Project Report
to the EU
28- 01

Rannsóknastofnun fiskiðnaðarins

NÓVEMBER 2001

QUALPOISS 2

THE EVALUATION OF A SIMPLE, CHEAP, RAPID METHOD OF NON-PROTEIN NITROGEN DETERMINATION IN FISH PRODUCTS THROUGH THE PROCESSING/MERCHANDISING CHAIN

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Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins
Icelandic Fisheries Laboratories Report Summary

Titill / Title	Qualpoiss 2: The Evaluation of a Simple, Cheap, Rapid Method of Non-Protein Nitrogen Determination in Fish Products Through the Processing/Merchandising Chain		
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Skýrsla Rf /IFL report	28-01	*Útgáfudagur / Date:*	Nóvember 2001
Verknr. / project no.	1304		
Styrktaraðilar / funding:	*European Community FAIR PL 96.3253*		

Ágrip á íslensku:

Helsta markmið verkefnisins var að þróa og betrumnbæta tækni til að mæla á auðveldan, ódýran og fljótlegan hátt rokgjörn köfnunarefnissambönd (TMA og TVB) í fiski og fiskafurðum frá því að fiskurinn er veiddur og þar til hann kemur í verslanir. Tæknin byggir á innsprautun á sýni, þar sem rokgjörnu köfnunarefnissamböndin hafa verið dregin út úr því yfir í þríklóracetiksýrulausn. Sýnið blandast natrium hydroxíð lausn sem leysri út köfnunarefnisgas úr sýninu sem flæðir því næst yfir teflonhimnu í indikatorlausn en við það breytist pH lausnarinnar en sú breyting er numin í ljósdíóðu sem litabreyting sem kemur fram sem toppur á rita. Þessi tækni fékk heitið "Flow Injection Gas Diffusion" (FIGD). Í verkefninu mældu þátttakendur, sem voru frá 6 stofnunum og fyrirtækjum í Evrópu, mismunandi fisktegundir og gerðu samhliða mælingum með FIGD mælingar á TMA og TVB með hefðbundum aðferðum auk þess sem skynmat og örverufræðilegar mælingar voru framkvæmdar til samanburðar. Helstu niðurstöður verkefnisins voru að P-hlutfall (TMA/TVB*100), sem í byrjun verkefnisins var talinn vænlegur mælikvarði á ferskleika, reynist ekki betur en ef einungis TMA eða TVB voru mæld. Þá reyndist ekki unnt að þróa aðferð með FIGD tækninni sem hægt var að nota nánast sjálfvirkt í fiskvinnslum. Íslenska þátttakendanum tóks þó að gera þær endurbætur á tækninni að auðveldarar var að nota hana utan rannsóknastofa. Þá var eitt undirmarkmiðana að reyna að nota "safa" úr fiskholdinu beint til innsprutunar í tækið en það reyndist ekki unnt. FIGD tæknin er mun fljótlegri og auðveldari en flesar aðrar aðferðir til mælinga á TMA og TVB en gangast fyrst og fremst á rannsóknstofum hjá stofnunum og stærri fyrirtækjum en síður sem hluti fiskvinnslunni sjálfri.

Lykilorð á íslensku: *fljótvirkni, mæling, fiskur, TMA, TVB*

Summary in English:

The project's overall objective was to test the proposition that the "determination of P-ratio (i.e. trimethylamine/total volatile base concentration) in fish flesh by the flow injection/gas diffusion technique is an appropriate product safety and quality on-line monitoring methodology for the requirements of industrial and legislative standards for consumer satisfaction and protection".

The determination of TMA and TVB is based on the Flow Injection Gas Diffusion technuque. Sample is injected into the FIGD mainfold. The flow of NaOH solution carries the injected liquid through the mixing coil alkalising it and releasing its contained nitrogen in the form of ammonia gas. The gas passes through a gas permeable membrane into a solution of bromothymol blue indicator. The colour change caused in the indicator is detected in a light-emitteing diode photometer connected to a chart recorder. Six principal researchers from five European Institutes and on industrial subcontractor collaborate in this study. Parallel to TMA and TVB measurements with FIGD technique TMA and TVB were also mesured by more conventional methods. Sensory analysis and microbial counts were also done for comparison. The data collected in this study suggests that the P-ratio is no better an index of seafood quality than its component TMA or TVB values. The FIGD technique, once its operators have become accustomed to its use, appears to be as sensitive and reliable as the long-established methods but much faster and cheaper. Despite the efforts of the laboratories of the QUALPOISS transnational partnership, it has not been possible to develop a FIGD methodology (based upon exudates, which would preclude the need for taking chemicals into processing areas, rather than extracts) to make it suitable for on-line or line-to-line use. The use of exudate from applying pressure to the fish muscle was shown to be unsatisfactory.

English keywords: *FIGD technique, TMA, TVB, cod, haddock, storage experiments*

EXECUTIVE SUMMARY FROM THE CO-ORDINATORS

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Final Report 01-10-97/ 31-1-2001

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TABLE OF CONTENTS

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1.1 Research hypothesis

The hypothesis to be tested was that *the determination of P-ratio (i.e. trimethylamine/total volatile base concentration) in fish flesh by the flow injection/gas diffusion technique is an appropriate product safety and quality on-line monitoring methodology for the requirements of industrial and legislative standards for consumer satisfaction and protection.*

1.2 Specific objectives

The specific objectives were:

(i) to investigate the relationship of the P-ratio [determined by the flow injection/gas diffusion technique and by other accepted standard methods] with sensory indices [as determined through organoleptic panel testing] for fish flesh [from marine demersal species: *Gadus morhua* (cod) *Sparus aurata* (gilt head bream) *Melanogrammus aeglefinus* (haddock) *Merluccius merluccius* (Atlantic hake) and *Merluccius mediterraneus* (Mediterranean hake) marine pelagic species: *Sprattus sprattus sprattus* (sprat) *Scomber scombrus* (mackerel) *Clupea harengus harengus* (herring) *Sardina pilchardus* (sardine) and *Engraulis encrasicolus* (anchovy) and marine bivalve molluscan species: *Mytilus edulis* (mussel) and *Pecten maximus* (coquille St. Jacques)] through typical, standard, post-harvest sequences of handling, processing, storage, distribution and retail display;

(ii) investigate the adaptation of the sample extract preparation methodology for flow injection/gas diffusion P-ratio determination towards a more convenient-for-on-line usage methodology whereby pressure is applied to the fish tissue in question to yield 0.5ml exudate which would be sufficient for P-ratio determination by the FIGD method and to validate its reliability against the presently-used sample extraction methods;

(iii) investigate the applicability of P-ratio determination by the FIGD methodology for on-line monitoring of fish freshness through post-harvest handling, processing, storage,

distribution and retail display through trial operation in factory and distribution chain situations in each of the partner countries.

1.3 Achievements of tasks

The work proposed consisted of three principal components addressing the three aspects of the development of the new technique for exploitation by industries (particularly SMEs), public and private laboratories:

(i) validation of P-ratio, determined by the FIGD methodology, as a reliable and sensitive indicator of fish freshness/spoilage;

(ii) examination of the possibility of using the exudate, which can be obtained from fish flesh by applying pressure to the sample, by comparing P-ratio data obtained for such exudates with P-ratio data obtained for standard methodology extraction of the same samples with the objective of making the whole analysis more convenient for on-line operation; and

(iii) factory and distribution chain trials of P-ratio determination by the FIGD methodology for on-line monitoring of fish freshness to determine its suitability in industrial/commercial situations.

1.4 **Results**

(i) The collation of P-ratio data on the different species investigated by different partner laboratories (which continued to the end of the project) suggests that P-ratio is a less reliable indicator of the end of shelf life than was suggested by the end of the first QUALPOISS project. This conclusion is the main result of the research project.

(ii) The development and standardisation of methodology to obtain an exudate has not been achieved, although experiments have been conducted.

(iii) The experience of working with industrial partners, has been important. It suggests in general that fish processors have an ongoing need for an applied research input to progress questions related to traceability, on-line monitoring, and assessment of grades. But although factory trials have taken place, the FIGD methodology is not yet suitable for commercial applications.

(iv) Partners have achieved a number of other important scientific and technological results. These are enumerated in the individual reports by partners (see Appendix 2). A very significant example is an improved version of the FIGD equipment developed by the Iceland partner. This is a valuable addition to laboratory equipment. A second example is the finding by TEI (Greece) that feed in cultured seabass and sea bream affects volatile bases.

1.5 Overall conclusion

The project's overall objective was to test the proposition that the "*determination of P-ratio (i.e. trimethylamine/total volatile base concentration) in fish flesh by the flow injection/gas diffusion technique is an appropriate product safety and quality on-line monitoring methodology for the requirements of industrial and legislative standards for consumer satisfaction and protection"*.

(i) The data collected in this study suggest that the P-ratio is no better an index of seafood quality than its component TMA or TVB values. Like them, its initial lack of change with time then sudden rise as the seafood product approaches the limit of fitness for human consumption is useful with respect to "*consumer protection"* but is insensitive with respect to "*consumer satisfaction"*. A proposed new index (H-factor) which takes into account the disappearance of TMAO as well as the changes in TMA and TVB content, appears worthy of further investigation as an index for both satisfaction and protection and should be a component of a future research project.

(i) The FIGD technique, once its operators have become accustomed to its use, appears to be as sensitive and reliable as the long-established methods but much faster and cheaper.

Despite the efforts of the laboratories of the QUALPOISS transnational partnership, it has not been possible to develop a FIGD methodology (based upon exudates, which would preclude the need for taking chemicals into processing areas, rather than extracts) to make it suitable for on-line or adjacent-to-line use. Considerable effort, however, from the Icelandic partner has resulted in the development of the equipment into a prototype which could much more easily be accommodated by the very restricted test kitchen/QC department–facilities typically available in fish processing companies. This compares with the original, prototype equipment which may only have been operable in the analytical chemistry laboratories of companies who provide this kind of service to the industry.

(iii) In order for the method to be used as an on-line measurement by industry it is essential that the method of obtaining samples for analysis be simplified. Currently the use of TCA extraction of fish muscle is destructive, time consuming and requires the use of “hazardous” chemicals not suited to the food environment in addition to requiring technical personnel and facilities. At the same time the sampling procedure must provide representative values of TVB and TMA from which P-ratio can be determined, reliably and accurately in a wide range of fish species.

The use of exudate from applying pressure to the fish muscle was shown to be unsatisfactory since the exudate also contained other material which either required high speed centrifugation to remove it, or components lipids, proteins that significantly interfered with the analysis. It was also shown that the resulting TVB and TMA values were not comparable with values obtained using the standard TCA extraction method.

