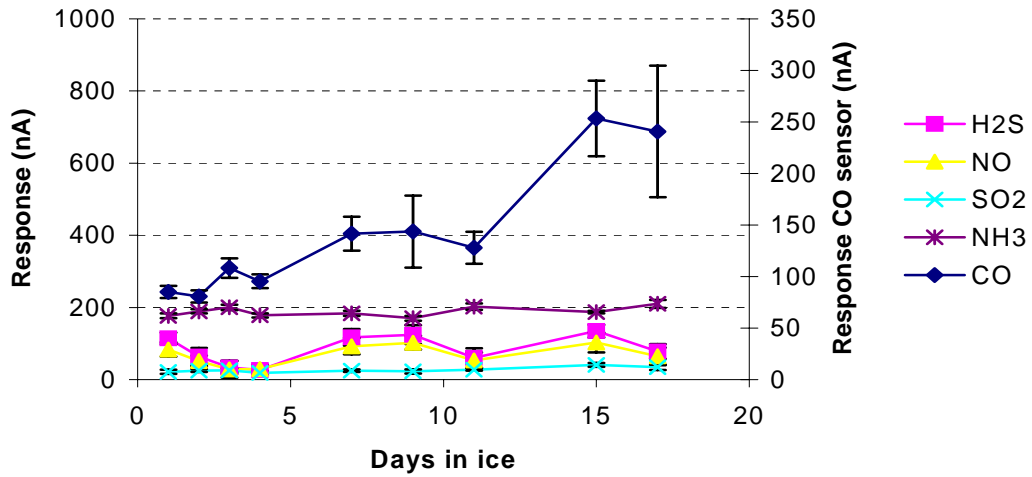


Figure 15. Electronic nose (FreshSense) measurements of cod fillets in Reykjavik, November 1999

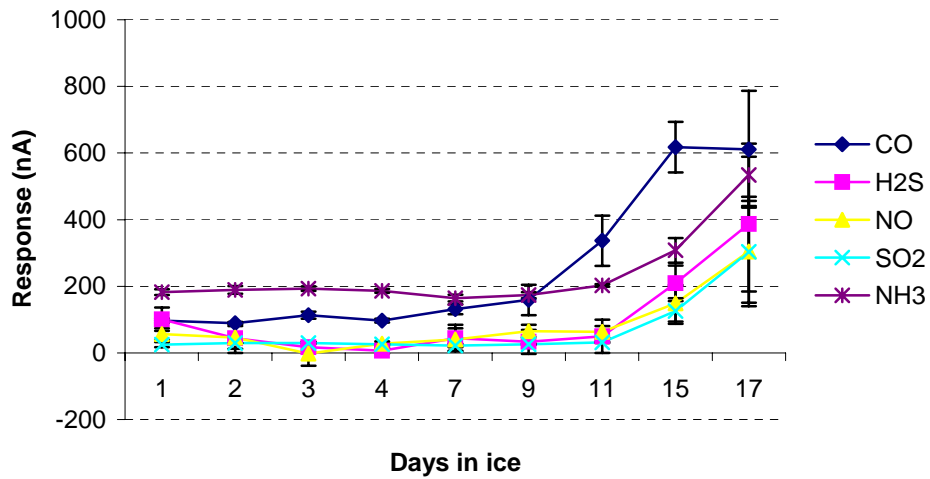


Figure 16. Electronic nose (FreshSense) measurements of cod heads in Reykjavik, 1999

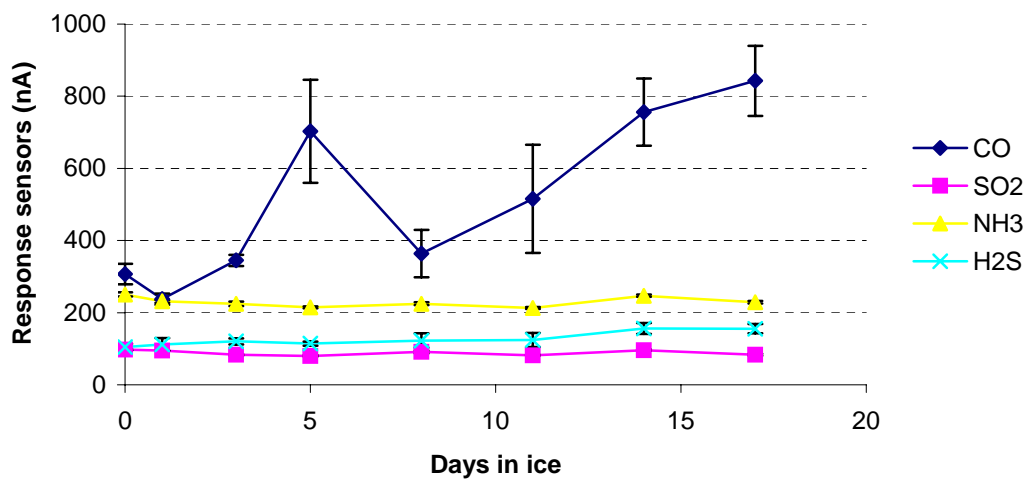
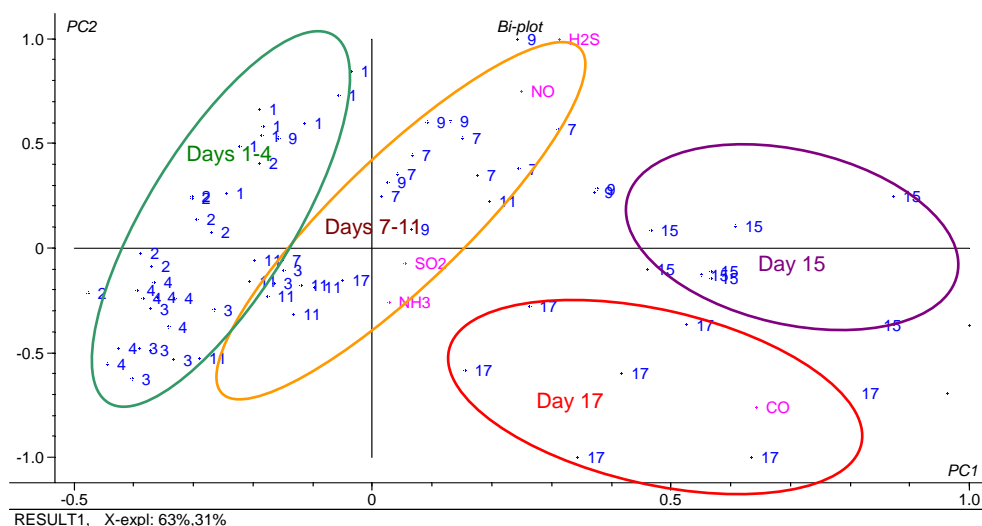


Figure 17. Electronic nose (FreshSense) measurements of cod fillets in Tromsø, May 2000

