Verkefnaskýrsla til RANNÍS
32 - 03

Desember 2003

ACCURATE PREDICTIVE MODELS

I . Bacterial growth experiments: effect of temperature and bacterial interaction
II. Storage studies of cod and haddock fillets at different temperatures
III. Modelling

Guðrún Ólafsdóttir
Hélène L. Lauzon
Rósa Jónsdóttir
Emilía Martinsdóttir
Jóhann Örlygsson

Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins
Icelandic Fisheries Laboratories Report Summary

Titill / Title	**Accurate predictive models**		
Höfundar / Authors	*Guðrún Ólafsdóttir, Hélène L. Lauzon, Rósa Jónsdóttir, Emilía Martinsdóttir, Jóhann Örlygsson*		
Skýrsla Rf /IFL report	32 -03	*Útgáfudagur / Date:*	Desember 2003
Verknr. / project no.	1434		
Styrktaraðilar / funding:	*RANNÍS*		
Ágrip á íslensku:	Áhugi á ferskum eða kældum matvælum fer vaxandi og íslensk fiskvinnslufyrirtæki hafa að undanförnu séð aukin sóknarfæri fyrir útflutning á ferskum flökum. Lágt hitastig við geymslu á ferskum fiski er frumskilyrði þess að varðveita neyslugæði hans. Í verkefninu um nákvæm geymsluspálíkön fyrir fisk var markmiðið að afla þekkingar um áhrif mismunandi hitastigs á vöxt sérhæfðra skemmdarörvera og myndun niðurbrotsefna í ferskum fisk. Tilraunir í verkefninu voru miðaðar að því að herma eftir þeim hitasveiflum sem geta orðið í raunverulegum ferli og nota framleiðsluferil þekktrar vöru. Gerðar voru umfangsmiklar geymslutilraunir á ferskum ýsu og þorskflökum við mismunandi hitastig og hitasveiflur þar sem líkt var eftir aðstæðum sem geta orðið við framleiðslu og dreifingu ferskra flaka. Breytingar á gæðum voru metnar með hefðbundnum aðferðum þ.e. heildartalningum á örverufjölda, magni af reikulum bösum (TVB-N) og skynmati (Torry einkunn), en megin áhersla var lögð á að nota og þróa nýjar hraðvirkar aðferðir til talninga (Malthus tækni) á sértækum skemmdarörverum (SSÖ) og niðurbrotsefnum þeirra með rafnefi. Ávinningur verkefnisins felst í öflun þekkingar á skemmdarferli fisks sem nýtist m.a. við þróun geymsluspálíkana fyrir fiskflök byggð á hraðvirkum mælingum þar sem tekið er tillit til áhrifa hitastigs á vöxt skemmdarörvera og framleiðslu þeirra á niðurbrotsefnum eða skemmdarvísum. Jafnframt sýna niðurstöðurnar vel nauðsyn þess að tryggja lágt hitastig í öllum ferlinum frá veiðum til neytenda þar sem hitasveiflur geta haft mikil áhrif á geymsluþol afurðanna. Upphaflegt ástand hráefnis og meðhöndlun fyrir vinnslu hafa einnig afgerandi áhrif á geymsluþol flakanna. Þannig getur varan haft allt að 12- 13 daga geymsluþol ef flökin eru framleidd úr vel ísuðu hráefni sem fyrst eftir veiði, en ekki er óalgengt að flök hafi um 8-9 daga geymsluþol ef hitasveiflur verða í ferlinu eða ef flökin eru framleidd úr nokkura daga gömlu hráefni. Þátttakendur í verkefninu voru Rannsóknastofnun fiskiðnaðarins og fyrirtækið Tros í Sandgerði, en jafnframt lagði fyrirtækið Tangi á Vopnafirði til hráefni til tilrauna. Upplýsingarnar nýtast fyrirtækjunum til að endurbæta vinnsluferil sinn og tryggja geymsluþol afurða til neytenda, jafnframt munu geymsluþolsspálíkön væntanlega geta nýst við dreifingu og sölu á ferskum fiski. Vísindalegur ávinningur var fólginn í frekari þekkingu á samspili skemmdarörvera og áhrifum hitastigs á vöxt þeirra og framleiðslu á niðurbrotsefnum. Gögnin eru grunnur að spálíkanagerð sem unnið verður að frekar og verða niðurstöðurnar birtar í alþjóðlegum vísindagreinum.		
Lykilorð á íslensku:	*spálíkön, skemmdarörverur,gæðavísar,rafnef,skynmat*		

Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins
Icelandic Fisheries Laboratories Report Summary

Summary in English:

The overall aim of the project was to study the influences of temperature fluctuations on the quality of fresh fish fillets from harvest through the processing chain until the finished product reaches the consumer. This was done by doing controlled storage experiments and investigating the effect of temperature fluctuations on the growth of spoilage bacteria and their metabolites**.** Rapid measurements of specific spoilage organisms (SSO) with the Malthus technique and microbial metabolites with an electronic nose will be used as data for models to predict the shelf life of fish stored at different temperatures. Microbial growth experiments of specific spoilage organisms were done to provide understanding of the spoilage potential of the individual organisms and their combined effect at different temperatures. Moreover, gas chromatography analysis provided detailed information about the identity of the microbial metabolites suggested as indicators of spoilage. This knowledge is the basis to develop further an electronic nose that can rapidly measure volatile spoilage indicator compounds.

The first year report included typical temperature profiles for the production of haddock fillets from catch through processing and transport via airfreight. Also, a temperature profile of raw material form the fish market to the fishmonger and for the process for production of frozen fillets was included.

Shelf life studies were done in the first year on raw material from different seasons and with different fishing gear which influenced the spoilage process of the products and their shelf lives. The experiments included in the final report were designed to imitate the temperature profiles and possible abusive conditions that could occur in the production, transport and retail. The raw material in the different experiments was from the same season, but caught in different catching areas. Moreover, time before processing was different (1 – 3 days) which obviously influenced the shelf life of the finished products. The experiments included studies on the influence of different constant temperatures during storage, and the effect of fluctuating temperatures in the processing and storage. The results show that the growth and development of the microflora is different under the different conditions and the development of specific spoilage organisms (*Pseudomonas spp*, H_2S producing bacteria and *Photobacterium phosphoreum*) is highly dependent on the temperature conditions. The results of the measurements of microbial metabolites with the electronic nose are in agreement with the microbial data and the traditional sensory and chemical measurements of TVB-N. Moreover the individual responses of the electronic nose sensors (CO and NH_3) appear to be highly correlated to the growth of the of Pseudomonas and *Photobacterium phosphoreum* spoilage organisms.

English keywords: *Predictive models, SSO, microbial metabolites, electronic nose,sensory*

TABLE OF CONTENT

CHAPTER I Bacterial growth experiments: effect of temperature and bacterial interaction

CHAPTER II Storage studies of cod and haddock fillets at different temperatures

CHAPTER III Modelling

TABLE OF CONTENTS

APPENDIX

1. Lauzon, H.L. & Ólafsdóttir, G. 2002. Spoilage potential and growth of specific spoilage organisms in fish model system at different temperatures. Erindi á 32nd WEFTA Meeting, 12-15 May 2002, Galway, Ireland. (Ágrip og Power-point skjal með glærum)

2. Guðrún Ólafsdóttir, Soffia Vala Tryggvadóttir, Sigurdur Einarsson and Hélène L. Lauzon, 2002. Prediction of sensory quality of haddock fillets using various instrumental techniques. 32nd WEFTA meeting, May 13th-15th, 2002, Ireland. (Ágrip og Power-point skjal með glærum).

3. Guðrún Ólafsdóttir, Rósa Jónsdóttir, Soffia Vala Tryggvadóttir, Sigurdur Einarsson, Hélène L. Lauzon: Prediction of freshness quality of haddock fillets using ELECTRONIC NOSE, TEXTURE AND TVN MEASUREMENTS. First Joint Trans-Atlantic Fisheries Technology Conference (TAFT) 33rd WEFTA Meeting and 48th Atlantic Fisheries Technology Conference, 11-14 June 2003, Reykjavik - Iceland 2003 (Veggspjald)

4. Guðrún Ólafsdóttir, Rósa Jónsdóttir, 2003. DETECTION OF VOLATILE COMPOUNDS BY AN ELECTRONIC NOSE TO MONITOR FRESHNESS OF HADDOCK STORED IN ICE. First Joint Trans-Atlantic Fisheries Technology Conference (TAFT) 33rd WEFTA Meeting and 48th Atlantic Fisheries Technology Conference, 11-14 June 2003, Reykjavik - Iceland 2003 (Veggspjald).

5. Guðrún Ólafsdóttir, Hélène L. Lauzon & Jóhann Örlygsson. 2000. Nákvæm geymsluþols spálíkön fyrir fisk. Fiskvinnslan, 12/2000, bls. 21-23.

6. Jóhann Örlygsson, 2000. Spálíkön fyrir geymslu á ferskum fiski. Stafnbúi Rit nemenda við Sjávarútvegsdeild HA.

7. Guðrún Ólafsdóttir, 2003. Rokgjörn efni í fiski – vísir að gæðum Fréttablað MNÍ Matur er mannsins megin okt 2003 1tbl., 15árg. ISSN1029-2691

Chapter 1
Bacterial growth experiments: Effect of temperature and bacterial interaction

Hélène L. Lauzon

Guðrún Ólafsdóttir

Rósa Jónsdóttir

TABLE OF CONTENTS

1 INTRODUCTION

It is well recognised that fish spoilage is caused by microbial action. Low temperature storage of fish is very important to maintain its freshness and quality. However, temperature control throughout the process from harvest to the consumer is often difficult. Research on predictive microbiology has shown that more realistic shelflife models for food can be achieved if temperature fluctuations are taken into account and specific spoilage organisms (SSO) are used rather than total viable counts. *Shewanella putrefaciens* and *Pseudomonas* spp. have been reported as the specific spoilage bacteria of iced, air-stored fresh fish (Gram & Huss, 1996). *Photobacterium phosphoreum* is considered as the main specific spoilage organism of marine fish caught from temperate waters and stored under modified atmosphere (Dalgaard *et al*., 1997). *P. phosphoreum* can be found on fresh fish fillets, and being a TMA-producer, its role in the spoilage of air-stored fillets may be of importance and should therefore not be overlooked.

Growth studies, involving strains of *Pseudomonas* spp. (groups I and II), *S. putrefaciens* and *P. phosphoreum*, were done in fish extract at 0.5 ± 0.4°C, 7.6 ± 0.1°C and 14.8 ± 0.1°C. The aim of the growth studies was to evaluate the spoilage potential/activity of the chosen bacteria species when grown alone and mixed together. Information about microbial interaction and metabolite formation was obtained at the different temperatures. Traditional microbiological and conductance methods were used to assess the development of the bacteria, while microbial metabolites were measured using GC-MS and an electronic nose.

2. MATERIALS & METHODS

2.1 Selection of selective media for the enumeration of bacteria

Modified Long & Hammer's medium (Van Spreekens, 1974) with 1% NaCl (spread-plated LH, 15°C) was used to assess total counts and Iron Agar (Gram *et al*., 1987) with 1% NaCl (spread-plated IA, 15°C) to assess counts of H_2S-producing bacteria, like *Shewanella putrefaciens*, (black colonies). CFC medium (Pseudomonas Agar Base

CM559 supplemented with CFC selective agar supplement SR103, Oxoid) was used to enumerate *Pseudomonas* spp. and differentiate between groups I and II using a UV-light, fluorescent colonies being *Pseudomonas* I and non-fluorescent colonies as *Pseudomonas* II. *S. putrefaciens* cannot grow onto CFC medium while some *P. phosphoreum* strains can grow at 15°C, but not at 22°C. Counts of *P. phosphoreum* were estimated by using the PPDM-Malthus conductance method (Dalgaard *et al*., 1996), as described by Lauzon (2003).

2.2 Development of fish extract

a) Preparation of fish extract of different concentrations: Thawed haddock fillets were used to prepare fish extract with different amounts of deionised water (1part of fish : 1 part of water, 1: 2, 1:3, 1:4 and 1:9). This was done to find the proper concentration of fish extract that would allow *P. phosphoreum* to grow. The fillets were minced, portions weighted, water added, the mixture boiled for 2 min and filtered to obtain a clear extract. Salt (1% NaCl) was added and the extracts sterilised at 121°C for 15 min. pH was measured prior to and after the salt addition. Ten ml of extracts were pipetted into sterile glass tubes, supplemented with 2.5 mg TMAO (filter-sterile solution prepared from TMAO*2H_2O, Sigma), inoculated with an overnight culture (10 µl) of *P. phosphoreum* and grown at 15°C for 3 days. Positive and negative growth was recorded.

b) Preparation of fish extract forgrowth experiments: Freshly caught haddock were used to prepare the fish extract (1part of fish : 4 parts of water). Fish were filleted, skinned and minced. Portions of mince were weighted, diluted with deionised water, boiled for 2 min, filtered, supplemented with 1% NaCl and 125 ml of the extract poured into a glass jar (590 ml; ORA, Kópavogur, Iceland). pH of the mince was measured, as well as pH of the extract prior to and after the salt addition. The jars were autoclaved at 121°C for 15 min., cooled and stored refrigerated till used. The extract (each jar) was aseptically supplemented with 31.25 mg TMAO, 12.5 mg L-cysteine HCL*H_2O (Sigma), 2.75 mg DL-serine (Fluka) and 2.75 mg L-methionine (Sigma) prior to inoculation.

2.3 Growth experiments

a) Bacterial cultures and inoculation: Strains of *Pseudomonas* spp. (groups I and II), *S. putrefaciens* and *P. phosphoreum* were used (Table 1). The strains were maintained on LH-slope agar and inoculated into Supermarine broth (15°C, overnight) prior to use. Supermarine broth is prepared from Marine broth supplemented with 10g Lab-Lemco, 5g Bacto-peptone and 2g yeast extract per liter.

Table 1. List of the different bacterial species and their origin

Monoculture	Bacterial species	Code	Origin
A	*Pseudomonas I*	54	spoiled cod (flesh) stored at 10°C
	Pseudomonas I	90	spoiled cod (flesh) stored at -2°C
	Pseudomonas I	127	spoiled cod (flesh) stored at 0°C
B	*Pseudomonas II*	38	spoiled cod (flesh) stored at 10°C
	Pseudomonas II	80	spoiled cod /flesh) stored at -2°C
	Pseudomonas II	131	spoiled cod (flesh) stored at 0°C
C	*S. putrefaciens*	92	spoiled cod (flesh) stored at -2°C
	S. putrefaciens	105	spoiled cod (flesh) stored at 0°C
	S. putrefaciens	126	spoiled cod (flesh) stored at 0°C
D	*P. phosphoreum*	S3	spoiled MAP[1] haddock fillet (0°C)
	P. phosphoreum	S6	spoiled MAP[1] haddock fillet (0°C)
	P. phosphoreum	S26	air-stored haddock fillet (0°C)

1: MAP = 60% CO_2 / 40% N_2

The strains were then grown separately in fish extract (3 ml) for 4 days (7-8°C) before being diluted and inoculated into the fish extract jars. A 1000-fold dilution was done in cooled Maximum Recovery Diluent (MRD, Oxoid), preparing a different bottle (100 ml) for each different species (using 3 strains per species). One ml of each respective dilution

bottle (monocultures A, B, C and D) was inoculated into each designated fish extract jar. A co-culture group, including all species (E), was prepared by adding 0.2 ml of each of the 4 dilution bottles to each designated fish extract jar. The inoculation level was expected to be log 2-3/ml. Uninoculated jars were used as a control for volatile studies. Upon completion of the inoculation, counts (CFU/ml) of the 4 dilution bottles were assessed by plating further dilutions onto LH medium, incubating at 15°C for 5 days. The jars were stored at 0.5 ± 0.4°C in a storage room, and at 7.6 ± 0.1°C and 14.8 ± 0.1°C in a SANYO incubator (MIR-552).

b) *Microbiological analysis:* Each group (A, B, C, D and E) was tested in duplicate regularly over the storage period at each chosen temperature, taking aseptically a 1-ml aliquot from each designated fish extract jar with further dilution into cooled MRD as required. The same jars were used throughout the storage period.
Dilutions of groups A, B, C, D and E were plated onto LH medium to assess their numbers, while group E was also plated onto IA (counts of black colonies, i.e. *S. putrefaciens*) and CFC (counts of *Pseudomonas* I-II) media. LH and IA media were incubated at 15°C for 5 days, while CFC was incubated at 22°C for 3 days. CFC medium was illuminated with a UV-light to assess the number of fluorescent colonies (*Pseudomonas* I), while non-fluorescent colonies were counted as *Pseudomonas* II. Counts of *P. phosphoreum* in the co-culture (E) were assessed using the PPDM-Malthus conductance method.

c) *Electronic nose analysis:* Each group (A, B, C, D and E) was tested regularly over the storage period at each chosen temperature, using a new jar at each sampling time. Uninoculated jars were used as a control to assess the background given by the fish extract itself. The electronic nose FreshSense (Bodvaki, Artorg1,550 Saudarkrokur, Iceland) was used for the electronic nose measurements. The instrument consists of an array of electrochemical gas sensors (CO, H_2S, SO_2 (Dräger, Germany) and NH_3 (City Technology, Portsmouth, Britain)), a glass container (2.3 L) closed with a plastic lid and a PC with a Labview measurement software. The static headspace sampling technique described earlier by Ólafsdóttir *et al*. (1997, 2000) was slightly modified. A separate measurement chamber was installed and a pump was used to allow continuous circulation

of the air between the measurement chamber and the sampling container while measuring. The sampled jars were allowed to stand at room temperature for a few minutes prior to measurement to reach a temperature of 10-14°C. The lid of each jar was removed just before being placed into the glass container which was closed immediately. Measurements were taken every 10 seconds for 5 minutes. In the data analysis, the reported value (current) is calculated as the average of 6 valuesduring the last minute of measuring time minus the base line value calculated as the average of 6 values before the measurement starts.

d) *Analysis of volatile compounds by gas chromatography:* The procedure for the GC analysis is described in section 2.6 in chapter 2 in this report

e) *Data analysis*: Microsoft Excel 97 was used to calculate means and standard deviations of microbiological data and to generate graphs.

2.4 Estimation of growth rate of bacterial strains

The growth data collected were fitted using the DMFit in-house program of IFR (UK) which is based on a reparameterised version of the model of Baranyi & Roberts (1994). DMFit is an Excel add-in program to fit curves where a linear phase is preceded and followed by a stationary phase. The main difference between this model and other sigmoid curves, like Gompertz, Logistic, etc, is that the mid-phase is really close to linear, unlike the classical sigmoid curves which havea pronounced curvature there.

DMFit has been used to model the time-variation of the logarithm of the cell concentrations of bacterial cultures. This model has 4 main parameters:

rate: potential maximum rate of the model

lag: length of the interval beginning at the first coordinate of the independent variable and ending at the point where the maximum slope drawn to inflexion crosses the level of y0

y0: initial cell concentration

yEnd: upper asymptote of the sigmoid curve

3 RESULTS & DISCUSSION

3.1 Microbiological data

Figures 1 to 3 illustrate how the different bacterial species grew in fish extract, either separately or in coculture within the temperature range of 0.5-14.8°C. At 0.5°C, rapid growth of *Pseudomonas* II spp. was observed in the monocultures, followed by a steady development of *S. putrefaciens* and *Pseudomonas* I spp. *P. phosphoreum* grew more slowly during the first 2 days, afterwhich a similarly rapid growth occurred. In coculture, *Pseudomonas* II spp. grew rapidly and was dominating, while the development of *S. putrefaciens* and *Pseudomonas* I spp. was slower than that seen in monocultures, perhaps due to bacterial competition occurring in the mixture. However, *S. putrefaciens* started to proliferate at a faster rate after day 3. Growth of *P. phosphoreum* occurred more rapidly in the coculture than that observed in monoculture, but did not reach as high levels.

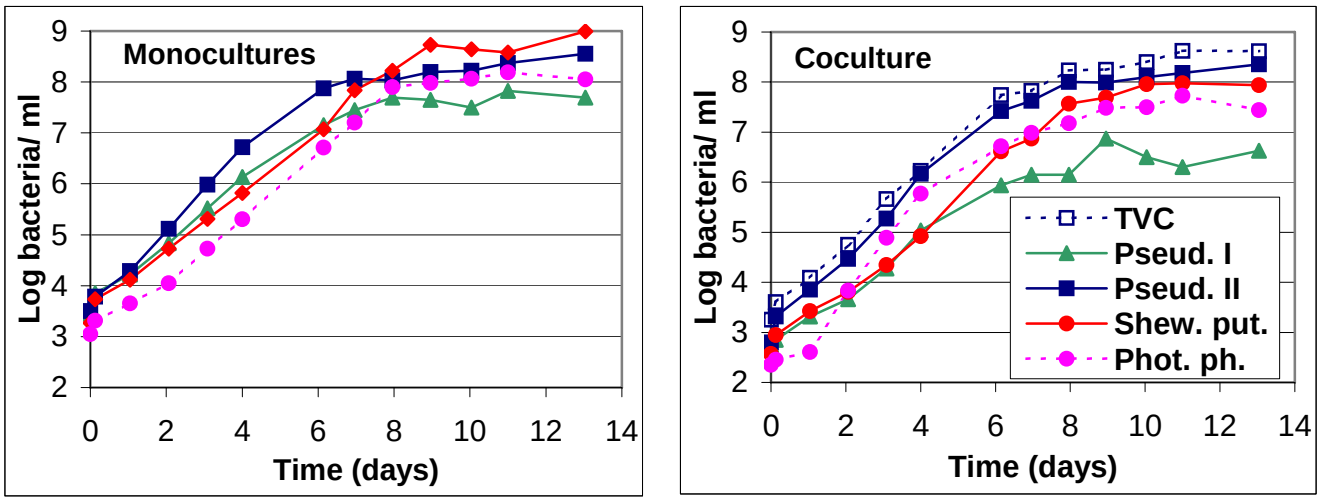


Figure 1. Bacterial proliferation of monocultures and coculture in fish extract at 0.5°C

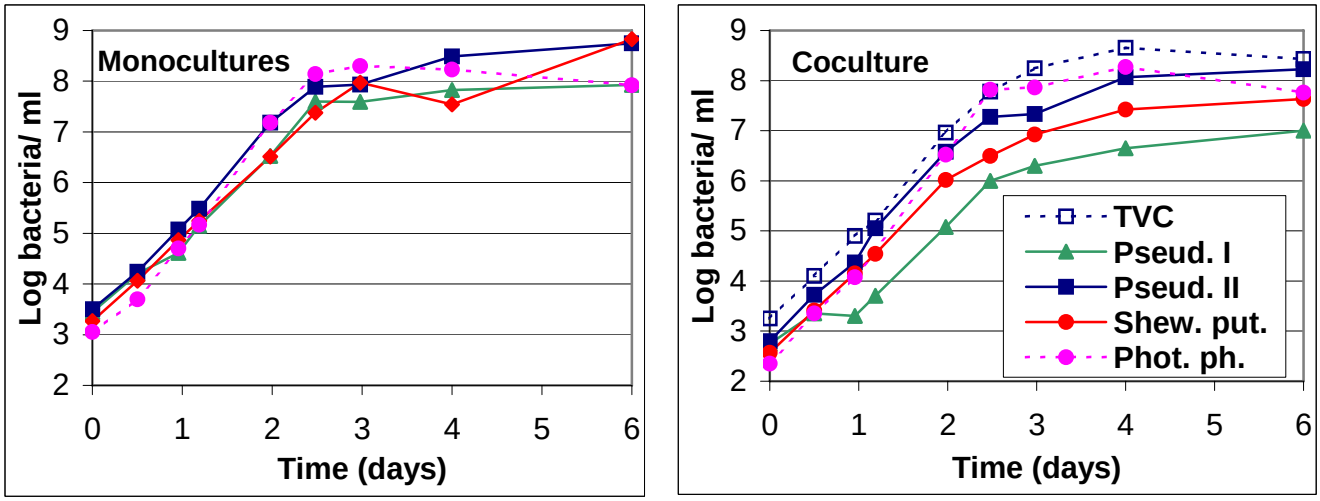


Figure 2. Bacterial proliferation of monocultures and coculture in fish extract at 7.6°C

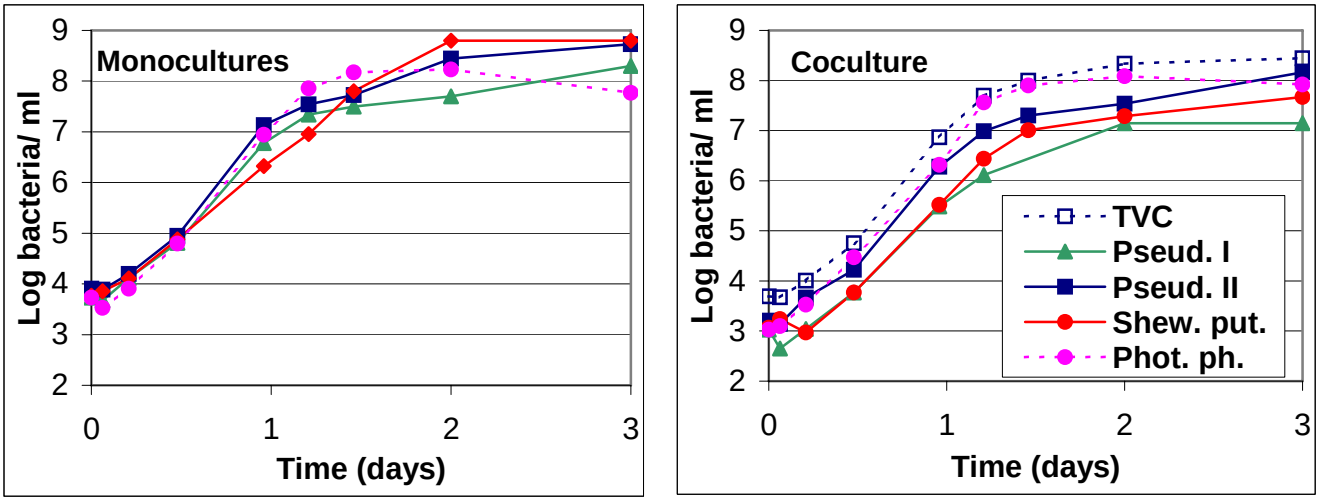


Figure 3. Bacterial proliferation of monocultures and coculture in fish extract at 14.8°C

At 7.6°C, similar growth was observed among the species tested separately. In coculture, *P. phosphoreum* and *Pseudomonas* II spp. grew rapidly and were dominating. *S. putrefaciens* grew to lower levels, while growth of *Pseudomonas* I spp. was delayed and reached the lowest level. Similar results were obtained at 14.8°C, except that *P. phosphoreum* was the dominating species in the coculture.

3.2 Analysis of volatile compounds by electronic nose and gas chromatography

Figures 4 to 6 show the results of the electronic nose measurements performed regularly on the inoculated fish extracts stored at 0.5, 7.6 and 14.8°C for several days, as well as the comparison to measurements of haddock fillets stored in EPS boxes (see Chapter 2, Experiment Nov. 2001). Such a comparison is meant to demonstrate what spoilage pattern is expected from the inoculated extracts based on the results obtained from spoiling haddock fillets. This can shed light on the importance of the different spoilage bacteria under investigation as well as how bacterial competition (coculture) can affect the spoilage pattern caused by certain bacteria.

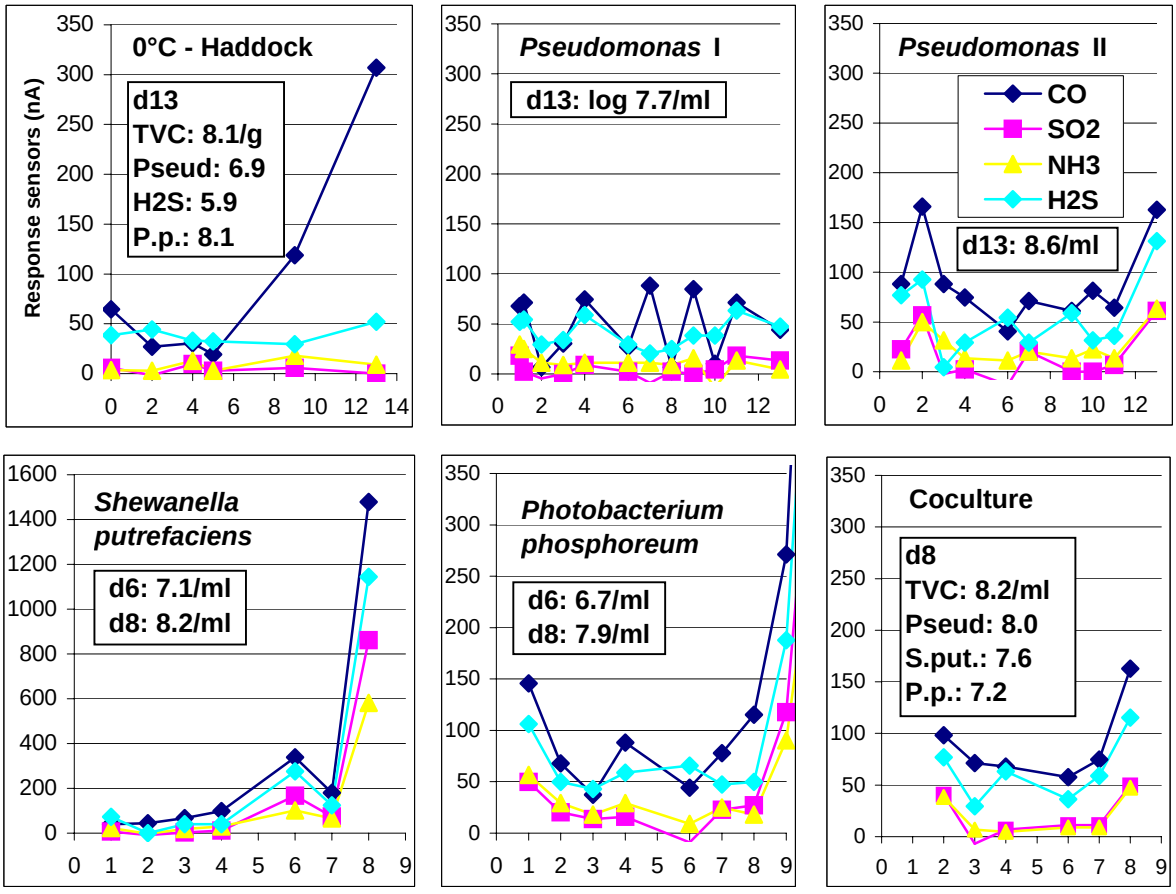


Figure 4. Electronic nose measurements of inoculated fish extracts stored at 0.5°C compared to measurements of haddock fillets (bacterial data in text boxes, log/ml or g)

However it should be pointed out that due to differences in the measuring methods, i.e. a few minutes headspace for haddock vs. an accumulated headspace for the fish extract in the jars, much higher responses can be expected for the fish extract. Also, initial bacterial counts and the composition of the microflora in coculture do not represent exactly what is found in fish fillets.

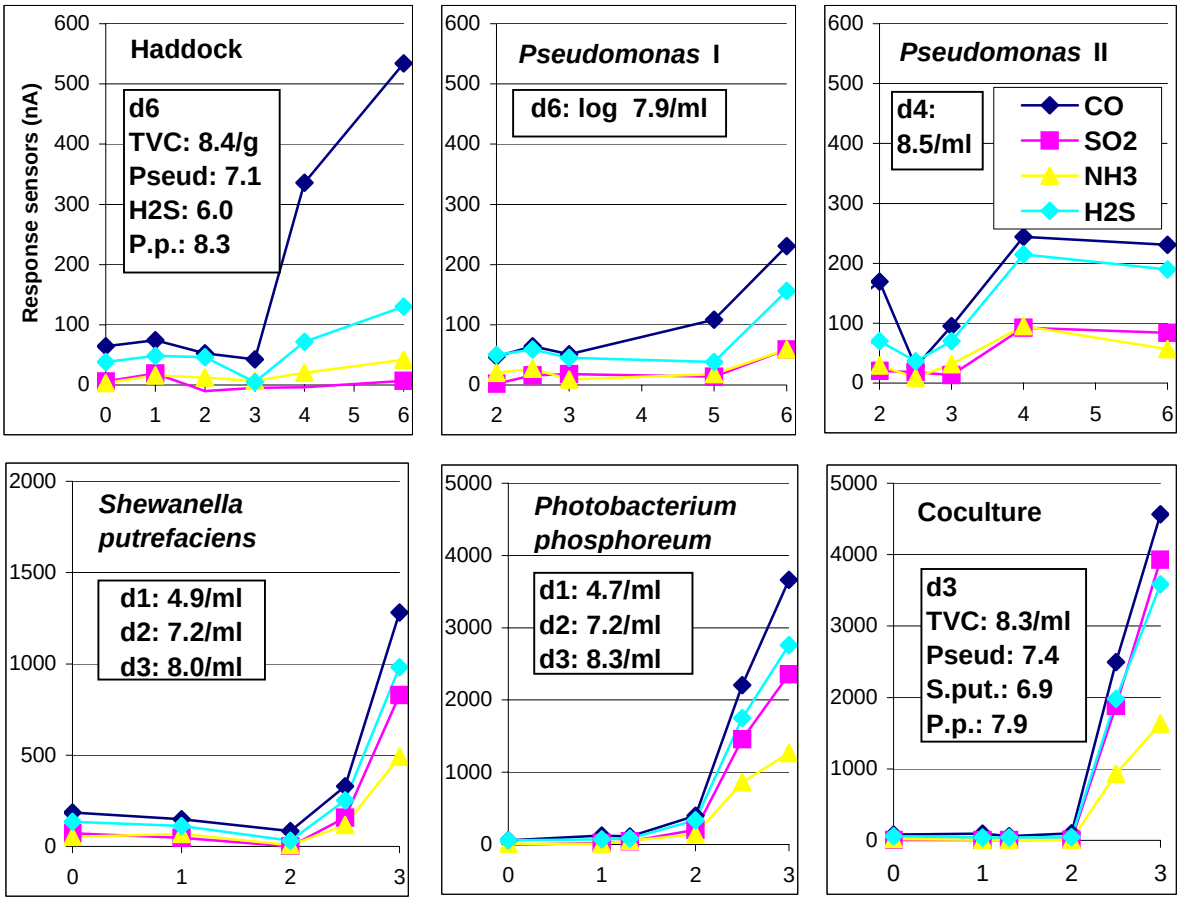


Figure 5. Electronic nose measurements of inoculated fish extracts stored at 7.6°C compared to measurements of haddock fillets (bacterial data in text boxes)

In Figure 4, a very rapid increase in the response of the CO sensor is observed for haddock fillets from day 5. On day 13, *Pseudomonas* spp. and *P. phosphoreum* accounted for almost 10% of the spoilage microflora while H_2S-producing bacteria (*S. putrefaciens* mainly) hardly reached 1%. In monocultures, it can be said that *Pseudomonas* I spp. did not show any potential production of metabolites under the conditions tested, while spoilage activity increased for the other bacterial groups evaluated in the following order:

Pseudomonas II spp., *P. phosphoreum* and *S. putrefaciens*. But high bacterial levels were required (log 8/ml). The CO sensor was mostly affected, followed by H_2S and SO_2, while NH_3 had the lowest response. In coculture, a similar pattern with a lesser response was observed. In contrast to the haddock response, the H_2S sensor was triggered, probably due to higher levels of *S. putrefaciens* and *Pseudomonas* spp. than what was observed in haddock fillets.