2. OBJECTIVES

The hypothesis to be tested was that *the determination of P-ratio (i.e. trimethylamine/total volatile base concentration) in fish flesh by the flow injection/gas diffusion technique is an appropriate product safety and quality on-line monitoring methodology for the requirements of industrial and legislative standards for consumer satisfaction and protection.*

The specific objectives were:

(i) to investigate the relationship of the P-ratio [determined by the flow injection/gas diffusion technique and by other accepted standard methods] with sensory indices [as determined through organoleptic panel testing] for fish flesh [from marine demersal species: *Gadus morhua* (cod) *Sparus aurata* (gilt head bream) *Melanogrammus aeglefinus* (haddock) *Merluccius merluccius* (Atlantic hake) and *Merluccius mediterraneus* (Mediterranean hake) marine pelagic species: *Sprattus sprattus sprattus* (sprat) *Scomber scombrus* (mackerel) *Clupea harengus harengus* (herring) *Sardina pilchardus* (sardine) and *Engraulis encrasicolus* (anchovy) and marine bivalve molluscan species: *Mytilus edulis* (mussel) and *Pecten maximus* (coquille St. Jacques)] through typical, standard, post-harvest sequences of handling, processing, storage, distribution and retail display;

(ii) to investigate the adaptation of the sample extract preparation methodology for flow injection/gas diffusion P-ratio determination towards a more convenient-for-on-line usage methodology whereby pressure is applied to the fish tissue in question to yield 0.5ml exudate which would be sufficient for P-ratio determination by the FIGD method and to validate its reliability against the presently-used sample extraction methods; and

(iii) investigate the applicability of P-ratio determination by the FIGD methodology for on-line monitoring of fish freshness through post-harvest handling, processing, storage, distribution and retail display through trial operation in factory and distribution chain situations in each of the partner countries.

3. PROJECT MILESTONES

12 months

Partner's familiarisation with equipment and methods.

This was achieved within the designated time limit, apart from difficulties outside the project's control in Portugal.

27 months

Relationship between P-ratio and time of storage to be established.

This has been achieved: for verification see Annex containing individual country reports.

30 months

Alternative sampling procedures that are factory friendly to be established

Various sampling procedures have been tested but factory friendliness for the FIGD methodology has not been completely achieved. The procedures are still too slow and costly for them to constitute a method for on-line monitoring, but they have proved their value for the laboratory.

36 months

Factory trials of methodology completed by seafood processing companies willing to act as pilot sites for using FIGD as a P-ratio quality monitoring procedure.

The partners have adopted various approaches to completing this milestone. The UK team's activities during the final period were an extensive industrial trial, the results of which will be analysed in collaboration with the company in the summer of 2001. Others have adopted other approaches which, in the light of the circumstances described in their reports (see Annex) seem appropriate.

4. ACHIEVEMENT OF TASKS AND SUBTASKS

4.1 The three principal tasks

The work proposed consists of three principal components addressing the three aspects of the development of the new technique for exploitation by industries (particularly SMEs), public and private laboratories:

(i) validation of P-ratio, determined by the FIGD methodology, as a reliable and sensitive indicator of fish freshness/spoilage;

(ii) examination of the possibility of using the exudate, which can be obtained from fish flesh by applying pressure to the sample, by comparing P-ratio data obtained for such exudates with P-ratio data obtained for standard methodology extraction of the same samples [in each case, ten replicates from each fish sample would be used to examine intra- and intersample variability] with the objective of making the whole analysis more convenient for on-line operation [because, at the beginning of the study, the sample preparation procedure was seen as the time obstacle for FIGD's usage in rapid, on-line, objective monitoring of freshness quality]; and

(iii) factory and distribution chain trials of P-ratio determination by the FIGD methodology for on-line monitoring of fish freshness to determine its suitability in industrial/commercial situations.

4.2 Task 1

4.2.1 Outline of task

Task 1 required partners to focus on the investigation of the relationship of P-ratio (determined by the FIGD method) with a decomposition index (determined through organoleptic panel testing) for selected species of fish through the post-harvest sequence of handling, processing, storage, distribution and retail display. In general terms it is quite

evident that this task was achieved by the project, as is evidenced in the periodic reports and attached final reports. The project has shown that the P-ratio can be reliably determined by the Flow Injection Gas Diffusion (FIGD) method. At present, FIGD appears satisfactory for TVB; for TMA, it still requires some methodology development.

4.2.2 Sub-tasks associated with Task 1

Sub-tasks 1.i (acquisition, installation and commissioning of FIGD equipment in partner laboratories) and 1.ii (familiarisation of partner laboratories with the FIGD technique) were completed by month 3 except for the case of the Portuguese partner (IPIMAR) who had to be sent a replacement FIGD detector box after repeated failure to make the original work properly after dismantling by customs in transit.

Sub-task 1.iii (inter-laboratory FIGD technique standardisation exercise to determine P-ratio for replicate sets of unknown fish extract samples supplied by industrial sub-contractor) was completed by month 6 and 1.iv by month 12 (except for part of 1.iii for the aforementioned reason in the case of the Portuguese partner).

Sub-task 1.iv (validation of FIGD technique for P-ratio determination against standards approaches), as adapted and expanded, has been a major component of the work by all partners throughout the project period.

Sub-task 1.v (inter-laboratory sensory analytical technique standardisation using replicate sets of unknown frozen fish (0-14 days *post-mortem)* was changed by partners because of doubts about practicability of, and legal position on, shipping food samples in various stages of decay across national frontiers. No two fish of the same species are the same anyway and panellists in the different countries have no experience in tasting the species which would have been prepared in Scotland. It was also felt that the exercise had no relevance to the rest of the project as each partner country would be following the spoilage of its own particular allocated species, although there was some experimentation with species prepared by the Scottish industrial partners. The standardisation that was,

therefore, agreed upon was to use both the Torry/EU schemes and the QIM schemes for the species concerned where these both existed and train the panels accordingly.

Sub-task 1.vi (the production of laboratory model simulations of fish handling/ processing/ merchandising sequence) was completed by month 15. The results are reported in University of Hull Annual Report for period 01-10-98 to 30-09-99 section 3.1 in Periodic report No. 2 as well as in country results.

The idea of using the industrial simulation model to show the effect of the temperature variations typically involved in the fish supply chain was pursued in depth by the UK partner, partly because chilled display of previously frozen fish was considered less common for the species and countries of the other partners, partly because most of the literature deals with fish spoilage at 0°C in melting ice and partly because each partner wished to extend this part of the investigation along avenues which the plenary meetings considered would better assist the validation of the technique and development of the methodology for use by the industry.

Sub-task 1.vii (relationship between P-ratio and decomposition index (for different species) to be established) was programmed for completion by month 21 was, in fact, continued to the end of the project – much of the experimental data presented in the periodic and final reports from each partner are concerned with this particular project sub-task. There has also been an agreed divergence of species studied from the original programme. This divergence is summarised in Table 4.1.

Table 4.1: Employment of Species by Partners

Partner	Planned species	Researched species	Notes
(1) University of (1) Hull Institute of Food Health Quality (Formerly International Fisheries Institute) and (1) Industrial Subcontractor – Alexander Buchan Ltd	Cod *(Gadus morhua)* Sprat *(Sprattus sprattus sprattus)* Scallop *(Pecten maximus)*	Cod *(Gadus morhua)* Sprat *(Sprattus sprattus)* Scallop *(Pecten maximus)*	The divergence between the planned species and the researched species arose through decisions taking by the research teams. There were three reasons for this: It was a means of enhancing collaboration between the scientists with the role of the industrial partner as a supplier of various fish species being important; (b) The scientists wished to test the main research hypotheses on a wider variety of fish species than originally envisaged in the research proposal in order to expand the scientific validity of the work; (c) In order to explore the commercial possibilities of the FIGD equipment and methodology, it was important to test them on all commercially relevant species as was feasible.
(2) Technological Institution of Athens (TEI)	Gilthead seabream *(Sparus aurata)* Mackerel *(Scomber japonicus)*	Gilthead seabream *(Sparus aurata)* European seabass *(Dicentrarchus labrax)* Hake *(Merluccius merluccius)* Sardine *(Sardina pilchardus)* Atlantic mackerel *(Scomber scombrus)* Chub Mackerel *(Scomber japonicus)* Scampi *(Nephrops norvegicus)* Horse mackerel *(Trachurus trachurus)* Shrimp *(Penaeus kerathurus)*	
(3) Icelandic Fisheries Laboratories	Haddock *(Melanogrammus aeglefinus)* Herring *(Clupea harengus harengus)*	Cod Gadus morhua) Haddock *(Melanogrammus aeglefinus)* Herring *(Clupea harengus harengus)* Shrimp Salmon	
(4) Instituto Portugues da investigacao das Pescas e do Mar (IPIMAR)	Atlantic hake *(Merluccius merluccius)* Sardine *(Sardina pilchardus)* Mussel (*mytilus edulis)*	Atlantic hake *(Merluccius merluccius)* Sardine *(Sardina pilchardus)* Atlantic horse mackerel *(Trachurus trachurus)* Gilthead seabream *(Sparus aurata)* Seabass *(Dicentrarchus labrax)* Haddock *(Melanogrammus aeglefinus)* Atlantic Mackerel *(Scomber scombrus)*	
(5) Departmento de Nutrició I Bromatologia, Facultat de Farmacia, Universitat de Barcelona	Mediterranean hake *(Merluccius mediterraneus)* Anchovy *(Engraulis encrasicolus)*	Mediterranean hake *(Merluccius mediterraneus)* Anchovy *(Engraulis encrasicolus)* Tuna Sardine	

Sub-task 1.viii (interlaboratory sample exchanges) was programmed for completion by month 27 was, in fact, repeated and only completed by the end of the project. The exchange of sample extracts from the different partner laboratories taken under this sub-task did not occur exactly as envisaged in the proposal. It was felt that the objective of the exercise could be achieved through the industrial partner preparing, coding and distributing the extracts, as in the first such exercise, then collating the data and identifying the samples for the partners to interpret the results. Herring was used to prepare the extracts which were distributed at the Barcelona meeting for the second collaborative study and at the Reykjavik meeting for the third collaborative study.

In respect of the various scientific investigations the project flourished, and proved an excellent tool for research. For example, the UK partner examined the disappearance of TMAO as a freshness/spoilage index using FIGD; the Greek partner examined additional species and the relationship of FIGD with microbiological data as well as sensory data through iced storage; the Icelandic partner developed the FIGD equipment so that it was more industry user friendly and the relationship of FIGD with electrical conductance data as well as sensory data through iced storage; the Portuguese partner examined in more detail the relationship of FIGD with sensory data obtained using the QIM schemes developed for their experimental species and with the same biochemical data obtained using the Conway Microdiffusion Technique; and the Spanish partner examined the relationship of FIGD (including TMAO data) with evolution of biogenic amines (determined using HPLC) Ph and microbiological data as well as sensory data through iced storage.

4.3 Task 2

4.3.1 Outline of Task 2

Task 2 required the project to adapt the sampling procedure to make the FIGD methodology more suitable for commercial, on-line operation for freshness monitoring.

The project has made some progress, but within the timescale it has not been possible to finalise the techniques. Two achievements which might be mentioned in this context are the development of an improved version of the FIGD box by the Icelandic partner and the industrial trial by the UK partner.

4.3.2 Sub-tasks associated with Task 2

Sub-tasks 2.i (the development of a methodology to obtain an exudate) and 2.ii (the investigation of P-ratios from exudates) continued to the end of the project as no methodology which would allow operation of the technique to be located in food processing areas had been found within the scheduled timescale. Development work focused upon the use of exudates (rather than trichloracetic acid extracts) which have been obtained by freezing then thawing the samples. Although it was possible to obtain exudates sufficient for analysis on line, none of the exudates proved representative of the whole fish with a reliability that would enable a line supervisor to make an accept/reject decision with a satisfactory degree of confidence. IFL refers to the difficulty of obtaining satisfactory exudate, an experience shared by others (TEI, IFHQ).