#### 4.3.2.3 PCA analysis of electronic nose data from the storage studies in Reykjavik and Tromsö

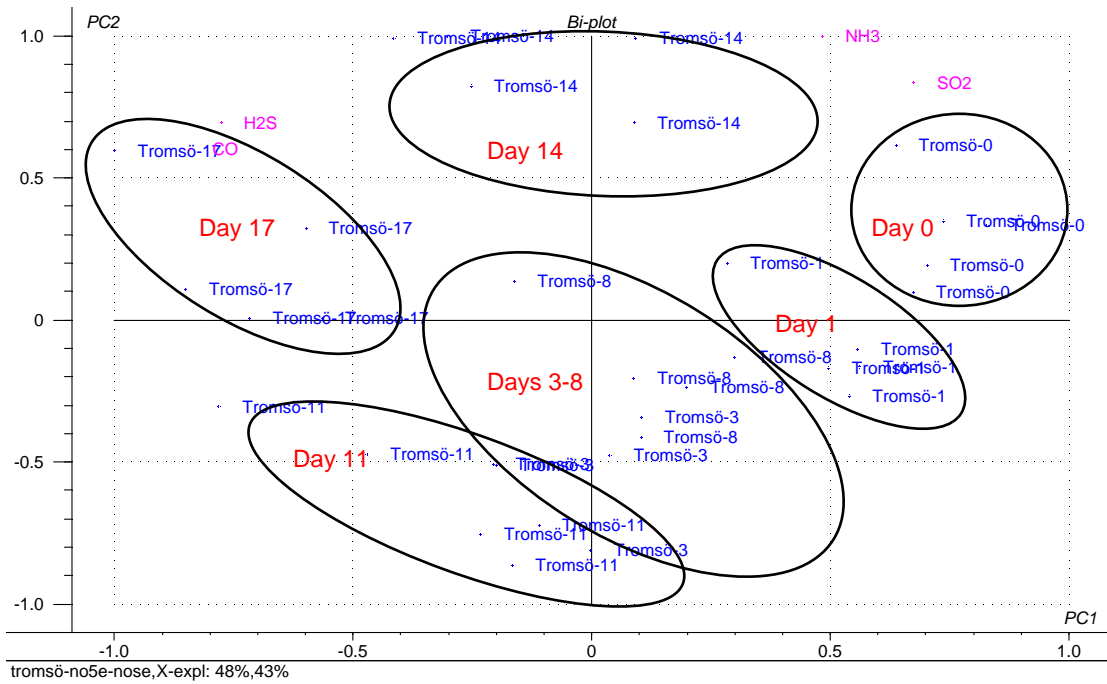
Principal component analysis (PCA) was performed to study the trend in the data set and see if samples could be discriminated based on spoilage level expressed as days of storage. In all PCA runs two principal components and full cross validation were used.



**Figure 18.** PCA biplot of FreshSense measurements of cod fillets after storage in ice in Reykjavik experiment. Sample scores are shown in blue and labeled with storage day. The variable loadings are shown in pink (CO, H<sub>2</sub>S, NO, SO<sub>2</sub> and NH<sub>3</sub> sensors).

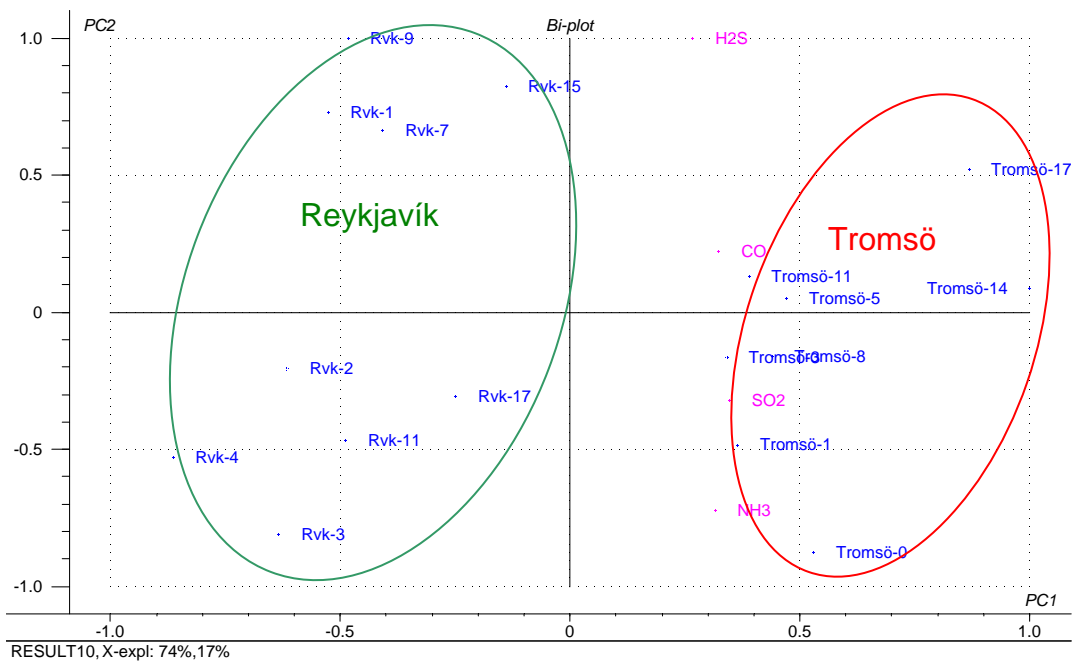
PCA biplot of the electronic nose data for fillets from the storage experiment in Reykjavik is shown in Figure 18. Samples are grouped together according to days of storage. The first two PCs describe 63% and 31% respectively, of the variation of the samples. The samples from days 1 and 4 are grouped together on the left side of the plot and the spoilage level or days of storage increases from left to right. The CO sensor is mainly influencing the first PC and the grouping of samples according to storage time is evident. The samples from day 15 had the highest response for the CO sensor and are therefore located furthest to the right on the plot.

Figure 19 shows the results of PCA analysis of the FreshSense data from Tromsö and better discrimination is noticed for the first days of storage than in the Reykjavik experiment. This may be because the sampling system has been improved and the technique is more sensitive. PC1 and PC2 explain 48% and 43% respectively of the variation in the data. The spoilage level of samples increases from right to left and the CO and H<sub>2</sub>S sensors are mainly influencing this trend. It appears that very slight decrease in the responses of the NH<sub>3</sub> and SO<sub>2</sub> sensors contribute to the grouping of the first days of storage. Here it should be emphasised that only very slight difference in low responses of these sensors appear to influence the PCA model considerably.



**Figure 19.** PCA of FreshSense measurements of cod fillets during storage in ice in Tromsø experiment. Samples scores are shown in blue and labeled with storage day. The variable loadings are shown in pink (CO, H<sub>2</sub>S, SO<sub>2</sub> and NH<sub>3</sub> sensors).

Direct comparison of the FreshSense data from the two experiments can not be done since modifications of the instrument were done between the experiments. This is illustrated in Figure 20 showing a PCA of the combined data from Reykjavik and Tromsø. The data do not overlap as it ideally should do if the measurement technique and the spoilage pattern was the same.



**Figure 20.** PCA of FreshSense measurements of cod fillets from storage studies in Reykjavik and in Tromsø. Samples scores (averages) are shown in blue and labeled with storage day. The variable loadings are shown in pink (CO, H<sub>2</sub>S, SO<sub>2</sub> and NH<sub>3</sub> sensors).



#### 4.3.2.4. *Conclusions of electronic nose measurements*

The results of the electronic nose measurements of cod from Reykjavik and Tromsø show the same overall trend. The CO sensor has the highest response and the response increases during storage. The CO sensor is sensitive to short chain alcohols (i.e. ethanol) and aldehydes that form during storage. The response of the CO sensor levels off at advanced stages of storage. The responses of the NH<sub>3</sub> and SO<sub>2</sub> sensors are very low for the fillets, but increase in the responses of these sensors is noticed for the cod heads 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.

*Improvements and modifications of the FreshSense instrument:* The use of a smaller sampling container resulted in more sensitive measurements and the FreshSense data from Tromsø, of cod stored in ice, shows better discrimination between storage days than the Reykjavik data.

The main *limitation* when using the FreshSense instrument in the fish industry is the sensitivity of the sensors to contamination from the environment. This is not surprising since these sensors are actually designed to detect hazardous gases such as carbonmonoxide and ammonia. Therefore, the instrument has to be in an environment free of any contamination such as solvents, chemicals, detergents and also exhaust from cars.

*Further development of the FreshSense instrument:*

Sampling conditions need to be standardised further including better control of temperature of samples. Further studies need to be done to determine the limit of detection for these measurements. Slightly different sample weights and differences in temperature of the individual fish samples influence the sample/headspace ratio and affect the precision of the measurements. Further studies are needed to verify these relations and more careful control of sampling conditions are necessary to improve the repeatability and the long term reproducibility of the measurements.

The *long term reproducibility* of the measurements has been monitored during the last year by measuring standard compounds and appears to be satisfactory for the concentration range selected. However, measurements of standard samples with lower concentrations are needed to study further the sensitivity of the sensors and the influences of different sample/headspace ratio.