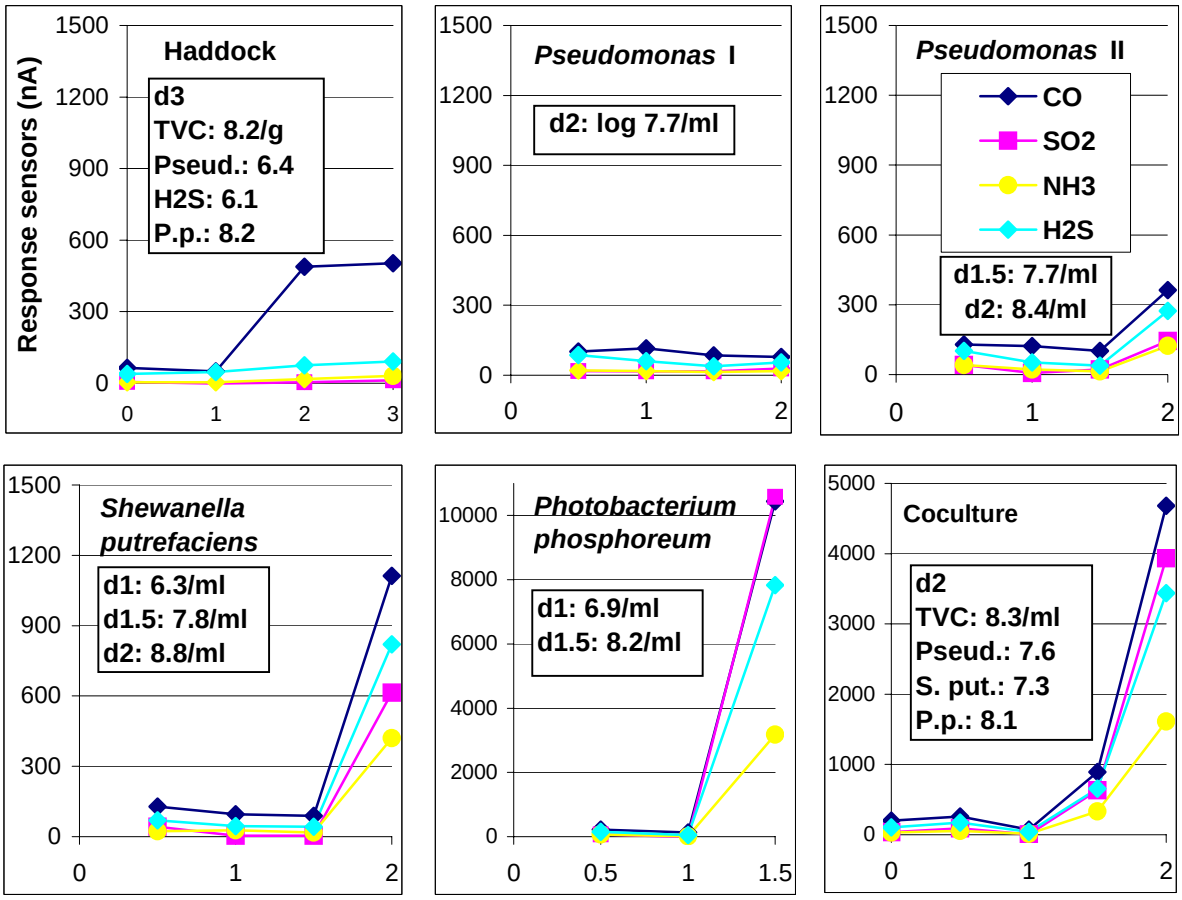


Figure 6. Electronic nose measurements of inoculated fish extracts stored at 14.8°C compared to measurements of haddock fillets (bacterial data in text boxes)

At 7.6°C, the haddock spoilage pattern (Figure 5) corresponded to that seen at 0°C, but with an increased response passed sensory rejection (day 6) despite the fact that similar levels of the different bacterial groups were found. In monocultures, all bacterial groups triggered the sensors but *P. phosphoreum* being the most active metabolites producer,

followed by *S. putrefaciens* and *Pseudomonas* spp. The coculture pattern resembled much that of *P. phosphoreum* monoculture. Based on the counts and corresponding responses of the different bacterial groups in monocultures, *P. phosphoreum* is believed to be responsible for the metabolites detected in the coculture. Similar findings were obtained at 15°C (Figure 6).

Table 2 summarises the preliminary results concerning the microbial metabolites detected by the electronic nose and GC-MS at 0°C and 15°C. Bold letters indicate the importantce of the compounds produced, while the boxes illustrate how influential are the bacterial strains to the spoilage pattern of the coculture at each respective temperature. This brings to conclude based on the results presented that at 0°C, *Pseud*omonas II, *S. putrefaciens* and *P. phosphoreum* all probably participate in the production of metabolites. However with increasing temperature, *P. phosphoreum* apparently becomes the major spoiler.

Table 2. Preliminary results on microbial metabolites detected by E-nose and GC-MS

Bacterial species	**E nose response**	**GC-MS (0-1°C)**	**GC-MS (15°C)**
Pseud. I	low: CO, H_2S	low amines	esters
Pseud. II	low: CO, H_2S	**DMDS, esters**	high DMDS, esters, DMTS, low amines
Shew. put.	**CO, H_2S, SO_2, NH_3**	**amines, DMS, acids, esters**	amines, esters, DMS, ketones
Ph. phosp.	**CO, H_2S, SO_2, NH_3**	**amines, esters, aldehydes, DMS**	**DMDS, acids, amines, esters, DMTS, al-OHs**
Coculture	**CO, H_2S,** SO_2, NH_3	amines, DMDS, acids, DMS, esters	amines, DMDS, esters, acids, DMTS, ketones
Haddock	**CO,** H_2S	**al-OHs** , amines, sulfides, ketones , esters	

Experiments in Feb 2001 GC analysis of haddock fillets showed that the compounds present in the highest concentration were . 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl acetate, 3-hydroxy-2-butanone, volatile sulfides and TMA which are all suggested as potential spoilage indicators because their concentration increases with time.

3.3 Estimation of growth rate of bacterial strains

The effect of temperature on growth and spoilage activity of selected bacterial strains previously isolated from spoiling fish was evaluated for temperature ranging from 0.5 to 14.8°C. The growth data were fitted using the DMFit program based on a reparameterised version of the model of Baranyi & Roberts (1994).

Table 3. Potential maximum growth rate (log_{10} N/ml/h) estimated by DMFit for bacterial cultures grown alone or in mixture in sterile fish juice at various temperatures

	Incubation temperatures		
Bacterial cultures	**0.5°C**	**7.6°C**	**14.8°C**
***Pseudomonas* I spp.**			
monoculture	0.0252	0.0712	0.1350
coculture	0.0219	0.0793 (22.1 h)*	0.1402 (3.7 h)*
***Pseudomonas* II spp.**			
monoculture	0.0316	0.0768	0.1331
coculture	**0.0301**	0.0751	0.1375
S. putrefaciens			
monoculture	0.0257	0.0705	0.1297 (3.6 h)*
coculture	0.0291 (24.7 h)*	0.0670	0.1561 (7.2 h)*
P. phosphoreum			
monoculture	0.0291 (26.1 h)*	0.1111 (9.9 h)*	0.1984 (5.6 h)*
coculture	0.0481 (60.5 h)*	**0.1067 (19.2 h)***	**0.2186 (5.4 h)***

* estimated lag phase (h)

The estimated growth rates are given in Table 3. Values given in parenthesis represent the lag phase (h) which was estimated by the DMfit program. It should be noticed that a rapid growth rate is often observed following a certain growth delay (lag phase).

Therefore rates should be carefully compared to other values where no lag phase occurred.

In parallel to what was described in the previous sections, the bacterial groups behaved differently whether cultured alone or in mixture. They most often had a slower rate in cocultures, except in the case where a lag phase occurred followed by a sudden growth. *Pseudomonas* group I spp. had the poorest growth rate when grown in coculture at low temperature (0°C), while *Pseudomonas* group II spp. grew fast and steadily. A lag phase was observed for both *S. putrefaciens* and *P. phosphoreum*, the latter being retarded the longest but reaching then a high growth rate. With increasing temperature, the lag phase occurring for *P. phosphoreum* decreased accordingly and reached the highest growth rates. It is interesting to point out that no obvious inhibition was observed for *Pseudomonas* group II spp. when grown in coculture, while with increasing temperature *Pseudomonas* group I spp. and *S. putrefaciens* became slightly inhibited as indicated by a lag phase or a slower growth rate. These results exemplify the behavior of the specific spoilage organisms in sterile fish extract incubated over the temperature range of 0.5°C to 14.8°C. The next chapter will provide data relating to the real behaviour of SSO in fish flesh.

4 CONCLUSION

Development of *Photobacterium phosphoreum* was evidenced within the temperature range of 0-15°C in fish extract and its important contribution to the spoilage pattern of air-stored fish should not be overlooked. *Pseudomonas* II, *S. putrefaciens* and *P. phosphoreum* all probably participate in the production of metabolites in spoiling fish stored aerobically at low temperatures. However with increasing temperature, *P. phosphoreum* apparently becomes the major spoiler.

.5 REFERENCES

Baranyi J. & T.A. Roberts (1994). A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23, 277-294.

Dalgaard P. (1995). Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int. J. Food Microbiol. 26, 319-333.

Dalgaard P., O. Mejlholm & H.H. Huss (1996). Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. J. Appl. Bact. 81, 57-64.

Dalgaard P, O. Mejlholm, T.J. Christiansen, H.H. Huss (1997). Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. Lett. Appl. Microbiol. 24: 373-378.

Gram L., G. Trolle & H.H. Huss (1987). Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int. J. Food Microbiol. 4, 65-72.

Gram L. & H.H. Huss (1996). Microbiological spoilage of fish and fish products. Int. J. F. Microbiol. 33 (1), 121-137.

Lauzon H.L. (2003). Notkun Malthus leiðnitækni til hraðvirkra örverumælinga. Verkefnaskýrsla 30-03, 30 síður (Icelandic).

Ólafsdóttir ,G., Á. Högnadóttir, E. Martinsdóttir and H. Jónsdóttir, 2000. Application of an Electronic Nose to Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production, J. Agric. Food Chem. 48 ,6, 2353-2359.

Ólafsdóttir , G., E. Martinsdóttir and E. H. Jónsson, 1997. Rapid gas sensor measurements to predict the freshness of capelin (*mallotus villosus*). J.Agric. Food Chem. 45,7, 2654-2659.

Van Spreekens K.J.A (1974). The suitability of Long & Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. Archiv fur Lebensmittelsh. 25 (10), 213-219.

Chapter 2

The influence of temperature fluctuations on the shelf life of haddock and cod fillets stored under chilled conditions

Guðrún Ólafsdóttir

Hélène L. Lauzon

Rósa Jónsdóttir

Emilía Martinsdóttir

TABLE OF CONTENTS

1 INTRODUCTION

Determination of freshness quality and shelf life of fish is based on monitoring autolytic, microbial, oxidative and physical changes occurring in fish *post mortem*. Different handling, processing and storage conditions influence the nature and rate of spoilage changes. Temperature is by far the most influential factor determining the rate of the deteriorative changes occurring in fish. Fish has a very limited shelf life when stored at refrigeration temperatures. Fluctuating temperatures, i.e. improper icing or higher environmental temperature during any stage of the processing or transport, will undoubtedly shorten the shelf life of the products. However, the effect of fluctuating temperatures on the deterioration rate as well as the spoilage pattern is not well known.

Reliable tools to determine the actual freshness quality of fish products are important for the fish industry for process management and for the buyers in the chain to verify the quality of the final products to ensure products of consistent freshness quality for consumers. Sensory analysis is generally recognised as the best method to evaluate freshness and spoilage level of fish. Microbial growth and oxidative changes are the main causes of quality changes in chilled fish causing the formation of volatile degradation compounds and the development of spoilage odors. However, microbial counts and laboratory techniques such as TVB-N analysis and gas chromatography to measure microbial metabolites are time consuming and expensive techniques. Electronic nose measurements of volatile compounds can be useful to measure simultaneously in a rapid way the various spoilage indicators that correspond to the odor changes.

Earlier storage experiments on whole haddock stored in ice and haddock fillets stored at 0-2°C have shown that microbial counts (TVC) are not accurate indicators of the spoilage level of samples when compared to sensory analysis (Olafsdottir *et al.*, 2000) Measurements of specific spoilage organisms (SSO) like *Shewanella putrefaciens*, *Pseudomonas* ssp. and *Photobacterium phosphoreum* are more reliable than TVC to predict the quality. It has also been pointed out that TVB-N does not always give the best information about the quality of the products. The importance of identifying and monitoring more than one indicator to monitor the complex changes occurring in fish during storage has been emphasised. Preliminary studies in this project using PLS

prediction based on electronic nose, microbial and sensory data from storage experiments of haddock showed that a better model was obtained when using electronic nose data to predict sensory scores than when the model was based on e-nose data to predict microbial counts (Olafsdottir *et al.*, 2000).

The aim of the studies reported herein is to monitor changes during storage of cod and haddock fillets stored at different temperatures to obtain data from rapid measurements of specific spoilage organisms with the Malthus technique and electronic nose measurements of microbial metabolites for the development of models to predict the microbial growth and shelf life of fish. Traditional sensory (Torry), chemical (TVB-N, pH) and microbial (TVC) analysis were used as reference methods. Research on predictive microbiology has shown that more realistic shelf life models for food can be achieved if temperature fluctuations are taken into account and specific spoilage organisms (SSO) are used rather than total viable counts. Collection of data to use for the development of models to predict the shelf life of fish has been done in the project by carrying out various storage experiments to study the effect of fluctuating temperatures on the deteriorative changes in fish fillets during storage. In this report the data from the following storage studies of both haddock and cod fillets will be presented.

- Preliminary storage experiments of haddock fillets stored at 0-1°C - February 2001
- Storage experiments of haddock fillets at 0°C, 7°C and 15°C – Tros / November 2001
- Storage experiments of cod fillets under different handling and storage conditions - Tangi / October 2003
- Storage experiments of cod and haddock fillets at different fluctuating temperature - Tros /November 2003

2 MATERIALS & METHODS

2.1 Storage experiments of cod and haddock fillets processed and stored under different conditions

2.1.1 Preliminary storage experiment of haddock fillets stored at 0-1°C - February 2001

Preliminary storage experiment of haddock fillets was done at 0-1°C to test the performance of the measurement techniques. Samples were obtained from the company TROS (Sandgerði, Iceland) as fillets packed in styrofoam boxes (EPS boxes 110 x 400 x 263mm) in the same way as the product is exported by air transport to the US and the UK (Olafsdottir *et al.*, 1999). Samples were analysed by electronic nose and gas chromatography on days 3, 7, 10 and 14, and microbial measurements were done on days 1, 3, 6, 9 and 14.

2.1.2 Storage experiments of haddock fillets at 0°C, 7°C and 15°C – Tros / November 2001

The fish was caught by long line and received at the processing factory within 12 hours from catch. After gutting, filleting, and skinning, the fillets were packaged in styrofoam boxes (EPS boxes) lined with a plastic bag and an absorbing pad at the bottom. Each box (110 x 400 x 263 mm) contained 11 fillets and a cooling mat was placed on top prior to closure. All the boxes were shipped to the laboratory and stored at different temperatures (0°C, 7°C and 15°C) until sensory rejection.

Table 1. Overview of sampling days for haddock samples from Tros stored at different temperatures - November 2001

	Storage Temperature		
Days of sampling	**0°C**	**7°C**	**15°C**
0d	x	x	x
1d		x	x
2d	x	x	x
3d		x	x
4d*	x	x	
6d	x	x	
9d	x		
13d	x		

***only microbial and electronic nose measurements performed**

Upon receipt, temperature loggers (Optic StowAway®, Onset Computer Corporation, US) were inserted underneath and above the fillets in one box for each temperature treatment, as well as on top of the box to follow the environmental variations of each storage condition. The initial quality of the raw material was assessed in the afternoon (day 0) by sensory, microbial, and chemical (TVB-N/TMA and pH) analysis as well as by electronic nose measurements. Further sampling occurred on days 1, 2, 3, 4, 6, 9 and 13, as shown in Table 1

2.1.3 Storage experiments of cod fillets under different handling and storage conditions - Tangi / October 2003

The fish was caught by bottom trawl on Friday afternoon (October 17th 2003) by the ice fish trawler Brettingur east off Iceland in the Berufjardarall catching zone. The fish was gutted on board and iced in tubs (400L). The fish was landed at the processing plant Tangi in Vopnafjordur on the following Monday morning (October 20th 2003) (three days after catch)

Preparation of samples

Approximately 150 fishes from the same batch were used to prepare four groups of samples to study the influence of different handling and processing practices and storage conditions.

Group A – traditional processing - storage at 0-1°C: After filleting, the fillets were deskinned in a Baader deskinning machine, packed (12 fillets per EPS box) and transported before further storage at 0-1°C at the laboratory.

Group B – traditional processing - temperature fluctuations and storage at 0-1°C: The fillets were deskinned in a Baader deskinning machine. After packing and transport, Group B was kept at 0-1°C for 3 days and then transferred into 15°C storage for 8 hours and finally transferred back to 0-1°C storage.

Group C – superchilling - storage at 0-1°C: After filleting, 80 fillets were prepared using a new deskinning technique. The fish was put in an ice-water cooling solution containing 0.85% NaCl for 45 minutes. The new deskinning technique involves partially freezing quickly the surface of the fillets by moving them through a freezing tunnel on a conveyor belt. The technique facilitates the removal of the skin and also

cools the fish effectively so the temperature of the fillets is around –1°C when packed in the styrofoam boxes.

Group D - uniced - traditional processing - temperature fluctuations and storage at 0-1°C: To simulate abusive temperature conditions, 30 fishes were selected from the same batch and kept uniced in the reception area (approximately 7-8 hours) and then transferred into the processing area (T=15-18°C) until the following morning when the fish was filleted, deskinned, packed and transported to the lab. The same temperature conditions during storage were used as for group B, which was kept at 0-1°C for 3 days and then tranferred into 15°C storage for 8 hours and then transferred back to 0-1°C storage.

All the samples were packed in plastic bags in styrofoam boxes (160 x 400 x 263 mm) containing 8-12 fillets per box, an absorbing pad at the bottom and a cooling mat (230 x 160 mm; 146 g) placed on top. Selected boxes contained temperature loggers (different types) to follow the temperature of the fillets at the bottom and in the middle of the box as well as above the fillets and on top of the box to record environmental variations. The samples were transported by a refrigerated truck (4-5°C) to Reykjavik and the samples arrived on Tuesday morning (October 21st (Groups A, B and C) and Group D on Wednesday morning (October 22nd).

Analysis of samples were done on days 1, 4, 7, 9, 11, (14) after processing which corresponds to days 4, 7, 10, 12, 14 after catch for groups A and C. Samples from groups B and D were analysed on days 3, 4, 5, 7 and 9 after processing, corresponding to days 6, 7, (8), 10 and 12 after catch.

Following analyses were done to monitor changes during storage and to determine the shelf life of the samples stored and processed under different conditions.

Sensory analysis using the Torry scheme, microbial analysis (TVC and SSO counts), chemical analysis of total volatile bases (TVB-N) and pH, analysis of volatile compounds using gas chromatography (GC-MS and GCO) and an electronic nose (FreshSense) with electrochemical sensors.

2.1.4 Storage experiments of cod and haddock fillets at different temperatures - Tros /November 2003

Additional storage experiments were done on cod and haddock fillets obtained from the company Tros to collect further data for models and study the influence of fluctuating temperatures during storage. The fish was caught by longline close to Sandgerði (November 10th) and processed according to the traditional process of the factory one day after catch. The cod was gutted on board the boat and stored in ice in the factory overnight, but the haddock was stored ungutted in ice slurry overnight. The fish was hand filleted the following morning and cooled in ice-water before deskinned in a machine (Baader) and packed in plastic bags in styrofoam (EPS) boxes (cod:135 x 400 x 263 mm; haddock:110 x 400 x 263mm). The boxes contained 11 fillets each and were transferred to the laboratory the same day. Four groups of samples were prepared (haddock groups A and C; cod groups B and D). Upon receipt, temperature loggers (Optic StowAway®, Onset Computer Corporation, US) were inserted underneath, in between and above the fillets in one box for each temperature treatment, as well as on top of the box to follow the environmental variations of each storage condition. All the sample groups were initially stored at 0-1 °C, but on the third day of storage groups C and D were transferred from the cooler and stored overnight (16 hours) at elevated temperatures (RT) and then moved back to the cooler.

The shelf life of the cod and haddock fillets was determined based on sensory analysis using Torry schemes. Spoilage changes of the different samples during storage were monitored by microbial analysis (TVC and SSO counts), chemical analysis of total volatile bases (TVB-N), pH and rapid measurement of volatile compounds by an electronic nose (FreshSense) with electrochemical sensors. Gas chromatography (GC-MS and GCO) analysis of volatile compounds was done for haddock fillets stored at 0°C (Group A) to identify the compounds present in the highest concentration in the headspace.

The first samples were taken on the day following processing (day 1, i.e. 2 days from catch). Samples were analysed regularly until sensory rejecion: group A (haddock) on days 1, 3, 6, 8 and 10 after processing; group B (cod) on days 1, 3, 6, 8, 10 and 13; group C (abused haddock) on days 1, 3, 4, 6 and 8; and group D (abused cod) on days 1, 3, 4, 6, 8 and 10.

2.2 Microbiological evaluation

Fillets were aseptically minced, assessing 2 pooled fillets for each sample. Twenty-five grams of minced flesh were mixed with 225 mL of cooled Maximum recovery diluent (MRD, Oxoid) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required. Total viable psychrotrophic counts (TVC) were evaluated by spread-plating aliquots onto modified Long & Hammer's medium (Van Spreekens, 1974) containing 1% NaCl, with aerobic incubation at 15°C for 4-5 days. H_2S-producing bacteria were evaluated on spread-plated Iron Agar (IA based on Gram *et al.*, 1987, but containing 1% NaC) after incubation at 15°C for 4-5 days. Presumptive *Pseudomonas* counts were obtained using modified CFC medium (Pseudomonas Agar Base CM559 supplemented with CFC selective agar supplement SR103, Oxoid; modifications as described by Stanbridge & Board, 1994) following incubation at 22°C for 3 days. Bacteria were counted using a Darkfield Quebec Colony Counter (Spencer). The detection limit was 20 colony forming units (CFU)/g. Counts of *Photobacterium phosphoreum* were estimated by using the PPDM-Malthus conductance method (Dalgaard *et al*., 1996), as described by Lauzon (2003).

2.3 Electronic nose measurements

Electronic nose measurements were performed using a gas sensor instrument called "FreshSense", developed by the Icelandic Fisheries Laboratories and Bodvaki (Maritech, Iceland). The instrument is based on electrochemical gas sensors (Dräger, Germany: CO, H_2S, and SO_2; City Technology, Britain: NH_3). The measurement technique was described earlier by Olafsdóttir *et al*.(2000) and Ólafsdóttir and Högnadóttir, (2000).

The instrument consists of a 2.3L closed glass sampling container and a dynamic sampling system. The headspace from the sampling container was transported with a pump into a small measurement chamber. The headspace was circulated between the sampling container and the measurement chamber and no extra air introduced into the system. Approximately 500g of fish fillets was weighted and put into the sampling container and sampling was done at room temperature. The temperature of the fillet was monitored and the influence of different temperatures (4-5°C and 14-18°C) during sampling was studied in the experiment in February 2001. The standard

procedure is to keep the fillets at room temperature for 30 minutes to allow the fillets to reach 8-12°C before measurements start. A Labview measurement and data acquisition software was used. Measurements were taken every 10 seconds for 10 minutes in the measurements in 2001, but in the experiments in 2003 the measurement time was shortened to 5 minutes. The reported value (current) is the average of last three measurements of the 10 minutes measurement cycle minus the average of 3 values before the measurement starts.

2.4 Chemical analyses

Chemical analyses were done with the rest of the flesh mince prepared for microbiological analyses within 30 min. of preparation.

2.4.1 pH measurements

The pH was measured in 5 grams of mince moistened with 5 mL of deionised water. The pH meter was calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

2.4.2 TVB-N analysis

Total volatile base content (TVB-N) was measured by the steam distillation method described by Malle & Poumeyrol (1989), using the rest of the flesh mince prepared for microbiological analysis in the experiments in 2003.

In November 2001 the TVB-N content was determined by steam distillation of TCA extract according to the 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.

2.4.3 Determination of TMA

TMA analysis was only done in the experiments in November 2001. Determination of TMA was done according to Dyer method (Dyer *et al*., 1945) modified by Tozawa (1971).

2.5 Sensory analysis.

The IFL sensory panel evaluated the freshness of the fillets in all the experiments to determine the shelf life of differently treated products. A total of 8-12 previously trained panellists participated at each session. Fish from each treatment was portioned into an aluminium box and cooked in a steam oven (98-100°C for 5 min.). Each treatment was assessed in duplicate and the samples were anonymously coded. A computerised system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording of cooked samples and their further processing. Average scores were calculated for each treatment and significant differences between corresponding treatments evaluated.

The modified Torry scheme (Shewan *et al*., 1953) was used to assess the freshness (odour and flavour) of cooked pieces. This scheme ranges from 10 = very fresh to 3 = very spoiled, with a rejection level at 5.5.

2.6 GC measurements

Gas chromatography analysis were done for selected samples in the storage studies to identify the main compounds present in the headspace of fish fillets during storage and contributing to the odor changes. Moreover the information about the identities of the compounds is useful to guide the future development of the electronic nose based on selecting sensors that are sensitive to the indictor compounds identified.

2.6.1 Sample preparation for GC-MS

Headspace of samples was collected by an air pump sampling (ALPIN-2, Air sampler, METEK). Approximately 500 g of fish (1-2 fillets) was placed in the glass container (2.3L, Ø 17 cm) and the headspace collected by sweaping volatiles from the surface of the fish by pumping for 2 hours with a flow rate of approximately 100 mL/min. Volatiles were collected on 250 mg Tenax 60/80 (Alltech, IL) in stainless steel tubes

(Perkin-Elmer, Buchinghamshire, UK) for the combined ATD 400 and GC-MS measurements. Heptanoic acid ethyl ester was used as an external standard by putting 25 mL beaker (Ø 3.5 cm) containing 1 mL of 10 ppm aqueous solution of the standard into the sampling glass container. Each sample was prepared in duplicate.

2.6.2 Sample preparation for GCO

Samples were prepared by weighing 100 ± 2 g of fish fillets and 100 ± 5 g of saturated aqueous solution of NaCl into a 250 mL round bottom flask. Saturated NaCl solution (200 ± 5 g) was prepared as a reference sample. Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 1 mL of 10 ppm aqueous solution of the standard to the 200 g fish /$NaCl_{sat}$ solution. The sample was purged at room temperature with nitrogen at about 100 mL/min for 2.5 hours. Volatiles were collected on 250 mg Tenax 60/80 (Alltech, IL) in stainless steel tubes (Perkin-Elmer, Buchinghamshire, UK) for the combined ATD 400 and GC-MS measurements or 150 mg Tenax in a Pasteur pipette for the GC-O measurements. Each sample was prepared in duplicate. Sampling was done at room temperature

2.6.3 GC-MS analysis

Volatile compounds were thermally desorbed (ATD400, Perkin Elmer) from the Tenax tubes and separated on a DB-5ms column (30 m × 0.25 mm i.d. × 0.25 µm, J&W Scientific, Folsom, CA) using helium as a carrier gas. The following temperature program was used: 50°C for 7 min, 50°C to 120°C at 5°C/min and from 120°C to 220°C at 10°C/min. Volatile compounds were identified with GC-MS (HP G1800C GCD, Hewlett-Packard, Palo Alto, CA) by matching retention indices (RI) of ethyl esters and mass spectra of samples with authentic standards (Sigma-Aldrich and Merck). Tentative identifications were based on standard MS library data (Hewlett-Packard Co, 1997) and manually checked against literature sources and the database Flavornet (Acree and Arn, 1997). Semi-quantitative evaluation based on the external standard was done for selected components using PAR (Peak Area Ratio).

2.6.4 GC-O measurements

Volatiles were extracted from the Tenax traps with 1-2 mL diethyl ether. The sample was then concentrated by passing nitrogen over the solution leaving a small amount of

sample, 20-30 µL. Headspace samples (1µL) were then injected splitless. Measurements were performed on a GC (HP 5890, Hewlett-Packard, Palo Alto, CA) with the same type of column and the same conditions as for the GC-MS measurements. The end of the column was split 1:1 between flame ionization detector (FID) and an ODO-1 olfactory detector outlet (SGE, UK). Nitrogen, bubbled through water to add moisture, was used to drive the sample up to the sniffer. Two persons sniffed the effluent and described the odor accordingly. Intensity (quality and duration/retention times) of each odor was determined using an intensity from 0 to 5, 0: not present; and 5: very strong.

2.7 Temperature recording

Various types of temperature data loggers were used in the experiments (Optic StowAway®, Onset Computer Corporation, US; StarOddi, Iceland; Leiðir, Iceland) to monitor the temperature of the samples as well as that of the environment. The loggers were located underneath, between and on top of the fillets inside the styrofoam packages

2.8 Data analysis

Microsoft Excel 97 was used to calculate means and standard deviations for all multiple measurements and to generate graphs.
Microbiological, chemical and physical data were processed using Microsoft Excel 97. Data from sensory evaluation was further analysed using the NCSS 2000 software. Duncan´s Multiple-Comparison Test was used to determine the statistical difference between samples, evaluated at the 95% level.

Multivariate analysis was performed by the Unscrambler (version 8., CAMO A/S, Trondheim, Norway). Principal component analysis (PCA) was performed on the data using values standardized to equal variance to study the main variance in the data set Cross validation was used in the validation method.

The statistical analysis was carried out with the Number Cruncher Statistical Software (NCSS) 2000, using ANOVA. In case of statistical significance, the Duncan's multiple range was performed. An effect was considered significant at the 5% level.

3 RESULTS & DISCUSSIONS

3.1 Preliminary storage experiment of haddock fillets stored at 0-1°C - February 2001

The aim of the preliminary experiments was to test the performance of the measurement techniques in particular: a) the microbial analysis using the Malthus conductance technique (Lauzon, 2003), b) a new sampling technique for gas chromatography using an air pump to collect samples on an adsorbent (TENAX).

3.1.1 Microbial and electronic nose analysis

The microbiological quality (TVC) of the haddock fillets was evaluated the day following processing and packaging (day 1) and was found to be satisfactory (5.420 psychrotrophic bacteria/g or log 3.7/g). After 3 days of storage rapid bacterial proliferation occurred, reaching log 8.0/g on day 9 as seen in Figure 1. Slower growth was observed towards the end of the storage period. Similarly, growth of *Pseudomonas* spp. was steady and rapid, hence dominating the other bacterial species evaluated towards the end of the storage period (log 8.4/g). Development of *Photobacterium phosphoreum* (PP) and H_2S-producing bacteria (*Shewanella putrefaciens* mainly) also occurred rapidly, but reached lower levels during late storage.

The sensors of the electronic nose typically do not show significant increases in their responses until after a few days of storage as seen in Figure 2. This trend and similar values for the sensors are in agreement with data presented from storage studies of haddock fillets in May 2000 (Olafsdóttir *et al*., 2000). The steady increase in the response of the CO sensor after day 7 from 180 nA (Day7) to more than 700nA on day 14 coincides with the increase in the count of specific spoilage bacteria, i.e. *Pseudomonas* counts from log 6/g to log 7.5/g (day 10) to reach log 8.4/g on day 14, H_2S-producing bacteria from log 5.5/g to log 7/g (d10) to reach log 7.7/g on day 14

and *Photobacterium phosphoreum* from log 6.4/g to 6.7/g (d10) to reach log 7.3/g on day 14. Increase in the response of the NH_3 sensor after day 10, detected on day 14, may be contributed by the growth of *P. phosphoreum* and H_2S-producing bacteria (*S. putrefaciens* mainly) which are both known as TMA producers.

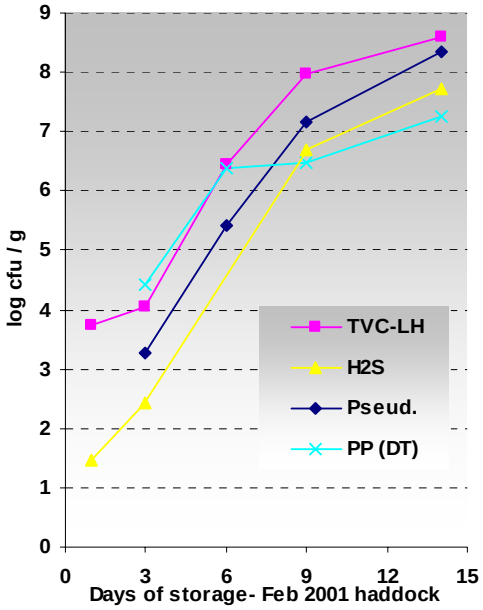


Figure 1. Total psychrotrophic, H_2S-producing bacteria, presumptive Pseudomonas spp., and P. phosphoreum counts of haddock fillets stored in EPS boxes at 0-1°C (February 2001)

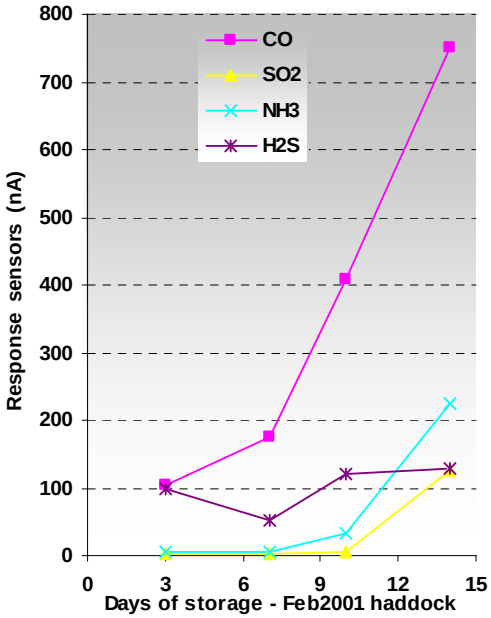


Figure 2. Response of CO, NH_3, H_2S and SO_2 sensors of the electronic nose of haddock fillets stored in EPS boxes at 0-1°C (February 2001)

3.1.2 Gas chromatography analysis

Gas chromatography analysis was done to obtain detailed information about identities and the level of the most abundant volatile compounds present in the headspace during storage of fish. Information on the identities and quantities of volatile compounds present in the headspace during storage of fish is essential to select the key indicator compounds for freshness quality and to understand the responses of the sensors of the electronic nose.

A TENAX tube and an air pump were used to collect the headspace volatiles of haddock fillets stored for 3, 7, 10 and 14 days. Table 2 shows the main classes and identities of compounds present in the highest concentration in the headspace. Quantities were estimated based on peak areas. The results are in agreement with earlier work of Lindsay *et al*. (1986) who reported that the main classes of compounds that are present in high concentrations in the headspace during storage of fish are

short chain alcohols, aldehydes, ketones, esters, sulphur compounds and amines. The identities of compounds analysed in refrigerated fish fillets are very similar as the volatiles in smoked salmon reported by Joffroud *et al*. (2001). They found that 2,3-butandione, 3-hydroxy-2-butanone, 2-methyl-1-butanol- and 3-methyl-1-butanol were the most abundant volatiles in the headspace and these could be used as indicators of spoilage for smoked fish. Our results show that some of these compounds or similar ones are present, i.e. 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl acetate, 3-hydroxy-2-butanone, volatile sulfides and TMA that are all potential indicators because their concentration increases with time. These findings indicate that a similar set of sensors can be used to evaluate the spoilage of different fish products.

For meaningful interpretation of electronic nose data it is useful to determine the sensitivities of the sensors towards the main quality indicator compounds. Analysis of selected standards, which are representative of the main classes of spoilage compounds have shown that the electrochemical gas sensors (CO, SO_2 and NH_3) in the electronic nose "FreshSense" have different sensitivities towards compounds from these classes (Ólafsdóttir *et al.,* 1998b; Olafsdóttir *et al.,* 2002). Figures 3 and 4 give a comparison of the analysis of volatiles by GC and sensor responses of the electronic nose FreshSense and demonstrate well the sensitivities of the sensors towards different classes of compounds. A similar trend of the sensor responses and the concentration of volatiles analysed in the headspace of the haddock fillets during storage is evident. Figure 3 shows a graph with the sum of the concentration of compounds representing each class of compounds from Table 2 and Figure 4 shows the results of the electronic nose measurements of the same samples with the FreshSense instrument. The ketones are not detected by the sensors and have therefore not been included in the graph.

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

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

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

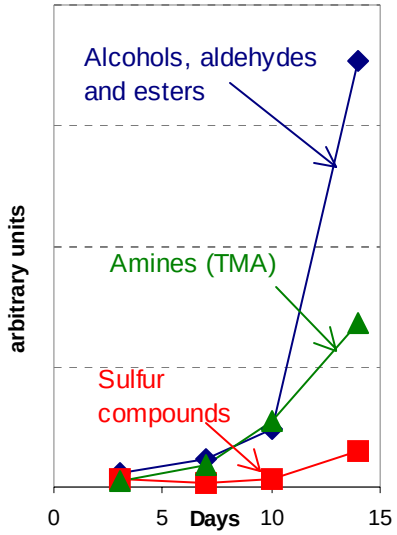


Figure 3. Sum of the peak areas of compounds representing the three different classes of compounds detected by GC in the headspace of haddock fillets during storage in ice

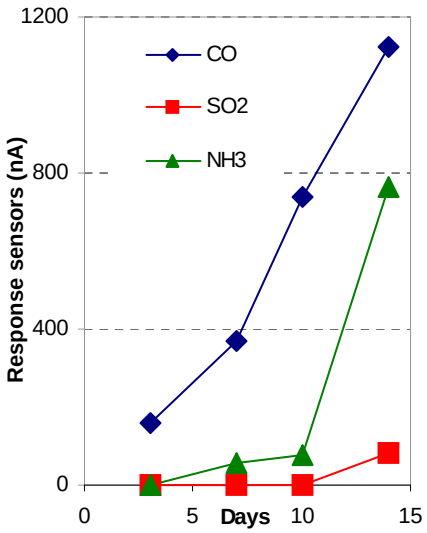


Figure 4. Responses of the CO, SO_2 and NH_3 sensors towards haddock fillets during storage in ice (From Olafsdóttir, 2003)

Figure 4 shows that the responses of the CO sensor detecting alcohols, aldehydes and esters is the highest and increases early in the spoilage process while the other sensors´ responses increase later in the spoilage process. The NH_3 sensor detects amines, mainly TMA and ammonia and the SO_2 sensor detects volatile sulfides. The TENAX technique does not detect the very volatile small molecules like ammonia, hydrogen sulfide, methyl mercaptan, and ethanol that are also known to be present in abundance in the headspace of spoiled fish and have been suggested as indicators of fish quality. The electrochemical sensors can however detect these compounds and therefore the slopes and shapes of the curves are slightly different.

3.2 Storage experiments of haddock fillets at 0°C, 7°C and 15°C – Tros / November 2001

The aim of these experiments was to monitor spoilage changes of haddock fillets stored at different constant temperatures (0°C, 7°C and 15°C) to obtain data for models taking into account the influence of different temperatures during storage. Fresh haddock fillets were obtained in EPS boxes from the company Tros.