4.4 Task 3

4.4.1 Outline of Task 3

Task 3 required the project to conduct on-line factory trials using the FIGD methodology.

In the context of what has been achieved by the project, each partner has attempted to test the FIGD approach in an industrial context are summarised in Table 3.2.

Table 4.2 The Industrialisation of the FIGD approach

Partner	Industrial Activity
(1) IFHQ	Extended trials in a Grimsby Fish Processing Factory (Freebooter Bluecrest – final results still to be analysed July-September 2001), and throughout with Alexander Buchan of Peterhead. The Grimsby company showed a strong interest in the FIGD approach, because it is seeking an objective test of fish freshness, but it is acknowledged that it is not yet ready for commercial use. A research student has been assigned to analyse the results of the industrial trial in the summer of 2001 as part of an MSc project.
(2) TEI	Industrial workshop carried out. Fifteen participants from major fish processing/merchandizing companies and competent authorities attended the four-hour workshop held in the Fisheries Laboratory, TEI Athens, on Wednesday 20 December 2000. Information about the FIGD equipment/technique and the newly developed QIM schemes was disseminated to the participants. During the course of the workshop it was demonstrated that the flow injection/gas diffusion technique could successfully be used to determine volatile bases in fish and fish products. For a number of crustacean shellfish (e.g. scampi and caramote prawn) and pelagic fish species (e.g. mackerels), determination of P-ratio by the FIGD technique could be an appropriate product safety and quality monitoring methodology for the requirements of industrial and legislative standards.
(3) IFL	Employment of the "Mustec" project (FAIR CT98-4076) to carry out trials – conclusion – IFL-FIGD equipment still basically a laboratory tool.
(4) IPIMAR	Limited interest by industry because equipment not sufficiently robust. Very successful workshops on 30 October 2000 and 9 November. Limited interest by industry for immediate industrial use because the equipment is not yet sufficiently robust. Successful workshops on 30 October 2000 and 9 November in which industry showed interest in the research project
(5) UBNB	Factory trials on anchovy and hake. TMA-N and TVB-N have been determined during the processing and distribution of anchovies marinated in vinegar. Chemical changes in salted anchovies during ripening have been studied. For hake the effect of evisceration and ice storage have also been studied

4.4.2 Sub-tasks associated with Task 3

Sub-task 3.i was to establishing agreements with and training appropriate industrial partners to conduct on-line trials of P-ratio determination using FIGD methodology and collect the data therefrom and Sub-task 3.ii was the assessment of the effectiveness of P-ratio determination using the FIGD and (if proved valid) the exudate sampling methodology as a routine fish freshness quality monitoring technique in industrial/commercial situations. As noted above partners approached these sub-tasks in different ways, appropriate to their own industrial situation.

5. DISCUSSION OF RESULTS

5.1 Principal general results

The collation of P-ratio data on the different species investigated by different partner laboratories under Task 1. (which continued to the end of the project) suggests that P-ratio is a less reliable indicator of the end of shelf life than was suggested by the end of the first QUALPOISS project. This conclusion is the main result of the research project.

From the first inter-laboratory collaborative QUALPOISS study, it appeared that a P-ratio of < 5% indicated that the fish is of EEC 'E' or 'A' grade freshness quality, whereas a P-ratio > 20% (P fitness upper limit) suggested that the fish is of EEC 'B' grade to unfit freshness quality. This conclusion from the first study arose from storage trials of white-fleshed, demersal species. When such fish are kept at higher storage temperatures, however, the use of this conclusion as a guide to freshness quality would tend to lead more frequently (12% of samples, cf. 6% of samples stored at 0°C on this data) to the rejection of fish before the expiry of its sensory fitness for processing into consumer products. Only 2% of fish stored at 0°C and 3% of fish stored at 4°C, however, would be likely to be rejected on sensory grounds before rejection on P-ratio grounds.

The following table compared the time taken for respective P-ratios and sensory scores to reach theses limits.

Table 5.1

Summary of P-ratio and Sensory Score Results from Qualpoiss1

SAMPLE	TIME @ 4°C to reach:		TIME @ 0°C to reach:	
	P-ratio 20%	Sensory score 5.5	P-ratio 20%	Sensory score 5.5
Hull Haddock	2days	4days	12days	12 days
Peterhead Haddock		8	12	12
Hull Whiting	3	6	12	11
Nantes Whiting	4	5	6	7
Hull Coley	4	5	7	8
Hull Cod	4	6	12	13
Reykjavik Cod			12	15
R'vik Cod Fillets			11	14
Athens Hake	9	12	16	15
Barcelona Hake	12	9	18	16
Lisbon Hake (u)			6	12
Lisbon Hake (l)			7	13

Source: Qualpoiss 1

In the second (current) study, levels of TMA in some species observed (anchovy, sardine, horse mackerel, sea bream, sea bass) remained very low (often <2mg TMA-N/100g flesh) throughout the storage period. The standard deviation as a percentage of the sample mean is very high for replicate observations on fish with such low levels which in turn makes the P-ratio a less reliable index. The P-ratio was, however, an index of similar usefulness (i.e. similar to the first study ranges of P-ratio being found) for other species: herring, shrimp, skate, monkfish and scallops.

An alternative index, the H-factor, which takes into account the disappearance of TMAO as well as the evolution of TMA and other volatile bases, has been proposed by the UK partner as worthy of further investigation.

On this basis, the P-ratio appeared to show potential usefulness as an objective indicator of the approach of the end of the "good" freshness quality shelf life of fish at 0°C. (The Lisbon Hake results were an exception to this, but the P-ratios, in this case, showed a widely distorted upward trend with time due to the large effect of experimental error/inter-individual variation in readings taken whilst TVB-N and TMA-N concentrations remained very low.) When the fish was stored at slightly higher (typical refrigerator) temperatures, however, the surpassing of the 20% upper limit for P-ratio usually (with the exception of the "Barcelona Hake" samples) preceded the sensory indication of loss of freshness quality.

Having completed this three-year programme, it appeared that the FIGD methodology could give data on the flesh concentration of these volatile bases and precursor in a wide range of different species of marine demersal and pelagic fish and shellfish which is at least as accurate and reliable as the long-established methods against which it was tested. Also data obtained for replicate samples amongst the different laboratories coincided more closely than the data obtained for the replicates using the established methods.

However the finding of this second phase is that TMA and TVB levels increase over the entire storage period but imperceptibly over the period when the fish could be deemed “fresh”. TMAO levels, on the other hand, appear to fall over the first days of storage but,

again, only gradually. The up and down variation in these results, obtained when different fish or different parts of the same fish are used to follow the changes in these substances with time of storage, over the first 7 days of storage, can be greater than the mean increase.

The use, therefore, of the concentration of any single one of these substances as an index of spoilage is limited by the lack of sensitivity over precisely the period up to when the fish appears obviously spoiled. Malle and Poumeyrol ( ) suggested that the % ratio of TMA to TVB (P-ratio) was somewhat more sensitive (than TMA, TVB or TMAO concentrations) as an index of spoilage over the first few days. The data accumulated through the activities of the six partner laboratories of this project suggests that P-ratio is no better an index of freshness than the level of TMA or TVB.

As TMA is largely, if not entirely, derived from the reduction of TMAO through prolonged storage of fish, the ratio of TMA to TMAO plus TMA (S-ratio) has also been examined for its potential as an index, but this appeared to promise no more sensitive an index of spoilage in the early period than did P-ratio.

Dividing the P-ratio by the S-ratio produces the ratio of TMAO plus TMA to TVB (H-factor). During the initial period of storage, theory has suggested that some of the TMAO is lost, not because it is reduced by spoilage bacteria to TMA, but, like in frozen storage, it is converted enzymically to DMA. The later is one of the volatile bases making up the TVB concentration. The value {[TMAN] + [TMAON]}/ [TVBN], with the numerator decreasing and the denominator increasing due to this suggested DMA production, consequently exaggerates the effect which, if it happens, could make the ratio more sensitive as an index of spoilage in the first few days of storage.

This period is the one which is of greatest concern to the chilled fish products industry. To be able to predict the remaining shelf-life (RSL) of packaged chilled fish by objective chemical means as well as by sensory analysis would be a bonus in marketing such products. The fish processing industry refers to the age of its incoming fish supplies as “Harvest plus however many days it has been kept since then. So H + 5 means the fish

has been kept in ice for 5 days since capture. For this reason, the ratio {[TMAN] + [TMAON]}/ [TVBN] has been referred to as the H-factor.

On the evidence of preliminary trials by the UK partner, the methodology looked more promising as a means of establishing an objective index of RSL It was, therefore, suggested that further investigation of H-factor by FIGD be pursued to establish its accuracy as a RSL predictor for the industry – especially for those species of fish which are being increasingly imported to meet EU demands as its own shortfall in fish supply widens.

IPIMAR's and results suggest that TVB and TMA-N are valuable indices in assessing the degree of fish deterioration, but do not accurately enough distinguish difference freshness grades, and that detailed information concerning the early stages of freshness and their differentiation cannot be obtained by these chemical indices. UB also found that TMA-N was more suitable as an indicator of spoilage rather than freshness. Also it appears from IPIMAR's results that, for horse mackerel and for sardine, the volatile nitrogen compounds change over the year, being low in January, increasing to a maximum between July and October and decreasing again towards the end of the year. TMAO-N appears to decrease as QIM (Quality Index Method) scores increase, but it does not appear to be a good freshness indicator due to different initial conditions. Similar problems with the development of the volatile bases emerged from the work by TEI. IFL concluded that TMA and TVB values do not increase significantly in most sea foods until after 8-10 days of storage, when the signs of spoilage become clearer using other methods of assessment. Therefore TMA and TVB add little new information about fish freshness or as predictors of the future shelf life of the fish. However, IFL believes that the IFL-developed FIGD equipment might be used to adjudicate when there is a disagreement about freshness. Work conducted at IFHQ demonstrated that the evolution of TVB and TMA during a typical processing time temperature profile could be seen to increase only after relatively long periods (>8 hours) of storage at temperatures >5°C which would not be encountered during commercial processing. The chief value of the IFL-FIGD equipment is in the laboratory.

5.2 Subsidiary results

5.2.1 Inter-laboratory sensory analysis of fish standardisation

The distribution of sensory analysis samples between the different partners was deemed to be impractical since not all sensory panels would be familiar or trained with an individual fish species and none of the species being investigated is common to all the partners' countries. It was therefore decided at the Athens plenary meeting that the standardisation of sensory analysis should be in the form of where a species was common between two or more partners, the sensory schemes and attributes to be used would be standardised. This has been carried out for the analysis of both raw and cooked fish, the sensory panels have been trained and assessments have been made during storage trials under standard conditions (refrigerated storage on ice).

The assumption that a standard model of time and temperature during the processing chain for all fish species being investigated could be established, although ideal, has been found to be impractical. The main reason is the differences in the time period that either the individual species are characterised as fresh, both by sensory and chemical analysis and the rate at which any significant changes in either sensory characteristic or chemical indices are noticeable. Table 11 illustrates the substantial variability in sensory characteristics between species. In addition the temperature and conditions experienced during processing within each country was shown to differ.