### 4.3.3 TMA and TVB measurements

Figure 21 shows the changes in TMA and TVB during storage of whole cod in ice. The TVB values were determined by the FIGD technique and the WEFTA Codex method, using steam distillation on TCA fish extract (Antonacopolus and Vyncke, 1989). Earlier studies have shown that TVB values, measured by the FIGD technique, gave on the average 65% lower values than was obtained when TVB was measured by the WEFTA Codex method (Einarsson, 2000). This was especially the case when the "white fish" species, cod and haddock, were measured but the difference between these techniques does not seem to be as much when TVB was measured on shrimp and herring.

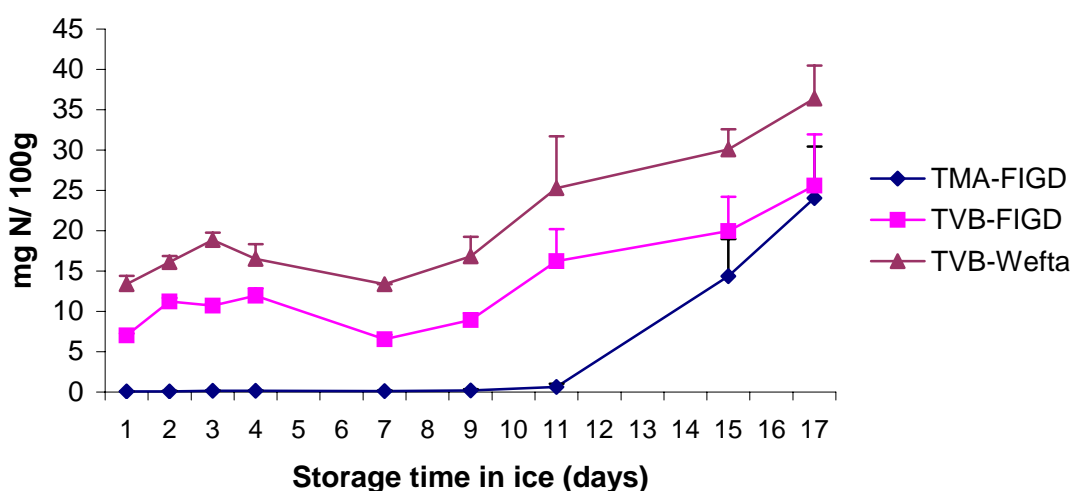


Figure 21. Determination of TMA and TVB (measured by FIGD and the Wefta steam distillation method) in “Reykjavik” cod during storage in ice.

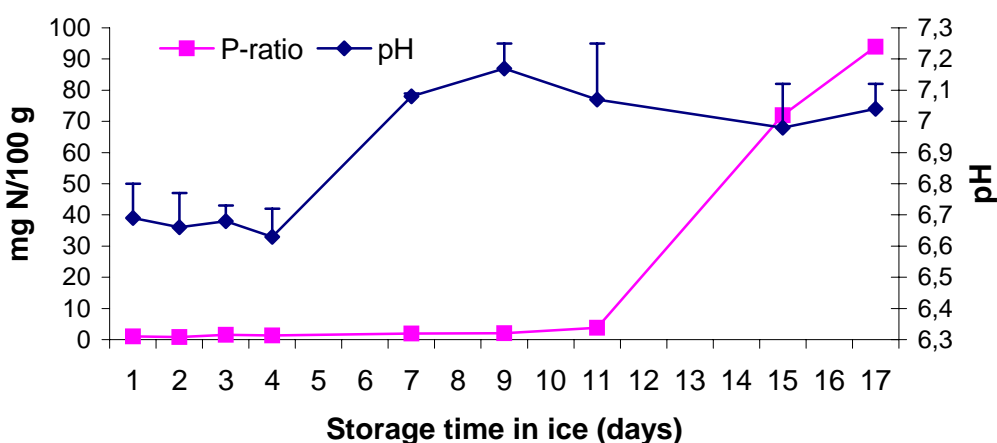


Figure 22. Determination of pH and P-ratio [(TMA/TVB)\*100] during storage of “Reykjavik” cod in ice.

The evolution of pH and the calculated P-ratio is shown in figure 6. The pH is under 7 for the first 4 days but over the neutral 7 at day 7 and levels off after that. In the case of the pH it was an indicator of the very fresh fish for the first 4 days but there were no measurements done on the fillets between day 4 and 7. However it could be stated that at day 7 the fish has lost its major freshness characteristics and the rise in pH was probably due to breakdown products from specific spoilage organisms such as *Shewanella putrefaciens* and *Pseudomonas* species (Gram *et al*, 1990). The P-ratio acts in a similar way as the TMA curve, stays very low from day 1 until day 11 but there is a gap between day 11 and 15 so it can not be confirmed if the low values would have stayed low up to day 14 but when the TMA and P-ratio were measured at day 15 the values had risen significantly.

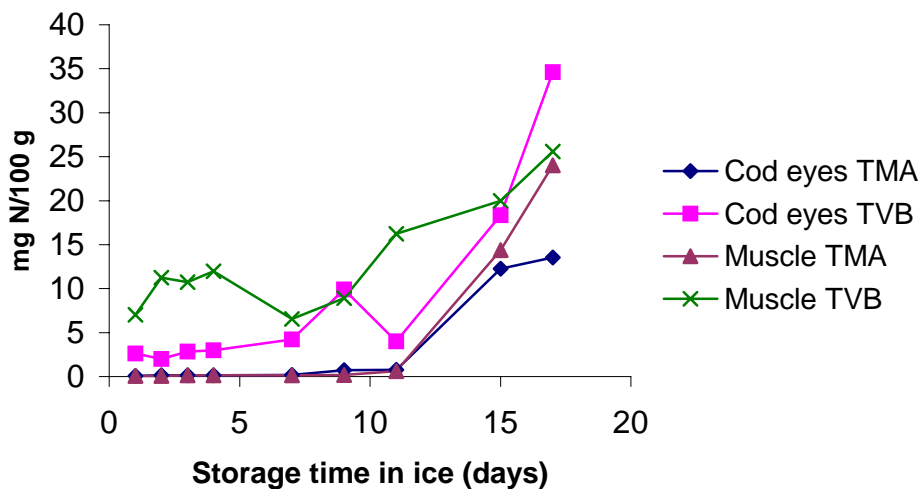


Figure 23. Comparison of TMA and TVB in the cod fillet muscle and cod eyes during storage of “Reykjavik” cod.

Figure 23 compares both TMA and TVB in fish fillet muscle and cod eyes. In order not to destroy valuable fish muscle it would be beneficial if measurements of fish muscle could be replaced by measuring TMA and TVB in the eyes that are of no value. Vyncke (1995) determined TVB in eye fluid and it appeared to be a valid alternative method for the same measurement in muscle. In this experiment the whole eyes were measured instead of only a few ml of eye fluid. The results show that the muscle TVB was higher than the eyes TVB except at the last storage day. The correlation between TVB in the muscle and the eyes was poor. The equation for this was expressed as:

$$[TVB_{Muscle}] = 0,91 [TVB_{Eyes}]; (R^2 = - 0,478)$$

There was a better correlation between  $TVB_{Muscle}$  and  $TVB_{Eyes}$  even though TMA showed higher values at the end of the storage experiment. The equation was expressed as follow:

$$[TVB_{Muscle}] = 0,91 [TVB_{Eyes}]; R^2 = 0,948$$

Figure 24 shows the TMA, TMAO, TVB and pH results for fresh cod during ice storage. The TMA and TVB patterns are similar as was shown in previous storage experiments and TMAO decreases during storage time as the TMAO is reduced to TMA when the bacteria population increases, but the *Shewanella putrefaciens* (Gram *et al*, 1987) responsible bacteria produce the enzyme that catalyse the reduction.

Table 3 shows the TMA, TVB, TMAO and pH values in the “Tromsö” salmon and the “Tromsö” thawed cod.

As was expected the TMA values were very low, even in the 13 days post-mortem age salmon (under 1 mg N/100 g where the TMA can not be detected) and the TVB did not change significantly between days 1 and 13. The TMAO values were though significantly higher at day 13 than at day 1 but the values were low in both cases in comparison with seawater white fish.

The thawed cod was measured on the second day after it had been taken out of frozen storage. The TMA and TVB values were very low but TMAO showed very high values indicating that the fish was very fresh when it was frozen and could therefore have spoiled in a similar way as fresh fish during storage if the TMAO reducing bacteria (*Shewanella putrefaciens*) had survived the freezing storage.