3.2.1 Sensory analysis and shelf life determination

Sensory evaluation was done to determine the shelf life of haddock fillets stored in EPS boxes. Figure 5 shows that the sensory rejection (based on Torry score of 5.5) of haddock fillets occurred after 12.5 days for samples stored at 0.9 ± 0.3°C; after 5.5 days for samples stored at 7.2 ± 0.2°C and after 3.5 days of storage for samples stored at 15.1 ± 1.1°C. Figure 5 shows that the deviation from a linear trend of the Torry score with storage days is more pronounced at higher storage temperatures.

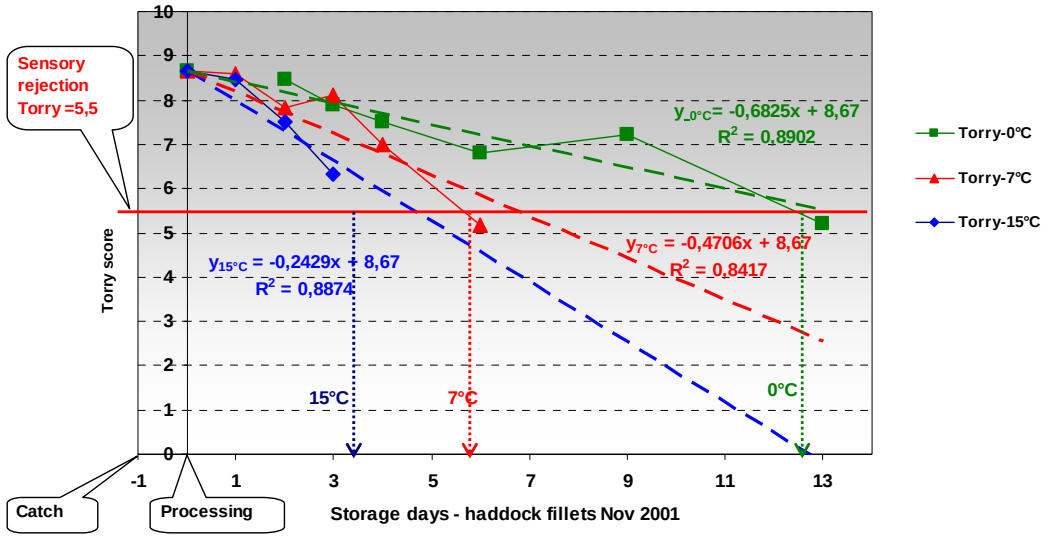


Figure 5. Results of sensory analysis (Torry) of haddock fillets stored in EPS boxes at 0°C, 7°C and 15°C - Tros (November 2001).

The rate of spoilage is highly dependent on the temperature. Figure 6 shows how temperature of the fillets increased due to environmental conditions. It should be pointed out that initial temperature of the fillets was around 3°C for the fillets stored at 15°C, but had reached 14°C after 1.5 day of storage after which rapid deterioration occurred as demonstrated on Figure 5. The initial temperature of fillets stored at 7°C was around 4-5°C and raised to 7°C within 3 days, coinciding with a rapid drop in Torry scores during the following days.

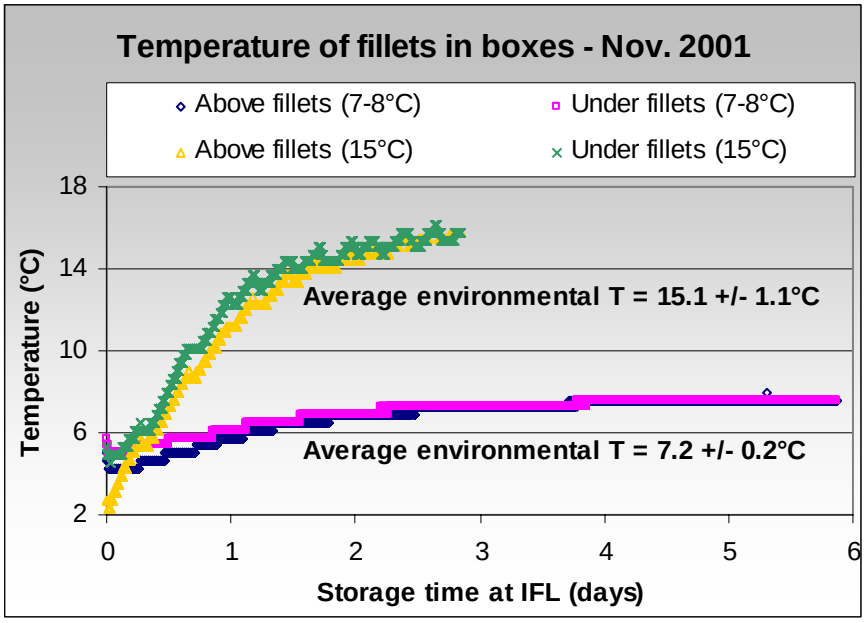


Figure 6. Temperature profiles of haddock fillets stored at 7°C and 15°C during storage

3.2.2 Electronic nose, TVB-N and pH measurements

Figures 7 and 8 show the same overall trend for the production of volatiles during storage at the different temperatures, measured by the electronic nose and TVB-N analysis, respectively. The spoilage rate is fastest at 15°C and slowest at 0°C as expected.

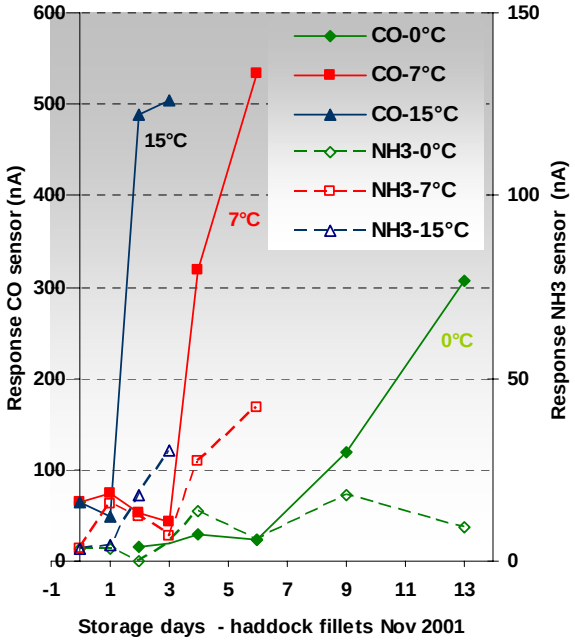


Figure 7. Response of CO and NH_3 sensors of the electronic nose to haddock fillets stored in EPS boxes at 0°C, 7°C and 15° C.

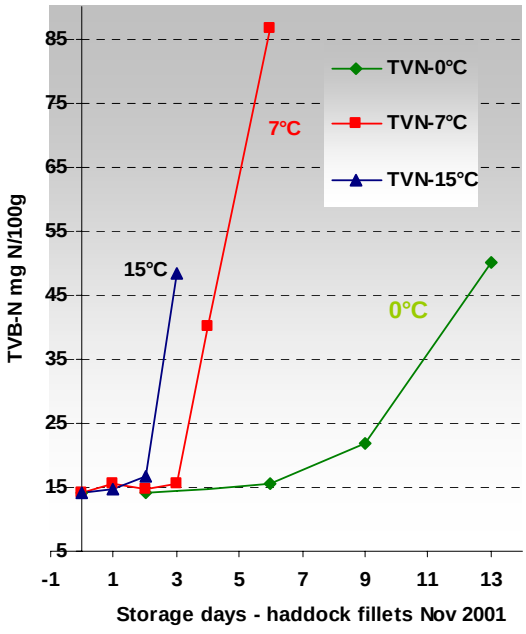


Figure 8. Results of TVN measurements to haddock fillets stored in EPS boxes at 0°C, 7°C and 15°C.

The TVB-N values and the responses of the electronic nose sensors show the development of microbial metabolites. The CO sensor values for haddock fillets (Figure 7) stored at 0°C are in general lower than in the experiment from February 2001 for haddock fillets stored at 0°C (Figure 2) (i.e. ca. 50 nA on day 6 in Nov 2001, instead of 180 nA on day 7 in Feb 2001 and increasing to about 300nA on day 13 in the Nov 2001 experiment, but increasing to more than 700nA on day 14 in Feb 2001). The steady increase in the response of the CO sensor coincides with the increase in the count of specific spoilage bacteria. The CO sensor response is in agreement with the microbial counts which are lower in general in this experiment compared with the experiment in Feb 2001, except for the counts of *P. phosphoreum* that levelled of in Feb 2001 and remained lower after 10 days of storage.

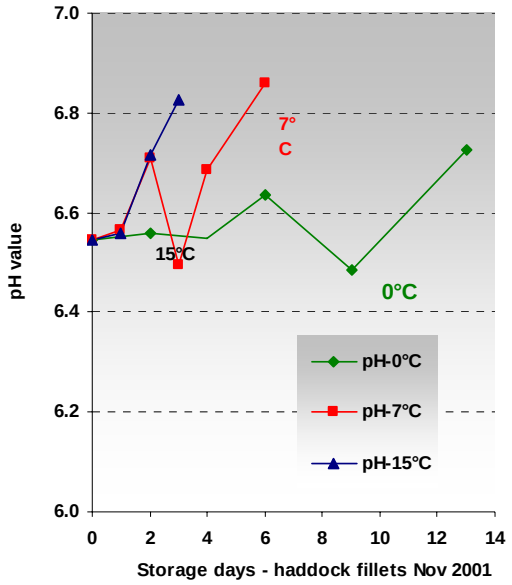


Figure 9. pH values of haddock fillets stored in EPS boxes at 0°C, 7°C and 15° C

Figure 9 shows a similar trend for the pH measurements as the electronic nose responses and the TVB-N values The pH value increases with storage time at the same days as the increases are noticed for the other measurements.

Following is a summary of the comparison of the microbiological evaluation, the TVB-N and electronic nose data to understand better the contribution of the individual spoilage bacteria to the production of metabolites. The increase in metabolites concentration measured with both the electronic nose and the TVB-N is noticed on the same days in most occasions and *Photobacterium phosphoreum* and *Pseudomonas* spp appear to contribute more to the spoilage of the fillets than the H_2S producers based on the counts:

TVB-N rapidly increased at:

15 C: from day 2 with Pp log7/g, H_2S log 3/g, Pseud log 3-4/g

7 C: from day 3 with Pp log6/g, H_2S log 2-3/g, Pseud log 4/g

0 C: from day 6 with Pp log5.5/g, H_2S log 3.5/g, Pseud log 4.5/g

NH_3 sensor rapidly increased at:

15 C: from day 1 with **Pp log 3.5/g**, H_2S log 2.5/g, Pseud log 3.5/g

day 2 with **Pp log 7/g**, H_2S log 3/g, Pseud log 3-4/g

7 C: from day 3 with **Pp log6/g**, H_2S log 2-3/g, Pseud log 4/g

day 4 with **Pp log 7.5/g**, H_2S log 4.5/g, Pseud log 6.3/g

0 C: from day 6 with **Pp log5.5/g**, H_2S log 3.5/g, Pseud log 4.5/g

day 9 with **Pp log7.5/g**, H_2S log 4.5/g, Pseud log 5.5/g

too low levels of H_2S-producing bacteria to be responsible for NH3 response…

CO sensor rapidly increased at :

15 C: from day 1 with **Pp log 3.5/g**, H_2S log 2.5/g, Pseud log 3.5/g

day 2 with **Pp log 7/g**, H_2S log 3/g, Pseud log 3-4/g

7 C: from day 3 with **Pp log 6/g**, H_2S log 2-3/g, **Pseud log 4/g**

day 4 with **Pp log 7.5/g**, H_2S log 4.5/g, **Pseud log 6.3/g**

0 C: from day 6 with **Pp log5.5/g**, H_2S log 3.5/g, Pseud log 4.5/g

day 9 with **Pp log7.5/g**, H_2S log 4.5/g, Pseud log 5.5/g

3.2.3 Microbiological evaluation

The initial microbiological quality (TVC) of the haddock fillets was evaluated upon receipt (d0) and was found to be satisfactory (5.000 psychrotrophic bacteria/g or log 3.7/g). Slow proliferation of the microflora occurred during the first 4 days of storage at 0-1°C, after which it grew steadily (Figures 10 and 11). A similar behaviour was observed for *Photobacterium phosphoreum* (Pp) reaching levels as high as the total psychrotrophic counts towards the end of the storage period. Initial load of spoilage bacteria differed between genera, with *Pseudomonas* spp, being highest (log 3.2/g), followed by *P. phosphoreum* (log 2.6/g) and H_2S-producing bacteria being at lowest level (log 1.4/g). Growth of *Pseudomonas* spp. and H_2S-producing bacteria was slower but steady throughout the storage period, reaching

10- and 100-fold lower counts, respectively, than TVC (log 8.1/g) at the last sampling day.

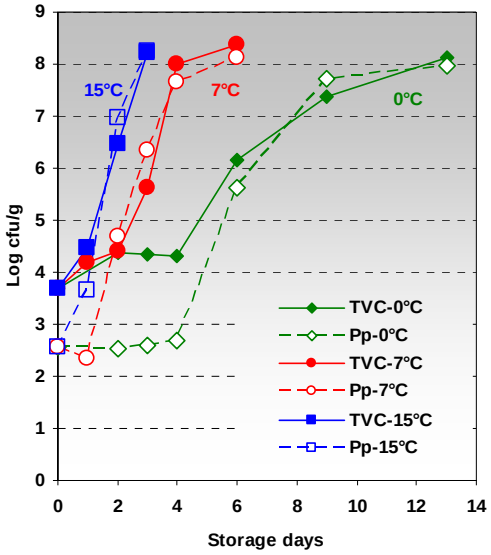


Figure 10. Total psychrotrophic (TVC) and P. phosphoreum (Pp)counts of haddock fillets from Tros (November 2001) stored in EPS boxes at 0°C, 7°C and 15°C.

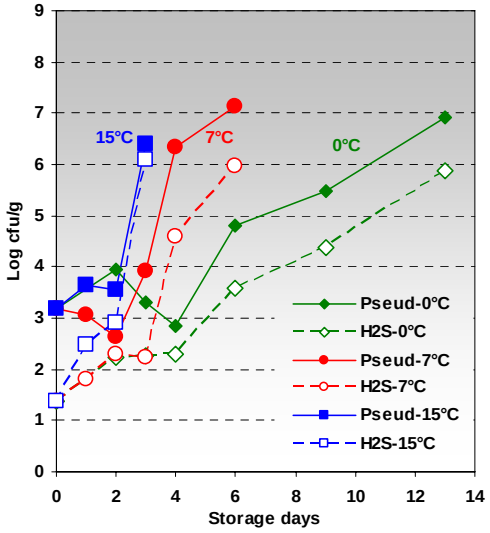


Figure 11. Counts of presumptive Pseudomonas spp. and H_2S-producing bacteria in haddock fillets from Tros (November 2001) stored in EPS boxes at 0°C, 7°C and 15°C.

For the fillets stored at 7.2 ± 0.2°C, slow bacterial growth was observed during the first 2 days, followed by a rapid increase to reach log 8.0/g (TVC) on day 4. In fact, the initial temperature of the fillets ranged from 4.2 to 5.0°C and reached 7°C after 2 days. For *P. phosphoreum*, a lag phase was observed during the first storage day after which a rapid increase took place to reach levels as high as the total psychrotrophic counts from day 2. Growth of *Pseudomonas* spp. did not progress until day 3, followed by a steep increase. A steady growth was recorded for H_2S-producing bacteria. Similarly to what was reported for fillets stored at 0-1°C, the composition of the microflora at the end of the storage period was found to dominate in *P. phosphoreum* (log 8.1/g), while *Pseudomonas* spp. and H_2S-producing bacteria reached 10- and 100-fold lower counts, respectively.

When the environmental storage temperature was 15.1 ± 1.1°C, the fillets temperature raised from 2.7-5.0°C to 15°C in one and a half day. Development of TVC occurred therefore rapidly, especially after 1 day of storage where the temperature was around 12°C. Growth of *P. phosphoreum* was also very rapid, reaching levels as high as the total psychrotrophic counts from day 2. Proliferation of *Pseudomonas* spp. was faster after the second day of storage, similarly to that of

H_2S-producing bacteria. Both reached levels slightly higher than log 6/g on day 3, i.e. counts 100-fold lower than TVC (log 8.2/g).

Table 3. Data at sensory rejection of haddock fillets stored in EPS boxes under different conditions

Information/ Analyses performed	**0-1°C**	**7.2 ± 0.2°C**	**15.1 ± 1.1°C**
End of sensory shelf life at day[1]	**(12.5+1) 13.5**	**(5.5+1) 6.5**	**(3.5+1) 4.5**
Average temperature of fillets	NA	6.9 ± 0.9°C	12.0 ± 3.7°C
TVC (log $_{10}$ CFU/ g)	**8.1**	**8.4**	**8.2**
H_2S-producer counts	5.9	6.0	6.1
% H_2S-producers / TVC	**0.6%**	**0.4%**	**0.8%**
***Pseudomonas* counts**	6.9	7.1	6.4
% *Pseudomonas* spp. / TVC	**6.3%**	**5.0%**	**1.6%**
***P. phosphoreum* counts**	8.0	8.1	8.2
% *Pp.* / TVC	**79.4%**	**50.2%**	**100%**
TVB-N content (mg N / 100 g)	45-50	~ 70	48+
pH	6.7	6.9	6.8+
CO sensor	~ 300	~ 500	500+
NH_3 sensor	~ 9	~ 40	30+
H_2S sensor	~ 50	~ 130	90+

1: total shelf life, including days from catch, based on the evaluation of cooked fish (Torry scheme)

Overall it can be said that the spoilage microflora became dominated by *P. phosphoreum* at all storage temperatures tested (Table 3). *Pseudomonas* spp. represented about 10% of the spoilage microflora at the lower temperatures (0-7.2°C) while at 15.1°C *Pseudomonas* spp. and H_2S-producing bacteria were found at levels as low as or lower than 1%. Microbial metabolites were produced in greater amounts with increasing storage temperatures as shown by the increasing sensors' response of the electronic nose and TVB-N content at sensory rejection (Table 3). This is especially true for the CO sensor. This occurred despite the fact that similar bacterial levels were found at sensory rejection. This indicates the greater ability og the spoilage microflora to produce metabolites at increasing temperatures. *P. phosphoreum* was found to dominate the spoilage microflora at all temperatures and is therefore importantly contributing to the metabolites produced.

3.3 Storage experiments of cod fillets under different handling and storage conditions - Tangi / October 2003

The aim of the experiment was to study the influence of temperature fluctuations on the shelf life of refrigerated cod fillets. The influence of bad handling of the raw material was simulated by storing whole cod uniced and temporarily temperature abused until processed the next morning. The finished product was also exposed to temperature fluctuations during storage to take into account possible abusive conditions during transport. Finally, to demonstrate good handling and low temperature conditions, a new superchilling technique was used for the fillets prior to deskinning.

3.3.1 Sensory analysis and shelf life determination

Comparison of groups A (traditional process) and C (new superchilling technique) showed that the shelf life was longer for group C according to sensory analysis using the Torry scheme. Torry scores were significantly different on days 7 and 9 for groups A and C (Figure 12). Torry score of 5.5 is the limit of sensory rejection and groups A and B reached that limit on day 9/8.5, resulting in a total shelf life of 12 days from catch, because the fish was stored whole on ice during the first three days onboard the vessel. It should be pointed out that group B had undergone an abusive temperature treatment at the post-processing stage only, where the average temperature of the fillets went from 0.8°C to 3.8°C in 8 hours and returned back to 1.1°C after 2.5 days (Figure 13b). As observed in Figure 12, quality deterioration was slightly more rapid for group B fillets than for group A fillets, as expected. However, the effect of the abusive temperature conditions at pre- and post-processing stages is reflected by rapid spoilage of group D (uniced raw material and abusive temperature conditions) and significantly shorter shelf life (about 5 days) than the other groups. This is explained by the temperature profile starting with the average fillet temperature of 2.9°C prior to abusive conditions, where it reached 4.8°C at the maximum and returned back to 2.9°C after 30 h (Figure 13b) but reached its lowest temperature (2.0°C) at the end of the storage period. Group C had the longest shelf life (9.5 days), which could be attributed to the lower temperature of the fillets (-0.9 to -1.4°C) at packaging (Figure 13a).

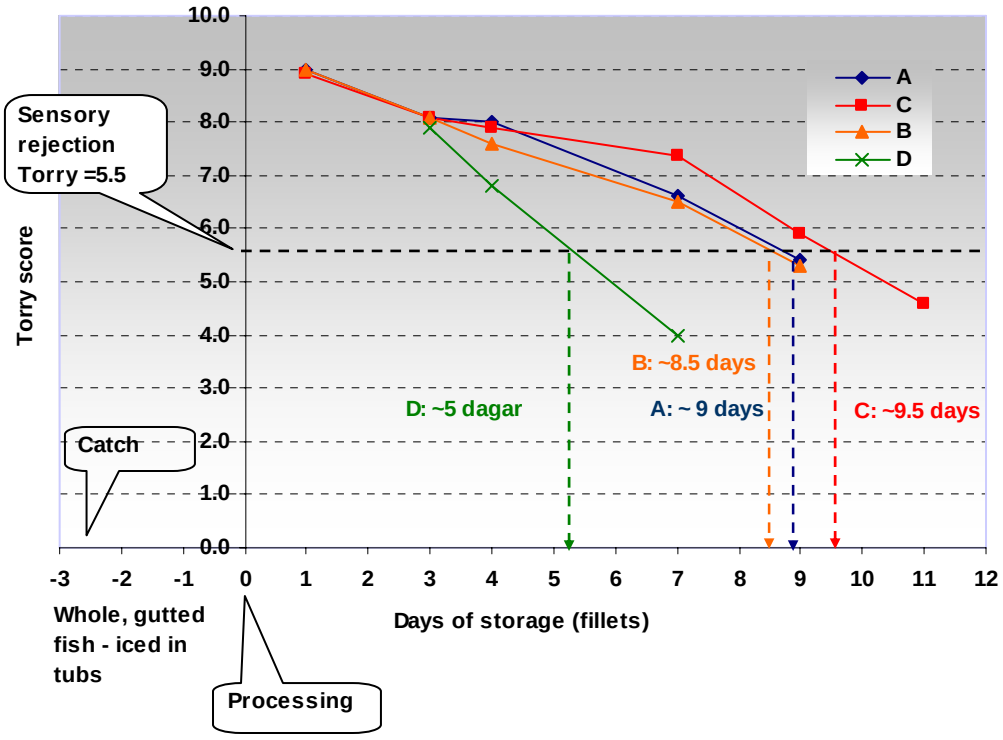


Figure 12. Results of sensory analysis (Torry) of cod fillets. Group A (traditional process), group B (temperature abuse on day 3), group C (new superchilling technique) and group D (not iced + temperature abuse on day 3).

3.3.2 Temperature recording

Initial temperature of the fillets varied at packaging based on the processing practice used. As shown in Figures 13a-b, the temperature of the fillets prepared conventionally (A and B) ranged from 4 to 7°C. Fillets of group A, being in the upper temperature range (6-7°C), took 4-5 days to reach a low temperature (1°C), while group B had reached a similar temperature after 3 days. Then after a temperature abuse (15°C for 8h) an increase in the temperature of the fillets of group B, reaching 3.8°C in 8 h. It then took 2.5 days to reach back a low temperature (1.1°C).

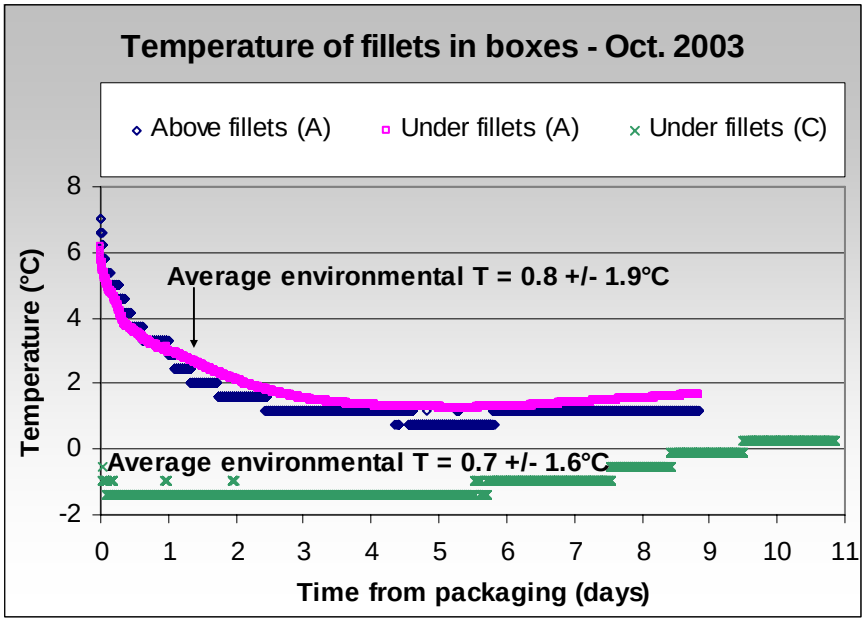


Figure 13a. Temperature profiles of samples of cod fillets. Group A (traditional process) and group C (new superchilling technique).

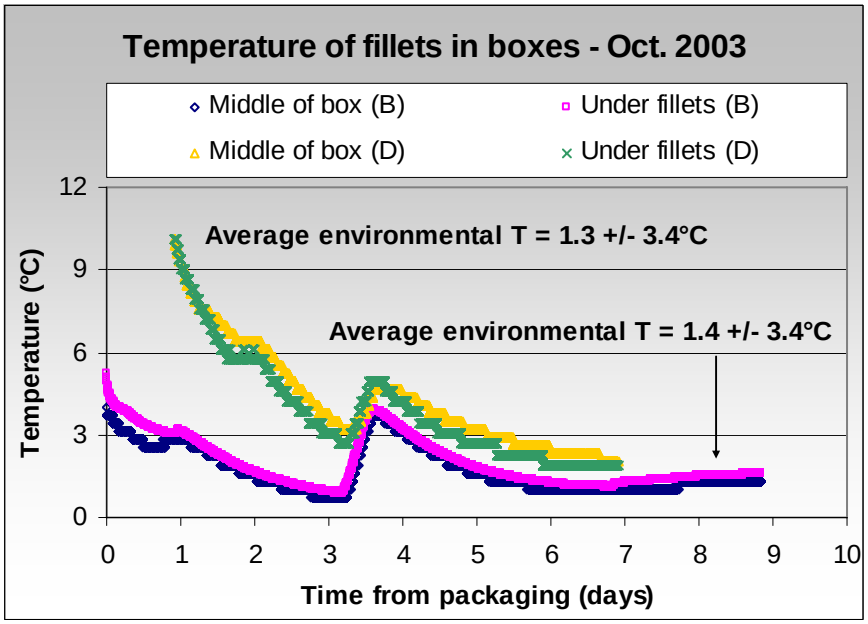


Figure 13b. Temperature profiles of samples of cod fillets. Group B (temperature abuse on day 3), and group D (not iced + temperature abuse on day 3).

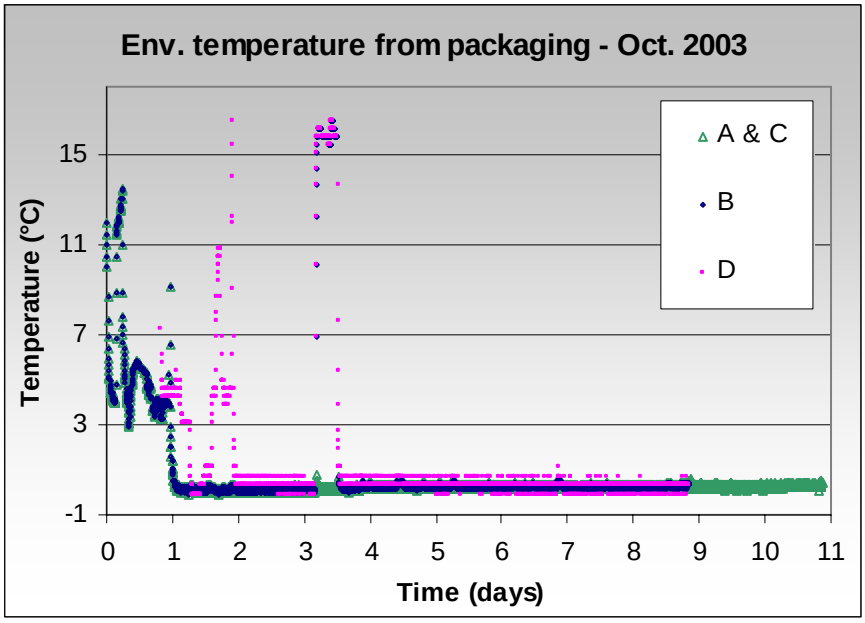


Figure 14. Environmental temperature profiles for samples of cod fillets (Group A: traditional process; B: temperature abuse on day 3; C: new superchilling technique; and D: not iced + temperature abuse on day 3)

3.3.3 Electronic nose, TVB-N and pH measurements

The results of the electronic nose measurements show that the responses of the CO and NH_3 sensors are the lowest when the new superchilling technique is used (group C) indicating that the spoilage process is slower because of the lower temperature (Figure 16). The electronic nose detects volatile microbial metabolites and the lower responses of the sensors indicate, that the growth of the spoilage flora is slower in group C when compared to groups A and B. The CO sensor can detect alcohols, aldehydes and esters which are known to be produced by specific spoilage organisms like *Pseudomonas* ssp. This is in agreement with the microbial analysis showing higher counts of *Pseudomonas* ssp. in group A than group C (see Figure 16). The response of the NH_3 sensor is higher for group C than A. This is unexpected because the overall spoilage appears to be slower, but it is possible that a specific TMA and NH_3 microbial flora may be dominant in the superchilled fillets. The results of the TVB-N measurements show slightly higher values for TVB-N in group C than in group A, supporting the electronic nose data (Figure 18).

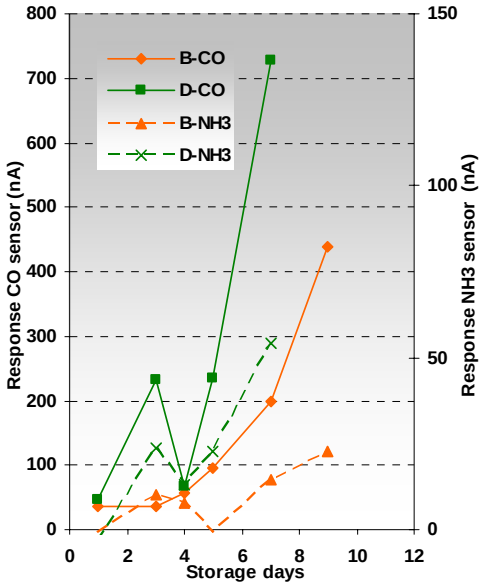


Figure 15. Response of CO and NH_3 sensors of the electronic nose to cod fillets stored at 0°C: group B (temperature abuse on day 3), group D (not iced + temperature abuse on day 3)

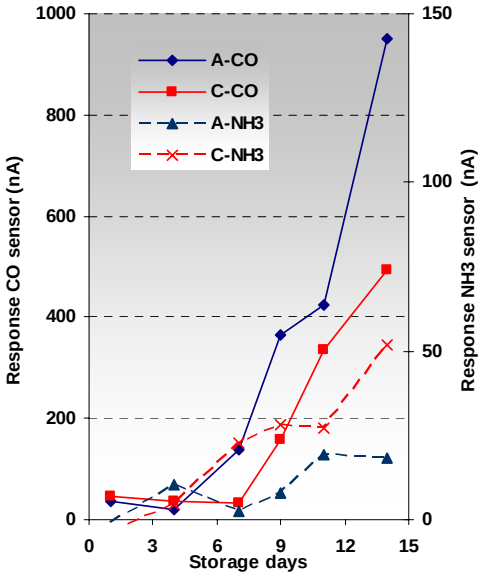


Figure 16. Response of CO and NH_3 sensors of the electronic nose to cod fillets stored at 0°C: group A (traditional process) and group C (new superchilling technique).

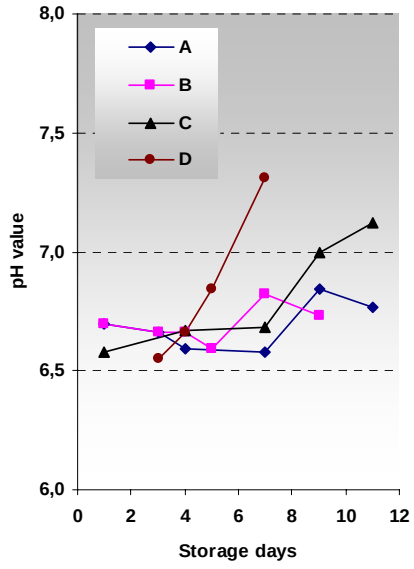


Figure 17. pH of cod fillets stored at 0°C. Group A (traditional process), group B (temperature abuse on day 3), group C (new superchilling technique) and group D (not iced + temperature abuse on day 3).

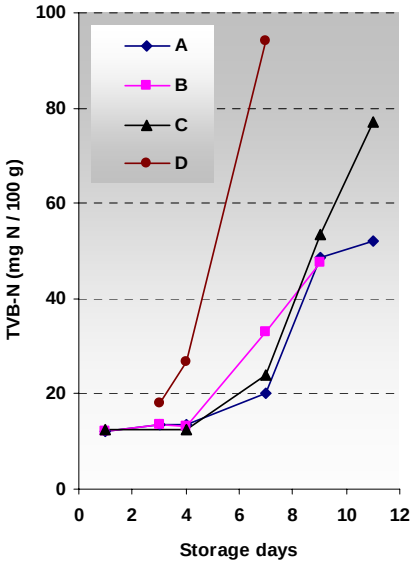


Figure 18. TVB-N value for cod fillets stored at 0°C. Group A (traditional process), group B (temperature abuse on day 3), group C (new superchilling technique) and group D (not iced + temperature abuse on day 3).

The electronic nose measurements show highest responses for group D as can be expected because of the influence of the abusive temperature conditions stimulating the microbial growth. The shelf life of group D is only about 5 days. The lower response of the CO sensor on day 4 than on day 3 may be explained because the temperature abuse at the end of day 3 may affect the development of the spoilage microflora and promote the production of different metabolites. Interestingly, the counts of H_2S-producing bacteria indicate that they become more dominant than the *Pseudomomans* ssp. (Figure 24) and metabolites start to increase again on day 5.

Groups A and B have a shelf life of about 9 days according to sensory analysis, but the higher responses of both the CO and NH_3 sensors for group B (Figure 15) compared with group A (Figure 16) indicate that the temperature abuse (15.7°C for 8 hours) on day 3 of storage influences the spoilage rate. This difference is not significant when measured by sensory analysis but the electronic nose can detect a difference between groups A and B.

pH and TVB-N measurements show the same trend as the electronic nose measurements as seen in Figures 17 and 18. The pH is slightly higher for group B than A and the TVB-N value on day 7 is similarily higher for group B on day 7 than for group A.

3.3.4 Comparison of gas chromatography and electronic nose measurements of headspace volatiles in cod

Table 3 shows the identities and concentration of headspace volatiles of cod fillets during storage collected by an air pump on a Tenax trap followed by thermal desorption, separation and detection by GC-MS. The evaluation of the concentration is based on a comparison with an internal standard. The following compounds: ethanol, 3-methyl-1- butanol, ethyl acetate, 3-hydroxy-2-butanone, 2-butanone, 3-pentanone, 2,3-butandiol, hexanal, decanal, sulfides and TMA appear to be increasing with time and can be monitored to indicate the changes during storage. The composition of the volatiles of cod during storage is slightly different from the volatiles identified in the highest concentration during the February 2001 experiment. This may because of the different species involved, but most likely the differences reflect the difference in the initial condition of the raw material, the origin, and difference in the handling practices in the different fish factories which

influence the growth of the spoilage microflora. Ketones (3-hydroxy-2-butanone, 2-butanone, 3-pentanone), appear to be in higher concentration in the headspace of cod while 2-methyl-1-propanol is present in high levels in the haddock fillets. Further analysis of the data and additional experiments of cod and haddock fillets is necessary to confirm these findings

Table 3. Headspace volatiles of cod fillets (Group A) during storage collected by an air pump on a Tenax trap followed by thermal desorption, separation and detection by GC-MS.