Table 5.2

Limits of Sensory Acceptability

	Days on ice
Anchovy	9-12
Cod	10-11
Haddock	10
Hake	10-14
Herring	7-9
Mackerel	15
Queen Scallop	14
Scampi	9.5
Seabass	20
Seabream	19
Shrimp *Pandalus borealis*	5
Sprat	9

In the absence of a standard time temperature profile applicable to all partners a storage standard was established for all species. This was defined as being surrounded by ice, stored at 0-5°C, in containers that permit the free drainage of melt water away from the fish.

5.2.2 Development and standardisation of methodology to obtain an exudate

The use of aqueous extracts was investigated, but without success. This method has not provided the on-line analysis that was originally hoped for, but it does reduce the use of hazardous chemicals in the analysis method.

5.2.3 IFHQ – Hull

The experience of working with industrial partners, in Peterhead (Alexander Buchan) and in Grimsby (Freebooter Bluecrest) was important. It suggested in general that fish processors have an ongoing need for an applied research input to progress questions related to traceability, on-line monitoring, and assessment of grades. IFHQ has assigned a research student to analyse the results of the collaboration with the Grimsby company during the summer of 2001. However, we do not expect the scientific results to alter materially the basic conclusion of the project set out under 5.1 above.

5.2.4 TEI - Athens

Sea bream is one of the study species. The TEI team found that feed affects TMAO levels. Sea bass is another important commercial species in Greece but the small amounts of TMAO in the species make it unsuitable for this study. Only 1.5 mg TMAN /100 g fish after 15 days storage, but the situation for sea bream is not much better. Nevertheless the FIGD equipment is capable of determining very small concentrations. The pH level important for TVB and TMA determinations - if it is too high, secondary deamination occurs producing a falsely high result.

5.2.5 IFL - Iceland

IFL investigated the use of fish eyes for the determination of TVB and TMA, since although the eyes may be used in freshness grading, they are not part of the final fillet. Although initial results show that the values obtained during storage trial were comparable although lower than values obtained from the fillet muscle, this method would only be suitable for large whole fish and could not therefore be employed with many of the fish species being investigated as part of this project. IFHQ has attempted several approaches to obtaining a suitable exudate, such as squeezing muscle, using "drip" and soaking muscle tissue, the latter two methods showing limited success.

5.2.6 IPIMAR - Lisbon

Sardine is one of the pelagic species to be studied but when stored at 0°C for the 8-10 days after capture, flesh TMA concentrations are still too low to measure.

Mussel is not an important species in Portugal therefore IPIMAR has little research experience with it. Could the study species be changed in this case? Shellfish have been included in the study due to an enquiry from Scofro Foods (Fort William) Christine Monzie via Alex Buchan. They are an industry who process scallops and wanted a standardised method for chemical assessment of scallop (*Pecten virens*) quality. Uglow (*pers.com*.) has shown that live mussels, when kept out of water prior to cooking show flesh concentration of TMA increasing with time of storage, so an in-depth study of this would be interesting. But, for the Portuguese study, octopus or squid would be a more relevant study subject for which it is possible to get fresher supplies (i.e. direct from the boat). It was, therefore, agreed to change the IPIMAR shellfish study subject from mussel to squid.

5.2.7 UBNB - Spain

Mediterranean hake and anchovy were the allotted study species, which could be obtained fresh and appeared suitable for a P-ratio study. Histamine analysis might also be useful in the case of anchovy. Dr Ruiz-Capillas is in the process of developing a FIGD methodology for this.

6. CONCLUSIONS

The project's overall objective was to test the proposition that the "*determination of P-ratio (i.e. trimethylamine/total volatile base concentration) in fish flesh by the flow injection/gas diffusion technique is an appropriate product safety and quality on-line monitoring methodology for the requirements of industrial and legislative standards for consumer satisfaction and protection"*.

(i) The data collected in this study suggests that the P-ratio is no better an index of seafood quality than its component TMA or TVB values. Like them, its initial lack of change with time then sudden rise as the seafood product approaches the limit of fitness for human consumption is useful with respect to "*consumer protection"* but is insensitive with respect to "*consumer satisfaction*". A proposed new index (H-factor) which takes into account the disappearance of TMAO as well as the changes in TMA and TVB content, appears worthy of further investigation as an index for both satisfaction and protection.

(ii) The FIGD technique, once its operators have become accustomed to its use, appears to be as sensitive and reliable as the long-established methods but much faster and cheaper. Despite the efforts of the laboratories of the QUALPOISS transnational partnership, it has not been possible to develop a FIGD methodology (based upon exudates, which would preclude the need for taking chemicals into processing areas, rather than extracts) to make it suitable for on-line or line-to-line use. Considerable effort, however, from the Icelandic partner has resulted in the development of the equipment into a prototype which could much more easily be accommodated by the very restricted test kitchen/QC department–type facilities typically available in fish processing companies. This compares with the original, prototype equipment which may only have been operable in the analytical chemistry laboratories of companies who provide this kind of service to the industry.

(iii) In order for the method to be used as an on-line measurement by industry it is essential that the method of obtaining samples for analysis be simplified. Currently the

use of TCA extraction of fish muscle is destructive, time consuming and requires the use of “hazardous” chemicals not suited to the food environment in addition to requiring technical personnel and facilities. At the same time the sampling procedure must provide representative values of TVB and TMA from which P-ratio can be determined, reliably and accurately in a wide range of fish species.

(iv) The use of exudate from applying pressure to the fish muscle was shown to be unsatisfactory since the exudate also contained other material which either required high speed centrifugation to remove it, or components lipids, proteins that significantly interfered with the analysis. It was also shown that the resulting TVB and TMA values were not comparable with values obtained using the standard TCA extraction method.

PROGRESS REPORT FROM THE ICELANDIC FISHERIES LABORATORIES
Progress Report 1: 01.10. 1997 – 30. 09. 1998.

SUMMARY

This FAIR PL. 963253 project "QUALPOISS 2: The evaluation of a simple, cheap, rapid method of non-protein nitrogen determination in fish products through the processing/merchandising chain" started in October 1997.

The organisations involved are: University of Hull International Institute (HIFI) U.K (co-ordinator), Alexander Buchan Ltd. Peterhead (AB) SCOTLAND (sub-contractor), Technological Educational Institution (TEI) of Athens, GREECE, Icelandic Fisheries Laboratories (IFL), Reykjavik, ICELAND, Instituto Portugues da investigacao das Pescas e do Mar (IPIMAR), Lisboa, PORTUGAL and Departmento de Nutrició I Bromatologia, Facultat de Farmacia, Universitat de Barcelona (UBNB-Barcelaona), Barcelona, SPAIN.

The main scientific objective for the first year was to show that P-ratio (TMA-N/TVB-N) in fish flesh could be determined reliably by the FIGD technique. One of the most important way to test that was to determine P-ratios for supplied unknown extracts, prepared by AB-Peterhead, using the FIGD methodology and forward the results to the industrial sub-contractor.

Due to incorrect type of LED’s in the detector part of the FIGD, which resulted in to low TMA-N results, P-ratio could not been determined reliably by the FIGD technique. TVB-N could however been determined successfully and with good correlation with steam distillation and picrate method.

1. INTRODUCTION

The main objective for Partner no 3, IFL, in the 3 years project, as described in the proposal, was:

"To investigate the relationship of the P-ratio with sensory index for *Melanogrammus aegelfinus* (haddock) and *Clupea harengus* (herring) through typical, standard, post-harvest sequences of handling, processing, storage, distribution and retail display; investigate the adaptation of the sample extract methodology for flow injection/gas diffusion P-ratio determination toward a more convenient-for- "on-line"usage; and investigate the applicability of P-ratio determination by the FIGD methodology for on-line monitoring of fish freshness through post-harvest handling, processing, storage, distribution and retail display through trial operation in factory and distribution chain situations in Iceland."

On the inaugural meeting in Hull in November 1997 it was agreed that *Pandalus borealis* (shrimp) would be and extra study subject additional to haddock and herring for IFL.

The workplan for the first year was as described in task 1:
Sub-task 1.i:a) *To employ a technician to carry out laboratory-based tasks.* The responsible scientist, Sigurdur Einarsson, acted as a technician for the majority of the time with some assistance from two technicians.

b) *To acquire FIGD equipment*. It was acquired at the inaugural meeting in Hull and transferred to IFL-Reykjavik.

c) *To attend an inaugural meeting in Hull*. The IFL participant received a training to install, commission and operate the FIGD equipment.

d) *To install and commission FIGD equipment at IFL-Reykjavik.* Done as planned.

Sub-task 1.ii: a) *To train a technician in the use of FIGD and other experimental methodologies*. As shown in the results a few methods to measured TMA and TVB was performed, such as steam distillation, picric acid method and of course FIGD.

Sub-task 1.iii: a) *To determine P-ratios for the supplied unknown extracts using the FIGD methodology and forward the results to the industrial sub-contractor.* Extracts of haddock and mackerel arrived to IFL from AB-Peterhead Scotland on May 12th 1998 in good condition. The TMA and TVB-N in the samples were as shown in the results chapter.

Sub-task 1.iv: a) *To employ and train a research assistant*. The responsible scientist carried out the laboratory-based tasks with an assistance form a technician who helped out with a technical problems regarding the FIGD and another technician who carried out some of the measurements.

b) *To determine P-ratio of fish extracts replicate samples using the FIGD and other methodologies*. Experiments were done to compare TVB-N results measured by the FIGD technique and steam distillation in shrimp and haddock as the results show in chapter 3. Due to technical problems with the FIGD the TMA was not measured during this period.

c) *To collate, evaluate and present the results of the inter-laboratory P-ratio*… This part has been postponed until the meeting in Athens in January 1999 where each participant will present his or her results. After that IFL will collate, evaluate and present the results and send to each participant.

2. MATERIAL AND METHODS

2.1. Raw material

For sub-task 1.iia, samples of different fish species at different freshness stages were analysed for TMA-N and TVB-N using FIGD, steam distillation and picrate method. For sub-task 1.ivb, deep water unpeeled red shrimp (*Pandalus borealis*) was caught in August 1998 north of Iceland and stored in ice at an ambient temperature of 1°C for up to11 days. Haddock (*Melanogrammus aeglefinus*) was caught SW of Iceland by day boat in August 1998 and stored in ice at ambient temperature of 1°C for 14 days. TVB-N was

measured in the samples on days 1, 3, 5, 8, 10, 12 and 14 by steam distillation, and picrate method and the FIGD technique.

2.2. Chemical testing

2.2.1. Preparation of sample:

The same preparations were done for all of the following methods except 2.2.6. Fish extracts for TVB-N and TMA-N determination were prepared by blending 100g samples of macerated flesh with 200 ml of 7,5% trichloroacetic acid (TCA, Merck 807) for 2 min. in Waring blender at high speed and filtered the homogenates to produce a clear solution.

2.2.2. Chemical analysis

Flow injection gas diffusion method (FIGD)

APPARATUS

- Ismatec (Reglo-Digital MS-4/8-100) peristaltic pump with variable flow rate (1-100 rpm), 4 channels and 8 rollers
- Detector (Made by HIFI, Hull)
- Chart recorder (Perkin Elmer 56)
- Gas permeable membrane. PTFE sealing tape nº reference 512-238 (RS Tel: +44 1536 201201, Fax: +44 1536 201501)

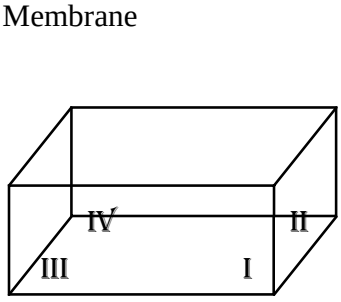

Fig. 1.- *Scheme for membrane*

IN (I)- Entry tubing for BTB from pump through detector.