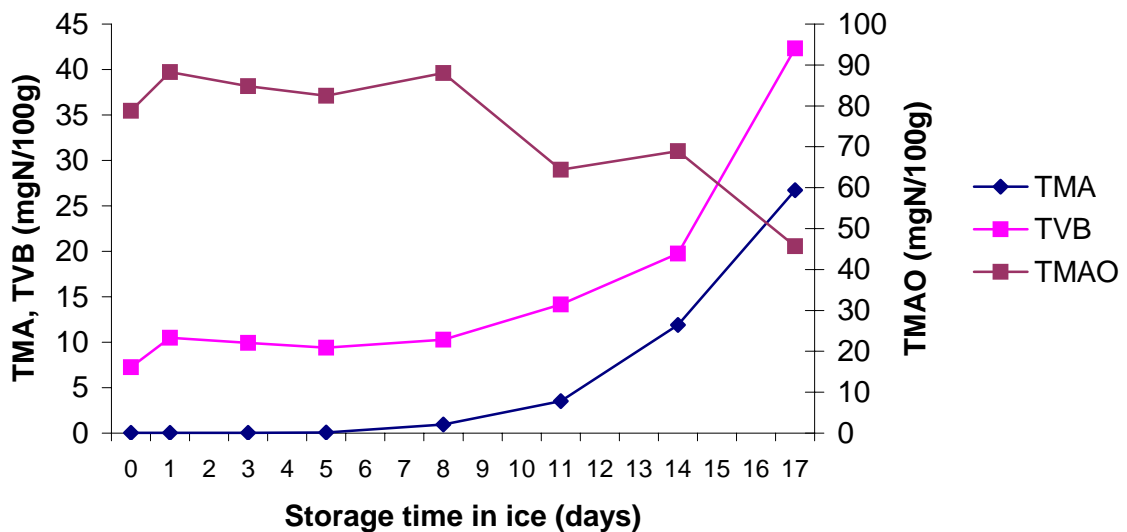


Figure 25. Determination of TMA, TVB and TMAO in “Tromsö” cod during storage in ice.

Table 3. TMA, TVB, TMAO and pH in “Tromsö” salmon and thawed cod during storage in ice

SALMON							
Days	TMA	s.d.	TVB	s.d.	TMAO	s.d.	pH
0	0,03	0	12,55	0,05	8,07	1,13	6,3
13	0,87	0	15,43	0,43	18,79	1,18	

**THAWED COD**

Days	TMA	s.d.	TVB	s.d.	TMAO	s.d.	pH
2	0,1	0	11,25	0,17	82,1	22,2	6,3

#### 4.3.4 Results of the RT-Freshmeter measurements

A comparison of the RT-Freshmeter measurements during storage of the “Reykjavik” and the “Tromsö” cod is illustrated in Figure 26. The “Tromsö” had a bit higher values than the Reykjavik cod, which is logical since the “Tromsö” cod was caught alive and kept alive until the experiment started and slaughtered under optimum condition. The “Reykjavik” cod was on the other hand slaughtered onboard the boat and the first sampling day was day 1 and no *pre rigor* sample was measured in Reykjavik. The correlation coefficient is higher in the “Reykjavik” cod and as Figure 27 shows the correlation coefficient between these two storage experiments is over 0,95.

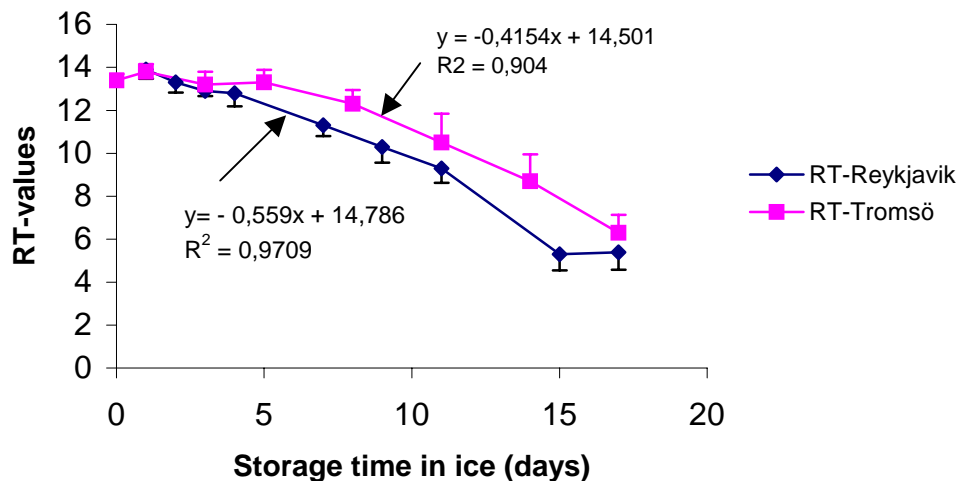


Figure 26. Comparison of RT-Freshmeter values during storage of “Reykjavik” and “Tromsö” cod.

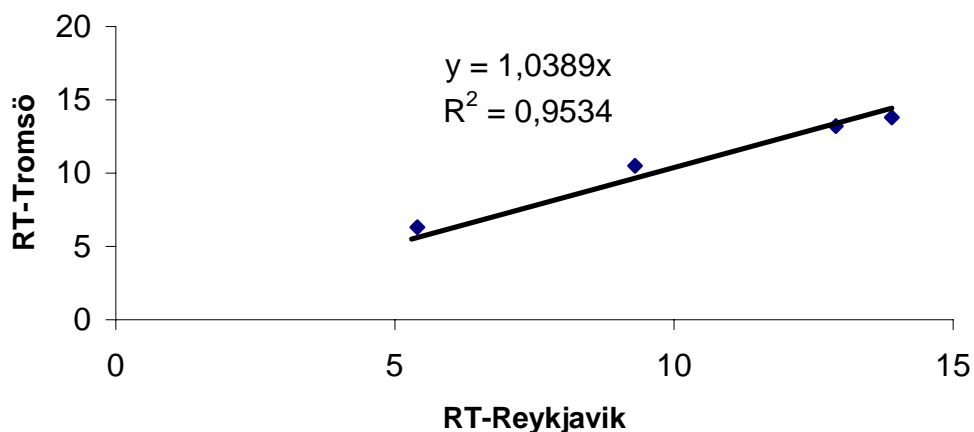
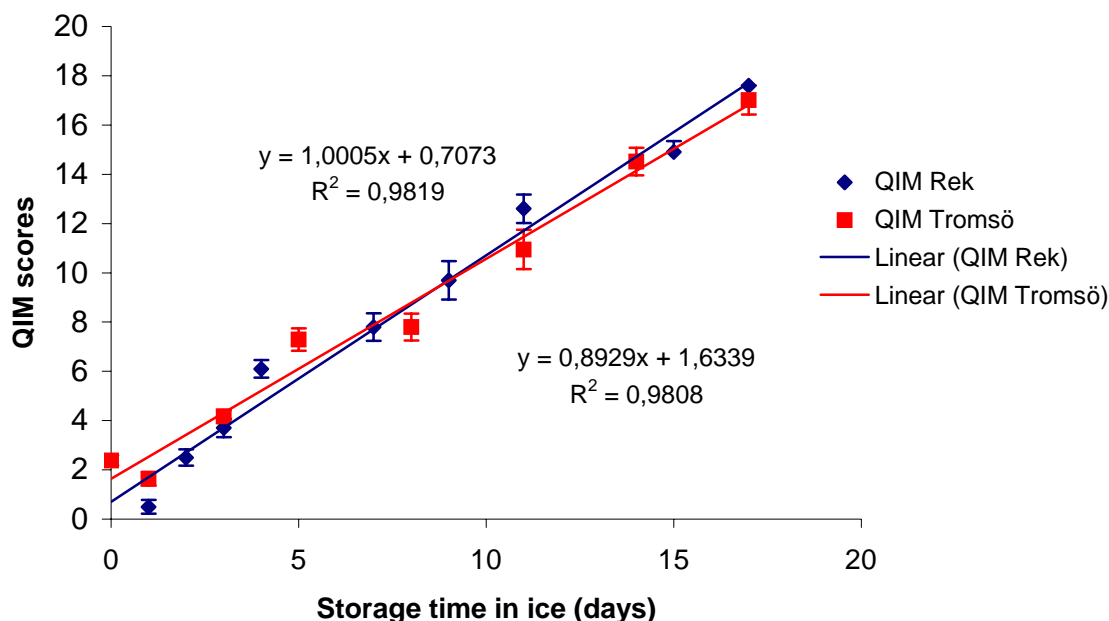


Figure 27. The least squares regression line for RT-values in “Tromsö” and “Reykjavik” cod for the same storage days during storage in ice.