	RT[a]	RI [a]	Day1	Day4	Day7	Day 9	Day 11	Day 14
acid								
acetic acid	2.23	191			6.3	20.4	8.3	
2-butenoic acid, methyl ester	3.08	273	2.6	16.8				
propanoic acid 2-methyl,3-h	24.50			0.5				
Sum	**29.8**		**2.6**	**17.3**	**6.3**	**20.4**	**8.3**	**0.0**
alcohol								
ethanol	1.89	<173	17.4		13.1			7.5
1-penten-3-ol	2.84	245	2.3		0.8	1.2	1.3	
3-methyl-1-butanol	3.44	300			2.7	8.5	13.9	31.6
2-methyl-1-butanol	3.50	306					3.4	
2,3-butandiol	4.43	359			1.3	5.1	0.7	23.5
1-octanol	4.59	371			0.5			
2-butanol	4.92	388				0.8		
2-ethyl-1-hexanol	14.08	633			2.3	1.8	1.4	2.5
Sum	**18.5**		**18.5**		**20.3**	**16.9**	**20.7**	**65.0**
aldehyde								
3-methyl-butanal	2.64	227				1.3	2.6	1.2
hexanal	4.82	382	1.9	2.0	3.2	2.0	4.3	3.5
heptanal	8.67	501		2.4	1.7	0.7	1.4	
octanal	13.06	606			1.4			
nonanal	16.95	710		4.3	6.3	3.7	7.1	
decanal	20.25	805	2.3	3.5	3.5		5.0	14.9
undecanal	20.28	808		0.6		2.4		
hexadecanal	23.13	907			0.6	0.4		2.0
Sum	**109.8**		**3.3**	**11.7**	**16.7**	**10.5**	**20.4**	**21.7**
alkane								
pentane	2.00	173		85.4	64.4	35.0	159.1	451.9
nonane	8.61	501	0.6	0.4	1.2	0.4		
2,6-dimethyl-octane	10.09	536	0.8	0.9	0.6			
3-methyl-2-methyl-heptane	10.32	541			0.3			
3-ethyl-2-methyl-heptane	10.52	545			0.1			
decane	12.95	602	2.6	2.5	3.6	2.1	2.9	
3,3-dimethyl-octane	13.83	626			0.7			
dodecane	20.22	805			0.8	0.5		

	Sum	**88.5**		**4.0**	**89**	**72**	**38.0**	**162**	**452**
amine									
TMA		1.90	<173				91.6	169.6	1721.4
	Sum	**1.90**					**92**	**170**	**1721**
ester									
ethyl acetate		2.33	200				0.6	5.7	258.9
	Sum	**2.3**	**0.6**				**0.6**	**5.7**	**259**
ketone									
isobutane		1.95	<173	14.0					
2-butanone		2.23	191	14.3	8.1	9.0		14.1	
3-pentanone		2.93	255	3.7	8.5	8.2	13.6	11.0	34.6
3-hydroxy-2-butanone		3.09	273			90.3	95.3	133.7	341.8
6-methyl-5-hepten-2-one		12.28	585			1.9	1.0	3.2	5.4
	Sum	**22.48**		**32.0**	**16.5**	**105**	**110**	**162**	**382**
S-containing									
dimethyl sulfide		2.00	173	4.5					
dimethyl disulfide		3.63	312						2.4
	Sum	**5.64**		**4.5**					**2.4**

[a]RT: retention time

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

Figure 19 shows the sum of the concentration of the compounds present in the highest amount in the headspace during storage of cod fillets at 0°C. The main groups of compounds identified earlier and suggested as indicators of spoilage are alcohols, aldehydes, ketones, esters, sulfides and amines, but alkanes and acids are also present in the headspace. The alkanes do not contribute to the odor development and have therefore not been suggested as indicators of spoilage. The electronic nose can not detect the alkanes, and neither the acids nor the ketones, but it may be of interst to find selective sensors for use for further development the electronic nose for selective detection of these compounds as indicators of quality.

Figure 20 shows a comparison of the analysis of volatiles by GC and sensor responses of the electronic nose FreshSense. A similar trend of the sensor responses and the concentration of volatiles analysed in the headspace of the haddock fillets during storage is evident as also seen in section 4.1.2. The summarised concentrations of alcohols and aldehydes analysed by the GC increase with time and shows a sharp increase after 11 days of storage. The response of the CO sensor increases earlier (day5) most likely because it is sensitive to i.e. ethanol that can not be detected by the GC technique used. This is in agreement with earlier result

showing that the composition of the volatiles changes with time and can be explained by the growth of the microflora and their competition for the available substrate for growth.

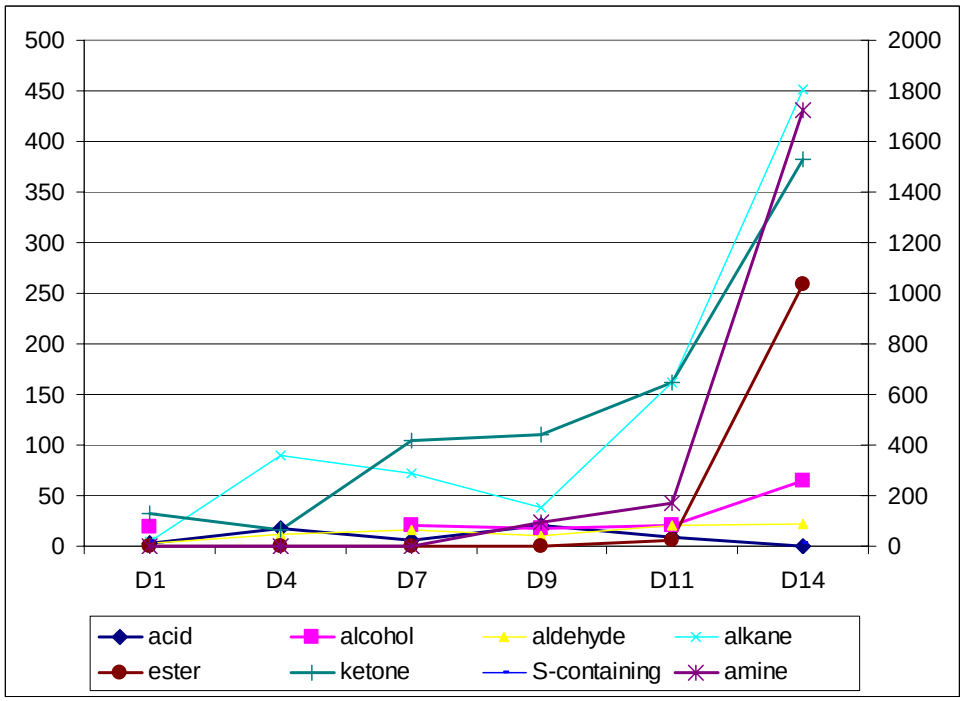


Figure 19. Development of the main classes of volatile compounds in cod fillets during storage at 0°C (group A). Concentrations estimated by comparison with and internal standard as peak area ratio (PAR)

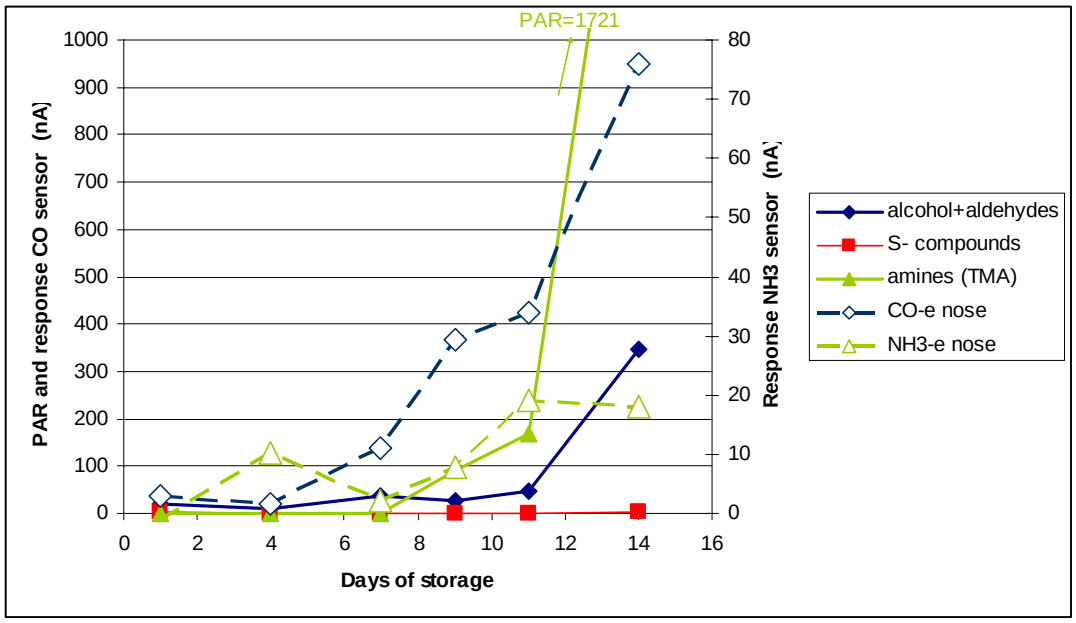


Figure 20. Sum of the peak areas of compounds representing the three different classes of compounds detected by GC and the responses of the CO, and NH_3 sensors towards cod fillets during storage in ice.

Analysis of the characteristic odor of the compounds was done by GCO odor and the flavour development during storage of the fillets can be explained by the odor description of the compounds.

Earlier results have shown that the spoilage odour development based on the odour characteristics of the compounds analysed and their increasing concentration with storage time can be rationalised based on the responses of the electronic nose sensors. The odor of the fresh fillet is very little or neutral and low responses of the sensors are observed on day 3. The first spoilage odors of the fillets are sweet like odors that are contributed by the alcohols that give sweet, solvent like odors in combination with the aldehydes giving sweet, oxidized-like odors (day 7). The amines contribute to salted fish or stock fish odor and in combination with the sulphur compounds, cheesy and foul odors develop and the fillets are stale as seen on day 11. Finally, the esters analysed in high levels in haddock and carbonyls in cod on day 14 have characteristic sweet, fruity odors. When these sweet odors are mixed with the foul smell of the sulphur compounds and ammonia- like stockfish character of the amines the odor of the fillet becomes TMA / ammonia-like and sour / putrid – like, signalling the overt spoilage.

In the fish industry the smell is one of the most important quality attribute for raw fillets. Standards can be selected based on GC analysis to evaluate the performance of different electronic nose instruments to monitor freshness quality of fish.

3.3.5 Microbiological evaluation

The microbiological quality of the raw material processed conventionally (filleted and deskinned mechanically) was satisfactory (7.400 psychrotrophic bacteria/g or log 3.9/g) after one day of cooled storage in EPS boxes. However, the total psychrotrophic counts (TVC) of the fillets deskinned by the new superchilling method was about tenfold higher (ca log 4.8/g). This is despite the fact that the raw material originated from the same haul and was treated similarly till processed. A possible explanation could be that the microbiological quality of the brine, into which the superchilled fillets were immersed for 45 minutes prior to partial skin freezing, was not sufficiently good and led to an increase in the bacterial load. Another possibility is that the traditional deskinning performed in the Baader machine, which involves a lot of water rinsing, decreased the bacterial load of the fillets treated conventially prior to packaging. It is therefore very important to

ensure that the brine and tub used are of excellent microbiological quality and will hence not cause any undesirable contamination of the fillets.

Comparison of groups A and C (Figures 21 and 23) demonstrates clearly the slower bacterial development occurring in superchilled fillets (C), where a bacterial increase of 1 log/g is reached after 3 days, as opposed to 2 log/g for group A. This is easily explained by the much lower temperature of the superchilled fillets (-0.9°C) upon packaging, in comparison to 6°C for the conventionally processed fillets. After a week storage at 0.2°C, there was less difference in the temperature of the fillets between groups A and C (about 0.5°C lower), and the total psychrotrophic counts had reached log 7.4/g for both groups. In group C, *Pseudomonas* count was slightly higher than that for H_2S-producing bacteria and *Photobacterium phosphoreum* on the first sampling day (day 1). Again, growth of specific spoilage organisms occurred at a slower rate in group C, but *P. phosphoreum* became dominant (about 1 log/g higher) among the spoilage organisms in both groups A and C. It is interesting to point out that the ratio of *P. phosphoreum* to TVC was higher in group A (21% vs. 2% for C) after 7 days of storage, but this difference became less as storage progressed (13% vs. 7% at sensory rejection). This difference could be explained by the sensitivity of this bacterium to sudden cooling/freezing, which can lead to a cold shock, damaging the cells and resulting in slower growth. Otherwise it was observed that the proliferation pattern and numbers of *Pseudomonas* spp. and H_2S-producing bacteria were similar during the whole storage period in either A, B or C group. They only represented 1-7% of the total microflora at sensory rejection of groups A and B, while *P. phosphoreum* was found at higher levels (13-16%).
Similarly, *Pseudomonas* spp. and H_2S-producing bacteria were found at much lower levels than *P. phosphoreum*, in group C at sensory rejection (0.2 and 0.5%, respectively).

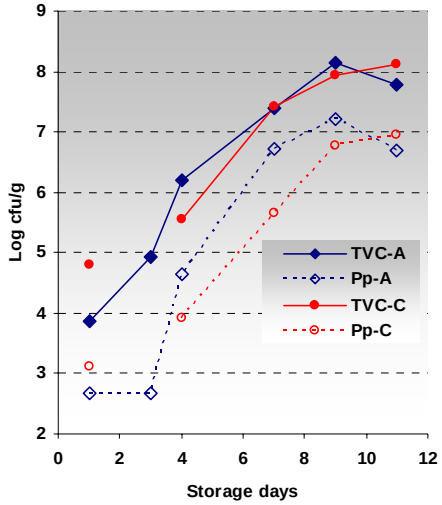


Figure 21. Total psychrotrophic and P. phosphoreum counts of cod fillets stored in EPS boxes at 0.2°C (group A: processed conventionally; and group C: partial skin freezing – superchilling technique)

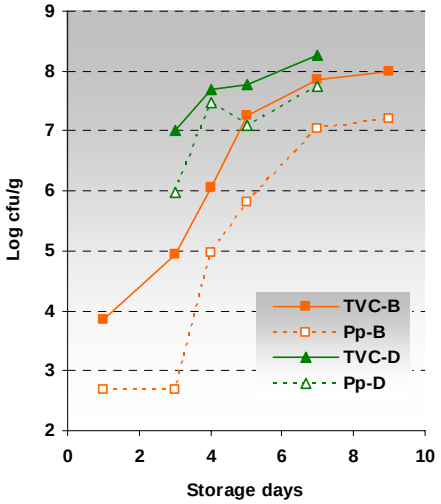


Figure 22. Total psychrotrophic and P. phosphoreum counts of cod fillets processed conventionally and stored in EPS boxes (group B: with post-processing temperature abuse; and group D: with pre- & post-processing temperature abuse)

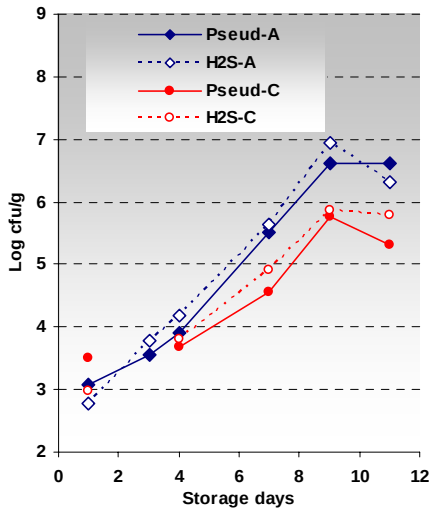


Figure 23. Counts of presumptive Pseudomonas spp. and H_2S-producing bacteria in cod fillets stored in EPS boxes at 0.2°C (group A: processed conventionally; and group C: partial skin freezing – superchilling technique)

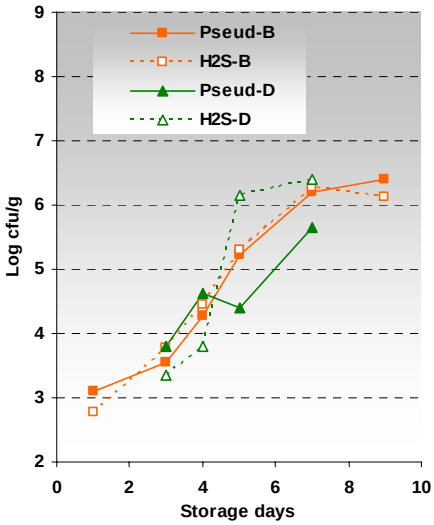


Figure 24. Counts of presumptive Pseudomonas spp. and H_2S-producing bacteria in cod fillets processed conventionally and stored in EPS boxes (group B: with post-processing temperature abuse; and group D: with pre- & post-processing temperature abuse)

Groups B and D were expected to demonstrate the bacterial behavior and proliferation following abusive temperature conditions at pre- and/or post-processing stages. The raw material had been processed conventionally, but that used for group D was stored uniced upon reception, kept for 7-8 hours in a cooled room until it was moved to the processing area to be stored overnight at 15-18°C and processed the next morning (4 days post catch). Both groups B and D were further temperature abused during the post-processing period, i.e. after 3 days of storage in EPS boxes at 0.2°C, where the boxes were moved to a 15.7°C area for 8 hours and then cooled again. As expected, total psychrotrophic counts were higher in group D (log 7.0/g) on its first sampling day (d3) compared to that of group B (log 4.9/g) which did not undergo pre-processing temperature abuses (Figures 22 and 23). The average temperature of the fillets was 0.9°C for B, but 3.6°C for D. Psychrotrophic counts had reached similar levels (log 8/g) at sensory rejection for both groups. *P. phosphoreum* load was considerable in group D (log 6.0/g, representing about 10% of microflora) on its first sampling day (d3) compared to that of group B (log 2.7/g). On the other hand, counts of *Pseudomonas* spp. and H_2S-producing bacteria were similar between these groups (B-D: log 3.6-3.8/g and 3.8-3.4/g, respectively) on that sampling day. This indicates that unproper icing and handling of raw material, leading to abusive temperature conditions, may especially influence the development of *P. phosphoreum* in the raw material.

Similar growth rates for *P. phosphoreum* were observed between days 3 and 4 in groups B and D, following post-processing temperature abuse. Development of *Pseudomonas* spp. and H_2S-producing bacteria was also comparable between the abused groups between days 3 and 4, after which a sudden increase of H_2S-producing bacteria occurred between days 4 and 6, leading to its dominance over *Pseudomonas* spp. Having a closer look at the growth curves of *Pseudomonas* spp. and H_2S-producing bacteria from group D and comparing them to group B, will demonstrate the possible inhibitive effect occurring towards some bacterial groups. For instance, *Pseudomonas* spp. did not proliferate at a steady rate despite the higher temperature profile of group D, while growth of H_2S-producing bacteria was accelerated after day 4 in group D. The reason for such differences could be linked to bacterial interaction (production of inhibitive substances) and competition for nutrients. It should be pointed out that *P. phosphoreum* and H_2S-producing bacteria

reached much higher levels in group D (early storage) and could have been responsible for some of the inhibition.

A comparison of groups A and B will show that *P. phosphoreum* grew more rapidly in group B, as demonstrated by its higher level reaching log 4.9/g on day 4. Similarly to group A, the development of *Pseudomonas* spp. and H_2S-producing bacteria was comparable during the first 7 days, but occurred at a faster rate in group B because of the temperature fluctuations.

Table 5 summarises the results of the experiment, emphasising the importance of the bacterium *P. phosphoreum* in fillets that has undergone a temperature abuse.

Table 5. Data at sensory rejection of cod fillets stored in EPS boxes under different conditions (Tangi- November 2003)

Information/ Analyses performed	**Conventional processing (A)**	**Conv. proc. + post-processing temp. abuse (B)**	**New proc. method (C)**	**Conv. proc. + pre/post-proc. temp. abuse (D)**
End of sensory shelf life at day[1]	**(9+3) 12**	**(8.5+3) 11.5**	**(9.5+3) 12.5**	**(5+3) 8**
TVC ($\log_{10}$ CFU/ g)	**8.1**	**8.0**	**ca 8**	**7.8**
H_2S-producer counts	7.0	6.1	5.8	6.2
% H_2S-producers / TVC	**7.0%**	**1.4%**	**0.5%**	**2.6%**
Pseudomonas counts	6.6	6.4	5.5	4.4
% *Pseudomonas* spp. / TVC	**4.9%**	**3.0%**	**0.2%**	**0.04%**
P. phosphoreum counts	7.2	7.2	6.9	7.1
% *Pp.* / TVC	**12.6%**	**16.2%**	**6.7%**	**12.7%**
TVB-N content (mg N / 100 g)	48.5	47.5	~60	~ 50
pH	6.9	6.7	~ 7.1	6.9
CO sensor	366	439	~ 450	~250+
NH3 sensor	8	23	~50	35

1: total shelf life, including days from catch, based on the evaluation of cooked fish (Torry scheme)

3.4 Storage experiments of cod and haddock fillets at different temperatures - Tros /November 2003

Additional storage experiments were done on cod and haddock fillets obtained from the company Tros to collect further data for models and to study the influence of fluctuating temperatures during storage.

3.4.1 Sensory analysis, TVN measurements and shelf life determination

Figure 25 shows that the influence of the temperature abuse of cod fillets in group D results in a shorter shelf of 9 days, compared with 12.5 days for group B. The figure also shows that the limit of sensory rejection based on the Torry score appears to be in agreement with the end of shelf life based on the TVB-N analysis with a rejection limit of 35 mg N/100g.

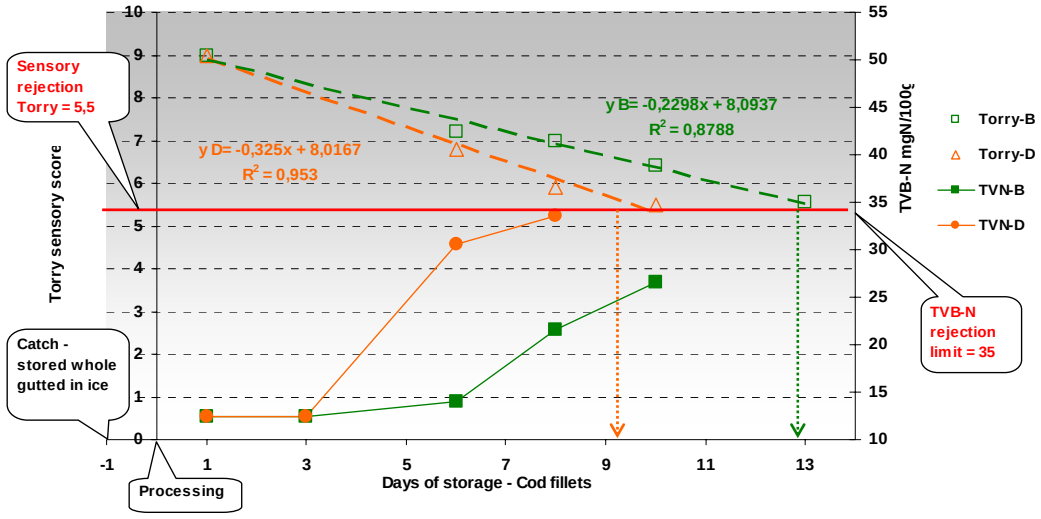


Figure 25 Torry sensory score and TVB-N value for cod fillets stored at 0°C. Group B (traditional process), Group D (traditional process and temperature abuse)

Similar results are observed in Figure 26 showing the Torry scores of the haddock fillets. The temperature abused fillets have shorter shelf life (8 days, group C vs 10.5 days group A). The end of shelf life based on the TVB-N values are estimated around 7 days for group C and about 10 days for group A. As seen by this the determination of the end of shelf life is dependent the on measurement technique used.

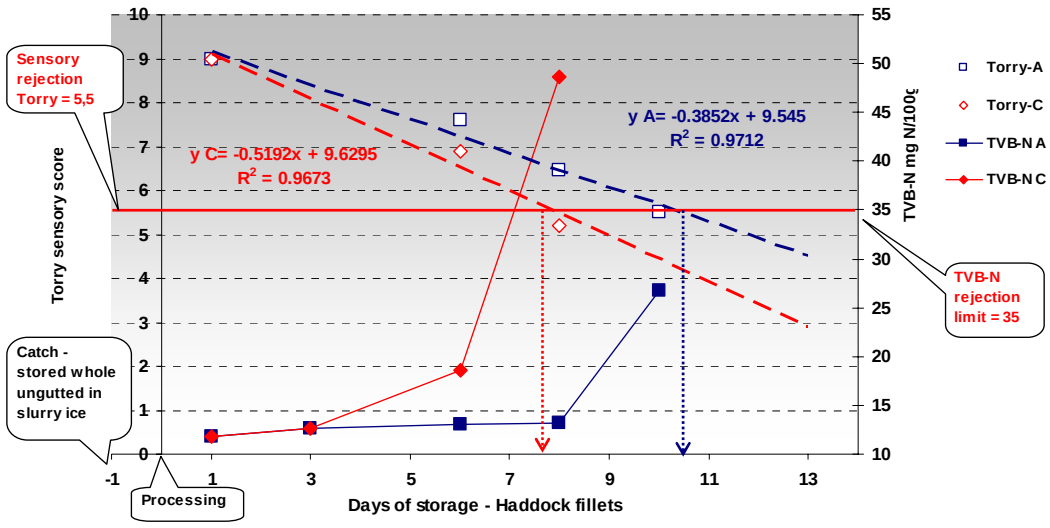


Figure 26. Torry sensory score and TVB-N value for haddock fillets stored at 0°C. Group A (traditional process) and Group C (traditional process and temperature abuse)

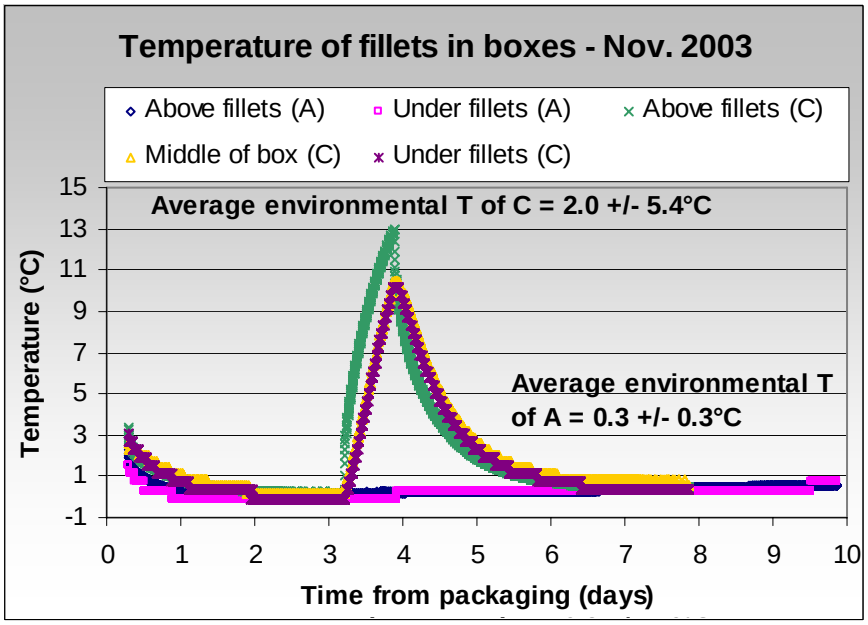


Figure 27a. Temperature profiles for haddock fillets stored at 0°C. Group A (traditional process) and Group C (traditional process and temperature abuse on day 3)

Figures 27 – 28 show the temperature profile for the sample groups and the temperature fluctuations. The average temperature is calculated for each sample group and will be used as identifiers for sample groups to differentiate between the different temperature treatments in Tables 7 and 8.

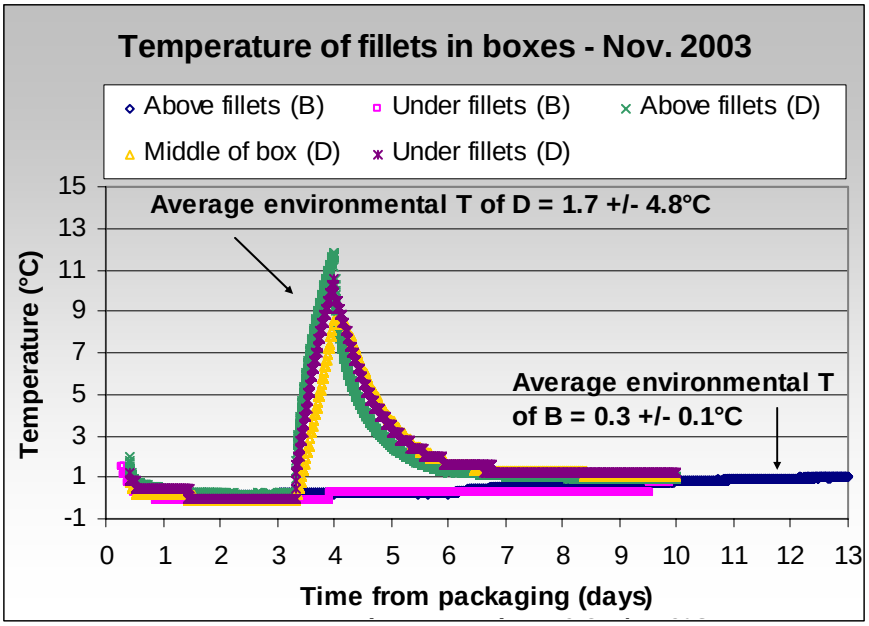


Figure 27b. Temperature profiles for cod fillets stored at 0°C. Group B (traditional process), Group D (traditional process and temperature abuse on day 3)

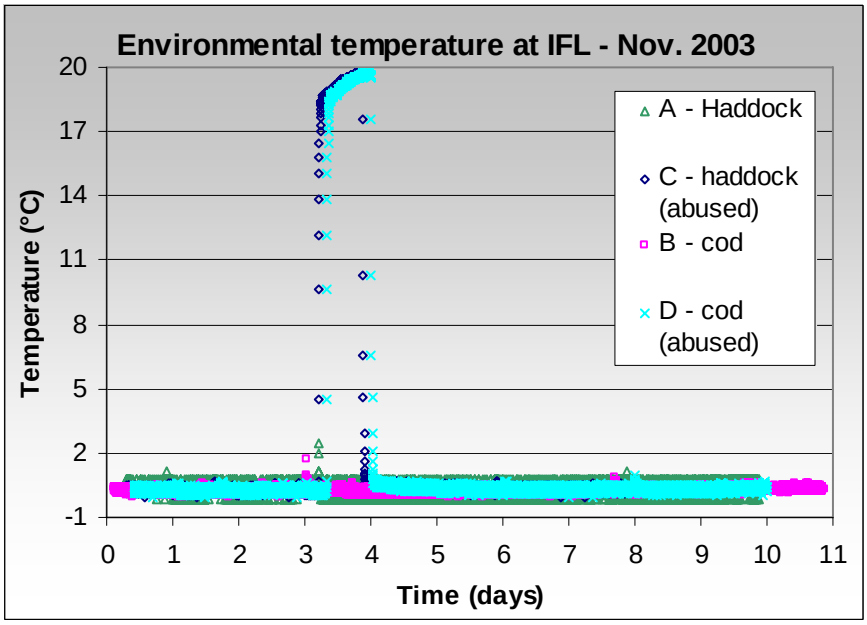


Figure 28. Environmental temperature profiles for haddock fillets (Group A (traditional process; Group C (traditional process and temperature abuse on day 3); Group B (traditional process); Group D (traditional process and temperature abuse on day 3)

3.4.2 Electronic nose, TVB-N and pH measurements

Figure 29 shows the responses of the electronic nose to the cod headspace during storage. The influence of the temperature abuse on the spoilage rate in group D is obvious as seen by the higher responses of both the sensors. The declining response of the CO sensor in group B is interesting and is reflected by a similar trend in the

microbialogial data (Figure 32) showing a decline in the counts of *Photobacterium phosphoreum* on day 13 and the Pseudomonas appear to level off as well.
Pseud. and Pp are found at similar levels (log 7.2-7.5/g) either conventionally stored or abused, and are therefore dominating the spoilage microflora (17-38%); H_2S-producing bacteria are found at higher levels (log 7.1/g) than in haddock (5.8/g); but did not become dominant.

Figure 30 shows the development of the volatiles in haddock fillets and here it is of interest to note that the responses of the CO sensors are much higher than for the cod. Similar high response for the CO sensors were also observed in the February 2001 experiment for haddock fillets. The difference in the spoilage profile of the two species is evident and counts of the *Photobacterium phosphoreum* and *Pseudomonas spp.* are higher in haddock than cod. However, the differences may also be because of the difference in the handling of the raw material. The haddock was stored ungutted in ice slurry before processing while the cod was stored ungutted in ice. The difference is probably not because of the different species because the experiments in November 2001 showed that the shelf life of haddock fillets was longer (12,5 days compared to 10 days in this experiment (Nov 2003)) and CO sensor's responses were much lower in that experiment

Haddock is dominated by Pp (60-100%) reaching levels of log 7.5 – 8 /g at sensory rejection; Pseud. spp. represent about 15% of the spoilage microflora reaching loads of log 7.3/g under abused conditions, while H_2S-producing bacteria are found in minority (1-2%) at levels of log 6/g. The low response of the SO_2 sensor shows that the H_2S-producing bacteria are not important in the development of spoilage odors for fish fillets.

Pseudomonas and H_2S-producing bacteria appear to have similar growth profiles in cod from both Tros and Tangi but when comparing the growth of Pseudomonas in cod from the two experiments the counts are notably lower in the cod from Tangi than from Tros: (log 6.6/8.1 vs. 7.2/8.0)

.

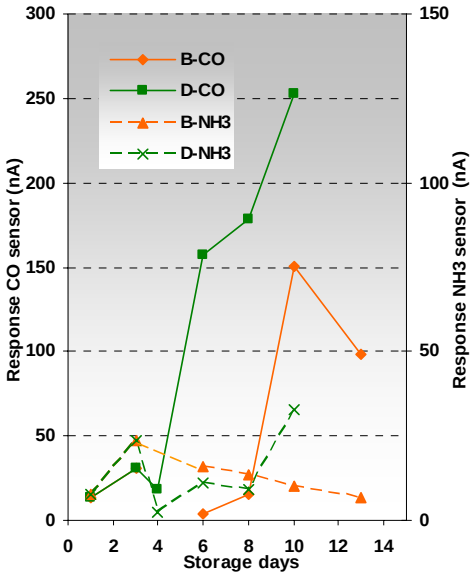


Figure 29. Response of the CO and NH_3 sensors of the electronic nose to cod fillets from Tros Nov 2003 stored at 0°C: group B (traditional process) and group D (traditional process + abuse)

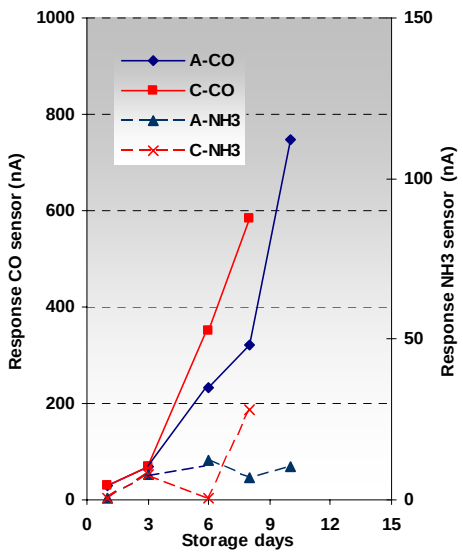


Figure 30. Response of the CO, and NH_3 sensors of the electronic nose to haddock fillets from Tros Nov 2003 stored at 0°C: group A (traditional process) and group C (traditional process + abuse)

3.4.3 Microbiological evaluation

The microbiological quality of the raw material processed conventionally was satisfactory, but with slightly higher psychrotrophic counts (TVC) than those found in the previous experiments. Cod fillets had lower TVC with an average of 17.000 psychrotrophic bacteria/g or log 4.2/g, while haddock fillets had a slightly higher average TVC with 52.500 psychrotrophic bacteria/g or log 4.7/g after one day of cooled storage in EPS boxes. Similarly to an earlier experiment in November 2001, H_2S-producing bacteria were found at low levels (log 0.7/g) in haddock fillets compared to *Pseudomonas* spp. (log 3.5/g). *P. phosphoreum* was not detected in haddock fillets on the first day of storage, while it was detected (log 2.6/g) on the first sampling day in November 2001. Comparison of the cod processed at TROS in November 2003 to that from Tangi (October 2003) indicates that despite the slightly higher TVC found in fillets from TROS, initial counts of specific spoilage organisms (SSO) were slightly lower, but proportionally similar per bacterial group. *Pseudomonas* spp. were found at higher levels (log 2.8/g), followed by H_2S-producing bacteria (log 2.3/g) and *P. phosphoreum* (log 1.3/g).

Measurements of temperature indicated that haddock fillets ranged from 1.5 to 3.4°C while cod fillets, which had been processed earlier the same day and stored

longer in a cooler prior to shipping to the laboratory, had a temperature of 0.7-2.0°C. The boxes were stored under refrigerated conditions while some boxes were temperature abused overnight (16 h) on day 3 and replaced under refrigeration the next day. The effect of these conditions on bacterial development are well illustrated in the following figures (31 to 34). Under refrigerated condition bacterial development occurred steadily in cod fillets (Figure 31), with all bacterial groups evaluated having reached high levels (log 7.1-7.5/g) at sensory rejection (day 13). In abused fillets (Figure 32), *P. phosphoreum* rapidly reached TVC levels (day 6) being hence dominating in the spoilage microflora, while *Pseudomonas* ssp. kept growing steadily to reach similar levels on day 10. H_2S-producing bacteria growth slew down from day 6 and had reached lower levels at sensory rejection (day 10). Spoilage occurred at a faster rate in haddock fillets and could be explained by the rapid development of *P. phosphoreum* in both refrigerated (Figure 33) and abused (Figure 34) fillets. In both cases it had rapidly reached TVC levels on day 6, hence dominating the spoilage microflora. At sensory rejection, *Pseudomonas* spp. represented 15% of the spoilage microflora, while H_2S-producing bacteria were found at lower levels (1-2%).