(II)- Entry tubing from T pieces (TMA analysis) or reodyne (2) (TVB analysis).

OUT (III)- Exit tubing for BTB and TMA/Ammonia to detector.

(IV)- Exit tubing to waste.

- Reodyne 5020 low pressure injection valve (ANACHEM Ltd.)

MANIFOLD TUBING

PVC tubing through peristaltic pump of 0.8 mm i.d. (ISMATEC) but others teflon tubing were of 0.6 mm i.d. (ANACHEM) Ltd. It is important is that the flow is uniform (not pulses) and at rate of 1ml/mim).

ANALYSIS OF TMA-N and TVB-N

Reagents

- NaOH (1.0 M)
- Formaldehyde 20% (v/v)
- Bromothymol blue water soluble (BTB): 0.3g/l (Merck 3026)
- Trichloroacetic acid (TCA): 7.5%.
- Trimethylamine Hydrochloride (Sigma T7630) stock solution (0.05 M) in TCA, 7.5%.

Standard curve (50µM/L, 100µM/L, 150µM/L, 200µM/L)

Calculations

TMA-N and TVB-N are determined as Nitrogen

$$\text{mg TVB-N /100 g sample} = \frac{\mu M/L(\text{TVB-N}) \times 10^{-3} \times 14.007 \text{ mg/mol} \times 100g}{10^{6} \mu mol/mol \times 333g \text{ fish/L}}$$

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

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

Preparation of the sample:

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

Determination of TVB:

The flow of 1,0 M NaOH (1 ml/min) from the peristaltic pump (Ismatec, 4-channel with variable flow rate) carries the injected liquid (standard or sample) through the mixing coil alkalising it and releasing its contained nitrogen in the form of ammonia gas. On flowing through the gas diffusion cell (a laboratory-built gas diffusion cell with channel dimensions 240mm x 1,5mm x 0,2 mm with a microporous, chemically inert and acid resistant PTFE membrane RS No. 8003525) this released ammonia passes through the gas permeable membrane into a 0,3 g/L (pH 6,5) solution of bromothymol blue (BTB)

indicator flowing from the peristaltic pump. The color change caused in the indicator produces a proportionate response from the detector; a photometer incorporating a red light-emitting diode connected to a Perkin-Elmer 56 recorder.

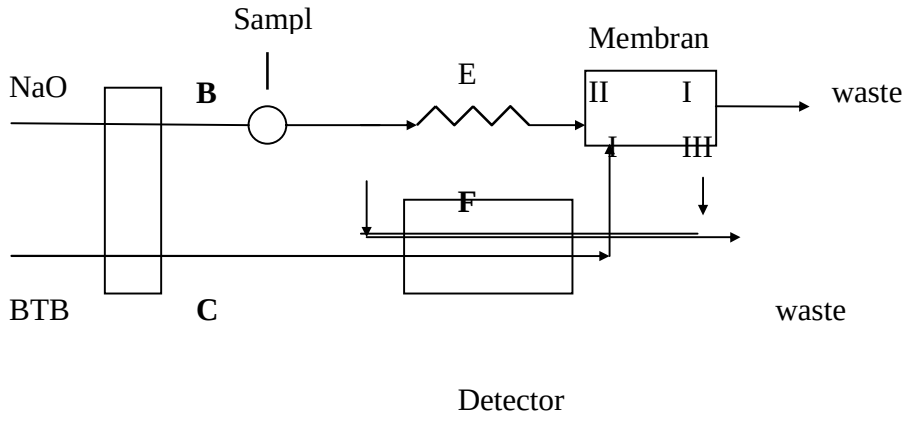

Fig. 1. *A schematic diagram of the Flow Injection/Gas Diffusion apparatus used for the determination of TVB-N.*

Determination of TMA:

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

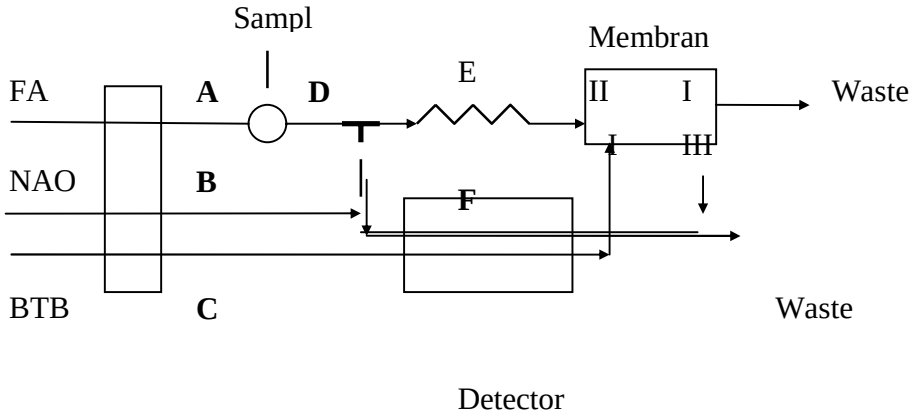

Fig. 2. *A schematic diagram of the Flow Injection/Gas Diffusion apparatus used for the determination of TMA-N.*

Determination of total volatile basic nitrogen (TVB-N) by steam distillation of TCA extract (Modified method of Malle and Tao, 1987).

One hundred gram of fish muscle was deprotinized as described above and the filtrate was collected. Steam distillation was carried our using a Struers-type distillator (Struers KEBO Lab, Denmark). Twenty-five ml of filtrate was placed into the distillation and 10 ml of 10% NaOH (Merck 6482). An Erlenmeyer flask containing 10 ml of 4% aqueous boric acid (Merck 165) solution and 0,04 ml of methyl red (Merck 6076)/bromcresol green (Merck 8121) mixed indicator, for titration of ammonia, was placed at the end of the condenser. Distillation was continued until a final volume of 90 ml was obtained in the beaker (80 ml of distillate). The boric acid solution turned green when alkalinised by the distilled TVB-N. This solution was titrated using a 0,1-ml graduated burette containing 0,025 N H_2SO_4 (Merck 713). Complete neutralisation was obtained when the colour turned pink on the addition of a further drop of sulphuric acid.

The quantity of TVB-N in mg was determined from the volume of sulphuric acid (N ml) added as follows: **TVB-N = (n)(4,2 mgN/100g)**

Determination of TMA-N by steam distillation of TCA extract (Modified method of Malle and Poumeyrol, 1989)

Twenty-five ml of the TCA sample-extract was placed into a distillation flask and 20 ml of formaldehyde (Fluka 47630) was added in order to block the primary and secondary amines. Steam distillation was then performed as for the determination of TVB-N. When the require amount of formaldehyde was added only the TMA was distilled. The TMA content was calculated from the volume of 0,025N H_2SO_4 used for titration (n ml) as follows: **TVB-N = (n)(4,2 mgN/100g)**

Colorimetric method for determination of Trimethylamine (Modified AOAC method, 971.14, 1990)

One ml of filtrate and 3 ml of deionized water were added to a test tube. Three other test tubes received 1, 2 and 3 ml of a standard TMA (Merck 8359) solution (concentration = 0,0085 mg/ml) and 3, 2, 1 ml of distilled water, respectively. A tube containing 4 ml of distilled water was used as a blank for colorimetry. One ml of a 20% magnesium carbonate treated formaldehyde, 10 ml of anhydrous toluene (Fluka 89675) and 3 ml of

45% KOH (Merck 5033) were placed in the tubes. The tubes were shaken vigorously by hand 80 times; 8 ml of the toluene phase was than transferred to a tube containing 0,2 g of anhydrous sodium sulphate (Merck 6649) and shaken until dehydrated. Five ml of the dehydrated toluene phase were mixed in another tube with 5 ml of 0,02% picric acid (Merck 623). Optical densities (OD) were measured using a Cecil Series 2 spectrophotometer at a wavelength of 410 N.M. A standard curve plotted from the standard solution values was used to determine the quantity of TMA in the samples in mg from the OD values.

The quantity per 100 ml of fish was given by: (x mg)(300 mg N/100g of fish).

Determination of total basic nitrogen (TVB-N) with the STRUER automatic distillation unit (Based on the method of N. Antonacopulus, 1968.

Ten grams of macerated fish flesh is placed in a distilling flask (Kjeldahl), 2 g of MgO (Merck 5862) and 100 ml of distilled water is added. The sample is distilled at a flow of 10 ml/min for 12 min. in a STRUER automatic distillation unit, which has been pre-heated. The distillate, containing the volatile basic nitrogen is collected in 100 ml of 0,27% boric acid containing 8 drops of Mixed indicator (Merck 6130) and titrated with 0,05 N H_2SO_4 so it stays at the neutral point during the 12 min. distillation.

Calculation: mg TVB-N = ml 0,1 N acid x 7

3. RESULTS AND; 4. DISCUSSION

Sub task 1.ii: a) Comparison was done on different fish samples, as a part of the familiarisation exercise, using two steam distillation methods, direct steam distillation of the fish using MgO (2.2.6) and steam distillation of TCA fish extract (2.2.3). Each sample was measured in triplicate. As shown in table 1 there was a good correlation between results obtained by using these two methods. It is demonstrated by the correlation coefficient r = 0,9975 and the regression equation is:

MgO = 0,988645 (TCA extract) -0,22629

Table 1. *Comparison of the levels of TVB-N in different samples of cod as measured by the MgO method and Steam distillation method.*

Sample	MgO (mgN/100g)	Steam distillation (mgN/100g)
1	11,6 ± 0,25	12,1 ± 0,18
2	15,6 ± 0,32	16,1 ± 0,30
3	19,5 ± 0,22	19,8 ± 0,15
4	25,6 ± 0,10	24,8 ± 0,33
5	29,6 ± 0,19	30,5 ± 0,26
6	33,9 ± 0,55	35,2 ± 0,10
7	37,7 ± 0,66	38,8 ± 0,22
8	44,5 ± 0,35	46,0 ± 0,46
9	55,4 ± 0,44	55,3 ± 0,20

Determination of TMA by the Picrate salt formation method and steam distillation method was also compared with different type of samples. Results from the steam distillation methods (TCA extract, 2.2.3) were similar when compared to the picrate

method (2.2.4) when TMA was above 1,5 mg N/100 g according to the picrate method as shown in table 2. Each sample was measured in triplicate The regression equation was: Picrate method = 1,040719 (Steam distillation) - 0,98085 R= 0,9982

Table 2: *Comparison of the levels of TMA-N in different samples of haddock as measured by the Picrate method and Steam distillation method.*

Sample	Picrate method (mgN/100g)	Steam distillation (mgN/100g)
A	0,2 ± 0,17	1,6 ± 0,11
B	0,8 ± 0,22	2,1 ± 0,21
C	1,4 ± 0,24	2,3 ± 0,23
D	2,4 ± 0,10	2,8 ± 0,31
E	3,8 ± 0,20	4,1 ± 0,21
F	6,9 ± 0,30	7,7 ± 0,18
G	9,5 ± 0,12	10 ± 0,12
H	17,5 ± 0,21	17,6 ± 0,15
I	23,5 ± 0,14	23,7 ± 0,50

Sub-task 1.iii: a) The TVB-N results from the determination of the extracted fish samples using FIGD and Steam Distillation are shown in fig. 1 and fig. 2.