### 4.3.5 Results of sensory evaluation with Quality Index Method (QIM)



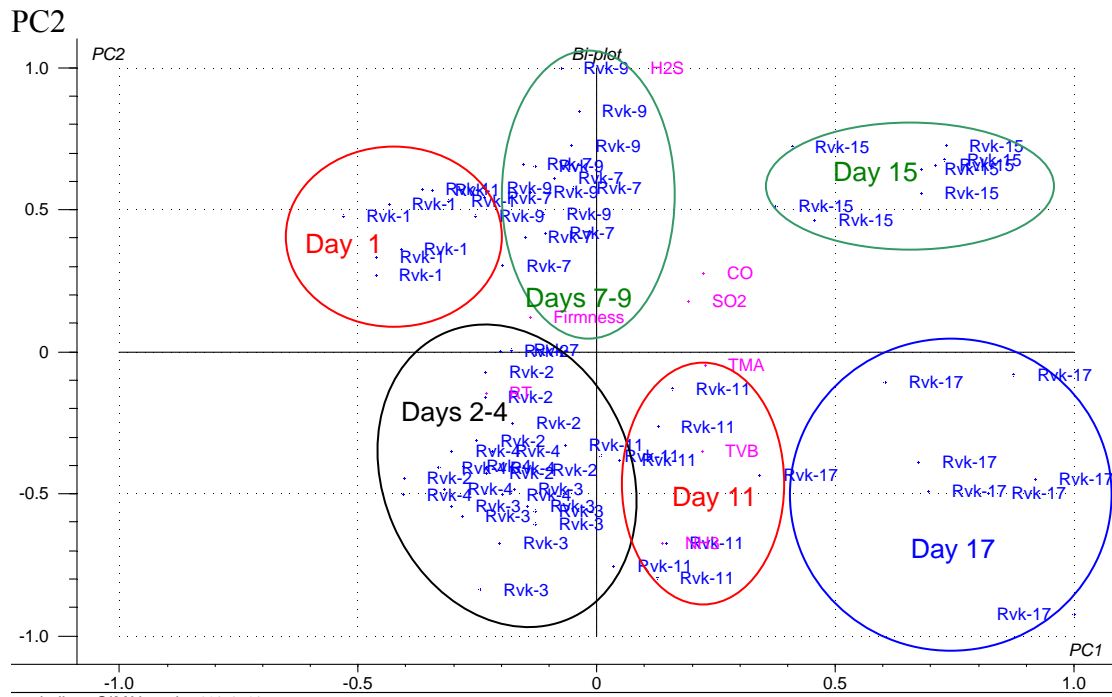
**Figure 28.** QIM scores for fresh cod stored in ice for 17 days from the Reykjavík and Tromsø work-ins.

Figure 28 shows that the results of the sensory analysis using the QIM scheme from the Reykjavík and Tromsø experiments are very similar. QIM scores show a linear increase during the whole storage time. The spoilage rate appears to be slightly faster in Reykjavik, but the *pre rigor* samples on day 0 influence the slope of the Tromsøline. Higher QI scores on day 0 than on day 1 are most likely observed because of the soft texture of the *pre rigor* samples in Tromsø, but in Reykjavik there were no *pre rigor* samples. The QIM scheme does not include a score for *pre rigor*.

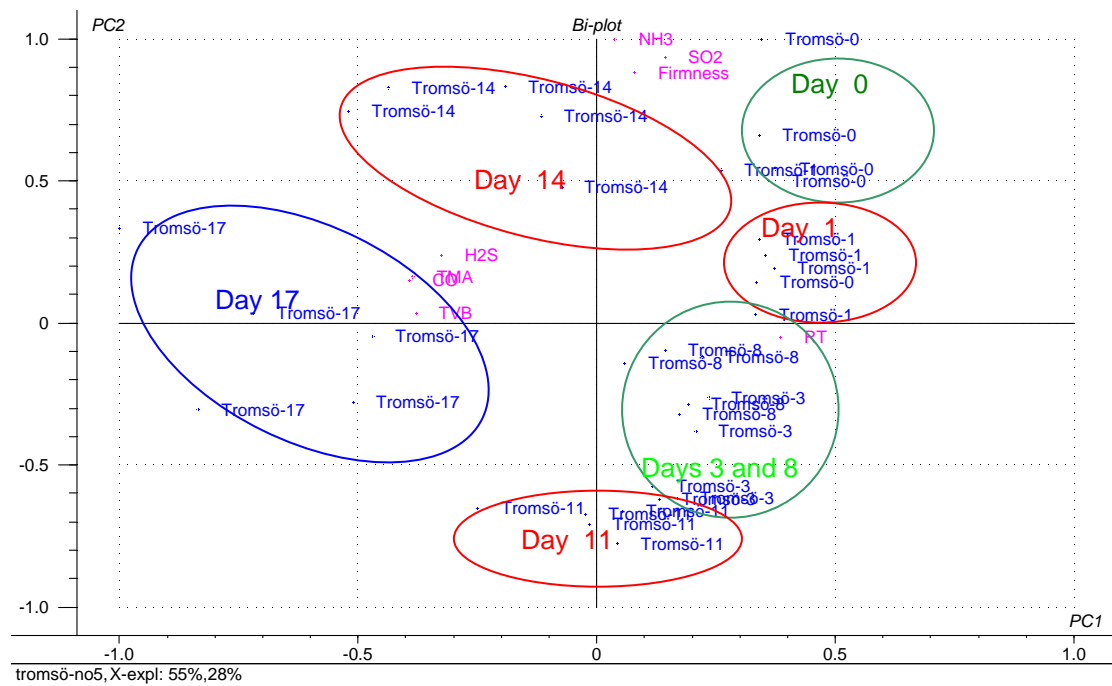
### 4.3.6 Comparison of data from the IFL measurements to evaluate freshness of cod

#### *Principal component analysis*

PCA was done to evaluate the ability of each of the measurement techniques to separate the samples based on storage days. Figure 29 shows a PCA biplot of all IFL data from the Reykjavik work-in. PC1 explains 59% of the variation in the data and the spoilage level of samples increases from left to right along PC1. Samples from day 1 are grouped together furthest to the left and day 17 furthest to the right. Samples from days 2-4 and days 7-9, respectively can not be discriminated. Most of the variable loadings are located on the right side of the plot and influence the separation of the samples based on spoilage level or days of storage. Firmness on the other hand is on the left side of the plot and contributes to the positioning of the day 1 sample on the left side. Also, the RT has loading on the left side because the scores decrease with storage and the freshest samples have the highest scores.



**Figure 29.** PCA of the IFL measurements of cod fillets stored in ice from Reykjavik work-in. Samples scores are shown in blue and labeled with storage day. The variable loadings are shown in pink (Firmness, RT, TMA/TVB, QIM, CO, H<sub>2</sub>S, SO<sub>2</sub> and NH<sub>3</sub> sensors).