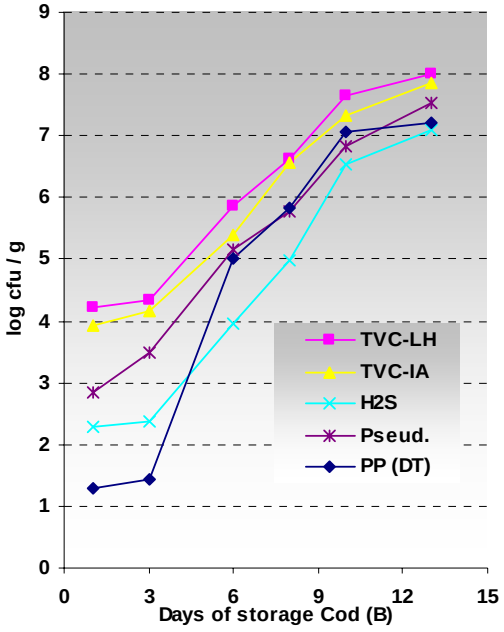


Figure 31. Total psychrotrophic, H_2S-producing bacteria, presumptive Pseudomonas spp., and P. phosphoreum counts of cod fillets stored in EPS boxes at 0.2°C (group B: processed conventionally)

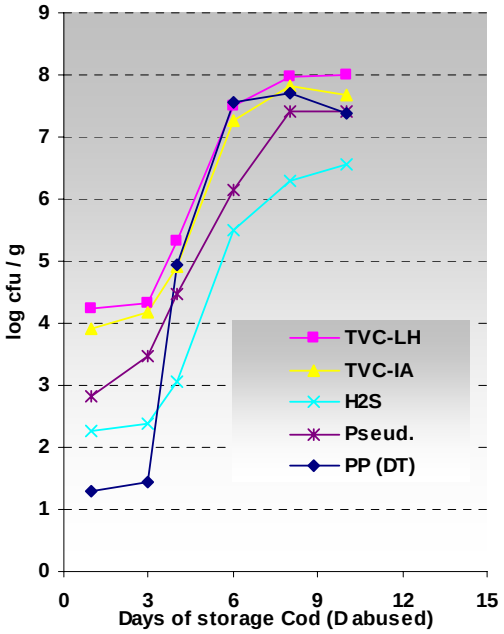


Figure 32. Total psychrotrophic, H_2S-producing bacteria, presumptive Pseudomonas spp., and P. phosphoreum counts of cod fillets stored in EPS boxes at 0.2°C (group D: processed conventionally; and abuse on day 3)

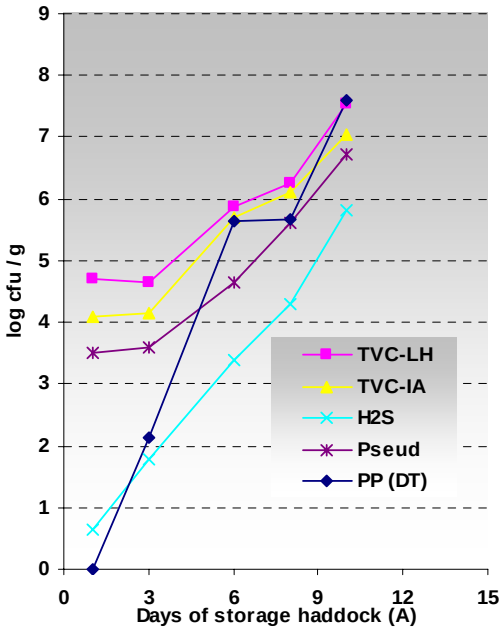


Figure 33. Total psychrotrophic, H_2S-producing bacteria, presumptive Pseudomonas spp., and P. phosphoreum counts of haddock fillets stored in EPS boxes at 0.2°C (group A: processed conventionally)

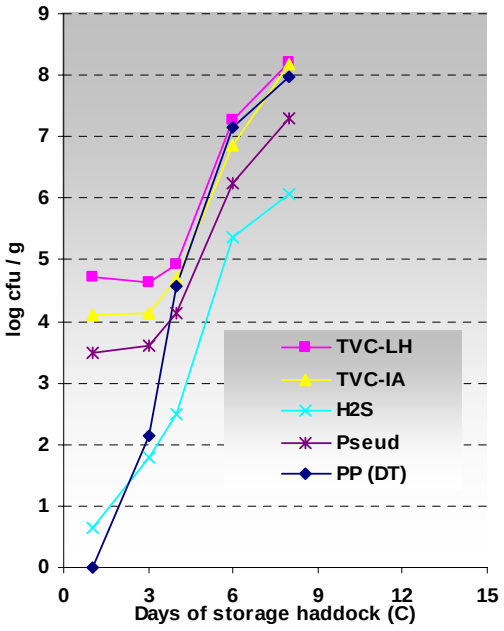


Figure 34. Total psychrotrophic, H_2S-producing bacteria, presumptive Pseudomonas spp., and P. phosphoreum counts of haddock fillets stored in EPS boxes at 0.2°C (group C: processed conventionally; and abuse on day 3)

Table 6 summarises the results of the different parameters tested and the data obtained at sensory rejection.

Table 6. Data at sensory rejection of fish fillets stored in EPS boxes under different conditions in the Tros Nov 2003 experiment

Information/ Analyses performed	Haddock (A)	Abused haddock (C)	Cod (B)	Abused cod (D)
End of sensory shelf life at day[1]	**(10+1) 11**	**(8+1)9**	**(12.5+1)13.5**	**(9.5+1)10.5**
TVC ($\log_{10}$ CFU/ g)	**7.5**	**8.2**	**8.0**	**8.0**
H_2S-producer counts	5.8	6.1	7.1	6.6
% H_2S-producers / TVC	**2.4%**	**0.8%**	**12.9%**	**3.7%**
Pseudomonas counts	6.7	7.3	7.5	7.4
% *Pseudomonas* spp. / TVC	**15.1%**	**15.2%**	**37.5%**	**25.4%**
P. phosphoreum counts	7.5	8.0	7.2	7.4
% *Pp.* / TVC	**100%**	**61.2%**	**17.2%**	**24.0%**
TVB-N content (mg N / 100 g)	26.8	40-45	26.6	33.6
pH	6.7	6.9	6.9	6.9
CO (nA)	750	580	98	250
NH3 (nA)	10	28	7	32

1: shelf life, including days from catch (days in storage from processing + days from catch until processed), based on the evaluation of cooked fish (Torry scheme)

3.5 Comparison of microbiological and chemical data (electronic nose, TVB-N and pH) at sensory rejection for the different storage experiments

Tables 7 and 8 summarise the microbial and chemical data at sensory rejection to gain an overview of the spoilage pattern and degree of spoilage in the different experiments. The values of the different variables are not comparable at sensory rejection and the shelf lives are different, underlining that different spoilage pattern occurs in the different experiments. Temperature influences these differences, but also different origin, handling and the different species.

Haddock: Lower levels of H_2S-producing bacteria were observed in haddock, both initially and at spoilage and therefore it is suggested that the H_2S-producing bacteria are probably of minor importance in the spoilage pattern of this fish species.

Table 7. Summary of microbiological data comparing initial and final counts at sensory rejection in the different experiments

	Experiment 1 **Haddock fillets** **TROS** 2001			**Experiment 2** **Cod fillets** **TANGI 2003**			**Experiment 3** **Cod fillets** **TROS2003**			**Experiment 4** **Haddock fillets** **TROS 2003**		
Microbiological analyses	**Temp. (°C)**	**Initial**	**Final**	**Temp. (°C)**	**Initial**	**Final**	**Temp. (°C)**	**Initial**	**Final**	**Temp. (°C)**	**Initial**	**Final**
TVC	**0-1**	3.7	8.1	**0.8**	3.9	8.1	**0.3**	4.2	8.0	**0.3**	4.7	7.5
H_2S counts		1.4	5.6	A	**2.8**	**7.0**	B	**2.3**	**7.1**	A	0.7	5.8
Pseud. counts		3.2	6.9		3.1	6.6		**2.8**	**7.5**		**3.5**	**6.7**
Pp. counts		**2.6**	**8.0**		**2.7**	**7.2**		**1.3**	**7.2**		**ND**	**7.5**
TVC	**7.2**	3.7	8.4	**1.4** [a]	3.9	8.0	**1.7** [a]	4.2	8.0	**2.0** [a]	4.7	8.2
H_2S counts		1.4	6.0	B	2.8	6.1	D	2.3	6.6	C	0.7	6.1
Pseud. counts		3.2	7.1		3.1	6.4		**2.8**	**7.4**		**3.5**	**7.3**
Pp. counts		**2.6**	**8.1**		**2.7**	**7.2**		**1.3**	**7.4**		**ND**	**8.0**
TVC	**15.1**	3.7	8.2	**1.9** [b]	7.0[c]	7.8						
H_2S counts		1.4	6.1	D	3.4[c]	6.2						
Pseud. counts		3.2	6.4		3.8[c]	4.4						
Pp. counts		**2.6**	**8.2**		**6.0**[c]	**7.1**						

a: abused at post-processing stage (on day 3); b: abused at pre- and post-processing stages;

c: counts on day 3

Responses of the CO sensors in the Tros 2003 experiment are higher for haddock than for the cod in both the Tros 2003 and Tangi 2003 experiments. Similar high responses of the CO sensor were also observed in the February 2001 experiment for haddock fillets. The difference in the spoilage profile of the two species is evident and counts of the *Photobacterium phosphoreum* and *Pseudomonas* are higher in haddock than cod. The differences may be because of the difference in the handling of the raw material. The haddock was stored ungutted in ice slurry before processing while the cod was stored gutted in ice. The short shelf life of haddock is not in agreement with the experiments in November 2001 which showed that the shelf life of haddock fillets was 12.5 days compared to 10 days in the Tros Nov 2003 experiment. The CO sensor's responses were accordingly much lower in the earlier experiment.

Cod: north-east of Iceland (Tangi) is high in SSO at initial stage (d1) but poorer growth of *Pseudomonas* spp., especially following temperature abuse (due to inhibitory effects, bacterial competition?).

The electronic nose measurements show highest overall responses for group D as can be expected because of the influence of the abusive temperature conditions (pre- and post-processing) stimulating the microbial growth. Groups A and B have shelf lives about 12 days according to sensory analysis, but the higher responses of both the CO and NH_3 sensors for group B compared with group A indicate that the temperature abuse (15.7°C for 8 hours) on day 3 of storage influences the spoilage potential. pH and TVB-N measurements have however similar values at the end of shelf life in all the groups.

Table 8 Summary of the results of chemical measurements comparing initial and final values at sensory rejection in the different storage experiments at different storage temperatures and temperature fluctuations (shown as average temperature)

	Experiment 1			**Experiment 2**			**Experiment 3**			**Experiment 4**		
	Haddock fillets			**Cod fillets**			**Cod fillets**			**Haddock fillets**		
	TROS Nov 2001			**TANGI Nov 2003**			**TROS Nov 2003**			**TROS Nov 2003**		
Chemical analysis	**Temp. (°C)**	**Initial**	**Final**	**Temp. (°C)**	**Initial**	**Final**	**Temp. (°C)**	**Initial**	**Final**	**Temp. (°C)**	**Initial**	**Final**
TVB-N	**0-1**	14.1	50	**0.8**	12	48.5	**0.3**	12.4	27	**0.3**	11.8	27
pH		6.5	6.7	**A**	**6.7**	**6.9**	B	**6.9**	**6.9**	A	6.7	6.7
CO		50	300		37	366		**13**	**98**		**30**	**750**
NH_3		**5**	**10**		**1**	**8**		**8**	**7**		**1**	**10**
	Shelf life*=13.5 days			**Shelf life=12 days**			**Shelf life=13.5 days**			**Shelf life=11 days**		
TVB-N	**7.2**	14	80	**1.4** [a]	12	47.5	**1.7** [a]	12.4	34	**2.0** [a]	11.8	45
pH		6.5	6.7	B	6.7	6.7	D	6.9	6.9	C	6.7	6.9
CO		50	500		37	~439		**13**	**250**		**30**	**580**
NH_3		**5**	**40+**		**1**	~28		**8**	**32**		**1**	**28**
	Shelf life=6.5			**Shelf life= 11.5**			**Shelf life =10.5 days**			**Shelf life =9 days**		
TVB-N	**15.1**	14	50+	**1.9** [b]	12	ca.50						
pH		6.5	6.8	D	6.7	6.9						
CO		50	500+		37	~250+						
NH_3		**5**	**30+**		**1**	~35						
	Shelf life =4.5 days			**Shelf life=8 days**								

* Total shelf life = days after catch

Cod: south-west of Iceland (Tros) has slightly lower levels of SSO initially, despite slightly higher TVC. All SSO seem of importance at spoilage, *Pseudomonas* spp. dominating, followed by *P. phosphoreum* and H_2S-producing bacteria in numbers. But under temperature abuse *P. phosphoreum* and *Pseudomonas* spp are dominating and probably similarly important.

The responses of the CO and NH_3 sensors to the cod fillets from Tros are lower than for Tangi The influence of the temperature abuse on the spoilage rate in group D is obvious as seen by the higher responses of both the sensors overall, however the value at sensory rejection is lower for group D than B.

Table 9. Potential maximum growth rate (log_{10} N/ml/h) estimated by DMFit for different bacterial groups assessed in fish fillets

Fish experiments / Bacterial species	**Temperature of fillets**			
Haddock – Nov. 2001 (Tros)	**0-1°C**	**6.9 ± 0.9°C**	**12.0 ± 3.7°C**	
TVC	0.0155	0.0371	0.0676	
Pseud. I-II spp.	0.0122	0.0326	0.0482	
S. putrefaciens	0.0141	0.0339	0.0632	
P. phosphoreum	0.0830 (105.8 h)*	**0.0456**	**0.0881**	
Cod – Oct. 2003 (Tangi)	**-0.9±0.6°C (C)**	**1.7 ± 1.0°C (A)**	**1.9 ± 0.9°C (B)**	**4.1 ± 1.9°C (D)**
TVC	0.0153	0.0274	0.0508 (28.2 h)*	0.0120
Pseud. I-II spp.	0.0095	0.0168	0.0370 (39.1 h)*	0.0176
S. putrefaciens	0.0130	0.0169	0.0269	**0.0343**
P. phosphoreum	**0.0176**	**0.1637** (60.1)	**0.1678** (60.3)	(0.0150)
Fish – Nov. 2003 (Tros)	**0.4 ± 0.4°C (Cod)**	**1.8 ± 2.2°C (Abused cod)**	**0.3 ± 0.2°C (Haddock)**	**1.9 ± 2.7°C (Ab. haddock)**
TVC	0.0148	0.0495 (49.9 h)*	0.0132	0.0237
Pseud. I-II spp.	0.0171	0.0386 (32.1 h)*	0.0218 (69.6 h)*	0.0466 (60.5 h)
S. putrefaciens	0.0187	0.0537 (59.5h)*	0.0231	0.0353
P. phosphoreum	**0.0238**	**0.1917** (52.9 h)*	**0.0342**	**0.0505**

* estimated lag phase (h)

Table 9 gives the potential maximum growth rate as estimated by the DMFit program (Baranyi & Roberts, 1994) for the different bacterial groups evaluated in fish fillets. This exemplifies which bacterial group(s) is (are) delayed (lag phase) initially or dominating during the storage of fish fillets, and how temperature influences their behaviour. At low temperatures, the potential maximum growth rate of the different bacterial groups is often slightly lower than that estimated for the corresponding total viable counts (TVC). However, the potential maximum growth rate of *P. phosphoreum* was generally higher than that of TVC and all other bacterial groups. This was true at all temperatures and in all fish products tested, despite the fact that a lag phase sometimes occurred after which very rapid growth of *P. phosphoreum* took place. But of course, these estimated growth rates do not give a complete picture of behaviour of the spoilage microflora, as initial bacterial loads and maximum levels reached at sensory rejection surely play an important role in the spoilage pattern resulting.

Nevertheless, it is pointed out once again that *P. phosphoreum* is certainly of importance in the spoilage of fresh fish stored aerobically. Danish scientists have already reported that this bacterium is the main spoilage organism in fish packed under modified atmosphere (Dalgaard *et al*., 1995 and 1997), but its role in aerobic spoilage of fish has not been demonstrated before. Similarly to the data presented in Chapter 1, discussing the effect of temperature and bacterial interaction on specific spoilage bacterial growth in a model fish substrate, temperature abuse of raw material and processed fillets clearly triggered the development of *P. phosphoreum*, as well as the other bacterial groups assessed but to a lesser extent. It should also be noticed that such undesirable environmental conditions appeared to affect the physiological state of the different bacterial groups evaluated as lag phases were commonly observed under abused temperatures. This unexpected behaviour could be attributed to the fact that bacteria need to readjust to current environmental conditions, independently to how beneficial these are to their development.

4 CONCLUSIONS

Different spoilage patterns were observed for haddock and cod fillets. Values of the different measurement variables are different for the different experiments at sensory rejection based on sensory scores. The effect of different catching areas, different handling and time before processing influences the spoilage rate. Fluctuating temperatures, i.e. improper icing or higher environmental temperature during any stage of the processing or transport, shortens the shelf life of the products.

The growth of specific spoilage organisms was different in cod and haddock fillets. The counts of *Photobacterium phosphoreum* and *Pseudomonas* were higher in haddock fillets than in cod fillets. Moreover the counts of *Pseudomonas* ssp. in cod caught northeast of Iceland were lower than the counts in fillets from cod caught southwest of Iceland.

P. phosphoreum and *Pseudomonas* spp were dominating under temperature abusive conditions and probably similarly important. The results show that *P. phosphoreum* appears to be very important in the spoilage of fresh fish stored aerobically. This has not been reported before. It is clear that the spoilage pattern varies depending on the various extrinsic factors. Therefore, it is necessary to include data on the different specific spoilage organisms and different chemical spoilage indicators to be able to develop reliable shelflife models for fish fillets. Analysis of volatile compounds during storage of cod and haddock fillets by gas chromatography showed that similiar spoilage indicators were present in both species and the responses of the electronic nose can be explained be the presence of these compounds and their inceasing levels during storage.

The response of the electronic nose supported the microbial data and the sensors showed higher reponsen towards haddock fillets than cod fillets. Their responses were lower towards cod fillets from the the southwest of Iceland (Tros) than from the southeast (Tangi) which is in agreement with the longer shelf life of the former.

Shelf life of haddock and cod fillets evaluated by sensory analysis was different in the different experiments. For example haddock fillets had shelf lives of 11 days and 13.5 days and cod fillets had shelf lives of 12 and 13.5 days. These differences can be explained by the fact that the fillets with the longer shelf lives for both species were processed one day after catch and ideal conditions for cooling were used after processing. On the other hand the cod fillets with the shorter shelflife was processed after 3 days from catch and the haddock fillets with 11 days shelf lfe were processed from raw material that had been stored ungutted in ice slurry prior to processing. This confirms the importance of initial handling and cooling conditons after processing will determine the shelf lives of the products.

5 ACKNOWLEDGEMENTS

The authors thank The Icelandic Centre for Research for partly financing the project. The staff of IFL is thanked for their valued contribution in chemical, microbial and sensory analysis of samples.

6 REFERENCES

Baranyi J. & T.A. Roberts (1994). A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23, 277-294.

Dalgaard P. (1995). Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int. J. Food Microbiol. 26, 319-333.

Dalgaard P., Mejlholm, O. & Huss, H.H. (1996). Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. J. Appl. Bact. 81, 57-64.

Dalgaard P, Mejlholm, O., Christiansen, T.J. & Huss, H.H. (1997). Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. Lett. Appl. Microbiol. 24: 373-378.

Dyer W.J., Dyer, F.E & Snow, M. (1945). Amines in fish muscle. I. Colorimetric determination of trimethylamine as the picrate salt. Journal of Fisheries Research Board of Canada, **6**, 351-358.

Gram L., Trolle, G. & Huss, H.H. (1987). Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int. J. Food Microbiol. 4, 65-72.

Lauzon H.L. (2003). Notkun Malthus leiðnitækni til hraðvirkra örverumælinga. Verkefnaskýrsla 30-03, 30 síður (Icelandic).

Lindsay, R.C., Josephson, D.B. & Ólafsdóttir, G. (1986). Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In Proceedings of an International Symposium, University of Alaska Sea Grant Program, Anchorage, Alaska, U.S.A., D.E. Kramer and J. Liston (Ed.), pp. 221-234. Elsevier Science Publishers B.V., Amsterdam.

Olafsdottir G.(2003). Developing rapid olfaction arrays for determining fish quality. In Ibtisam E Tothill (Ed) RAPID AND ON-LINE INSTRUMENTATION FOR FOOD QUALITY ASSURANCE. Woodhead Publishing Ltd, Cambridge, England, pp. 339-360

Olafsdóttir, G, Soffia V. Tryggvadóttir og Rósa Jónsdóttir, (2002). Multisensor for fish: Storage studies of frozen hake in Madrid and fresh cod in Hamburg. Electronic nose, Texture and sensory analysis. Third individual progress report for European Commission (Devolopment of multi- sensor techniques for monitoring the quality of fish, CT-98-4076). RF report 10-02

Ólafsdóttir, G, Jóhann Örlygsson, Sigrún Jónsdóttir, Ágúst Vilhjálmsson, Hélène L. Lauzon og Rósa Jónsdóttir (2000). Nákvæm geymsluþolsspálíkön - -Hitaferlar, örveruvaxtatilraunir, rafnefsmælingar, geymsluþolsrannsóknir. Rf Verkefnaskýrsla 05-00.

Ólafsdóttir G., Á. Högnadóttir, E. Martinsdóttir & H. Jónsdóttir (2000). Application of an Electronic Nose to Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production, J. Agric. Food Chem. 48 ,6, 2353-2359.

Ólafsdóttir G. & Áslaug Högnadóttir (2000). FreshSense rafnef - nákvæmni mælinga og svörunareiginleikar. Rf skýrsla, október 2000.

Joffraud J J, Leroi F, Roy C, Berdague J L (2001), 'Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon', *Int. J. Food Microbiol.* 66, 3, 175-184.

Malle, P. & Poumeyrol, M. (1989). A New Chemical Criterion for the Quality Control of Fish: Trimethylamine/Total Volatile Basic Nitrogen (%). *Journal of Food Protection*, Vol 52, No 6, pp. 419-423.

Shewan, J.M., Macintosh, R.G., Tucker, C.G. & Ehrenberg, A.S.C. (1953). The development of a numeric scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J. Sci. Food Agric. 4: 283-298.

Stanbridge, L.H. & Board, R.G. (1994). A modification of the *Pseudomonas* selective medium, CFC, that allows differentiation between meat pseudomonads and Enterobacteriaceae. *Letters in Appl. Microbiol*. **18**, 327-328.

Tozawa, H., Enokihara K & Amano K. (1971). Proposed modification of Dyer's method for trim ethylamine determination in cod fish. In: *Fish Inspection and Quality Control* (edited by R. Kreuzer). Pp. 187-190. London: Fishing News Books Ltd.

Van Spreekens K.J.A (1974). The suitability of Long & Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. Archiv fur Lebensmittelsh. 25 (10), 213-219.

Vyncke, W. (1995). The determination of total volatile bases in eye fluid as a non-destructive spoilage assessment test for fish. *Archiv fur Lebensmittelhygine*, 46, 4, pp. 96-98. Verlag M. & H: Schaper.

APPENDIX

Chapter 2

The influence of temperature fluctuations on the shelf life of haddock and cod fillets stored under chilled conditions

PCA - preliminary analysis

PCA analysis

PCA analysis of the overall data is useful to gain an overview of the general trend in the data set. Figures 35 – 39 show PCA analysis of the overall haddock data to give an idea how the different variables explain the variation in data set. PCA analysis was done based on electronic nose data (CO and NH3 sensors) to see if samples could be discriminated based on their spoilage level. Grouping of the data according to spoilage level of samples is observed on the PCA plot. PC1 explains 81% of the variation of the data and PC2 explaines 19%.

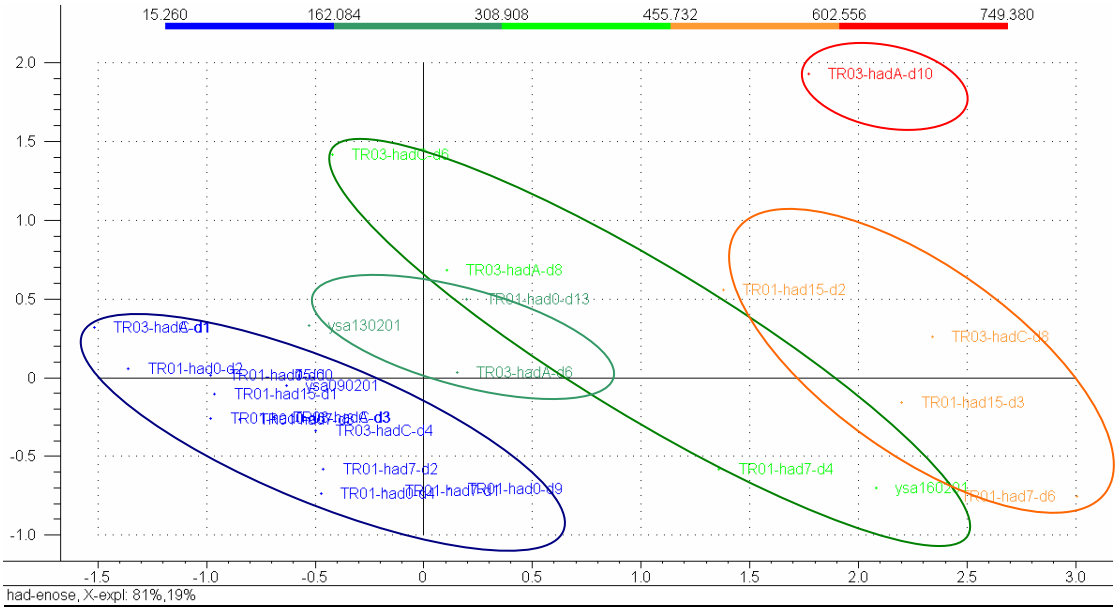


Figure 35. PCA scores plot based on electronic nose (CO and NH_3 sensors) and the haddock samples (Tros 2001 0°C, 7°C and 15°C; Tros 2003 Groups A and C and; samples from Feb 2001). Sample grouping (shown as different colors) based on CO sensor values.

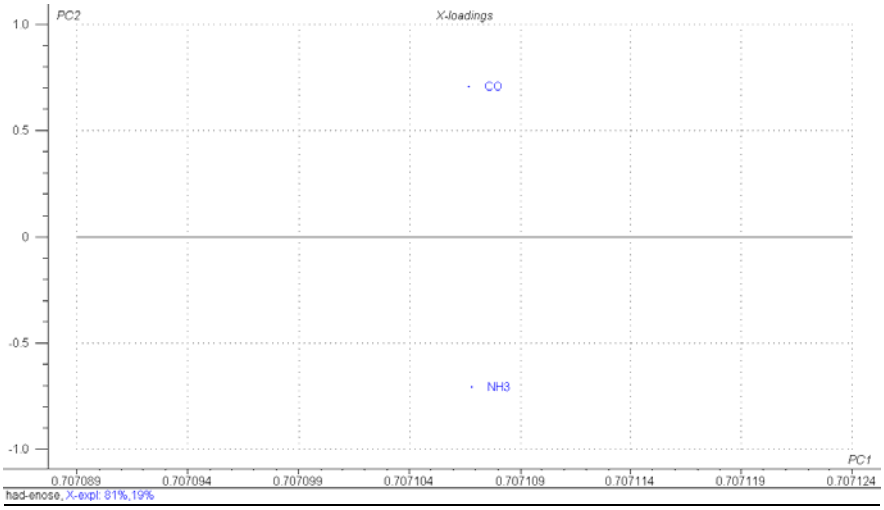


Figure 36. PCA loadings plot showing loading of the variables (electronic nose sensors;CO and NH_3) for haddock samples from different experiments (Tros 2001 0°C, 7°C and 15°C; Tros 2003 Groups A and C and; samples from Feb 2001).

In Figure 36 the loading of the two sensors CO and NH_3 are shown.

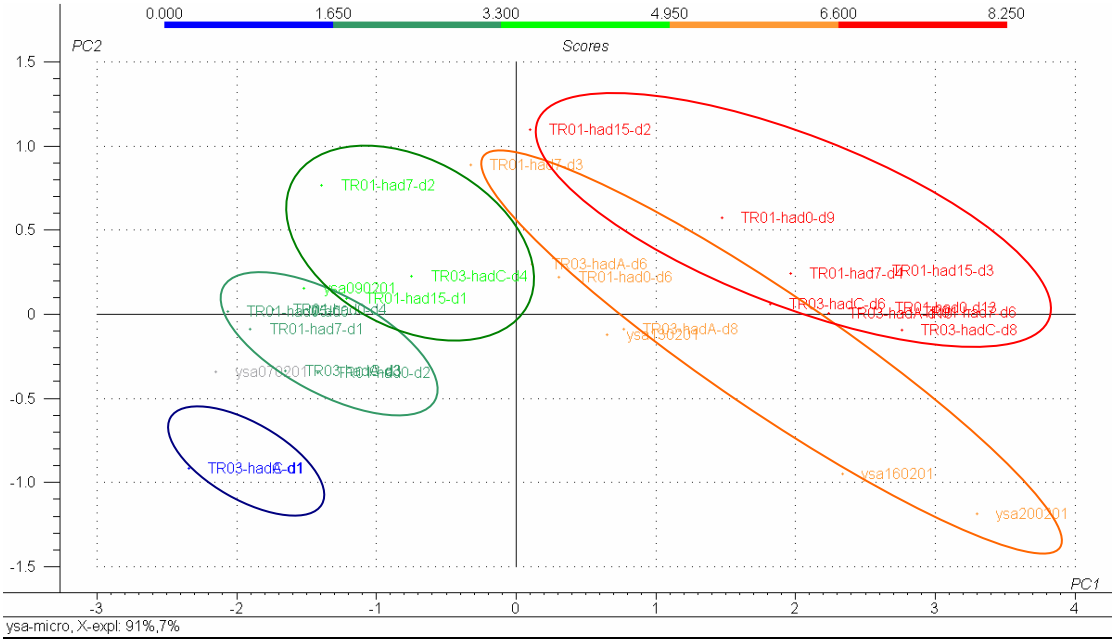


Figure 37. PCA scores plot based on all the microbial data for the haddock samples (Tros 2001 0°C, 7°C and 15°C; Tros 2003 Groups A and C and; samples from Feb 2001). Sample grouping (shown as different colors) based on counts of P. phosphoreum.

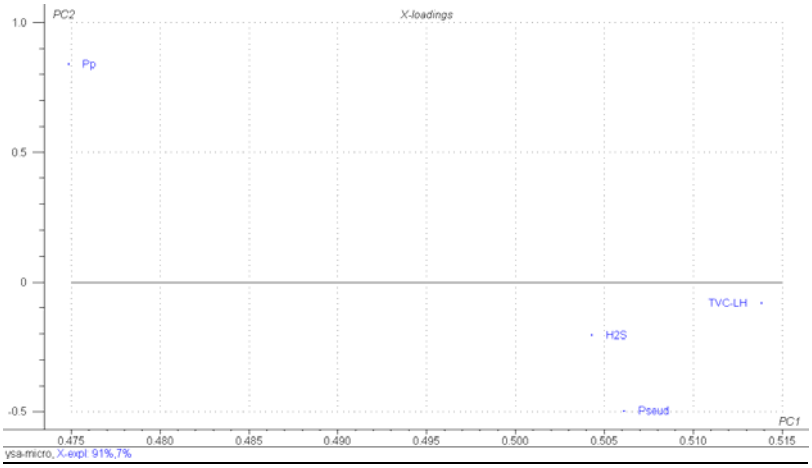


Figure 38. PCA loadings plot based on all the microbial data of the haddock samples (Tros 2001 0°C, 7°C and 15°C; Tros 2003 Groups A and C and; samples from Feb 2001). Sample grouping (shown as different colors) based on counts of P. phosphoreum

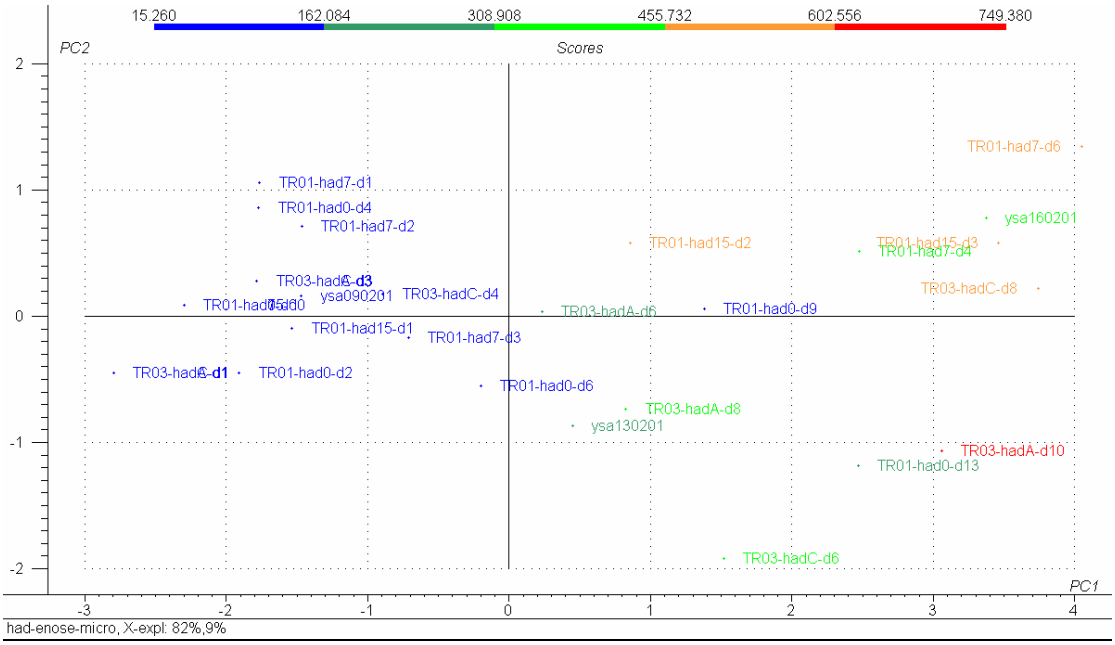


Figure 39. PCA scores plot based on electronic nose (CO and NH3 sensors) and all the microbial data for the haddock samples (Tros 2001 0°C, 7°C and 15°C; Tros 2003 Groups A and C and; samples from Feb 2001). Sample grouping (shown as different colors) based on CO sensor values.