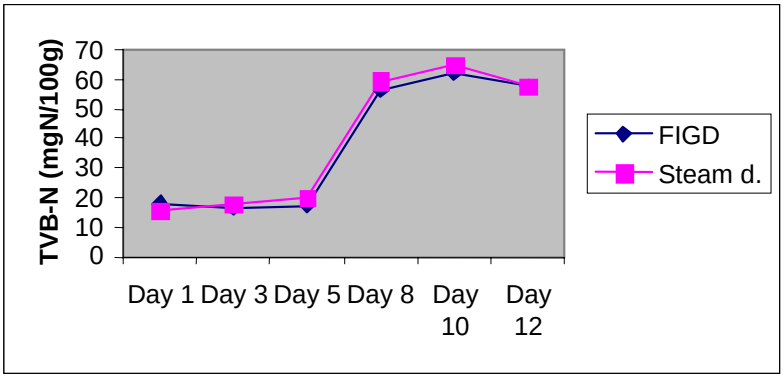

Fig. 1. *Comparison of TVB-N results measured by two different methods in samples of haddock extracts (n=5)*

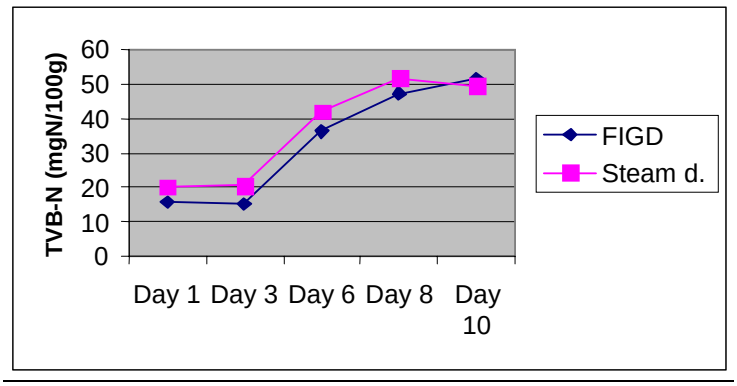

Fig. 2. *Comparison of TVB-N results measured by two different methods in samples of mackerel extracts (n=5)*

As the results show in fig. 1 and fig 2. there is not a statistical difference ($p<0,5$) between these two methods. The standard deviation is high in some of the samples and higher as the TVB-N gets higher.

The TMA results from the determination of the extracted fish samples using FIGD, Steam Distillation and picrate method are shown in fig. 3 and 4.

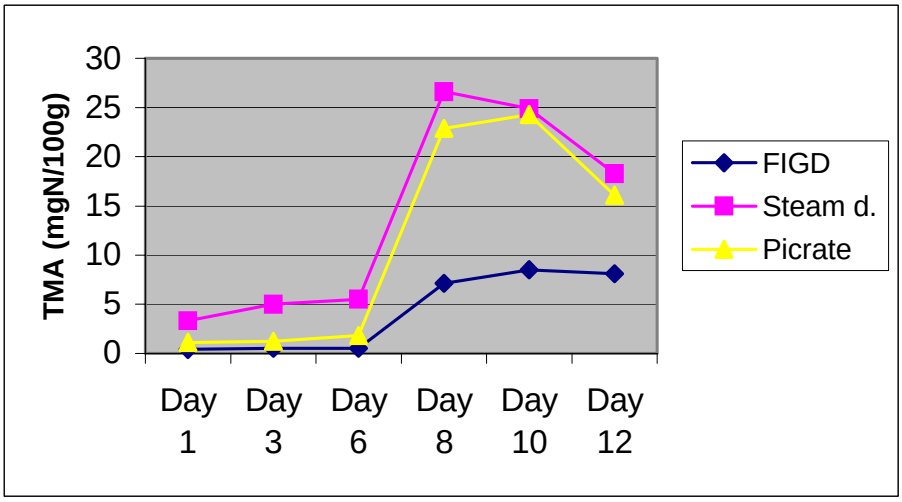

Fig. 3.

Comparison of TMA-results measured with three different methods in samples of haddock extracts (n=5):

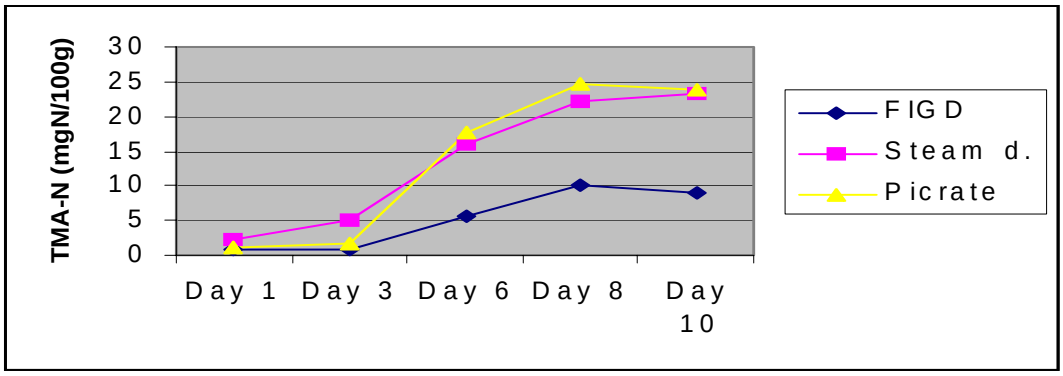

Fig. 4.

Comparison of TMA-results measured with three different methods in samples of mackerel extracts (n=5):

The results in fig 3 and 4 for the FIGD indicate a failure because TMA is about 3 times lower compared to the results measured by the other methods. Additionally the standard

deviation is very high. Replicate samples were not reliable either because the peaks could change 2-3 fold from one injection to the other, independent if the sample or the standard was injected. The results for TMA measured by FIGD are therefore based on a standard curve where the standard deviation is very high thus not dependable. However the trend line is similar as TMA measured my the other two methods but the values are much lower. This did not happened when TVB-N was measured on FIGD. Another problem that was a great problem was an unsteady baseline on the chart recorder. The low standards for TMA could therefore not be measured because of the fluctuation when only reagents passed by the diodes. The producers at HIFI, and it has been confirmed by a specialist working for IFL, have found out that the diodes, that were provided, are too strong but weaker diodes could solve this problem and probably give reliable TMA results.

The TVB-N standard, NH4Cl (Merck, pa), was checked by steam distillation and the recovery was 99,0 % . The regression equation was:
Peak height (mm) = 0,9184(µmol TVB-N/L) – 0,17 R = 0,9993

Table 3 *: Standard values for TVB-N measured on NH4Cl by FIGD (triplicate standards)*

200µmol/L = 186 mm ± 2
150µmol/L = 135 mm ± 3
100µmol/L = 90 mm ± 1
50µmol/L = 45 mm ± 4
20µmol/L = 19 mm ± 5
0µmol/L = 1 mm ± 0

Determination of TMA by FIGD technique

The standard was prepared by weighing 23,9 mg TMA-HCl into 500 ml of trichloroacetic acid (TCA) to give 0,5 mM TMA-HCl stock solution. This standard was checked by steam distillation:

%N of C3H9N-HCl = 14,66 $\Rightarrow$ $\frac{14,01 \text{ g/mole x } 0,475 \text{ ml x } 0,0262 \text{ mole/L x } 100\text{g}}{(25\text{ml}/500\text{ml})\text{x } 0,0239\text{gx}1000\text{ml/L}}$ = 14,59%

Recovery: 14,59/14,66 = 99,5%

Table 4 : *Standard values for TMA measured on TMA-HCl by FIGD (triplicate standards)*

200µmole = 191 mm ± 9
150µmole = 128 mm ± 14
100µmole = 74 mm ± 10
50µmole = 48 mm ± 12

Peak height (mm) = 0,9184(µmol TMA-N/L) – 0,17 R = 0,9993

Sub-task 1.iv b)

It was not possible to get reliable results for TMA using The FIGD method and therefore not to determine the P-ratio. TVB-N could on the other hand be determined by FIGD even though the baseline was not as strait and stable as it should have been. A comparison study was done on samples of shrimp to evaluate the reliability of TVB-N determination by FIGD compared to TVB-N determinations by steam distillation. As the results show in table 5 there was a fairly good correlation between these two methods. Each sample was measured in triplicate The regression equation was:

Steam distillation = 1,215997(FIGD) + 0,925677 R = 0,99214

Table 5. *Comparison of the levels of TVB-N in different samples of shrimp as measured by the FIGD method and Steam distillation method.*

Sample no.	FIGD TVB-N (mgN/100g)	Steam distillation TVB-N (mgN/100g)
Day 0	10,8 ± 0,32	12,1 ± 0,15
Day 1	12,4 ± 0,30	13,6 ± 0,24
Day 2	14,3 ± 0.24	14,5 ± 0,26
Day 3	16,6 ± 0,44	16,9 ± 0,36
Day 4	18,5 ± 0,12	18,1 ± 0,66
Day 7	25,6 ± 0,11	26,9 ± 0,12
Day 8	33,8 ± 0,25	35,8 ± 1,20
Day 9	39,5 ± 0,11	39,2 ± 0,24
Day 10	46,6 ± 0,65	47 ± 0,43
Day 11	44,4 ± 0,34	48,9 ± 0,55

A similar study was done on haddock as is demonstrated in table 6. The results are in similar manner as for the shrimp or that the steam distillation gave slightly higher results than the FIGD method but there was though no statistical difference between these two methods ($p < 0,05$) for both seafood species. The regression equation for the shrimp was:

Steam distillation = 1,0359 (FIGD) + 0,1066 R = 0,9911

The regression equation for the haddock was:

Steam distillation = 1,0556 (FIGD) + 0,1360 R = 0,9973

Table 6. *Comparison of the levels of TVB-N in samples of haddock as measured by the FIGD method and Steam distillation method.*

Sample no.	FIGD TVB-N (mgN/100g)	Steam distillation TVB-N (mgN/100g)
Day 1	10,2 ± 1,5	11,3 ± 0,32
Day 3	13,6 ± 0,65	13,9 ± 0,65
Day 5	15,8 ± 0,9	16,8 ± 0,36
Day 8	18,8 ± 0,56	19,5 ± 2,4
Day 11	22,3 ± 2,3	24,5 ± 3,6
Day 14	37,8 ± 4,5	39,9 ± 1,4

5. DISSEMINATION

An important part of the project for each participant was to find an industrial partner who was willing to set up a simulation model in his factory. "Frostfiskur" is such a factory, located in Reykjavik that processes both fresh and frozen fish of the species IFL will be working with or haddock, herring and shrimp. Frostfiskur will provide us with the raw product and the facility to do the experiments on the site as will be needed.

The FIGD technique has been introduced to many Icelandic and foreign visitors at IFL as a cheap, rapid and a reliable choice to determine TVB-N. This September, for example the United Nation University - Marine Division was established in Iceland. The students come from different countries in Africa and some of them are interested to take a special project in HACCEP and rapid methods to determine fish freshness where FIDG could be incorporated.