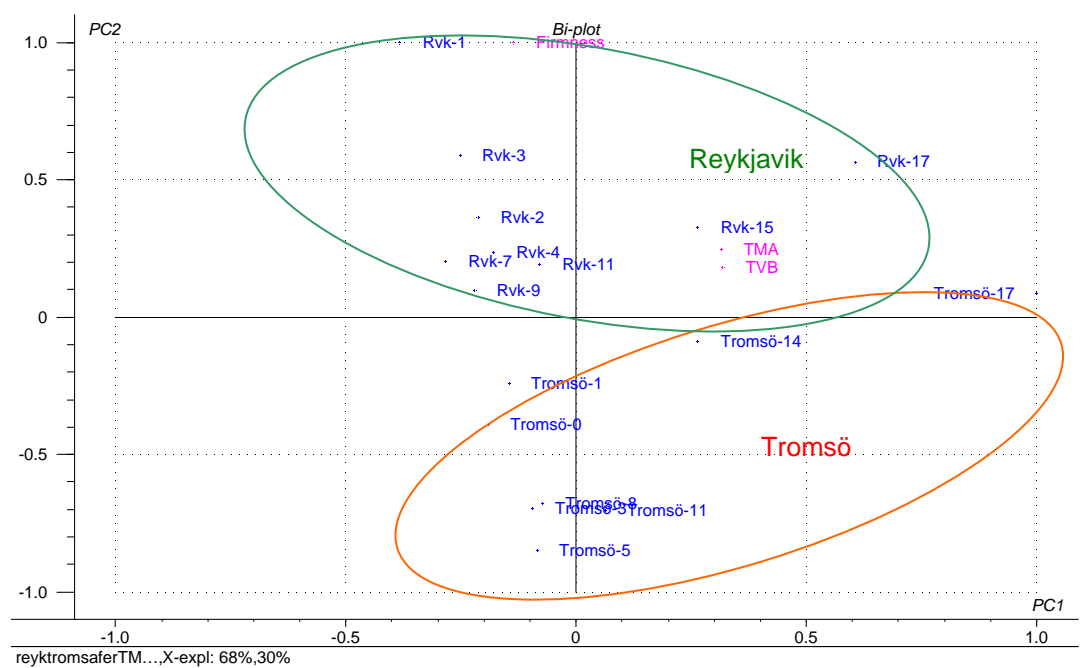


**Figure 30.** PCA of the IFL measurements of cod fillets stored in ice from Tromsø work-in. Samples scores are shown in blue and labeled with storage day. The variable loadings are shown in pink (Firmness, Creep, RT, TMA/TVB, QIM, CO, H<sub>2</sub>S, SO<sub>2</sub> and NH<sub>3</sub> sensors).

Figure 30 shows a PCA biplot of the IFL data from the Tromsø work-in and similarly PC1 is explaining most of the variation in the data (55%) but in this case the days of storage or spoilage level of samples increases from right to left and the day 0 and day 1 samples are clearly separated and located furthest to the right. Samples from day 17

are on the other hand located furthest to the left on the plot. The texture measurements (firmness and creep), the NH<sub>3</sub> and SO<sub>2</sub> sensors and the high RT scores contribute to the positioning of the day 0 and day1 samples on the right side of the plot.

Ideally if the measurement techniques and experimental conditions were identical the data from the Reykjavik and Tromsø work-ins should be comparable. However spoilage rate can be influenced by different season, origin, handling conditions and method of catching. When the data from Reykjavik and Tromsø are combined (Figure 31) it is clear that the data do not completely overlap when the texture measurements and the TMA/TVB analysis are used as variables in the PCA plot. The Tromsø data is located on the lower part of the plot while the Reykjavik data is on the upper part, but both show similar trend and storage days increase in a curve like pattern from left to right along the PC1 which explains 68% of the variation in data. This lack of reproducibility can partly be explained because of the different experimental conditions for the texture measurements and also the TMA/TVB results were slightly lower in the Reykjavik experiment.



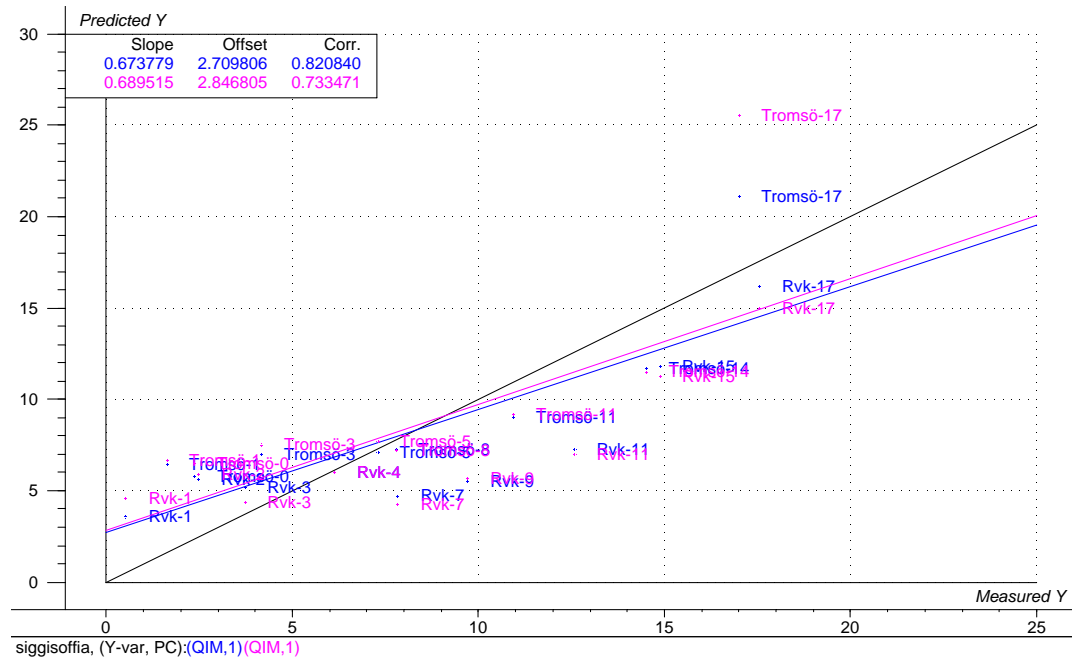
**Figure 31** PCA of texture and TMA/TVB data from Reykjavik and Tromsø experiments. Samples scores are shown in blue and labeled with storage day. The variable loadings are shown in pink (Firmness, TMA/TVB).

*Partial Least Squares regression*

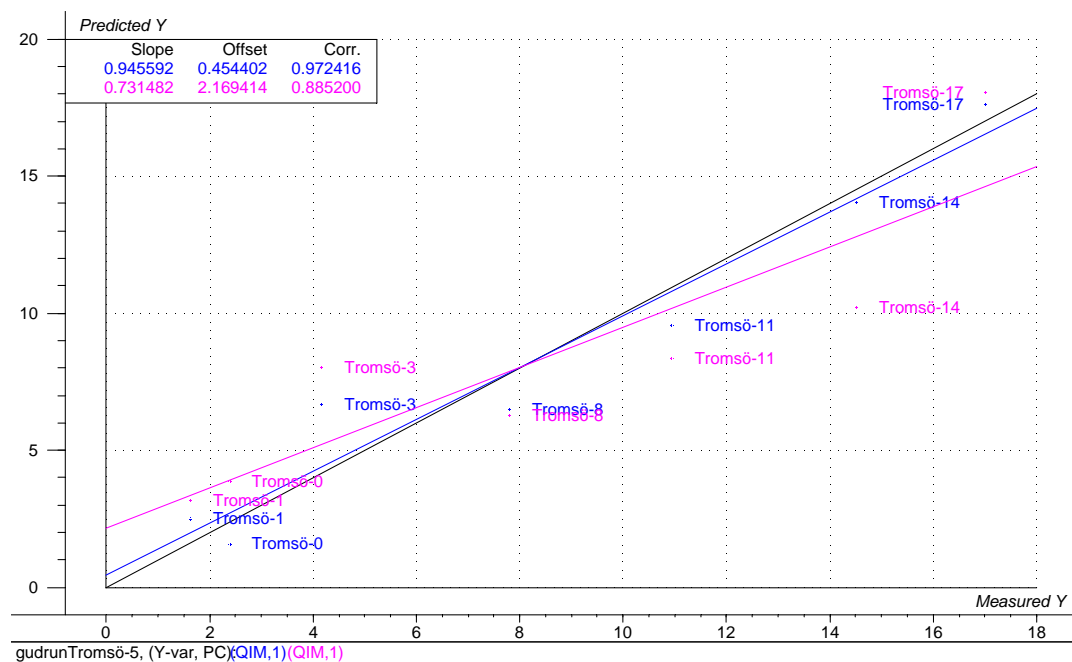
The separation of storage days can be achieved by combining all the different measurement techniques but the actual goal is to use only a few selected instrumental techniques to predict the spoilage level of the samples. The sensory analysis is the method that best describes the freshness or spoilage status of the samples and therefore it is of interest to evaluate the ability of the instrumental techniques to predict QI scores. An attempt was made to establish a model based on texture and TMA/TVB measurements because these techniques give different information about the spoilage level. The texture measurements show pronounced changes in the beginning while the TMA/TVB measurements are showing increasing signals at the end of the storage time. Figure 32 shows that the PLS model based on data from texture and TMA/TVB to predict QI scores has a correlation of 0.82 for the



calibration. This model is based on data from both Reykjavik and Tromsø data. Better model would be achieved if the measurement techniques were exactly the same in both experiments. Figure 33 shows a PLS model based on the FreshSense data from Tromsø as X- variables to predict QI scores as Y-variables and a model with a correlation of 0.97 for the calibration was obtained. The RMSEP (root mean square error of prediction) is rather high for both models or 3.81 and 2.62 (QI values), respectively, which can be translated into 3.1 days for the texture and TMA/TVB model and 1.9 days for the FreshSense model.