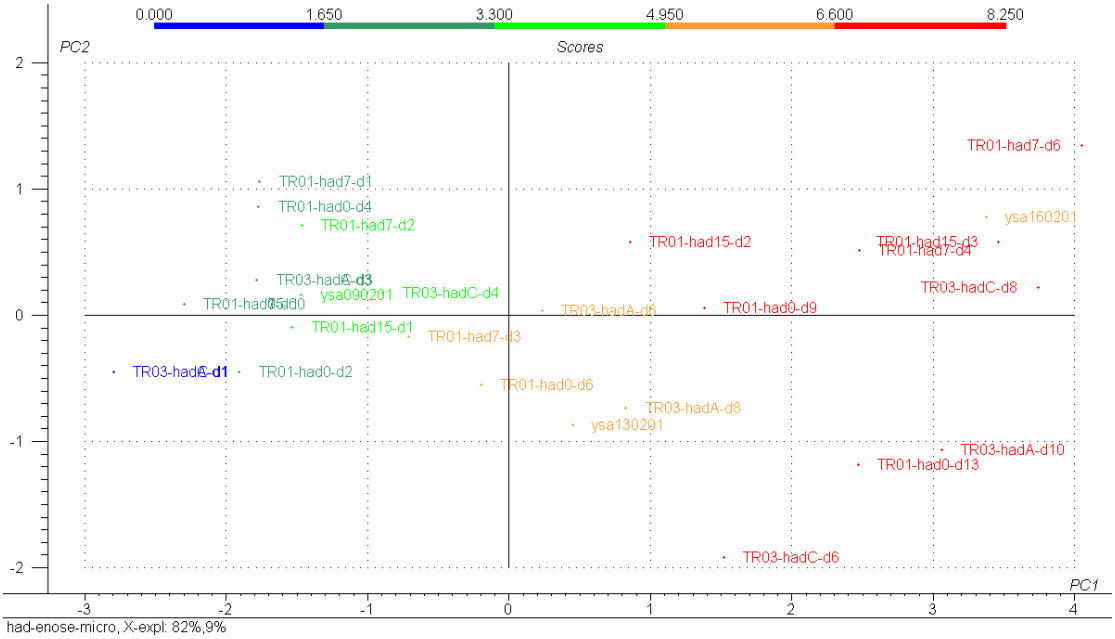
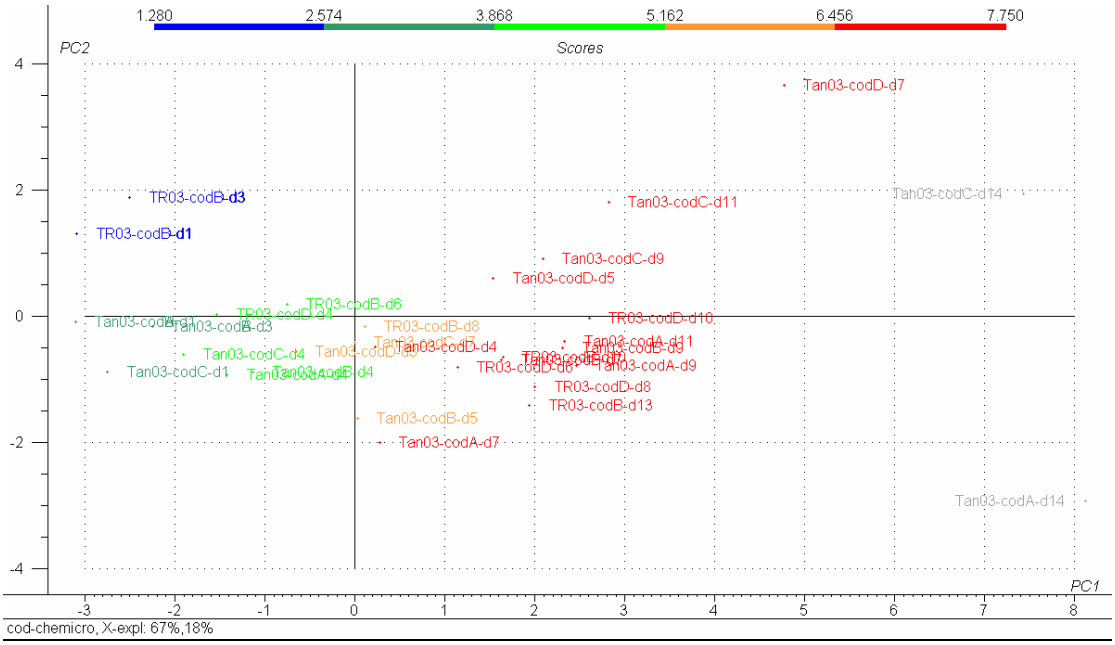
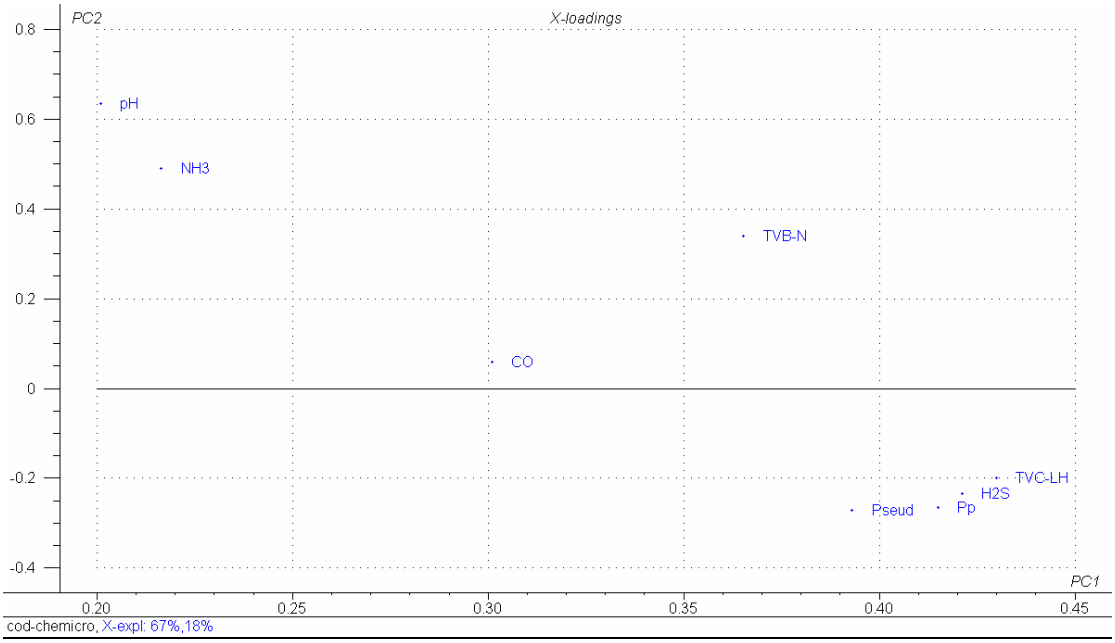
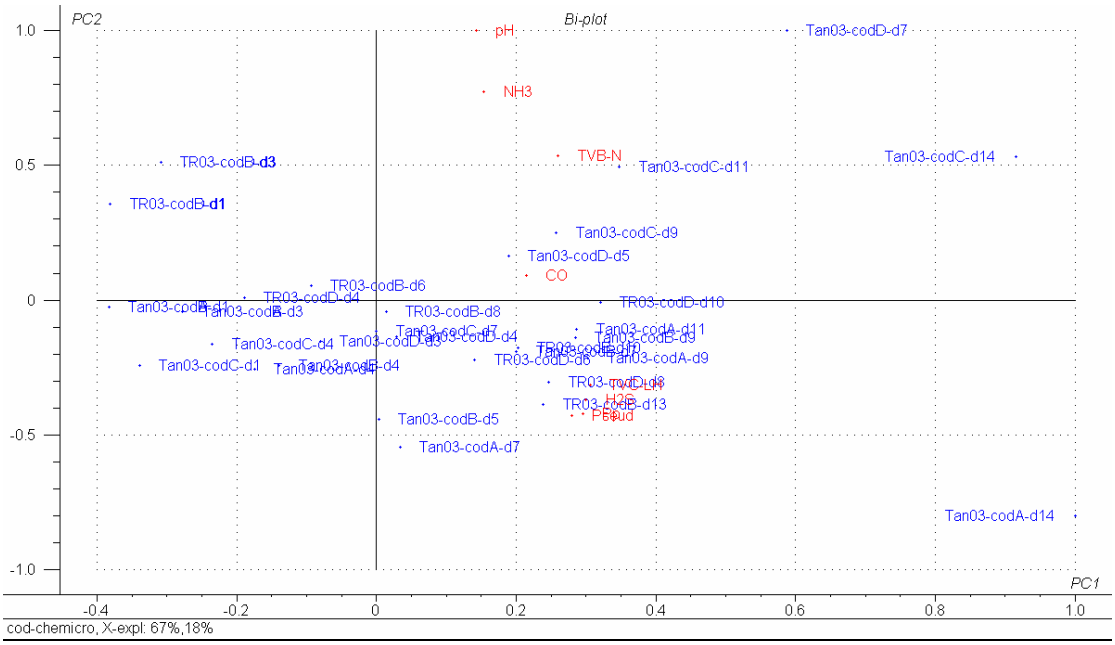


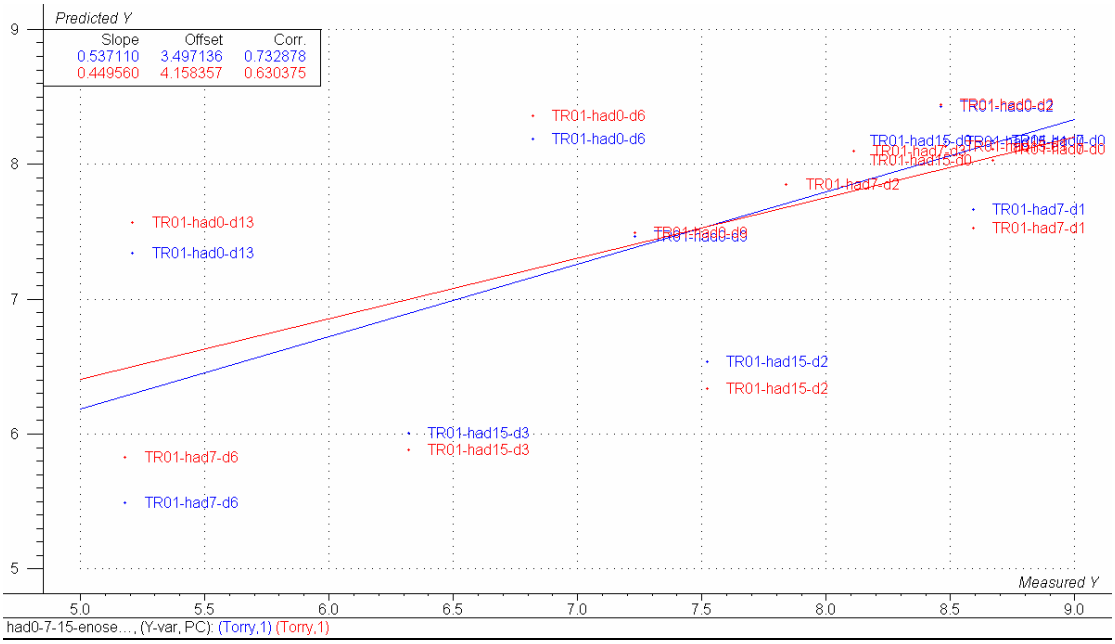
Figure 40. PCA based on electronic nose (CO and NH_3 sensors) and all the microbial data for the haddock samples (Tros 2001 0°C, 7°C and 15°C; Tros 2003 Groups A and C and; samples from Feb 2001). Sample grouping (shown as different colors) based on counts of P. phosphoreum

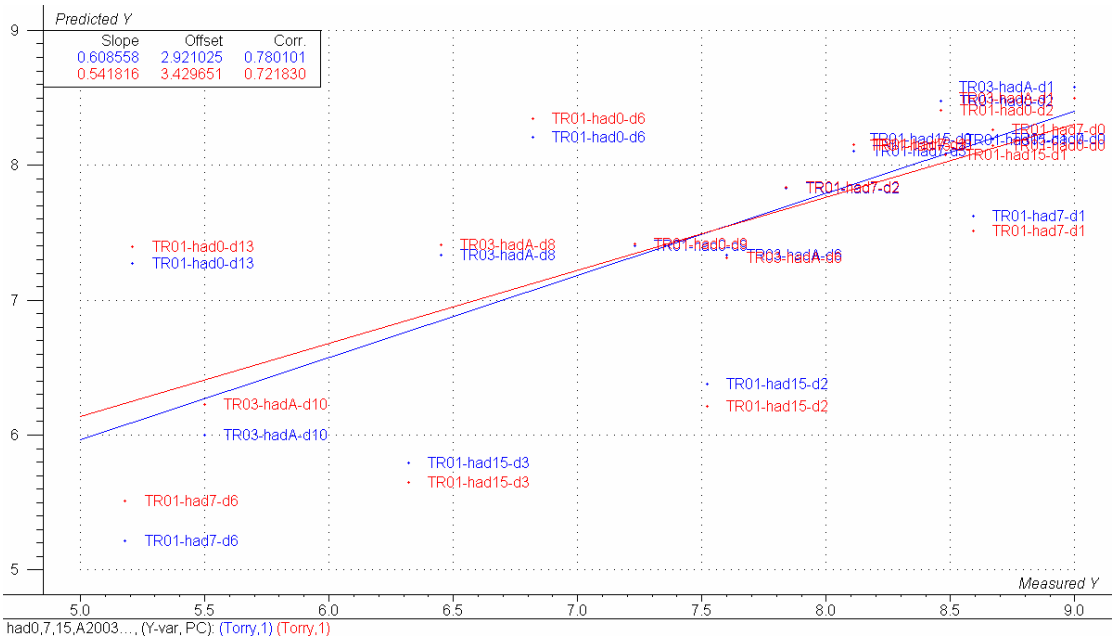




All the microbial variables have similar loadings on the plot and appear to be highly correlated, but the chemical measurement variables are located on the upper half of the plot and appear


Bi-plot
PC2
PC1
pH
NH3
TVB-N
CO
Tan03-codD-d7
Tan03-codC-d14
Tan03-codC-d11
Tan03-codC-d9
Tan03-codD-d5
Tan03-codB-d6
Tan03-codD-d4
Tan03-codB-d8
Tan03-codD-d10
Tan03-codA-d11
Tan03-codB-d5
Tan03-codA-d7
Tan03-codA-d14
cod-chemicro, X-expl: 67%,18%


Predicted Y
Slope Offset Corr.
0.537110 3.497136 0.732878
0.449560 4.158357 0.630375
Measured Y
TR01-had0-d6
TR01-had0-d13
TR01-had7-d2
TR01-had7-d1
TR01-had15-d2
TR01-had15-d3
TR01-had7-d6
had0-7-15-enose..., (Y-var, PC): (Torry,1) (Torry,1)


Predicted Y
Slope Offset Corr.
0.608558 2.921025 0.780101
0.541816 3.429651 0.721830
TR03-hadA-d1
TR03-had0-d2
TR01-had0-d2
TR01-had0-d6
TR01-had0-d6
TR01-had7-d2
TR01-had7-d1
TR01-had7-d1
TR01-had0-d13
TR01-had0-d13
TR03-hadA-d8
TR03-hadA-d8
TR01-had0-d9
TR03-hadA-d9
TR01-had15-d2
TR01-had15-d2
TR03-hadA-d10
TR03-hadA-d10
TR01-had15-d3
TR01-had15-d3
TR01-had7-d6
TR01-had7-d6
Measured Y
9
8
7
6
5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
had0,7,15,A2003..., (Y-var, PC): (Torry,1) (Torry,1)

Chapter 3
Modelling
Predictive microbiology

Summary by Hanna Miettinen

Prepared during an exchange visit at the Icelandic Fisheries Laboratories and the University of Akureyri under the supervision of Jóhann Örlygsson

TABLE OF CONTENTS

Predictive microbiology

History

The use of mathematical models in food microbiology is not new. Models for the thermal destruction of microorganisms by heat and modelling the fermentation are well established in literature and industry. The application of mathematical modelling techniques to the growth and survival of microorganisms in foods, however, did not receive wide attention until the 1980's (Ross and McMeekin, 1994).

The willingness to consider predictive modelling was influenced by marked increase in the incidence of major food poisoning outbreaks during the 1980's, which led to an acutely increased public awareness of the requirement for a sage and wholesome food supply. The second reason was the realisation by many food microbiologists that traditional and many rapid microbiological results had little predictive value. Third factor was the development of computing power (Ross and McMeekin, 1994).

Overview: Predictive food microbiology: where do we go from here?
Whiting, R.C., et al. 1997. Food Technol. 51 (4), 81-103.

Background

Traditionally the microbiological safety of foods has been established via challenge tests. These tests simulated the effects of environmental conditions on food, in terms of growth and proliferation of spoilage and pathogenic microorganisms. Challenge test can provide data useful in determining the safety and shelf life of food under set conditions. However, challenge tests have been criticised as an expensive, labour intensive, time-consuming and non-cumulative research tool. More recently challenge tests have been considered as only giving modest assurance on product safety in the food chain (McDonald and Sun, 1999, others).

Predictive food microbiology (PFM) is a promising and rapidly developing area of food microbiology, which has gained significant scientific attention in recent years. It encompasses such areas as mathematics, engineering, chemistry and microbiology to give microbial behavioural predictions in specific foods under defined conditions (McDonald and Sun, 1999, others).

The rapid development of microbial models and their ability to predict microbial growth makes modelling an invaluable research tool. Use of models can quickly provide information and, therefore, it is important to appreciate the real value and usefulness of predictive models. PFM has potential to be used in the development and maintenance of HACCP systems (McDonald and Sun, 1999).

The large variety and number of spoilage organisms encountered in foods means that spoilage models are less straightforward to develop than pathogen models and their application is much more limited (McDonald and Sun, 1999, others).

PFM can provide a means to quickly evaluate the consequences of any changes in formulation or processing of products. However it cannot avoid but can reduce the need for expensive, time-consuming challenge tests. Problems in production of existing products can also be evaluated in terms of out-of-specification circumstances (McDonald and Sun, 1999).

Development of predictive model

In the development of a model, it is essential to know the requirements of the model. Experiments must be designed in such a way as to make the best use of time and resources. Requirements of models can depend on whether the modeller needs to understand the effects of variables on microorganisms or the upper and lower limits for preservation or growth. Experiments must be designed to encompass as much of variability as possible. Many modellers use a mixed culture of the most commonly encountered strains in food for their experiments. In this way the growth or survival predicted by the model will correspond to the fastest growing strain present (McDonald and Sun, 1999, others).

A model development requires data from experimentation. The greater the quantity of data collected, the better the accuracy and reliability of the model derived. Methods of data collection vary, but the standard method is the total viable count. However, the total viable count is very labour-intensive, while rapid and automated methods of data collection have been used. However, automated methods can have a larger risk of misinterpretation than total viable counts (McDonald and Sun, 1999, others).

Spoilage bacteria are important in foods when the growth occurs in high levels. For such microorganisms the absorbance techniques will most often be of considerable practical importance (Dalgaard and Koutsoumanis, 2001).

Flow cytometry has been used to generate data for predictive modelling. The technique enables estimation of microbial population concentration at population levels considerably lower than achievable with optical density methods and much more rapidly than determinations of colony forming units (Sørensen and Jakobsen, 1997).

A model must be validated in real situations. This is critical to placing confidence in a model. Validation studies must demonstrate that microorganisms behave in similar ways both in the laboratory and in a real food system. Users of specific models must be aware of the boundary of model performance and understand the applicability of the model range. In practice, the issue is not necessarily how well a model fits to data, but the accuracy with which it mimics the microbial response (McDonald and Sun, 1999, others).

Errors made using predictive models in relation to industry and public health could have detrimental effects on the future research and development of PFM. Its use in real situations should not be applied unless a defined level accuracy and validation is achieved (McDonald and Sun, 1999).

Models

A predictive food microbiological model is a mathematical expression that describes the growth, survival, inactivation or biochemical process of food borne micro-organisms (McDonald and Sun, 1999).

Whiting and Buchanan (1993) (in McDonald and Sun, 1999) suggested a classification system that groups most model types together into primary, secondary and tertiary based models.

Kinetic models can differ in their approach. One approach is to model the growth rate of an organism and use it to make predictions based on the exponential growth of that microbial population. Another approach is to fit a sigmoid function or curve to a microbial population growth data, and then model the effects of various environmental factors such as temperature on this function (McDonald and Sun, 1999). Variables like temperature, pH, a_w, gaseous atmosphere, redox potential, biological structure, relative humidity, nutrient content and antimicrobial properties can also be included in kinetic models (McDonald and Sun, 1999, others).

The use of probability in PFM takes advantage of the likelihood that a particular event will occur under prescribed conditions, as that ability to predict likely occurrences in food systems has obvious advantages. **Probability-based models** have tended to be used to model spore-forming bacteria such as probability of *Clostridium botulinum* survival in canned corned beef (McDonald and Sun, 1999, others). The problem with probability is that probability changes with time, so probability models are in fact a combination of both probability and kinetics and that can make them confusing (McDonald and Sun, 1999).

Empirical models such as the Gompertz function are concerned with practical consequence and simply describe data under experimental conditions in the form of a convenient mathematical relationship. Polynomial equations are the common empirical models. These models are easy to use, straightforward and no knowledge of a particular process is required. However, polynomials often have no theoretical foundation and are non-linear, which are valid only for the range of variables of the underlying data and have numerous parameters without biological meaning. Therefore, polynomial models do not contribute any knowledge to mechanisms underlying a process (McDonald and Sun, 1999, others).

Understanding underlying mechanisms governing cellular metabolism, which produces data, may in time allow the construction of **mechanistic models**. Such model will represent that mechanism more accurately and will serve as a vehicle for generating prediction from hypotheses. Interpretation of the modelled response in terms of known phenomena and processes may then be possible. Indicated that mechanistically derived models would be easier to develop further, as the quantity and quality of information from the analysed system increases. However completely mechanistic models, which incorporate all intrinsic and extrinsic variables, that affect growth, have not been developed (McDonald and Sun, 1999, others). Most researchers agree that mechanistic models are inherently superior to empirical models for the above reasons (McDonald and Sun, 1999, others).

Primary models describe the change of the bacterial number with time under particular environmental and cultural conditions. Response can be measured directly by total viable count, toxin formation, substrate level or metabolic products or indirectly by absorbance, optical density or impedance (McDonald and Sun, 1999, others).

Secondary models describe the response of one or more parameters of a primary model (lag phase duration) changing to one or more changes in cultural or environmental conditions (pH, a_w, Eh, temperature).

Tertiary models basically take modelling to its final form. They are applications of one or more primary and secondary models, incorporated into a user-friendly computer software package. These models are incorporated into various function integrators such as temperature, a_w, or pH. A time/function integrator history of a product can then be used in conjunction with the secondary model to determine the extent and rate of growth of the organism. Microbial responses to variable conditions and the comparison and contrasting of these effects on several species of microorganisms can also be undertaken, using a pertinent database. End-users of these systems need not be aware of modelling techniques or the underlying primary and secondary models used. Tertiary models make predictive microbiology an easily accessible and powerful tool to all areas of food industry and research (McDonald and Sun, 1999, others).

Availability of many different models can make selection of the best model for a particular use difficult. In specific situations, some data can work better with one model than another. However, using the simplest available model without compromising too much on accuracy is a rational policy (McDonald and Sun, 1999).

The definition of model performance is sometimes difficult. Ross (1996, in Ross et al. 2000) introduced measures of model performance called the Bias factor (B_f) and Accuracy factor (A_f). Modifications of the factors were proposed by Baranyi et al. (1999, in Ross et al. 2000). Ideally, predictive models would have $A_f = B_f = 1$ but typically, the accuracy factor will increase by 0.10-0.15 for every variable in the model (Ross et al. 2000).

Satisfactory B_f limits are more difficult to define because limits of acceptability are related to the specific application of the model. B_f is a measure of the extent of under- or over-prediction by the model of the growth rates observed. Thus, a bias factor of 1.1 indicates not only that the model is 'fail-dangerous' because it predicts longer generation times than are observed, but also that the observations exceed the prediction, on average, by 10% in terms of log CFU. Conversely, $B_f<1$ indicates that a model is, in general, 'fail-safe'. However, when applied to rate-based data, $B_f>1$ indicates that the model under-predicts the observed rate, potentially leading to 'fail-dangerous' prediction (Ross et al. 2000).

Evaluation of predictive models has shown that lag times are less reliably predicted than generation times. This has usually been attributed to the effect of the prior history of cells on the duration of the lag time (Ross et al., 2000).

Prototype dynamic models to describe growth and inactivation of a microbial population as a function of time and temperature.
Presented: A combined model for growth and subsequent thermal inactivation of *Brochothrix thermosphacta*. Baranyi et al. 1996. Appl. Environ. Microb. 62. 1029-1035.

Predictive microbiology in a dynamic environment: a system theory approach.
Van Impe, et al. 1995. Int. J. Food Microbiol. 25, 227-249.

Primary models

Monod model

Monod model is an early model describing growth rate:

$N = N_0 e^{kt}$ where,

N = number of organisms at time t $(CFU)^{-1}$
N_0 = initial number of organisms at time $t = 0$
k = growth rate constant $(s)^{-1}$
t = time (s)

The disadvantage of this model lies in the fact that the lag time has to be determined from the data and cannot easily be determined using regression (McDonald and Sun, 1999, others).

The Gompertz equation

Models for microbial growth curves can also be obtained by non-linear regression techniques. Gibson et al. (1987) (in McDonald and Sun, 1999) introduced for the first time in food microbiology the Gompertz equation.

$y = A + C\exp\{-\exp[-B(t-M)]\}$ where,

y = logarithm of relative population size
A = asymptotic log count as t decreases indefinitely $(CFU)^{-1}$
C = asymptotic amount of growth that occurs as t increases indefinitely log (CFU)-1
B = relative growth rate at time M (s^{-1})
M = time at which absolute growth rate at maximum (s)
t = time (s)

The logistic model

The logistic sigmoidal relationship model is derived from the Gompertz equation (McDonald and Sun, 1999, others):

$$y = \frac{A + C}{\{1 + \exp[-B(t - M]\}} \quad \text{where,}$$

y = logarithm of relative population size
A = asymptotic log count as t decreases indefinitely $(CFU)^{-1}$
C = asymptotic amount of growth that occurs as t increases indefinitely log (CFU)-1
B = relative growth rate at time M (s^{-1})
M = time at which absolute growth rate at maximum (s)
t = time (s)

The Modified Gompertz equation

(Zwietering et al. 1990. Modelling of the bacterial growth curve. Appl. Environ. Mircobiol. 56: 1875-1881). The modified Gompertz equation is commonly accepted as a possible static equation to describe the growth of microorganisms as a function of time (Geeraerd et al., 1998). A dynamic extension of this model is developed by Van Impe et al. (1992) and Van Impe et al. (1995) (kts. Geeraerd) and makes it possible to describe accurately microbial growth in time-varying environmental conditions, which is important for chilled, prepared food products (Geeraerd et al., 1998).

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \quad \text{, where}$$

y = logarithm of relative population size
A = asymptotic log count as t decreases indefinitely $(CFU)^{-1}$
μ_m = maximum-specific growth rate (s^{-1})
λ = lag phase duration (s^{-1})
t = time

At present, the Gompertz function has became the most widely used sigmoid curve in PFM due to its simplicity and effectiveness. It has been used to describe growth curves for many organisms including at least 10 food borne pathogens. The lack of biological basis for the parameters used makes interpretation of parameters difficult. The calculation of lag time with the Gompertz equation can be wrongly used as growth can occur before the predicted lag time (McDonald and Sun, 1999, others).

Baranyi model

Baranyi model is a dynamic model for predicting microbial growth, combined with an adjustment function A(t) that depends on the physiological state of the microbial cells.

$$y(t) = y_{\max} - \ln\left[1 + (e^{-y_{\max} - y_0} - 1)e^{\mu_m A_n(t)}\right] \quad \text{where,}$$

y_{max} = logarithm of relative population size
y_0 = logarithm of initial population size
μ_m = maximum-specific growth rate (s^{-1})

A(t) = precise integral of the adjustment factor

The value of the adjustment function together with the post-inoculation conditions can predict the duration of the lag phase. Many researchers have used Baranyi model in specific microbial modelling applications and found in comparison to the Gompertz function and other models that it gives satisfactory results (McDonald and Sun, 1999, others).

The modified Gompertz model is known to overestimate μ_m values of typical microbial growth cultures by approximately 10-20% (Dalgaard and Koutsoumanis, 2001, others). Baranyi model provides μ_m values which are practically identical to those obtained from the less complicated Logistic model. For estimation of lag time from viable count data, very similar values have been obtained by the Baranyi model and the Logistic model whereas the modified Gompertz models may provide negative lag time estimates (Dalgaard and Koutsoumanis, 2001, others).

Primary models have relative advantages and disadvantages but there is a good general agreement between models for microbial growth (McDonald and Sun, 1999, others).

Secondary models

The vast majority of secondary models are kinetically based, with the most commonly used models being Arrhenius, modified Arrhenius, square root and polynomial models.

Arrhenius equation

The Arrhenius equation derived empirically based on thermodynamic consideration:

$k = k_0 e^{\frac{-E_A}{RT}}$ where,

k = growth rate constant (s^{-1})
k_0 = Arrhenius equation constant (pre-exponential factor)
R = universal gas constant (8.314 J mol^{-1} K^{1-})
T = temperature (K)
E_A = activation energy (314 J mol^{-1})

The equation cannot fit data well below optimum or above minimum temperature for growth. The plots are normally only accurate over a limited temperature range for microbial growth (McDonald and Sun, 1999, others).

Modification of Arrhenius equation has been made, e.g. Schoolfield, Davey and CRR models, to overcome the problems and enhance the original Arrhenius models in fitting data at microbial temperature extremes (McDonald and Sun, 1999, others, Daughtry et al., 1997).

Square-root model

The Belehradek model was proposed for the first time to model food microbiology by Ratkowsky et al. (1982) (in McDonald and Sun 1999). The model is also known as the square-root model.

$\sqrt{k} = b(T - T_{\min})$ where,

k = growth rate constant
b = regression coefficient for temperatures below optimal (K^{-1})
T = temperature (K)
T_{min} = notional minimum growth temperature (K)

The equation has been successfully used to model the effects of temperature on bacterial growth rate.

Ratkowsky model

The square-root model was later extended to cover the entire biokinetic temperature range. With terms T_{min} and T_{max}, classification of microbes can be achieved in a more objective manner as psychrophiles, mesophiles or thermophiles. The model was reasonably effective in predicting effects of constant storage temperatures on microbial growth rates (McDonald and Sun, 1999, others).
Various alterations and modifications have been made to Ratkowsky model. Parameters for water activity and temperature, pH and temperature, or all together have been suggested. Also a parameter to describe for dissolved CO_2 in modified atmosphere has been proposed (McDonald and Sun, 1999, others).

Artificial neural networks (ANN)

ANN is a low complexity non-linear modelling technique, capable of describing accurately experimental data in the field of secondary models in predictive microbiology (Geeraerd et al., 1998).

The development of artificial neural networks is inspired by the elementary principle of the human nervous system: an interconnection between neurons leading to a new nerve which causes a weighted, non-linear response. As such, not all the information is amplified in the same way. Each neuron performs the simple operation of adding a weighted sum (weights w_j) of incoming input signals p_j, to a bias term (or threshold) β_r and feeding the result to a non-linear activation (or transfer) function $\sigma(\cdot)$ (e.g., sigmoidal unit or binary threshold unit) which results in the out value y_i of the neuron (Geeraerd et al., 1998).

$$y_i = \sigma\left(\sum_{j=1}^{m}\left(w_i p_j + \beta_r\right)\right)$$

Different neurons can be connected to a neuron layer and different neuron layers can be placed behind each other forming a complete neural network. Interactions between different inputs p_j can be modelled without specifying them in advance. (Geeraerd et al., 1998).

ANN is adaptable and can be trained to recognize associations among complex data without requiring any prior assumptions of the mechanistic associations among the data (Almeida and Noble, 2000).

The technique could make it possible to describe more accurately the interaction of intrinsic and extrinsic variables in chilled meats when compared to more familiar predictive microbiological models. The model was more accurate than the polynomial relationship found in literature. This is explained by the flexible basis functions used in artificial neural network modelling compared to the fixed basis functions of polynomial or any other linear modelling approach (Geeraerd et al., 1998).

Tertiary models

There are several microbiological modelling software packages currently available.

Pathogen Modelling Program
The Department of Agriculture (USDA) Food Safety Research Unit has developed the Pathogen Modelling Program in the US. The software uses multivariant models based on the use of the Gompertz function in combination with response surface analysis. It was developed using extensive experimental data on the behaviour of microorganisms in liquid media. The program contain e.g. a growth model for *Clostridium perfringens*, thermal inactivation model for *Cl. botulinum* and gamma irradiation model for *Salmonella*, *E. coli* O157:H7 and normal flora in meats (McDonald and Sun, 1999, others). The software is available free at:
http://www.arserrc.gov/mfs/PATHOGEN.HTM.

Food MicroModel
The United Kingdom´s Ministry of Agriculture, Fisheries and Food (MAFF) has created the Food MicroModel software package, which was launched in 1992. This modelling software uses the results of predictive microbiological research in the context of an expert system. The Food MicroModel provides a range of predictive models for at least 12 implicated food borne pathogens (McDonald and Sun, 1999, others). http://www.lfra.co.uk/lfra/micromod.html

Pseudomonas Predictor
The Pseudomonas predictor was developed by a group of scientist at the University of Tasmania in Australia. The software package is a spread sheet based and can be used to predict the growth of *Pseudomonas* species. It is also capable of quantifying effects of storage temperature on dairy products, and it can predict how quickly pseudomonads will grow. In addition the software is able to correlate shelf life of a

product to its temperature history. However the Pseudomonas Predictor requires the user to have some computer literacy, which can offset the flexibility of the package (McDonald and Sun, 1999, others).

Seafood Spoilage Predictor (SSP) software

SSP software has been developed to facilitate the practical use of mathematical seafood spoilage models. SSP was designed to predict the effect of constant and fluctuating temperatures on different types of seafood. The SSP software can read and evaluate temperature data as collected by different types of loggers.
SSP contains relative rates of spoilage (RRS) models and microbial spoilage (MS) models. Models included in the software should only be used in products stored within the range of conditions where they have been successfully validated. Information about this range of applicability is found within SSP under 'Help' for the individual models (http://www.dfu.min.dk/micro/ssp/Default.htm).

Models for different microorganisms

Spoilage

Because of the variety and number of spoilage organisms, spoilage models are less straightforward to develop than pathogen models and their application is much more limited (Pin and Baranyi, 1998).

McMeekin and Ross (1996) (in (Pin and Baranyi, 1998) emphasised that spoilage models, based on the responses of the dominant organism, are valid only in a specific range of conditions. Out of this range, different bacteria or metabolites may be responsible for the spoilage, making the model no longer valid.

Pin and Baranyi (1998) studied the interactions of meat spoilage bacteria groups in the range of temperature 2-11°C and pH 5.2-6.4. The interactions of the organisms were analysed by comparing their growth parameters obtained in pure culture with those obtained in mixture by multivariate quadratic polynomial. The dominant organisms in the mixed population were one or more of the group *Pseudomonas* (Pin and Baranyi, 1998).

Neumeyer et al. (1997), studied the effects of temperature and a_w on the growth of spoilage pseudomonads and found that modified Ratkowsky model can be used in sub-optimal temperatures to calculate the predicted generation times.

Indicator organisms

A log-linear model was used to evaluate the microbiological quality and acceptability of baby clams in terms of faecal coliforms and *Escherichia coli* by Gardini et al. (2000). The evaluation of quality of the samples in relation to their geographical origin and season of harvesting and the prediction of the probability of having an unacceptable sample in given geographical origin and season were achieved.

Moulds
Sautor et al. (2001) proposed in their study with four moulds that the shape of the growth curve should determine the choice of the model rather than the organism type or the environmental parameter. The Rosso type model represented well the relationship between water activity and the growth of four moulds (*Penicillium chrysogenum, Aspergillus flavus, Cladosporium cladosporioides* and *Alternaria alternata*) (Sautor et al., 2001).

Rosso and Robinson (2001) have also proposed a model deduced from the cardinal model family to describe the effect of water activity on the radial growth rate of moulds.

Yeasts
Modified Gompertz model was used to model the combined effects of temperature, pH and NaCl on the growth of *Debaryomyces hansenii*. Model validation showed a good agreement between observed and predicted maximum specific growth rates whereas predicted lag phases were shorter than the observed lag phases (Sørensen and Jakobsen, 1997).

Pathogens
Especially the following question should be answered when modelling pathogen growth (Shimoni and Labuza, 2000).

- Can models be used that were developed for initial counts of 100 cells per gram or more for predicting growth in systems that contain less than four cells per 100 g?
- Will the time to detect pathogens follow the same temperature dependence as found for the lag phase when using higher inocula levels?
- What will be the effect of inoculum's physiological state (lag/log or stationary phase) on the growth parameters below the detection level?
- What is the effect of temperature fluctuations on pathogen growth in food in the lower lag phase, i.e. is there a temperature history effect, and what direction does it take?

The emergence of low infectious dose pathogens, i.e. those that may cause disease at 1-10 organisms ingested, presents a significant challenge to predictive microbiology (Shimoni and Labuza, 2000). Before a predictive microbiology model can be used in risk assessment, the validity of its predictions must be demonstrated in the product of concern under realistic conditions of contamination and handling. If it cannot, or has not been demonstrated, this applicability of the model should be identified explicitly as an assumption (Ross et al., 2000).

Listeria monocytogenes
Dalgaard and Vigel Jørgensen (1998) tested the performance of the Pathogen Modelling Program and the Food MicroModel for growth of *L. monocytogenes* by comparison with data from 100 seafood challenge tests and data from 13 storage trials with naturally contaminated sliced vacuum-packed cold-smoked salmon. In general the prediction made by the Pathogen Modelling Program and the Food MicroModel gave too high growth rates and too long lag phases.

Food MicroModel predictions of *L. monocytogenes* in smoked fish products were compared also by Thurette et al. (1998, in Ross et al., 2000). The Food MicroModel predictions underestimated the generation time and overestimated the lag time duration. The predictions came less reliable as storage time increased.

Accurate prediction of seafood may be obtained by expanding the predictive models to include the effect of temperature, NaCl/a_w, pH, lactate and phenol. To be accurate and thereby useful, predictive models should be product-related and studies of naturally contaminated products should be included in the development and validation of predictive models (Dalgaard and Vigel Jørgensen, 1998).

Augustin and Carlier (2000a and 2000b) have tested a multiplicative secondary model (Rosso, 1995) to *L. monocytogenes* growth data collected from the literature. The model independently described the effects of environmental factors on the growth rate and lag time. The growth rates calculated with the model were consistent with the published ones, but the fit was poor near the limits of growth of the microorganism. The model was also less accurate to describe the lag time. The improved version of the model had a rate of fail-safe growth predicted 12.1% and a rate of fail-dangerous no growth predicted 7.1% (Augustin and Carlier, 2000b).

Also other studies have shown that the temperature-growth rate relationship of *L. monocytogenes* is not as well described by existing models as are for other organisms, particularly at low temperatures that cause slow growth rates (Bajard et al., 1996; Ross, 1999b, in Ross et al., 2000)

Several recent reports indicate that log-linear models, i.e. based on D-values, are inadequate to describe the death kinetics of *L. monocytogenes*, and that more complex (e.g. sigmoidal) functions are needed (Ross et al., 2000).

Future

The issue of variability in responses between strains, or due to uncontrolled variables, is currently a major theme in 'predictive microbiology'. It is expected that predictive microbiology models will, increasingly, also incorporate such information and provide predictions not only of the most likely response, but also of the range of responses that may be expected (Ross et al., 2000).