6. REFERENCES

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PROGRESS REPORT FROM THE ICELANDIC FISHERIES LABORATORIES

Progress Report 2: 01. 10. 1998 - 30. 10. 1999

SUMMARY

The main objective of the study during this period was to determine TMA and TVB in cod, haddock, herring and shrimp by the FIGD method and to compare it with other standard methods (TVB WEFTA Codex and TMA Dyer methods). The fish/shrimp extract was injected into the FIGD manifold that was then closed. The flow of 1,0 M NaOH from a peristaltic pump carried the injected liquid through a mixing coil alkalinising it and releasing its contained nitrogen in the form of ammonia gas. On flowing through the gas diffusion cell this released ammonia passed through the gas permeable membrane into a bromothymol blue indicator solution flowing from the peristaltic pump. The colour change caused in the indicator produced a response from the detector to a chart recorder. Storage studies were done on the fish at two to three different seasons of the year and on shrimp that were kept in ice under standardised conditions. For two of the studies the cod and haddock eyes were also measured for TMA and TVB and compared to the results determined on the fish muscle. Sensory evaluation, by the Quality Index Method and the Torry scheme using trained panel and bacterial counts, were also a part of some of the storage experiments.

TMA measured by the FIGD method and the WEFTA Codex method gave similar results for all species (R^2 > 0,96). TVB measured by the FIGD method gave at average 60% lower results than if measured by the Dyer method for all species. TMA and TVB values in the cod and haddock eyes were lower than in the fillets but showed a similar exponential pattern. The detection limit for TMA and TVB by the FIGD method is 0,1 mg N/100 g wet tissue and spiked samples gave at average 99,9 % recovery.

The advantages of the FIGD method:

- ✓ It was faster and easier to use than comparable methods.
- ✓ It only required 0,1 ml of extractable sample.
- ✓ The FIGD equipment is inexpensive (<3000 EURO).

The disadvantages of the FIGD method:

- ✓ The extraction process and the dilutions of "strong" extracts were limiting factors.

- ✓ The TMA baseline was not as stable as the TVB baseline.
- ✓ The FIGD- TVB method gave about 60% lower values than the WEFTA Codex TVB method.

1. INTRODUCTION

The actions in the second year of this project was as is described in the Technical Annex sub task 1v to 1viii and sub-task 2 i.

Sub-task 1. v to viii: Due to difficulties of transportation and the cost of shipping frozen fish samples from the subcontractor, AB in Peterhead Scotland, to all the partners throughout Europe as planned, it was determined, at the meeting in Athens in Feb. ´99, to use fresh fish samples that each partner was more familiar with. The IFL partner investigated the relationship between sensory scores from trained panel and the freshness indicators TMA and TVB using the FIGD technique. The decomposition index had already been established at IFL for the investigated species, cod, haddock, herring and shrimp so more effort was put into the validation of the FIGD method against the known decomposition index and against other methods to determine TMA and TVB.

Sub-task 2.i: The effort to investigate methodologies to obtain exudate from fish samples instead of using extracts of trichloroacetic acid was carried on.

The TMA and TVB values were determined by the FIGD method to evaluate the spoilage stage of the most important fish species in Iceland, cod (*Gadus Morhua*), haddock (*Melanogrammus aeglefinus*), herring (*Clupea harengus harengus*) and Northern shrimp (*Pandalus borealis*) and to compare this method with other commonly TMA and TVB methods. Parallel to this, sensory analysis and microbial counts were also conducted to determine the freshness quality of the seafood species.

2. MATERIAL AND METHODS

2.1. Experiments

The cod was caught with long-line Southwest of Iceland. It was brought bled, gutted and iced into 90L boxes on board and brought ashore a few hours after catch. The fish was kept for storage trial in ice at 2°C. Samples of the fillets and the eyes were taken at 3 to 4 days interval during the storage period. On each day of sampling 3-5 fish were examined.

Three experiments were done on haddock. The haddock was caught SW of Iceland in December 1998, March 1999 and May 1999, NE of Iceland in floating nets. The fish was caught one day before the experiment started except in the March trial where the fish was caught 8 days before the experiment started. It was brought bled, gutted and iced into 90L boxes on board and brought ashore a few hours after catch. The fish was kept for storage trial at 2°C in ice. Samples of the fillets and the eyes were taken at 3 to 4 days interval during the storage period. On each day of sampling 3-5 fish were examined.

The herring was caught in Héraðsflói, NE of Iceland in floating nets and brought ashore in Neskaupsstaður where it was transferred by flight to IFL in Reykjavík, iced in insulated boxes. The fish was kept for storage trials at 0°C and 5°C. Samples were taken at 3 to 4 days intervals during the storage period. On each day of sampling 5 fish were examined.

The shrimp was harvested NW of Iceland in a shrimp-bottom-trawler net on August 11th and September 19th 1999. It was brought on shore and samples were iced and packed and shipped by air to the laboratory in Reykjavík where it was analysed 14-16 hours after catch.

2.1. Chemical analysis

a) Flow Injection analysis of TMA and TVB: See Project Report 1

b) Determination of TVB by steam distillation of TCA extract according to the WEFTA Codex Method (Vyncke et al. 1987).

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

2.2. Microbial counts

When examined cod- and haddock fillets and the whole shrimp, total viable counts and selective counts of H_2S-producing bacteria were done on iron agar as described by Gram et al. (1987). The plates were incubated at 22°C for 3 days. Bacteria forming black colonies on this agar produce H_2S from sodium thiosulphate and/or cysteine.

2.3. Sensory evaluation

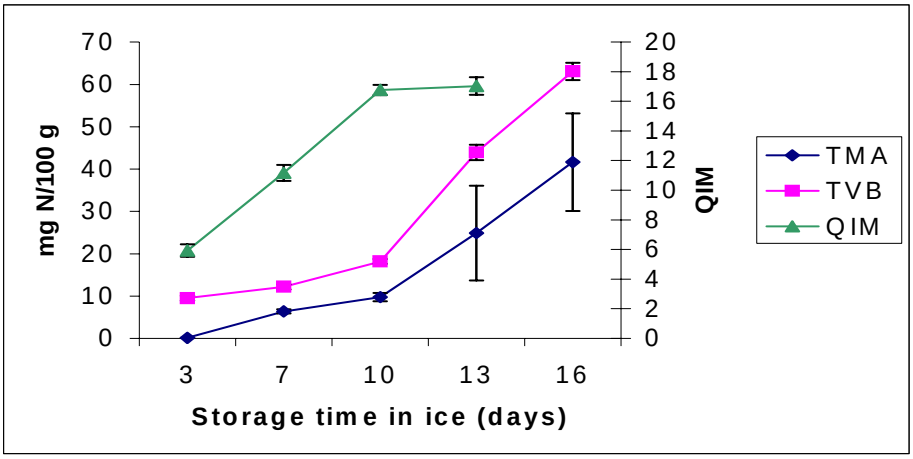
On each sampling day 10-12 members of the internal panel of IFL evaluated the haddock. Each panel member evaluated 5 fish according to the Quality Index Method (QIM) and 3 steam-cooked samples were evaluated according to Torry scale. The QIM grading scale for cod and haddock is form 0 for highest score to 20 for the lowest score. The Torry grading is 10 for the highest score and 3 for the lowest score for the shrimp was evaluated by the QIM, specified for shrimp.

3. RESULTS AND DISCUSSION

Sub-task 1v - 1viii

3.1 Cod (*Gadus Morhua*)

Figure 3 shows that the TMA and TVB values, measured by the FIGD method, can be very different between individual fish. That is very obvious when the fish has been stored

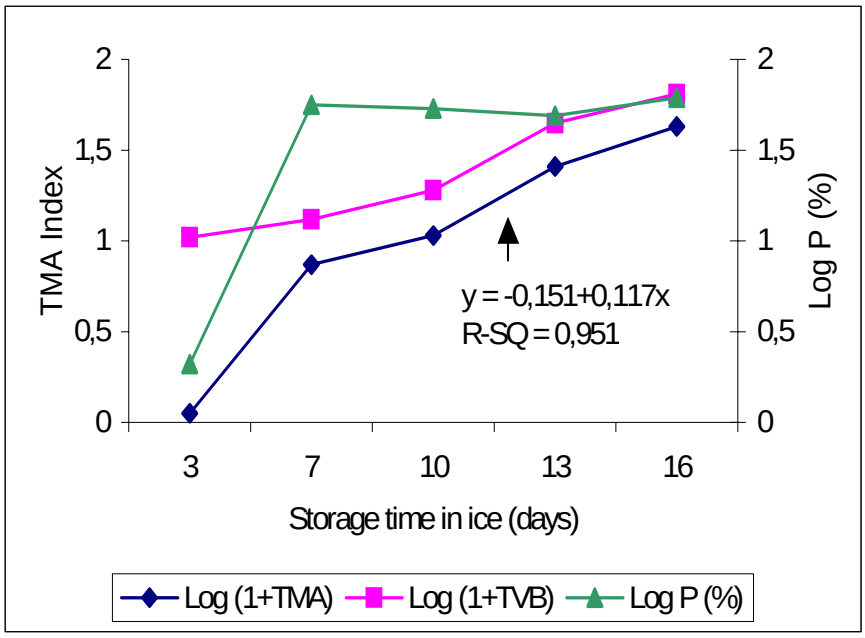


for 13 days in ice where the TMA values ranges from 5 to 50 mg N/100g and

Fig. 3. *TMA, TVB and Quality Index values for cod during storage in ice measured with the Flow Injection/ Gas Diffusion method.*

30 to 60 mgN/100 g for TVB. At day 10 the TMA-values are about 10 mgN/100g but fish containing more that that is usually regarded unfit for further production (Connel, 1990). The TVB values at day 10 indicate though that the fish was still in good condition since the values are only from 16 to 20 mgN/100g but it has been demonstrated that TVB values are at average 60% lower when TVB is measured by FIGD than if it is measured by the WEFTA Codex steam distillation method. If that is taken into the account the TVB values are about 30 mgN/100g, but 25-35 is usually the limits for marketable groundfish (Botta, 1995; 95/149/EEC).

P-ratio [(TMA/TVB)100] was not a good indicator of freshness since it was over 40% (log 40 = 1,60) already at day 7 when the fish was still good according to TMA and TVB values alone or the sensory and the microbial values as shown in figures 3-6. The P-ratio was high after that as well but very different between individual fish at the same storage time. Figure 4 demonstrates that the TMA index is a better indicator of freshness than



measured TMA with R^2 = 0,951 and the correlation between TVB index is in fairly good correlation with storage time

Fig. 4. *Correlation between the TMA index (log (1 + TMA value) and storage time for cod in ice and changes in TVB Index and log P-ratio with time.*

At IFL sensory laboratory it has been decided through repeated sensory testing that cooked white fish is fit for human consumption when the average Torry grade is 5,5 and QIM grade is 11. The Torry grades ranges from 3-10 where the best grade is 10 and the worst is 3 and the QIM goes from 0 for the highest grade and 20 for the lowest grade. The sensory scores (figure 5) indicate that the fish was fit for human consumption up to day 10-11.