**Figure 32.** PLS results of texture and TMA/TVB data to predict QI scores for fish stored in ice from Reykjavik and Tromsø work-ins. X-axis and Y-axis are the measured and predicted QI scores, respectively. — Calibration (prediction), ---- Validation



**Figure 33.** PLS results of FreshSense data to predict QI scores for fish stored in ice from Tromsø work-in (day 5 not included). X-axis and Y-axis are the measured and predicted QI scores, respectively. — Calibration (prediction), ---- Validation

Further analysis of the data using different type of models need to be explored. A generalised linear model (GLM) with electronic nose (FreshSense) data from a storage study of capelin gave a good model to predict TVB value for capelin stored under different conditions (Olafsdóttir *et al.*, 2000). Furthermore, Di Natale *et al.* (2000) used PLS-DA which is a supervised classification method where the search for optimal discriminant directions is performed using PLS to predict storage days of cod from the Reykjavik experiment. The merged electronic nose (FreshSense and LibraNose) shows improved classification performances, reducing the amount of misclassified samples to 4%. On the other hand, this small error remains qualitatively not negligible because samples of days 7-9 are classified as samples of day 1.

### 4.3 Conclusions

The sensory Quality Index scores (QI) and the RT Freshmeter measurements show a good linear trend ( $R^2=0.98$  and  $R^2>0.90$ , respectively) with storage time and the results of the two experiments in Reykjavik and Tromsø are in good agreement.

The texture measurements show changes in the beginning of the storage time while the TMA/TVB analysis and the FreshSense measurements have higher signals at the end of the storage time.

The FreshSense instrument and the texture measurements are still under development and the experimental conditions were not identical in Reykjavik and Tromsø. Therefore, the results can not be directly compared, but the improvements and modifications made resulted in better sensitivity of the FreshSense measurements and the texture measurements improved and became non-destructive.

The combined data from texture and TMA/TVB can be used to predict QI scores and similarly the FreshSense can be used to predict QI scores.

Further development of the measurements techniques and data analysis are needed to verify the use of these techniques together in a multi-sensor instrument to evaluate fish freshness and quality.

### 4.4 References

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## 5 Other activities during the reporting time

The third project meeting was during the work-in on Nov. 12-20,1999. The meeting was at the Icelandic Fisheries Laboratories, Reykjavik, Iceland. The personnel from IFL that participated in the work-in were: Soffía Vala Tryggvadóttir, Guðrún Ólafsdóttir, Sigurdur Einarsson, Luca Laghi, Emilia Martinsdóttir, Asa Thorkelsdóttir and Ósvaldur Thorgrímsson.

The fourth project meeting was held in Bremen in March 2000 in connection with the Seafood Exhibition. On behalf of IFT the project meeting was attended by Guðrún Ólafsdóttir. The results from the Reykjavik work-in were presented at the meeting.

The fifth project meeting was during the work-in at the Fiskeriforskning in Tromsø in May, 2000. During the work-in simultaneous measurements were carried out on cod at different storage time. Two batches of fresh farmed salmon and one batch of frozen-thawed cod was also measured. On behalf of IFL, Soffía Vala Tryggvadóttir, Sigurdur Einarsson and Sigrún Jónsdóttir attended the project meeting

The sixth project meeting and the third work-in was in Madrid, Spain (Task 2.2.) on November 12th-21st, 2000. During the work-in simultaneous measurements were carried out on frozen hake and cod at different storage time. The data analysis is not complete and the results of the experiment will be included in the next annual report.

## 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 difficulties or delays have been during the second year.

## 7 Dissemination of results

1. All participants contributed to the presentation made by Jörg Oehlenschläger in Bilbao: The MUSTEC (Multisensor for Fish) project, Jörg Oehlenschläger (Federal Research Center for Fisheries, Germany). European plenary meeting of the CA-FQLM (FAIR CT98-4174 project 18-20 May, Bilbao, Spain
2. Corrado Di Natale presented results at a meeting in Basel in July, 2000. The paper was about electronic nose measurements and FIGD analysis of cod done at Reykjavik work-in

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 8<sup>th</sup> IMCS 8th International Meeting on Chemical Sensors*, Bazel Switzerland 2-5 July, 2000, Elsevier.

3. Paper on the overall results of the questionnaire sent to the journal Food Quality and Preferences

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, A survey of the needs of the fish sector for quality control and labelling in Europe. *Food Quality and Preferences*, 2001 *submitted*.

4. A summary about the project and pictures from the work-in in Reykjavik are on the homepage of IFL:

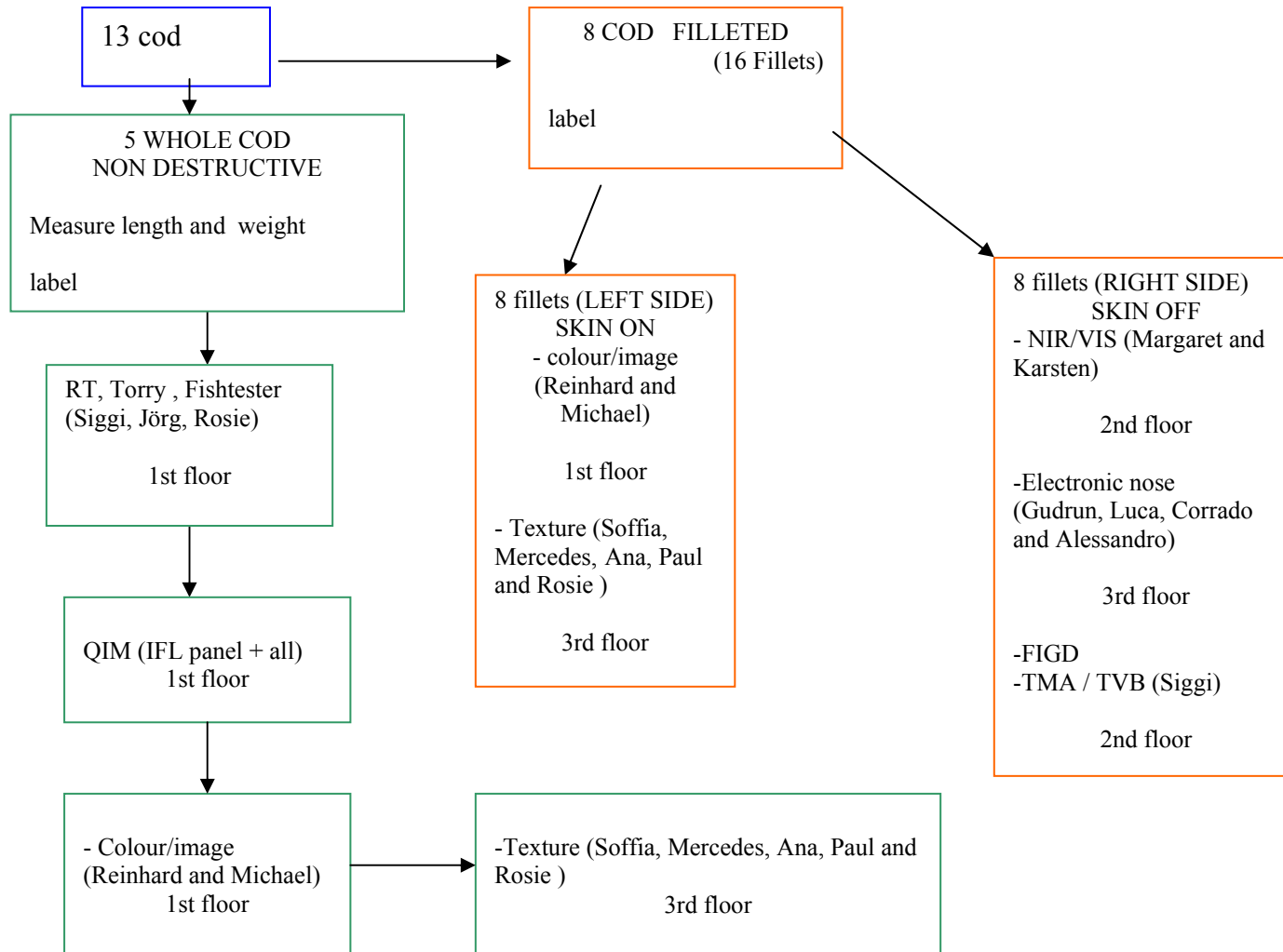
<http://www.rf.is/almennt/fraedsla/radstefnur/nosesense/NoseSense-adalsida.htm>