APPENDIX

Fylgiskjöl

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

Spoilage potential and growth of specific spoilage organisms in fish model system at different temperatures

Guðrún Ólafsdóttir and Hélène L. Lauzon
Icelandic Fisheries Laboratories, PO Box 1405, Skúlagata 4, 121 Reykjavík, Iceland
Tel.: 354 562 0240, Fax: 354 562 0740, e-mail: gudrun@rfisk.is, helene@rfisk.is

It is well recognised that fish spoilage is caused by microbial action. Low temperature storage of fish is very important to maintain its freshness and quality. However, temperature control throughout the process from harvest to the consumer is often difficult. Research on predictive microbiology has shown that more realistic shelflife models for food can be achieved if temperature fluctuations are taken into account and specific spoilage organisms (SSO) are used rather than total viable counts. *Shewanella putrefaciens* and *Pseudomonas* spp. have been reported as the specific spoilage bacteria of iced, air-stored fresh fish. *Photobacterium phosphoreum* is considered as the main specific spoilage organism of marine fish caught from temperate waters and stored under modified atmosphere. *P. phosphoreum* can be found on fresh fish fillets, and being a TMA-producer, its role in the spoilage of air-stored fillets may be of importance and should therefore not be overlooked.

Growth studies, involving strains of *Pseudomonas* spp. (groups I and II), *S. putrefaciens* and *P. phosphoreum*, were done in fish extract at 0.5 ± 0.4°C, 7.6 ± 0.1°C and 14.8 ± 0.1°C. The aim of the growth studies was to evaluate the spoilage potential/activity of the chosen bacteria species when grown alone and mixed together. Information about microbial interaction and metabolite formation was obtained at the different temperatures. Traditional microbiological and conductance methods were used to assess the development of the bacteria, while microbial metabolites were measured using GC-MS and an electronic nose.

Key words: specific spoilage organisms, electronic nose, volatile compounds

WESTERN EUROPEAN FISH TECHNOLOGISTS´ ASSOCIATION

32nd WEFTA meeting, May 13th-15th, 2002, Ireland

32nd WEFTA Meeting
12-15 May 2002, Galway (Ireland)

Spoilage potential and growth of specific spoilage organisms in fish model system at different temperatures

Hélène L. Lauzon and Guðrún Ólafsdóttir
Icelandic Fisheries Laboratories (IFL)
Reykjavík, Iceland

Introduction

- Increasing market share of **fresh or chilled products**
  - **Low temperature storage** of fish to maintain its freshness and quality
  - **Temperature control** throughout process from harvest to the consumer is often difficult.
- **Predictive microbiology:** realistic shelf life models should take into account **temperature fluctuations** and knowledge of **specific spoilage organisms** (SSO), **microbial interaction** and **metabolite formation**.
- **Spoilage bacteria of marine fish:**
  - **air storage:** *Shewanella putrefaciens* & *Pseudomonas* spp.
  - **MAP storage:** *Photobacterium phosphoreum*

Aim of the study

- to collect information about microbial interaction at different temperatures (0-2°C, 7-8°C, 14-15°C)
- to measure microbial metabolites by electronic nose and GC-MS
  - **The overall aim of the project is to use rapid measurements of specific spoilage organisms and/or microbial metabolites as data for models to predict the shelf life of fish.**
  - Detailed information about the production of microbial metabolites as spoilage indicators can be used to develop further the electronic nose.

Materials and methods - 1

Haddock fillets in EPS boxes:

- fresh from fish processor
- 11 fillets per box with a cooling mat
- Ambiant temperatures:
  - ✓ 0.9 ± 0.3°C
  - ✓ 7.2 ± 0.2°C
  - ✓ 15.1 ± 1.1°C
- Fillet temperatures:
  - ✓ < 5°C (2-4°C)
  - ✓ 6.9 ± 0.9°C
  - ✓ 12.0 ± 3.7°C

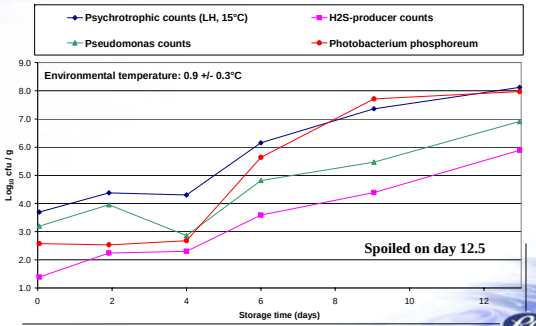
Fish model system:

- haddock extract prepared from 1 mince : 4 water
  - ✓ boiled for 2 min.
  - ✓ filtered and NaCl added
  - ✓ autoclaved (121°C, 15 min.)
  - ✓ supplemented with TMAO, cysteine, serine and methionine
- Bacteria: *Pseud.* I and II, *Shew. put.* and *Photobacterium phosp.*
- Inoculation level: log 2-3/ml in 125 ml extract in 590 ml jars
- Temp: 0.5 ± 0.4°C, 7.6 ± 0.1°C, 14.8 ± 0.1°C

Materials and methods - 2

- **Sensory evaluation of fillets** by a trained panel:
  - **Modified Torry scale:** cooked fillets (flavour & odour)
    - ✓ determination of shelf life (Torry score = 5.5)
- **Microbiological analyses**:
  - **Total Viable Counts** (TVC, 15°C) using Long & Hammer's medium and Iron Agar (both with 1% NaCl, spread-plating)
  - **H_2S-producer counts** (black colonies on Iron agar)
  - **Presumptive *Pseudomonas* counts** (22°C) using CFC medium
  - ***Photobacterium phosphoreum* counts** (15°C) using PPDM-Malthus method (Dalgaard, 1996)
- **Evaluation of microbial metabolites in headspace:**
  - **electronic nose** (FreshSense)
    - ✓ electrochemical sensors: CO, SO_2, H_2S, NH_3
  - **GC-MS**
    - ✓ purge and trap TENAX

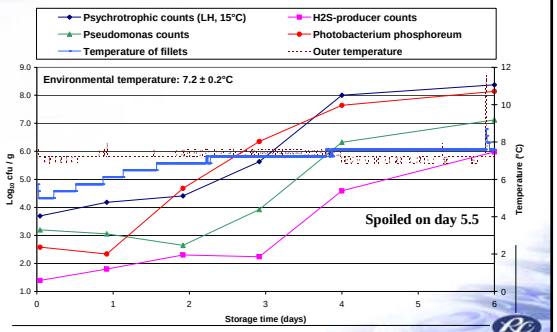
Microbiological counts of haddock fillets stored in EPS boxes



WESTERN EUROPEAN FISH TECHNOLOGISTS´ ASSOCIATION

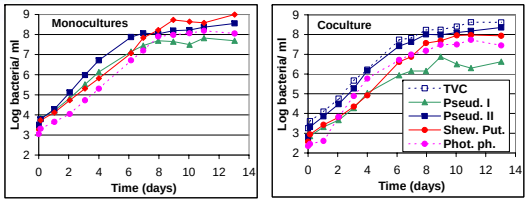
32nd WEFTA meeting, May 13th-15th, 2002, Ireland

Microbiological counts of haddock fillets stored in EPS boxes from 5 to 8°C



7

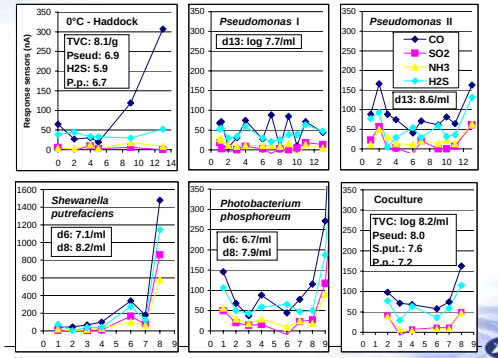
Bacterial proliferation in fish extract at 0.5°C



- In monocultures: *Pseud.*II (rapid), *Phot.ph.* (slower), *S.put.* (high)
- In coculture: *Pseud.* II (dominating), *S.put.* (late growth)

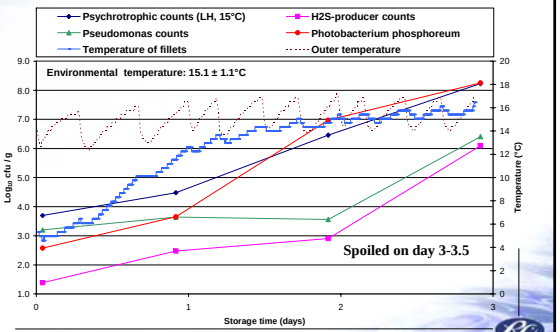
9

0°C – Electronic nose measurements



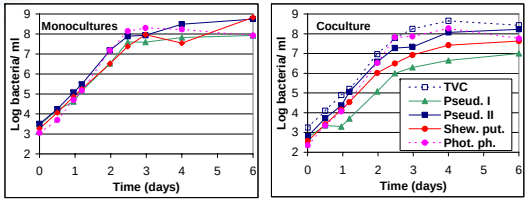
11

Microbiological counts of haddock fillets stored in EPS boxes from 5 to 16°C



8

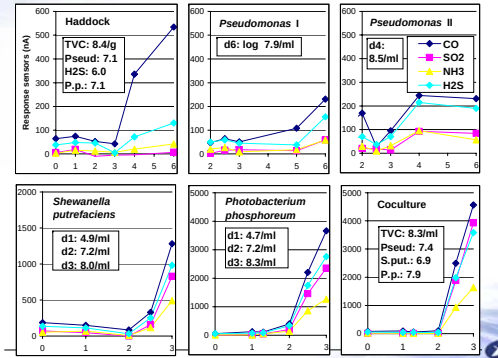
Bacterial proliferation in fish extract at 7.6°C



- In monocultures: similar growth among different species
- In coculture: *Photobacterium phosp.* & *Pseud.* II dominating
- Similar results for 14.8°C trials: *Phot. ph.* dominating

10

7-8°C – Electronic nose measurements

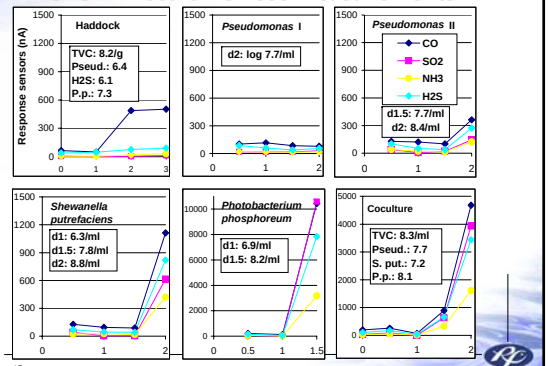


12

WESTERN EUROPEAN FISH TECHNOLOGISTS´ ASSOCIATION

32nd WEFTA meeting, May 13th-15th, 2002, Ireland

15°C – Electronic nose measurements



13

Conclusion

- **Development of *Photobacterium phosphoreum*** evidenced at 0-15°C in air-stored fresh fillets.
- **0°C:** *Pseud*. II, *Shew. put.* and *Phot. ph*. may all participate in the development of metabolites.
- **Increasing importance of *Photobacterium phosphoreum*** on spoilage pattern of air-stored fillets as temperature increased.

- **Acknowledgements:**
  - ✓ Icelandic Research Council
  - ✓ Tros ehf., fish processor

15

Metabolites detected - preliminary

	E nose response	GC-MS (0-1°C)	GC-MS (15°C)
***Pseud.* I**	low: CO, H2S	Low amines	Esters
***Pseud.* II**	low: CO, H2S	DMDS, esters	High DMDS,esters, DMTS, low amines
Shew. put.	CO, H2S, SO2, NH3	Amines, DMS acids, esters	Amines, esters, DMS, ketones
Phot. ph.	CO, H2S, SO2, NH3	~~Amines, esters~~ **aldehydes,DMS**	DMDS, acids, amines, esters, DMTS, al-OH
Coculture	CO, H2S, SO2, NH3	Amines,DMDS acids, DMS, esters	Amines, DMDS, esters, acids, DMTS, ketones

14

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

Guðrún Ólafsdóttir, Soffia Vala Tryggvadóttir, Sigurdur Einarsson and Hélène L. Lauzon
Icelandic Fisheries Laboratories, PO Box 1405, Skúlagata 4, 121 Reykjavík, Iceland
Tel.: 354 5620240, Fax: 354 5620740, e-mail: gudrun@rf.is

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.

WESTERN EUROPEAN FISH TECHNOLOGISTS´ ASSOCIATION

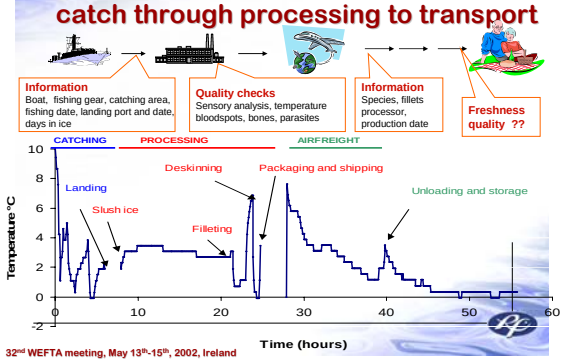
32nd WEFTA meeting, May 13th-15th, 2002, Ireland

Prediction of freshness quality of haddock fillets using instrumental techniques

Guðrún Ólafsdóttir, Soffía Vala Tryggvadóttir, Sigurður Einarsson and Hélène L. Lauzon

Icelandic Fisheries Laboratories, PO Box 1405, Skúlagata 4, 121 Reykjavík, Iceland
Tel.: 354 5620240, Fax: 354 5620740, e-mail: gudrun@rf.is

Temperature profile of haddock fillets from catch through processing to transport



The aim of the study

- To study the spoilage pattern of haddock
  - different seasons
  - different catching methods (longline and Danish seine).
  - influence of different handling
    - whole fish in ice
    - fillets in styrofoam boxes in cold storage (0-2°C)
- To monitor the freshness of haddock during storage using:
  - different instrumental techniques
  - traditional sensory and microbial methods
- To develop models to predict sensory quality by instrumental methods

Three storage experiments of haddock at 0-2°C for 15 days

- May 1999
  - Danish seine
  - whole haddock stored on ice
- Sept 1999
  - Longline
  - whole haddock stored on ice
- May 2000
  - Longline
  - haddock fillets stored in styrofoam boxes at 0-2°C

Samples measured on days:

1, 4, 6, 8, 11, 13 and 15 after catch

Freshness quality evaluation - measurements of haddock fillets

Traditional techniques:

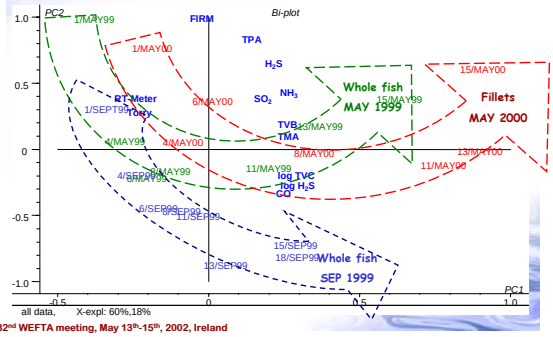
- Sensory analysis
  - Torry
- Microbial counts
  - TVC/H2S prod./*Pseud*
- TMA / TVB
  - FIGD analysis

Rapid instrumental techniques:

- Electronic nose FreshSense
  - electrochemical sensors (CO, H_2S, SO_2 ,NH_3)
- Texture (TA.XT2i)
  - TPA -Texture profile analysis
  - Firmness
- RT Freshmeter*
  - conductivity

* measurements of whole fish

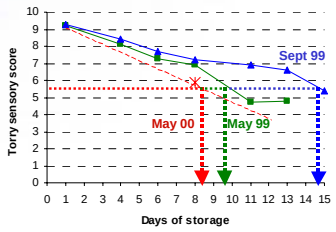
PCA of all data - 3 storage studies of haddock different spoilage pattern



WESTERN EUROPEAN FISH TECHNOLOGISTS´ ASSOCIATION

32nd WEFTA meeting, May 13th-15th, 2002, Ireland

Shelf life determined by sensory analysis



SHELF-LIFE
Torry score = 5.5

Sept 99: 14-15 days
(whole fish in ice)

May 99: 9-10 days
(whole fish in ice)

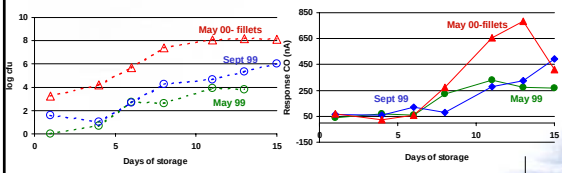
May 00: 8-9 days
(fillets 0-2°C)

Days of storage is not a good indicator to estimate the freshness quality

Electronic nose (CO sensor) and traditional microbial (TVC) measurements

TVC total viable count

CO sensor

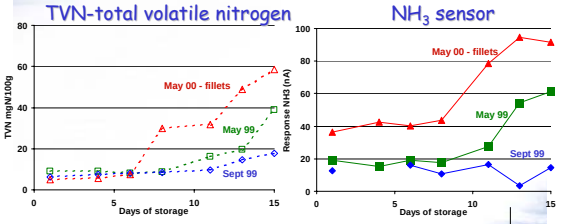


Similar overall trend for total microbial counts and the CO sensor

Electronic nose (NH_3 sensor) and TVN chemical measurements of fillets

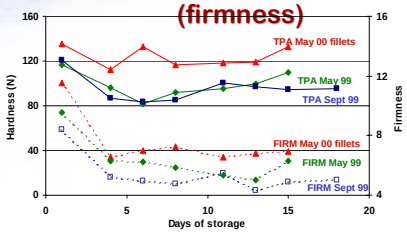
TVN-total volatile nitrogen

NH_3 sensor



The same overall trend for TVN and the NH_3 sensor

Texture analysis of fillets TPA (hardness) and puncture test (firmness)



Fish stored as fillets has slightly higher values for hardness and firmness

Seasonal difference is observed for firmness

PLS models - prediction of Torry scores based on data from whole fish stored in ice from two different seasons (May 1999 and Sept 1999)

X variables	Corr. Cal.	Corr. Pred.	RMSEC	RMSEP
Microbes, e-nose, texture TMA/TVB RT (12)	0,97	0,92	0,39	0,60
E-nose, texture TMA/TVB (8)	0,95	0,89	0,45	0,72
CO, TVB, TPA (3)	0,95	0,92	0,47	0,62

PLS prediction of Torry scores for fillets May 2000 based on a PLS models from May99 and Sep99

	PLS model 12 variables		PLS model CO,TPA,TVB 3 variables		
	predicted	deviation	predicted	deviation	measured
1/MAY/00	8,43	1,15	10,34	0,58	
4/MAY/00	7,57	0,87	9,43	0,33	
6/MAY/00	7,40	1,21	9,84	0,53	
8/MAY/00	4,00	1,49	4,33	1,61	5,9
11/MAY/00	2,19	1,66	2,41	0,82	
13/MAY/00	-0,18	2,31	-1,11	1,99	
15/MAY/00	-0,82	2,47	-0,52	4,01	

WESTERN EUROPEAN FISH TECHNOLOGISTS´ ASSOCIATION

32nd WEFTA meeting, May 13th-15th, 2002, Ireland

Conclusions

- Spoilage pattern is different
  - seasonal variation
    - spoilage more rapid in the spring (recently spawned fish)
  - fillets spoil faster than whole fish
- information on storage days is not a reliable estimate of the freshness quality
- Instrumental techniques (electronic nose, TVB/TMA, texture and microbial analysis) are consistent with sensory analysis

Conclusions

- PLS models show good performance to predict sensory freshness scores by selected instrumental techniques
  - electronic nose (CO), texture analysis (TPA) and TVB
- Prediction of sensory scores of fillets (May2000) using different PLS models tends to overestimate the values and shows large error at the end of the storage period
  - non-linearity of the data - signals level off
  - analysis of more samples would improve the model

Acknowledgements

This project was partly financed by:

- The Icelandic Research Council
  - Accurate predictive models – The effect of temperature fluctuations on microbial growth and metabolites in fish
- The European Commission
  - MUSTEC project FAIR98 4076

The raw material and the temperature profiles of the haddock fillets was supplied by :

- TROS Sandgerði
  - Ágúst Vilhjálmsson

PREDICTION OF FRESHNESS QUALITY OF HADDOCK FILLETS USING ELECTRONIC NOSE, TEXTURE AND RAPID TVN MEASUREMENTS

Guðrún Ólafsdóttir, Rósa Jónsdóttir, Soffia V. Tryggvadottir, Hélène L. Lauzon and Sigurdur Einarsson

Objectives

The main objective of the study was to develop models based on instrumental methods to predict sensory quality of haddock fillets.

The models are based on results from three different storage studies of haddock. Seasonal variation, different fishing gear and storage conditions were studied.

Changes of various properties related to freshness quality of haddock stored in ice were monitored during storage by using novel instrumental techniques, traditional sensory and microbial methods

Raw material is often labelled with days from catch, however because of the effect of various extrinsic and intrinsic factors, the information about days from catch is not always adequate to determine the quality or freshness of the raw material.

Materials and Methods

Three storage experiments of haddock at 0-2°C for 15 days

- Spring - May 1999
  Danish seine
  whole haddock stored on ice
- Autumn - Sept 1999
  Longline
  whole haddock stored on ice
- Spring -May 2000
  Longline
  haddock fillets stored in styrofoam boxes at 0-2°C

Samples measured on days:

1, 4, 6, 8, 11, 13 and 15 after catch

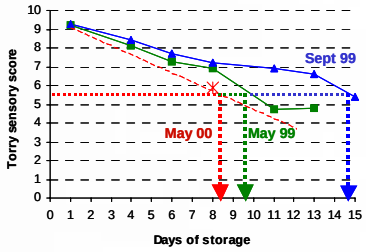
Traditional techniques:

- Sensory analysis
  Torry
- Microbial counts
  TVC/H2S prod./*Pseud*
- TMA / TVB
  FIGD analysis

Instrumental techniques:

- Electronic nose FreshSense
  electrochemical sensors
  (CO, H_2S, SO_2 ,NH_3)
- Texture (TA.XT2i)
  TPA -Texture profile analysis
  Hardness - Puncture test
- RT Freshmeter
  (measurements of whole fish)
  conductivity

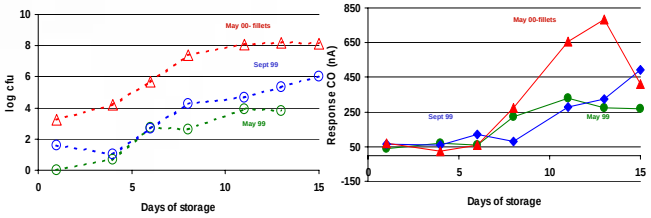
Results



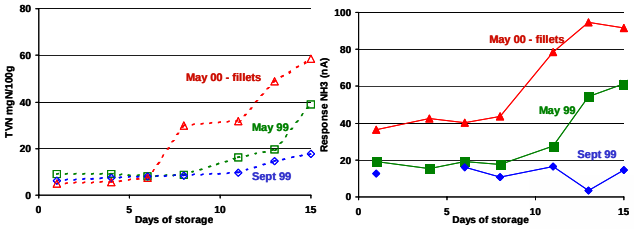
SHELF-LIFE Torry score = 5.5

Sept 99: 14-15 days (whole fish in ice)
May 99: 9-10 days (whole fish in ice)
May 00: 8-9 days (fillets 0-2°C)

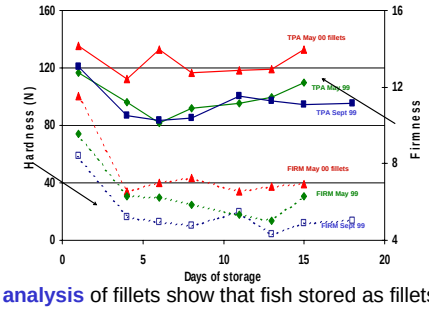
Shelf life of haddock in the three studies is different based on **sensory analysis** using **Torry scores**



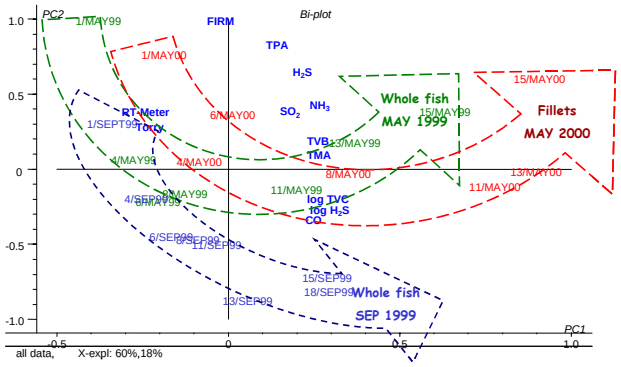
Results of **electronic nose** (CO sensor) and **traditional microbial** (TVC) measurements show similar overall trend



Results of **electronic nose** (NH3 sensor) and **TVN** chemical measurements of fillets show similar overall trend



Texture analysis of fillets show that fish stored as fillets has slightly higher values for hardness (TPA) and firmness (puncture test) than fish stored as whole fish



PCA of all data from 3 storage studies of haddock. Different spoilage pattern is evidents indicating that days of storage is not a good estimate of freshness quality

X variables	Corr. Cal.	Corr. Pred.	RMSE C	RMSEP
Microbes, e-nose, texture TMA/TVB RT (12 variables)	0,97	0,92	0,39	0,60
E-nose, texture TMA/TVB (8 variables)	0,95	0,89	0,45	0,72
CO, TVB, TPA (3 variables)	0,95	0,92	0,47	0,62

PLS models - prediction of Torry scores based on data from whole fish stored in ice from two different seasons (May 1999 and Sept 1999)

Conclusions

Spoilage pattern is different depending on seasons and fillets spoil faster than whole fish as expected. Information on storage days is therefore not a reliable estimate of the freshness quality.

Instrumental techniques (electronic nose, TVB/TMA, texture and microbial analysis) are consistent with sensory analysis

Partial least squares regression models (PLS) based on data from instrumental measurements show that these can give more reliable prediction of the freshness sensory score of haddock than traditional microbial counts and information about storage days in ice.

References

Guðrún Ólafsdóttir, Soffia Vala Tryggvadóttir, Sigurdur Einarsson and Hélène L. Lauzon 2002. Prediction of sensory quality of haddock fillets using various instrumental techniques. Presented at the 32nd WEFTA meeting, May 13th-15th, 2002, Ireland

Soffia V. Tryggvadóttir and Guðrún Olafsdóttir, 2000. Multisensor for fish: Questionnaire on quality attributes and control methods -Texture and electronic nose to evaluate fish freshness. Project report for European Commission (Development of multi- sensor techniques for monitoring the quality of fish, CT-98-4076). RF report 04-00.

The project was partly financed by:
The Icelandic Centre for Research: Accurate predictive models – The effect of temperature fluctuations on microbial growth and metabolites in fish
The European Commission: MUSTEC project FAIR98 4076
The project is in collaboration with **TROS /SIF** Sandgerði

DETECTION OF VOLATILE COMPOUNDS BY AN ELECTRONIC NOSE TO MONITOR FRESHNESS OF HADDOCK STORED IN ICE

Guðrún Ólafsdóttir and Rósa Jónsdóttir

Objectives

The odor of fish is one of the most important quality attributes to determine the freshness quality.

An electronic nose based on electrochemical gas sensors has the potential to be applied for quality monitoring in the fish industry by measuring rapidly the volatile compounds contributing to the spoilage odour of fish (Olafsdottir et al., 2000, 2002, DiNatale *et al*., 2001).

The aim of the study was to identify and determine the level of the most abundant volatile compounds produced in haddock fillets during storage by analysing the headspace volatiles by gas chromatography and comparing the results to electronic nose analysis.

Results

The results show that the compounds present in the highest concentration during storage are alcohols, carbonyls, esters, amines and sulfur compounds (Table 1).

Table 1. Headspace volatiles of haddock fillets during storage collected by an air pump on a Tenax trap followed by thermal desorption, separation and detection by GC-MS. (From: Ólafsdottir, 2003)

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

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

Conclusions

Ethanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 3-hydroxy-2-butanone, ethyl acetate and butanoic acid ethyl ester were the most abundant volatiles in the headspace. Similar volatile compounds were found in cold smoked salmon during refrigerated storage (Joffraud *et al*., 2001). This is expexted since similar profiles of microflora emerge in different food products under the same conditions despite heterogenity in the outset (Gram *et al*., 2002). A similar set of sensors can therefore be used for freshness evaluation of a variety of fish products and other muscle food that are processed in a different way but stored at same temperatures and conditions.

Materials and Methods

Haddock fillets were stored in styrofoam boxes in cold storage (0-2°C) for 3, 7, 10 and 14 days.

Sampling: TENAX tube, air pump, thermal desorption

GC analysis: GC/MS

Electronic nose: FreshSense (Bodvaki, Iceland)

CO, SO_2, NH_3 electrochemical sensors

Sensory analysis: odor evaluation of raw fillets

The overall trend in the responses of the electronic nose sensors is comparable to the sum of GC peak areas of spoilage indicator compounds.

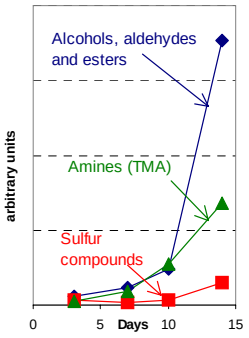


Figure 2 Sum of the peak areas of compounds representing the three different classes of compounds detected by GC in the headspace of haddock fillets during storage in ice

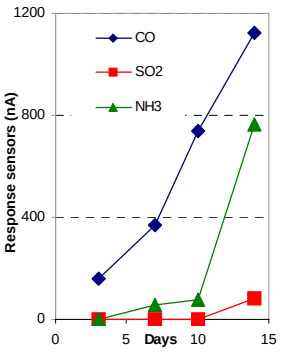


Figure 3 Responses of the CO, SO_2 and NH_3 sensors towards haddock fillets during storage in ice (From: Ólafsdottir, 2003)

The development of spoilage odor with storage time can be rationalised based on the increasing concentrations of spoilage indicator compoundsand increased responses of the electronic nose sensors are observed

FRESH FLAT — The odour of the fillet is FRESH or FLAT and low responses of the sensors are observed on day 3.

SWEET — The first spoilage odors of the fillets are SWEET like odors that are contributed by alcohols that give sweet, solvent like odors in combination with aldehydes giving sweet, oxidized-like odors. Increasing response of CO sensor is observed on day 7.

STALE — The amines contribute to salted fish or stock fish odor and cheesy and STALE odors develop as seen on day 10 in agreement with the increased NH_3 sensor response.

PUTRID — The esters analysed in high levels on day 14 have characteristic fruity odors. When these sweet and fruity odors are mixed with the foul smell of the sulphur compounds and stockfish- like character of the amines the odor of the fillet becomes PUTRID and the response of the SO_2 sensor is also increased.

Elliot 1947

References

Di Natale C, Olafsdottir G, Einarsson S, Mantini A, Martinelli E, Paolesse R, Falconi C, D'Amico A, 2001. Comparison and integration of different electronic noses for the evaluation of freshness of cod fish fillets. Sensors and Actuators B77,572-578.

Gram L, Ravn L, Rasch M, Bruhn JB, Christensen AB, Givskov M, 2002. Food spoilage - interactions betwen food spoilge bacteria. Int J Food Microbiol 78, 79-97.

Joffraud J J, Leroi F, Roy C, Berdague J L (2001), 'Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon', Int. J. Food Microbiol. 66, 3, 175-184.

Olafsdottir G. 2003. Developing rapid olfaction arrays for determining fish quality. In Francis Dodds (Ed) Rapid and on-line instrumentation for food quality assurance. Woodhead Publishing Ltd, UK (in print)

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

Ólafsdóttir G, Högnadóttir Á, Martinsdóttir E and Jónsdóttir H, 2000. Application of an Electronic Nose to Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production, J. Agric. Food Chem. 48 ,6, 2353-2359.

This project was partly financed by:

The **Icelandic Centre for Research**: Accurate predictive models – The effect of temperature fluctuations on microbial growth and metabolites in fish

The **European Commission**: MUSTEC project FAIR98 4076

The raw material and the temperature profiles of the haddock fillets was supplied by **TROS/SIF** Sandgerði

Nákvæm geymsluþolspálíkön fyrir fisk

Guðrún Ólafsdóttir[a], Hélène L. Lauzon[a] og Jóhann Örlygsson[b]

Lágt hitastig við geymslu á ferskum fiski er frumskilyrði þess að varðveita neyslugæði hans. Vegna þess hvernig veiðum og dreifingu á fiski er háttað, er oft erfitt að stjórna hitastigi vörunnar. Undanfarin ár hefur komið fram mikill áhugi á því að nota spálíkön til að meta geymsluþol viðkvæmrar vöru eins og fisks. Rannsóknir á möguleikum þess að nota örveruvaxtalíkön til að spá fyrir um geymsluþol matvæla hafa sýnt fram á að við gerð spálíkana er nauðsynlegt að hafa gögn þar sem tekið er tillit til hitasveifla og sérhæfðra skemmdarörvera í hráefninu.

Skemmd á ferskum fiski er fyrst og fremst af völdum örvera. Mat á geymsluþoli á ferskum fiski hefur því stuðst við talningar á heildarörverufjölda í hráefninu. Þetta er hins vegar þeim takmörkunum háð að hefðbundnar örverutalningar eru seinvirkar og heildarfjöldi örvera gefur ekki alltaf sambærilegar niðurstöður um ástand hráefnis eins og skynmat, sem er sú aðferð sem mest er notuð í fiskiðnaði til að meta ferskleika hráefnis. Ein ástæða fyrir þessu er sú að upphafsfjöldi örvera er breytilegur í mismunandi hráefni vegna ólíkrar meðhöndlunar og vinnslu. Bent hefur verið á að einungis hluti af upphaflegri örveruflóru veldur skemmdum. Þær örverur sem skipta mesta máli sem skemmdarvaldar í ferskum fiski eru kallaðar sérhæfðar skemmdarörverur (SSÖ). Komið hefur í ljós að vinnsluferlið og aðferðir sem notaðar eru við geymslu á ferskum fiski hafa mikil áhrif á samsetningu örveruflórunnar. *Pseudomonas* tegundir og *Shewanella putrefaciens* eru taldar vera sérhæfðar skemmdarörverur hefðbundins ísaðs fisks, óháð uppruna hans (Gram & Huss, 1996). Rannsóknum ber ekki alltaf saman um mikilvægi einstakra örverutegunda í skemmd og ljóst er að forsaga hráefnisins svo sem veiðiaðferðir og hitasveiflur sem hráefnið kann að lenda í við meðhöndlun og vinnslu ásamt pökkunaraðferðum getur skýrt þennan mismun. Samkeppni milli örvera er vissulega mjög mikilvægur þáttur í skemmdarferli fersks fisks, en einnig getur verið að aðrar örverutegundir eigi þátt í skemmdarferli ísaðs fisks. Nýlegar rannsóknir hafa sýnt að *Photobacterium phosphoreum* er mjög mikilvægur skemmdarvaldur í fiski (þorski) sem pakkaður er í

loftskiptar umbúðir (MAP) (Dalgaard, 1997), en hins vegar er lítið vitað um virkni hennar í fiski á ís.

Gerð spálíkana

Hugtakið "Predictive Food Microbiology" hefur fengið mikla athygli upp á síðkastið. Það sem við er átt er það að með því að mæla annað hvort magn ákveðinna örvera í matvælum eða niðurbrotsefna sem þær mynda þá má áætla líftíma (geymsluþol) hráefnisins. Gerð hafa verið líkön þar sem þetta hefur verið notað á hin ýmsu matvæli og er þá oftast verið að leita af ákveðnum sjúkdómsvaldandi örverum. Hins vegar hefur það færst í vöxt að reynt er að varpa þessu á skemmdarörverur, og þá þær örverur sem eru mikilvægastar í því hráefni sem við á hverju sinni (sjá m.a. http://www.dfu.min.dk/micro/ssp/). Þannig skiptir miklu máli hvort um sé að ræða kjöt, grænmeti, fisk eða eitthvað annað hráefni og við hvaða aðstæður varan er geymd (hitastig, bætiefni, gaspökkun, lofttæming). Það sem skiptir mestu máli við notkun líkana er að finna fljótvirka aðferð til að meta ástand vörunnar. Að einangra örverur sem eru sérhæfðir skemmdarvaldar (SSÖ) tekur oft langan tíma og er því ekki æskileg aðferð þar sem hráefnið hefur ekki nema takmarkaðan geymslutíma. Í fiski hefur verið reynt að áætla geymsluþol hráefnis með því að nota leiðnimælingar og sérhæfð bakteríuræti sem eru mun fljótvirkari aðferðir til að greina og telja örverur. Almennt má segja að annaðhvort sé myndun niðurbrotsefna eða vöxtur skemmdarörvera notaðar við gerð spálíkana.

Rafnefið FreshSense - fljótvirk aðferð til að meta skemmd.