# Appendix 1

## Work-in schedules

1. Sampling plan for Reykjavík work-in, Novemeber, 1999
2. Sampling plan for Tromsö work-in, May, 2000

## Sampling plan for the MUSTEC work-in, Reykjavík Nov 11-21, 1999



## Work-in schedule in Reykjavik Nov.1999

Following measurements were done:

**QIM:** The QIM analysis was done by the MUSTEC participants

**Torryster, RT meter, Fishtester:** Rosie, Paul, Sigurdur, Jörg

**Visible/NIR:** Karsten and Margarethe.

**Colour/Image:** Reinhard and Michael

**Electronic Nose:** Gudrun, Luca, Corrado and Allsessandro

**TVN -TMA / FIGD (Flow-Injection-Gas-Diffusion) :** Sigurdur, IFL

**Texture:** Soffia , Mercedes, Ana, Paul, Rosie and Reinhard

### Suggestion for texture measurements.

Some fish from batches A and C were frozen and will be used for thawing experiments measuring texture (Soffia)

### Fish batches:

Three batches of fish (A, B,C) were obtained from small boats from Reykjanes which fish in catching grounds in Faxaflói southwest of Iceland. The first batch of fish was caught with longline on Monday 1.Nov (A)(Fiskanes). The second batch was caught on Monday 8.Nov.(B) (Sigurthor)and the last batch on Tuesday Nov 16 (C) (Nonni). The fish was caught with longline, gutted and iced and brought to IFL the following day (Day 1). The fish was kept in ice at 0°C until analysed. Fish with the following storage times on ice was measured: 1,2,3,4, 7,9,11, 15 and 17

For each storage time 5- 8 fishes are measured.

### Timetable :

	Nov12	Nov13	Nov14	Nov15	Nov16	Nov17	Nov18	Nov19	Nov20
Batch / Day	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat
	Instr	Test	Off						
A (1. Nov)					15		17		
B (8. Nov)				7		9		11	
C (15. Nov)						1	2	3	4

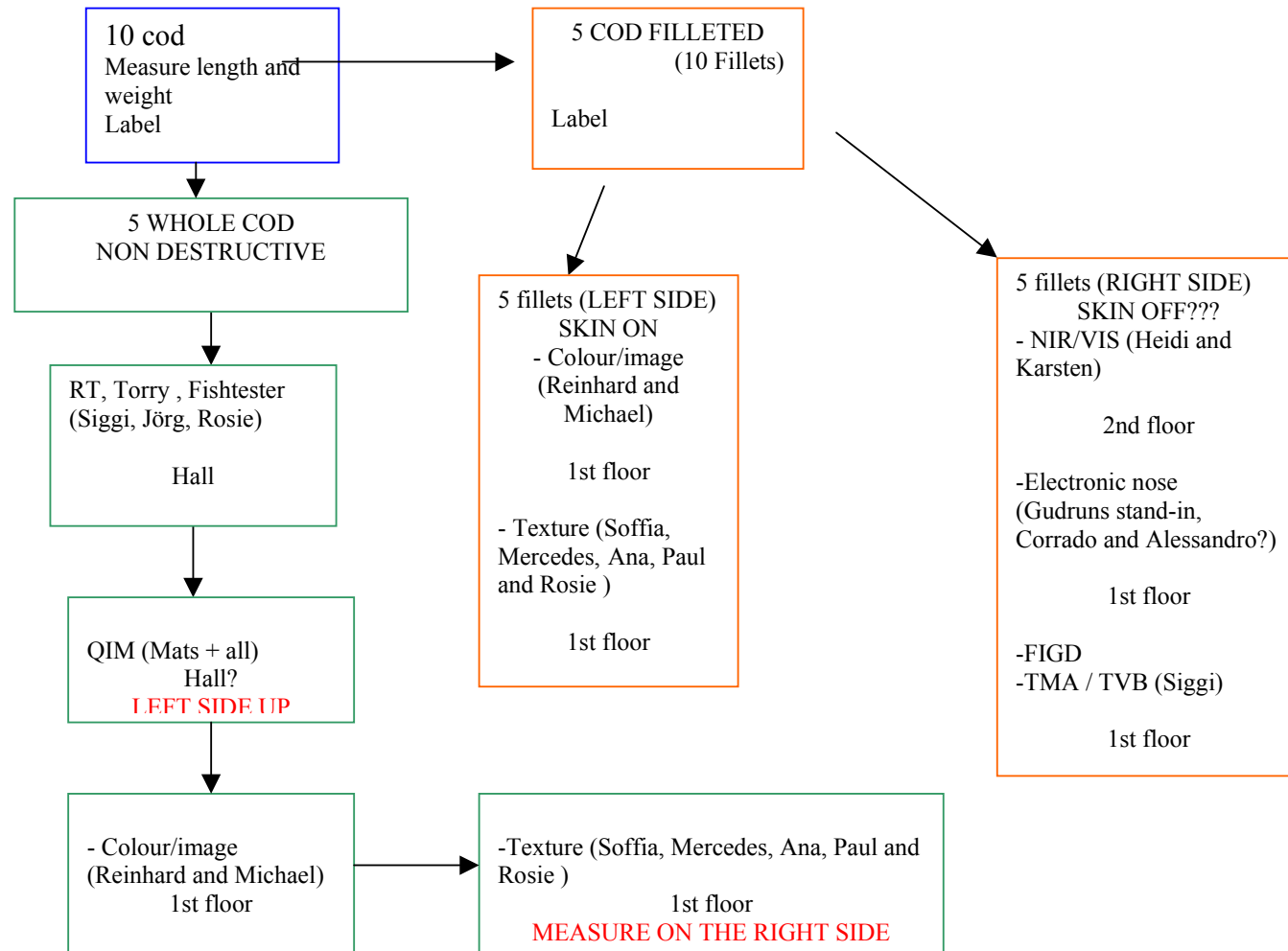
## Sampling plan for the MUSTEC work-in, Tromsø MAY 24– 31, 2000

Experimental plan

Day	Before lunch	After lunch
Wednesday 24 <sup>th</sup>	<i>Instrumental set up</i>	
Thursday 25 <sup>th</sup>	Salmon A	Thawed cod
Friday 26 <sup>th</sup>	Cod A	Cod B
Saturday 27 <sup>th</sup>	Cod C	Cod D
Sunday 28 <sup>th</sup>	Day off	
Monday 29 <sup>th</sup>	Cod E	Cod F
Tuesday 30 <sup>th</sup>	Cod G	Salmon B
Wednesday 31 <sup>st</sup>	Cod H	Make ready to leave



**Experimental design**  
**Sampling plan for MUSTEC work-in, Tromsø May 24-31, 2000**



# Appendix 2

Raw data from work-ins in Reykjavik and Tromsö