Gerðar hafa verið rannsóknir á Rf á undanförnum árum þar sem skoðað hefur verið vöxtur og virkni mismunandi sérhæfðra skemmdarörvera. Í þessum rannsóknum hafa niðurbrotsefni verið mæld með svokölluðu rafnefi sem þróað hefur verið í samvinnu við fyrirtækið Boðvaka. Rafnefið sem kallast FreshSense hefur verið notað í rannsóknum á Rf undanfarin ár og niðurstöður mælinga á rokgjörnum efnum í loðnu með tækinu sýna mjög góða fylgni við TVN-mælingar (heildarmagn reikulla basa) sem er sú aðferð sem notuð er í fiskimjölsiðnaðinum til að meta hráefnið (Ólafsdóttir o.fl. 1997a,b; 2000). Einnig hefur FreshSense-tækið verið notað fyrir þorsk og ýsu þar sem sýnt hefur verið fram á gott samræmi við hefðbundnar aðferðir til að meta ferskleika hráefnis. Rafnefið inniheldur rafefnanema (electrochemical sensors) sem

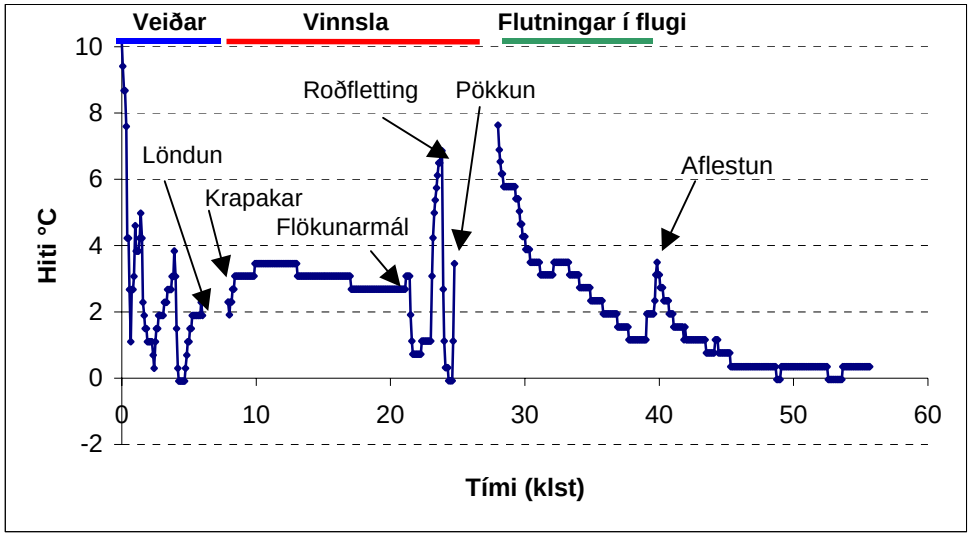
eru notaðir til að skynja einfaldar gastegundir eins og kolmónoxíð (CO) og vetnissúlfíð (H_2S). Jafnframt geta þessir skynjarar greint önnur efni ef þau eru nógu rokgjörn og hafa svipaða raffræðilega eiginleika og þau efni sem skynjararnir eru hannaðir fyrir. Þannig geta þessir skynjara greint niðurbrotsefni örvera sem myndast við skemmd eins og ýmis alkóhól, brennsisteinsefni og amín eins og TMA (trímetylamín).

Rannís verkefni um nákvæm geymsluþolspálíkön fyrir fisk

Meginmarkmið verkefnisins er að safna upplýsingum um vöxt sérhæfðra skemmdarörvera (SSÖ) og myndun niðurbrotsefna í fiski. Upplýsingarnar verða notaðar til þess að stuðla að þróun hraðvirkra aðferða til þess að meta gæði fisks og gera geymsluþolsspálíkön. Raunhæf spálíkön eru háð því að geta notað fljótvirkar mæliaðferðir til að meta ástand hráefnis. Rannsóknir á Rf benda til þess að fljótvirk mæling með rafnefi á niðurbrotsefnum örvera geti nýst við spálíkanagerð og notkun þeirra. Í verkefninu eru einnig skoðaðar fljótvirkar aðferðir til að telja bakteríur. Sérstök áhersla er lögð á að athuga notkunarmöguleika slíkra líkana á fisk sem hefur orðið fyrir hitasveiflum í framleiðsluferlinu.

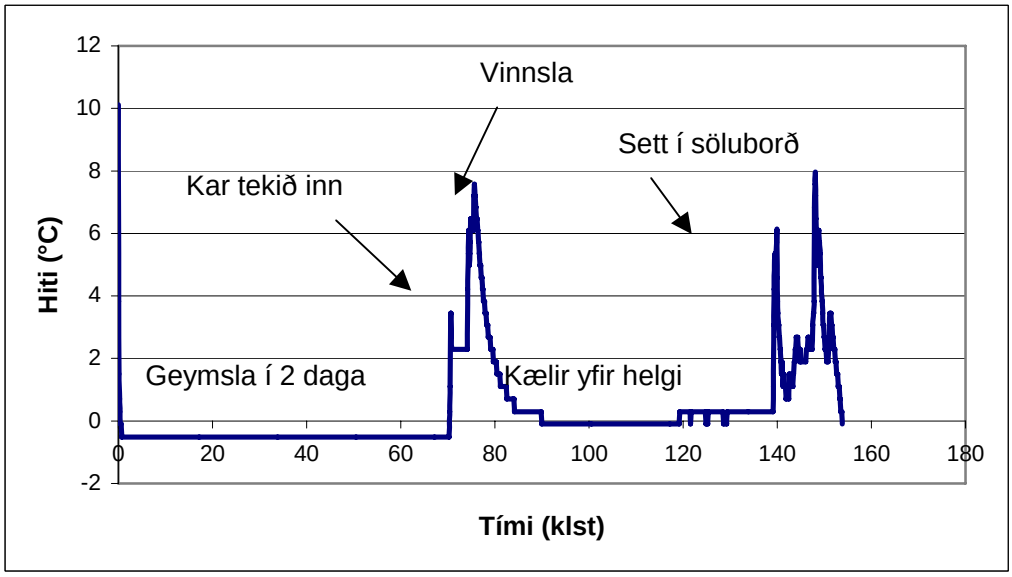
Í verkefninu var í fyrstu gerð úttekt á dæmigerðum hitaferli fyrir ferskan fisk þar sem fyrirtækið Tros ehf, Fiskbúðin Nethyl og Útgerðafélag Akureyringa aðstoðuðu við öflun gagna. Notaðir voru hitasíritar sem mæla yfirborðshita hraéfnisins og voru þeir lagðir ofan á hráefnið eða á milli. Hitasveiflur sem verða á ferlinum eru ekki ýkja miklar, en helsta hættan er sú að biðtími geti verið langur á þeim stöðum þar sem hitastigshækkanir voru í úttektinni þ.e. við flökun, roðflettingu og snyrtingu, þar sem hitinn fór upp í allt að 8°C. Einnig var hækkun á hitastigi í flutningum við lestun og aflestun, en hráefnið er að jafnaði snöggkælt aftur og er mestallan tímann við 0 - 4°C (sjá mynd 1). Hráefni sem fór í frystingu var allan tímann undir 4°C. Hitaferill frá fiskmarkaði til neytenda sýndi að hitastigshækkun var við vinnslu og í söluborði þar sem hitinn fór upp í 7-8°C við yfirborð hráefnisins, en að öðru leyti var hitinn að jafnaði við 0°C. Vitað er að stundum er fiski landað óísað og á sumrin þegar sjávarhiti er hár getur hráefnið verið allt að 12-13°C. Ljóst er að hitasveiflur geta haft mikil áhrif á geymsluþol hráefnisins og í verkefninu er ætlunin að framkvæma

geymsluþolstilraunir á fiski þar sem þessar upplýsingar verða notaðar til að setja upp tilraunaaðstæður sem líkjast þessum raunverulegu hitasveiflum.

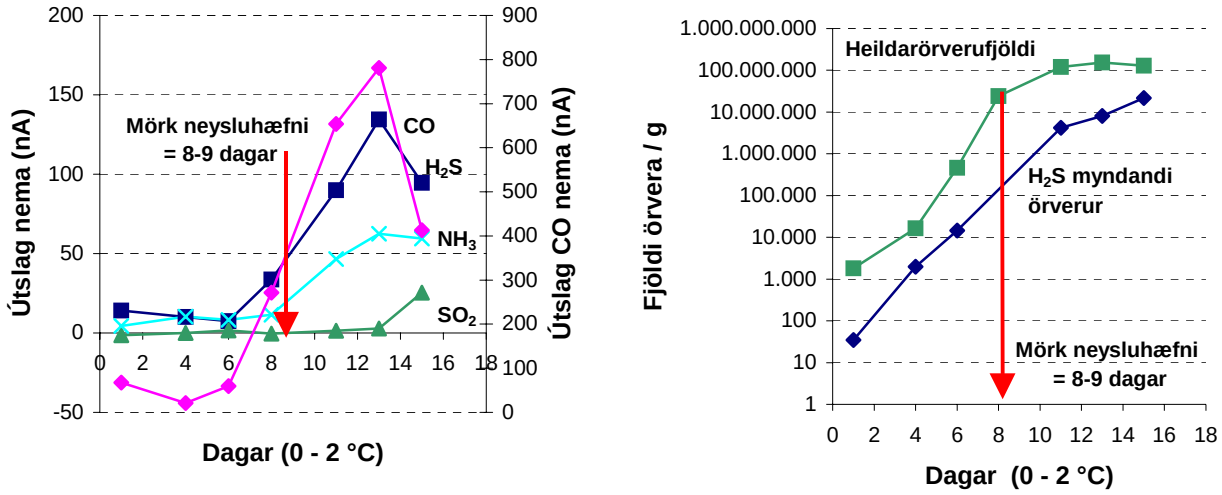


Mynd 1. Dæmigerður hitaferill á sumartíma fyrir framleiðslu á ferskum flökum til útflutnings með flugi

.



Mynd 2. Dæmigerður hitaferill fyrir hráefni hjá fisksala. Hráefni keypt á fiskmarkaði geymt vel ísað þar til það fer í vinnslu hjá fisksala og síðan í sölu.



Mynd 3. Niðurstöður rafnefs- og örverumælinga í geymslutilraunum á ýsuflökum (maí 2000)

Mynd 3 sýnir niðurstöður geymslutilrauna á ýsuflökum sem geymd voru í frauðplastkassa í kæli við 0-2°C, en þetta er sambærileg vara og flutt er út með flugi eins og hitaferill á mynd 1 sýnir. Niðurstöðurnar benda til þess að geymsluþol metið með skynmati sé um 8-9 dagar (stuðst var við soðið mat skv. Torry einkunnaskala = 5,5). Á mynd 3 sést vel að svörun einstakra skynjara rafnefsins (CO, H_2S, NH_3 og SO_2) er lítil á fyrstu sex dögum geymslutímans, sem gefur til kynna að lítið hefur myndast af niðurbrotsefnum örvera á þeim tíma. Hins vegar verður greinileg aukning á útslagi skynjaranna eftir 6. dag og á sama tíma er heildarfjöldi örvera orðinn um 1.000.000 /g. Þessar niðurstöður benda til þess að hægt sé að nota fljótvirkar mælingar með rafnefi til að meta neysluhæfni vörunnar.

Hugmyndin í Rannís verkefninu er sú að þróa spálíkön sem byggjast á fljótvirkum mælingum á gæðum eins og rafnefsmælingum. Þannig væri mögulegt að gera rafnefsmælingu á hráefni hvar sem er í vinnsluferlinum til að meta gæðin og áætla síðan geymsluþol vörunnar með hjálp spálíkana. Hin hugmyndin er sú að nota fljótvirkar örverumælingar sem þó taka um 1-2 daga, en hefðbundnar örverumælingar taka allt að 5 daga.

Lykillinn að því að slík spálíkön geti nýst fyrir fiskiðnaðinn er að þekkja vel hitaferil vörunnar og geta mælt ástand hráefnis á fljótvirkan hátt. Unnið er að frekari þróun

hraðvirkra mæliaðferða til að meta gæði fisks og líklegt er að fiskiðnaðurinn geti innan tíðar farið að nýta sér slíka tækni ásamt spálikönum.

Heimildir

G. Ólafsdóttir, Á. Högnadóttir, E. Martinsdóttir and H. Jónsdóttir, 2000. Application of an Electronic Nose to Predict Total Volatile Bases in Capelin (*Mallotus villosus*) for Fishmeal Production, J. Agric. Food Chem. 48 ,6, 2353-2359.

G. Ólafsdóttir, Á. Högnadóttir, E. Martinsdóttir and H.L. Lauzon, 1999. Application of an "Electronic Nose" to Detect Microbial Metabolites During Spoilage of Fish and Fish Products. COST 914 Predictive modelling of microbial growth and survival in foods. Prepared for publication by T.A Roberts. EUR 19103 European Commission Directorate - General Science, Research and Development, pp. 305-313.

G. Ólafsdóttir, Á. Högnadóttir and E. Martinsdóttir, 1998. Application of gas sensors to evaluate freshness and spoilage of various seafoods. *In* Methods to Determine the Freshness of Fish in Research and Industry, Proceedings of the Final meeting of the Concerted Action "Evaluation of Fish Freshness" AIR3 CT94 2283. Nantes Nov 12-14, 1997. International Institute of Refrigeration, 100-109.

G. Ólafsdóttir, E. Martinsdóttir and E. H. Jónsson, 1997. Rapid gas sensor measurements to predict the freshness of capelin (*mallotus villosus*). J.Agric. Food Chem. 45,7, 2654-2659.

Hélène L. Lauzon. 1997. Meistaranámsritgerð við matvælafræðiskor, Háskóli Íslands.

Gram, L., H.H. Huss. 1996. Int. J. Food Microbiol. 33, 121-137.

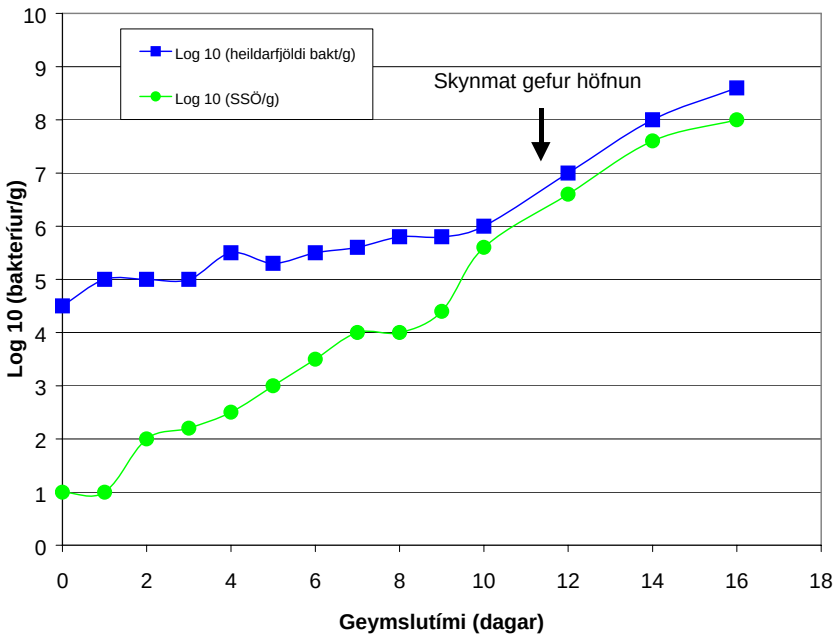
Bartlett, P.N., J.M. Elliott, J.W. Gardner. 1997. Electronic noses and their application in the food industry. Food Technology, 51 (12), 44-48.

Jóhann Örlygsson, 2000. Spálíkön fyrir geymslu á ferskum fiski. Stafnbúi Rit nemenda við Sjávarútvegsdeild HA

Spálíkön fyrir geymslu á ferskum fiski

Skemmd á ferskum fiski er fyrst of fremst af völdum baktería sem mynda margvísleg skemmdarefni við niðurbrot á lífrænum efnum (aðallega próteinum). Á fyrstu stigum skemmdarferilsins myndast margvísleg efni (aðallega karbónýl og alkóhól með kolefnisgrind sem í eru 6 - 9 kolefni) sem gefa "ferska" fiskilykt. Þessi efni gefa einkennandi græna, plöntulykt og myndast vegna ensímniðurbrots á fjölómettuðum fitusýrum. Á seinni stigum skemmdarferilins myndast m.a. stutt alkóhól (etanól og bútanól) í miklu magni og einnig esterar en þessi efni valda sætri lykt. Samhliða þessu myndast efni sem valda einkennandi skemmdarlykt, úldnun og rotnun. Þessi efni eru vel þekkt eins og TMA (trímetylamín), ammóníak og rokgjörn brennisteinsefni (H_2S).

Mat á geymsluþoli á ferskum fiski hefur því oftast stuðst við heildarbakteríufjölda í hráefninu. Þetta er hins vegar þeim takmörkunum háð að fjöldi örvera og skynmat hráefnis fer ekki alltaf saman. Ein ástæða fyrir þessu gæti verið að upphafsfjöldi baktería er breytilegur í mismunandi hráefni vegna ólíkrar sögu (meðhöndlun og vinnsla) þess. Einnig hefur komið í ljós að erfitt er að staðla heildarbakteríutalningar. Bent hefur verið á að einungis hluti af upphaflegri örveruflóru veldur skemmdum. Þær örverur sem skipta mestu máli sem skemmdarvaldar í ferskum fiski eru kallaðar sérhæfðar skemmdarörverur (SSÖ). Komið hefur í ljós að vinnsluferlið og geymsluaðferðir sem notaðar eru til geymslu á ferskum fiski hafa mikil áhrif á samsetningu örveruflórunnar.



Mynd 1. Mælingar á heildarörverufjölda og fjölda sérhæfðra skemmdarvalda í geymsluþolstilraun á ferskum fiski. Örin sýnir hvenær skynmat gefur til kynna að hráefnið sé óhæft til neyslu.

Mynd 1 sýnir geymsluþolstilraun á ferskum fiski. Greinilegt er að upphaflegt magn baktería í hráefninu samanstendum að mestu leiti af bakterium sem ekki eru taldar sérhæfðir skemmdarvaldar. Hins vegar, á seinni tímum geymslutímans, eru sérhæfðar skemmdarbakteríur orðnar allt að 90-95% af heildarörveruflóru hráefnisins.

Pseudomonas tegundir og *Shewanella putrefaciens* eru taldar vera sérhæfðar skemmdarörverur hefðbundins ísaðs fisks, óháð uppruna hans. Því hefur jafnvel verið haldið fram að *S. putrefaciens* væri aðalskemmdarvaldur í fiski sem geymdur er á ís í loftgeymslu. Þetta er aðallega vegna eiginleika þessarar bakteríu við að mynda rokgjörn brennissteins- og amínefnasambönd. Aftur á móti stangast sumar rannsóknir á. Í fyrsta lagi vegna þess að fjöldi *S. putrefaciens* í skemmdum fiski hefur verið breytilegur, allt frá því að vera 0-10% af heildarflórunni í það að vera ríkjandi hluti hennar. Í öðru lagi vegna þess að mjög mikill fjöldi *S. putrefaciens* (10^{8-9}/g) verður að nást í fiski áður en illa lyktandi rokgjörn efni myndast. Slíkur fjöldi er langt yfir þeim mörkum þar sem fiskur er talinn neysluhæfur. Þetta gæti bent til þess að forsaga hráefnisins, svo sem hitasveiflur, kunni að skýra þennan mismun sem hefur komið fram í hinum mismunandi rannsóknum. Einnig gæti verið að önnur örverutegund valdi skemmdum í ísuðum fiski. Samkeppni milli örvera er mjög mikilvægur þáttur í skemmdarferli fersks fisks en ytri þættir eins og hitastig og samsetning lofttegunda skipta einnig verulega máli.

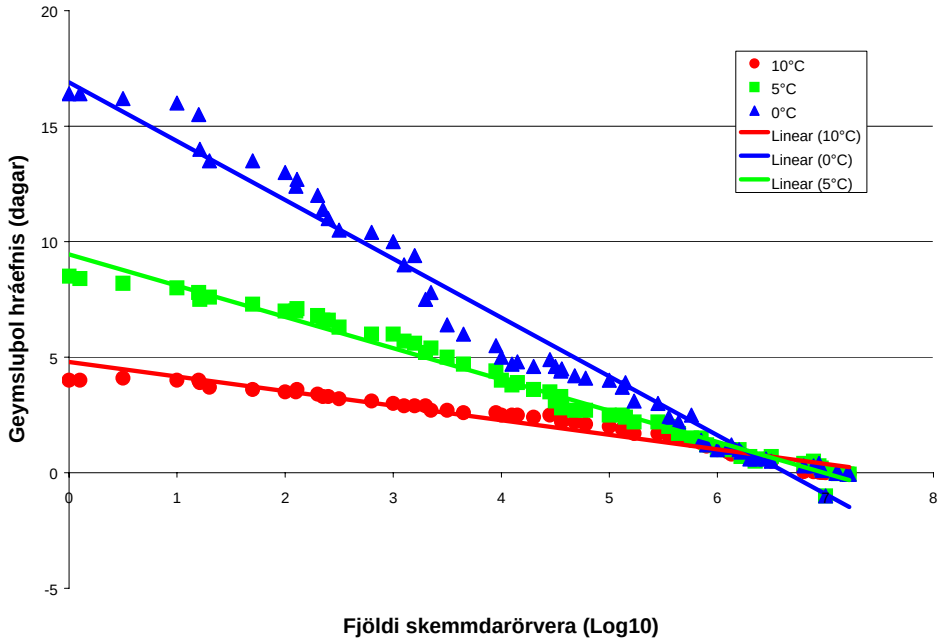
Komið hefur í ljós að með því að nota gaspökkun eða lofttæmda pökkun á fiski þá verði skemmdir aðallega af völdum bakteríunnar *Photobacterium phosphoreum*. Þessi baktería er mjög öflugur TMA-framleiðandi, hver fruma framleiðir allt að því 80 sinnum meira en t.d. *S. putrefaciens*. Aðrir hafa fundið að mjólkursýrubakteríur og þá sérstaklega *Lactobacillus* tegundir verði ráðandi örveruflóra við slíkar aðstæður.

Mikilvægi þess að skilja skemmdarferlin í fiski og fiskafurðum eru augljós. Um leið og búið er að greina hvaða afurðir hinar sérhæfðu skemmdarbakteríur mynda við ákveðnar aðstæður þá er hægt að gera líkan að vexti þeirra og þar með meta geymsluþol viðkomandi hráefnis. Þetta á við þegar aðstæður eru þekktar þ.e. hitastig er stöðugt og gasumhverfi þekkt. Hins vegar getur verið erfiðara að gera slík spálíkön ef þessi þættir eru ekki stöðugir, og þá sérstaklega ef hitastigbreytingar verða á geymslutímanum. Þess vegna er mikilvægt að safna upplýsingum hvað varðar vaxtarhraða sérhæfðra skemmdarörvera og myndun niðurbrotsefna við raunverulegar ástæður til þess að meta hvaða mæliþættir eru nothæfir við gerð geymsluþolsspálíkana.

Gerð spálíkana

Hugtakið “Predictive Food Microbiology” hefur fengið mikla athygli upp á síðkastið. Það sem við er átt er það að með því að mæla annað hvort magn ákveðinna baktería í matvælum eða niðurbrotsefna sem þær myndi þá má áætla líftíma (geymsluþol) hráefnisins. Gerð hafa verið líkön þar sem þetta hefur verið notað á hin ýmsu matvæli og er þá oftast verið að leita af ákveðnum sjúkdómsvaldandi örverum. Hins vegar hefur það færst í vöxt að reynt er að varpa þessu á skemmdarbakteríur, og þá þær bakteríur sem eru mikilvægastar í því hráefni sem við á hverju sinni (sjá m.a. http://www.dfu.min.dk/micro/ssp/). Þannig skiptir miklu máli hvort um sé að ræða kjöt, grænmeti, fisk eða eitthvað annað hráefni og við hvaða aðstæður varan er geymd (hitastig, bætiefni, gaspökkun, lofttæming). Það sem skiptir mestu máli í slíkum líkönum er að finna aðferð sem er fljótvirk. Að einangra bakteríur sem eru sérhæfðir skemmdarvaldar (SSÖ) tekur oft langan tíma og er því ekki æskileg þar sem hráefnið hefur ekki nema takmarkaðan geymslutíma. Í fiski hefur verið reynt að áætla geymsluþol hráefnis með því að nota leiðnimælingar og sérhæfð bakteríuræti sem eru mun fljótvirkari aðferðir til að greina örverur. Í slíkum ætum eru stærri efnasambönd

brotnuð niður og myndun smærri einiga veldur leiðnibreytingum í ætinu. (Til dæmis hefur TMAO æti verið notað vegna oxunar eiginleika ákveðinna örvera í TMA en myndun þess er mælt á tímaeiningu.) Almennt má segja að annaðhvort sé myndun skemmdarafurða eða vöxtur skemmdarörvera notaðar í þessum við gerð spálíkana. Mynd 2 sýnir mælingar á sérhæfum skemmdarörverum í hráefni sem hafa gefið því ákveðinn líftíma við þrjú mismunandi hitastig. Augljóst er að þeim mun hærra hitastig sem hráefnið er geymt í, þeim mun styttri verður geymsluþolið.



Mynd 2. Mælingar á fjölda sérhæftra skemmdarörvera og því geymsluþoli sem ferskur fiskur á eftir við hverja mælingu.

Augljóst er hins vegar að með því nota aðrar flótvirkari aðferðir eins og t.d. leiðnimælingar eða aðrar mælingar á niðurbrotsefnum sem skipta mestu máli varðandi skemmd hráefnisins mætti nýta hráefnið mun betur (koma því fyrr á markað).

Undirritaður tekur nú þátt í rannsóknum á þessu sviði þar sem sérstakleg er reynt að meta hitastigssveiflur og áhrif þeirra á geymsluþolsspálíkön.

Jóhann Örlygsson er doktor í örverufræði við Landbúnaðarháskólann í Uppsölum. Hann er dósent við Háskólann á Akureyri og sérfræðingur Rannsóknastofnunar fiskiðnaðarins á Akureyri.

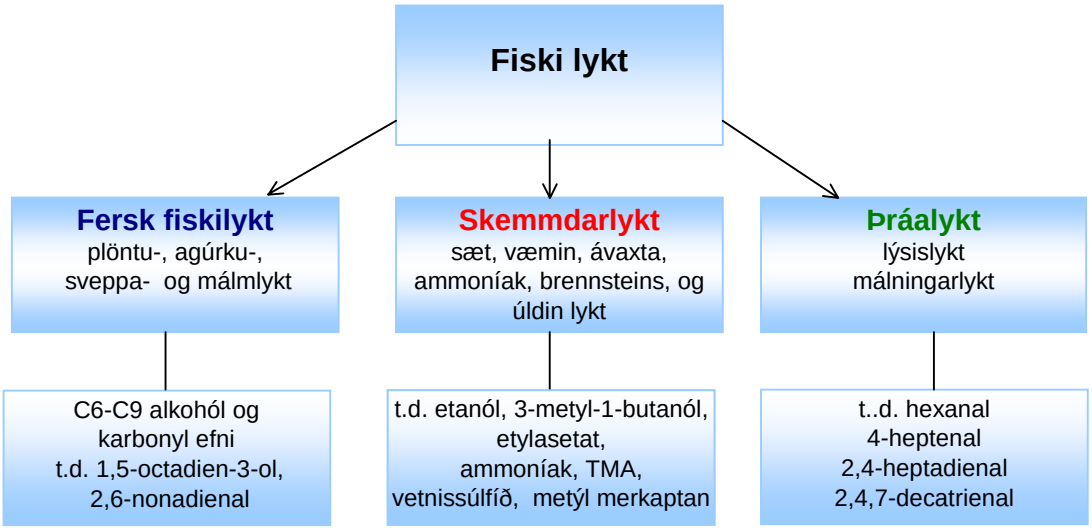
Rannsóknir

Rokgjörn efni í fiski - Vísir að gæðum

Grein eftir Guðrúnu Ólafsdóttur

Eftirfarandi grein er byggð á doktorsverkefni höfundar við Matvælafræðiskor Raunvísindadeildar Háskóla Íslands. Verkefnið er um rannsóknir sem styrktar hafa verið af Rannís og Evrópusambandinu og unnar hafa verið á Rannsóknastofnun fiskiðnaðarins. Um er að ræða rannsóknir á rokgjörnum efnum í fiski sem myndast vegna örveruniðurbrots og oxunar í fiski við geymslu og áhrif mismunandi geymsluaðstæðna á myndun þessara efna. Jafnframt fjallar verkefnið um möguleika þess að þróa spálíkön sem byggja á niðurstöðum fljótvirkra mælinga með rafnefi á niðurbrotsefnum í fiski, til að spá fyrir um geymsluþol og gæði fisks.

Fiskur er viðkvæmt matvæli og breytingar sem verða við geymslu í kæli eru aðallega til komnar vegna vaxtar skemmdarörvera, en oxunarferli og ensímvirkni stuðla einnig að niðurbroti fiskholdsins. Þessar breytingar leiða til þess að fiskur missir ferskleikaeinkennin og skemmdareinkenni koma fram í bragði, lykt, áferð og útliti.
Lykt af ferskum fiski er einkennandi fyrir hverja tegund og minnir á sjávarlykt eða málmkennda lykt eins og t.d. lykt af heilli ýsu og þorski. Vatnafiskar og lax hafa einkennandi agúrku eða melónulykt og loðnan sem er skyld laxfiskum er með sömu lyktareinkenni þegar hún er fersk.



Efnasamsetning fisklyktar

Lyktarbreytingar sem verða í fiski við geymslu eru vel þekktar og fiskifýla er eitt af því sem neytendur setja fyrir sig þegar velja á fisk í soðið. Lyktarbreytingum fisks sem geymdur er í ís má skipta í fimm stig: ferskt, hlutlaust, sætt, skemmt og úldið. Gasgreinirannsóknir á rokgjörnum efnum hafa sýnt að á hverju stigi eru ákveðin lyktarefni yfirgnæfandi og einkenna lyktina. Fersk fiskilykt er aðallega vegna niðurbrotsefna frá löngum ómettuðum fitusýrum sem eru einkennandi fyrir fiskmeti. Ensímvirkni lípoxygenasa veldur niðurbrotinu og efni myndast eins og t.d. 2,6-nonadienal, sem hefur einkennandi gúrkulykt og 1,5-octadien-3-ol sem hefur málmkennda sveppalykt. Einkennandi lykt hverrar tegundar er háð magni og tegund

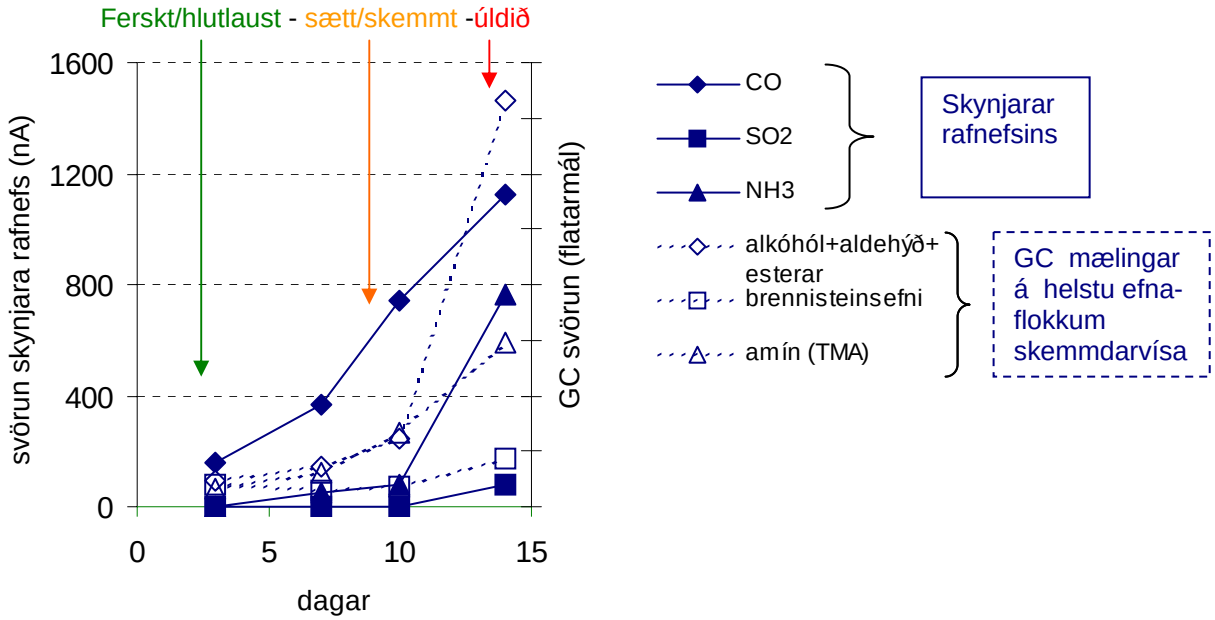
niðurbrotsefna og nákvæmlega hvaða efni myndast er síðan háð efnasamsetningu fisksins.

Skemmdarvísar í fiski

Nauðsynlegt er að standa vel að meðhöndlun fisks strax eftir að hann er veiddur og er góð ísun lykilatriði til að tefja vöxt skemmdarörvera. Fyrstu breytingar sem verða á lyktinni er að ferskleikalyktin tapast og lyktin verður lítil eða hlutlaus. Á seinni stigum þegar skemmdarörverur hafa náð sér á strik myndast skemmdarlykt vegna örveruniðurbrots á efnum í fiskholdi eins og fríum amínósýrum, fitusýrum og öðrum efnum. Nauðsynlegt er að þekkja efnasamsetningu fiskilyktar til hægt sé að velja skemmdarvísa til að meta gæði eða ferskleika fisks. Rannsóknir á myndun rokgjarna efna við geymslu á fiski hafa sýnt að þau efni sem myndast vegna örveruniðurbrots eru til staðar í mestu magni og hægt er að velja ákveðin efni sem skemmdarvísa. Skemmdavísar eru m.a. stutt alkóhól eins og etanól og bútanól sem myndast í miklu magni og einnig esterar, en þessi efni valda sætri lykt. Samhliða þessu myndast efni, sem valda einkennandi skemmdarlykt. Þessi efni eru vel þekkt eins og TMA (trímetylamín) og ammoníak, sem myndast vegna niðurbrots örvera á TMAO (trimetylamímoxíð) og amínósýrum og valda einkennandi lykt af t.d. harðfiski. Rokgjörn brennisteinsefni sem myndast frá amínósýrum myndast einnig í miklu magni og valda ýldu- og rotnunarlykt.

Mælingar á skemmdarvísum með rafnefi

Skynmat er mest notað til að meta gæði fisks, en lykt er einn mikilvægasti þátturinn í gæðamati. Fiskiðnaðurinn hefur lengi vel haft áhuga á að geta mælt gæði fisks með fljótvirkum mælingum, sem mæla ákveðnar breytingar sem verða við geymslu. Niðurstöður slíkra mælinga þurfa að vera sambærilegar við skynmat eða aðrar hefðbundnar efna- eða örverumælingar.



Mynd 1. Samband milli svörunar skynjara (CO, SO_2 og NH_3) í rafnefinu og svörunar gasgreininema á samanlögðu magni helstu efnaflokka skemmdarvísa (alkóhól, aldehýð og esterar; brennisteinsefni og amín) í ýsuflökum við geymslu á ís.

Rafnef er dæmi um fljótvirka mælitækni til að meta gæði, en slíkt tæki hefur verið þróað á Rf í samvinnu við fyrirtækið Boðvaka (Maritech). Í rafnefinu eru skynjarar sem eru næmir fyrir mismunandi skemmdarefnum sem myndast í fiski við geymslu. Þannig er CO skynjari næmur fyrir alkóhólum, aldehýðum og esterum, NH_3 skynjari greinir amín og TMA, og SO_2 skynjari er næmur fyrir brennsisteinsefnum, en þetta eru helstu efnaflokkar rokgjarnra skemmdarefna í fiski. Mynd 1 sýnir dæmi um skemmdarferil rokgjarna efna í ýsuflökum við geymslu í kæli ásamt því hvernig lyktin breytist. Á myndinni sést góð samsvörun á milli mælinga með hefðbundnum gasgreinimælingum (GC) á helstu efnaflokkum skemmdarvísa og svörunar skynjara rafnefsins sem næmir eru gagnvart viðkomandi efnaflokkum.

Í verkefninu hafa á þennan hátt verið rannsakaðir skemmdarferlar ýmissa fisktegunda sem geymdar hafa verið við mismunandi aðstæður og rokgjörn niðurbrotsefni hafa verið mæld á geymslutímanum og niðurstöðurnar bornar saman við hefðbundnar efna- og örverumælingar ásamt skynmati. Í ljós hefur komið að sömu skemmdarefni myndast í mismunandi fisktegundum sem geymdar eru við svipaðar aðstæður. Magn skemmdarefna sem myndast er mismunandi en það er háð örveruflórunni sem nær að vaxa og ræðst af geymsluaðstæðum, aðallega hitastigi.
Safna þarf upplýsingum um skemmdarefni sem myndast í hverri tegund fyrir sig með rafnefsmælingum og hefðbundum mælingum til að ákvarða hvað felst í viðkomandi mæligildi rafnefsins.

Þessar rannsóknir eru gagnlegar til að skilja betur skemmdarferil fisks. Slík þekking getur nýst fyrir fiskiðnaðinn við að stjórna aðstæðum til að koma í veg fyrir eða hægja á skemmd í fiski og þannig tryggja betri vöru fyrir neytendur. Einnig nýtast upplýsingar um magn og eðli skemmdarefna í fiski til að þróa tækni eins og rafnef til þess að mæla þessi efni og meta þannig ástand eða gæði fisks.

Rafnef eru ekki enn notuð í fiskiðnaðinum við gæðamat. Ýmislegt bendir þó til þess að vegna aukinna krafna kaupenda um upplýsingar varðandi gæði, öryggi og rekjanleika matvæla verði gerðar kröfur um mælingar til að staðfesta t.d. gæði við kaup og sölu á fiski. Sérstaklega gæti rafnef verið gagnlegt til að staðfesta gæðin þegar verslað er með fisk á fiskmörkuðum á rafrænan hátt.

Guðrún Ólafsdóttir er matvælafræðingur með BS próf frá HÍ og MS próf frá University of Wisconsin, í Bandaríkunum og hefur starfað sem sérfræðingur á Rannsóknarstofnun fiskiðnaðarins síðan árið1988.