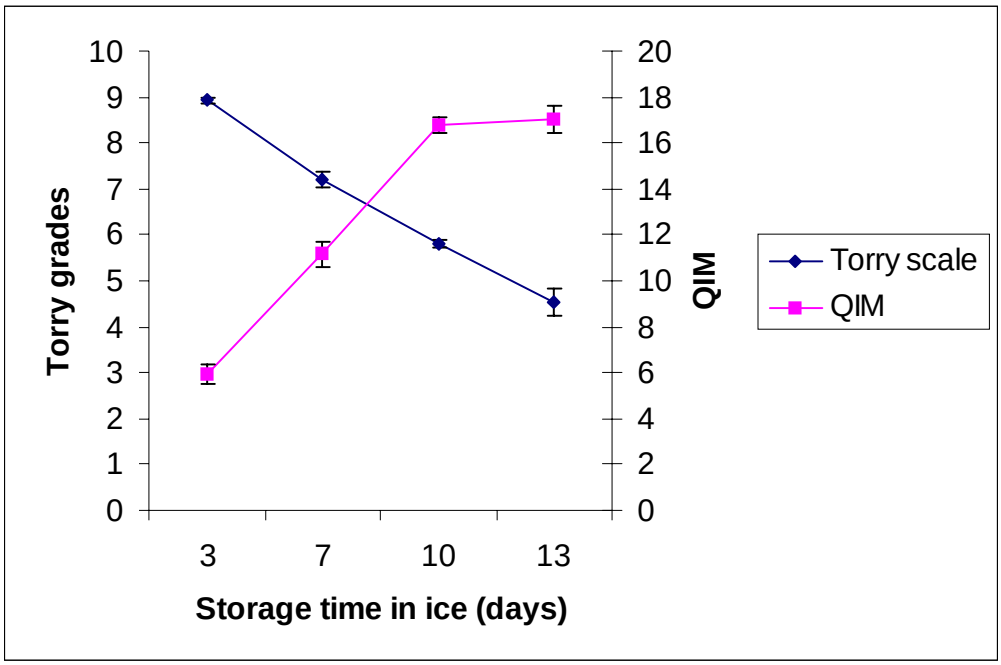


Fig. 5. *Sensory scores as Torry grade on cooked cod fillets and Quality Index on raw cod during storage of cod in ice.*

The microbial counts, as shown in figure 6, confirm the results found from the sensory analysis using the equation above that the micro-organisms begin to grow very fast at day 10 but the increase is not as fast after that. The chemical results indicate however that the fastest increase takes place at day 13. This is probably due to that the micro-organisms that are responsible for the breakdown of TMA-O to TMA, mainly H_2S producing *Shewanella putrefaciens*, have not produced enough enzymes to form TMA at day 10 but have apparently done so at day 13.

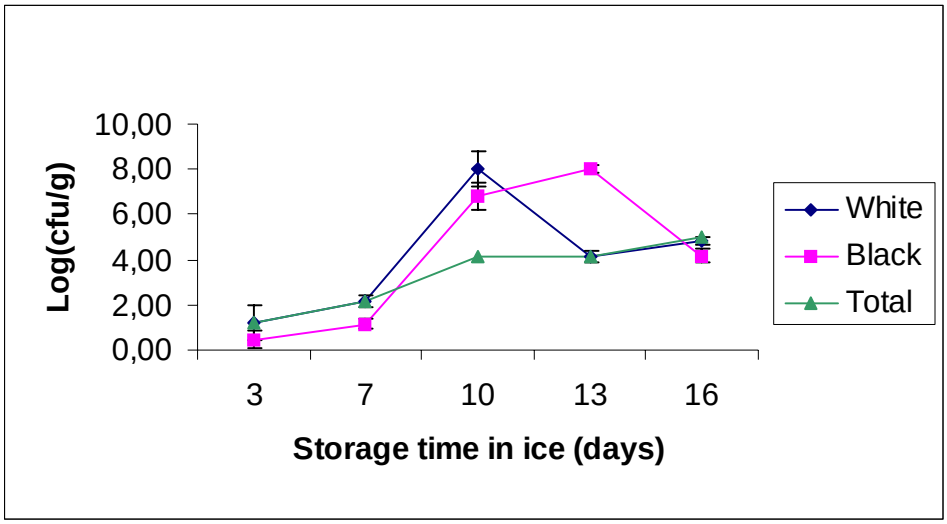


Fig. 6. *Bacterial counts in cod tissue as white CFU/g, representing total viable counts (TVC) and black CFU/g representing H_2S producing bacteria.*

The development of TMA-N and TVB-N in the cod-eyes is a bit different from cod-fillets. Between days 7 and 10 the TMA-N increased very fast but stayed stable after that or around 19 mgN/100g whereas it continues to increase in the fillets.

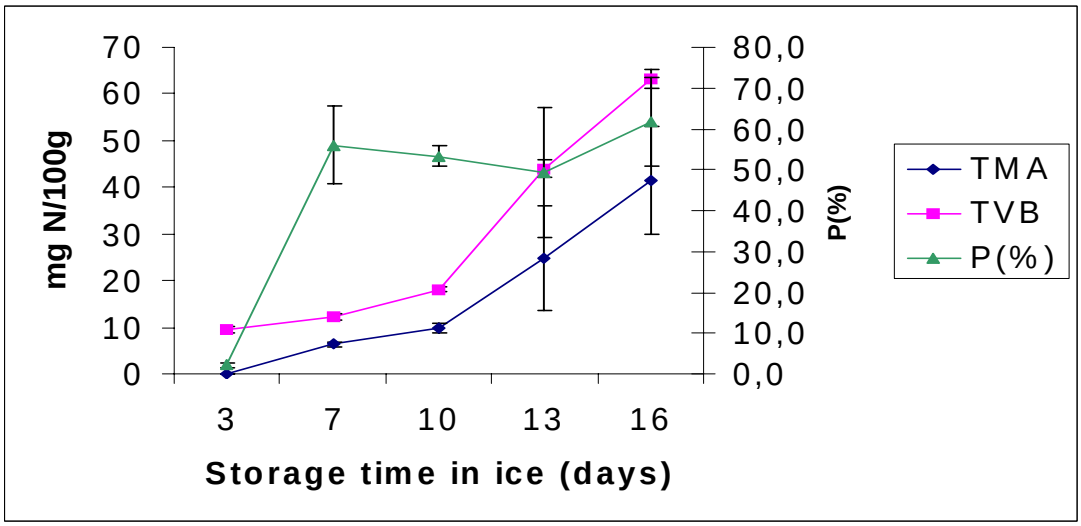


Fig. 7. *TMA, TVB and P-ratio for cod eyes, taken from 3 fish each time, during storage of cod in ice.*

3.2. Haddock (*Melanogrammus aeglefinus*)

The TMA and TVB results for haddock stored in ice at 0°C for up to 18 days, determined by different methods are shown in figures 8 and 9. The correlation between the two TMA methods (Dyer and FIGD) for haddock stored in ice is fairly good (R^2 = 0,974) but not satisfactory for TVB in haddock:

TVB: WEFTA = 1,06 + 1,49(FIGD), R^2 = 0,817
TMA: Dyer = 0,18 + 0,73(FIGD, R^2 = 0,974

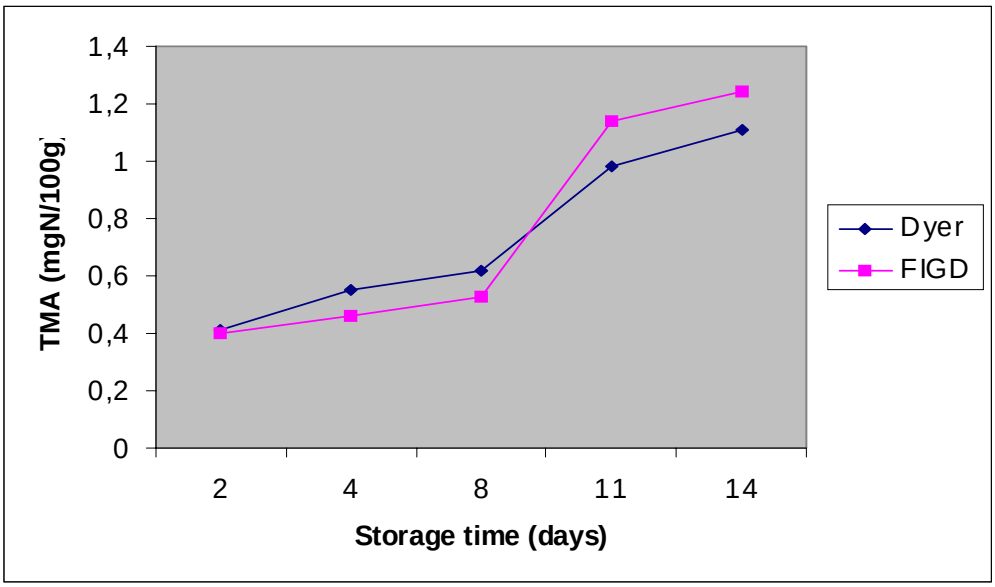


Fig. 8. *Changes in TMA during storage at 0°C of haddock measured by Dyer and FIGD methods.*

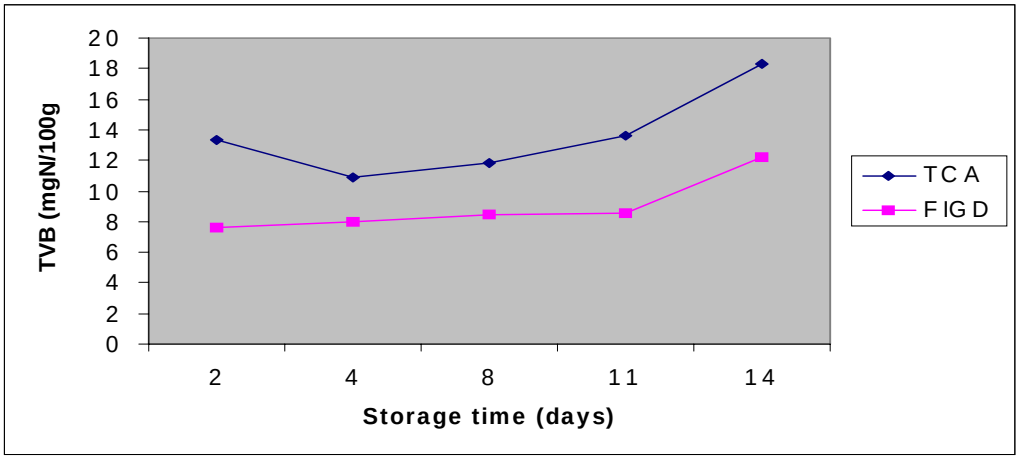


Fig. 9. *Changes in TVB during storage of haddock in ice measured by FIGD-method and the Dyer method.*

A comparison is done on different sampling dates, in figures 10 and 11, on sensory scores evaluated for the raw fish with the Quality Index Method and on the cooked fish according to the Torry scale and on TMA. According to the December trial. analysis of TMA and TVB (not shown) using the FIGD-method showed a slow increase during storage and the TMA and TVB were still very low at the last sampling day. Fish containing more than 10 mgN/100g is usually regarded as unfit for further production (Connell, 1990). The fish in this storage experiment did not even reach 2 mgN/100g at the last sampling day (day 15) even though the sensory panel had judged the fish unfit for consumption as soon as at day 10. The storage life of fish is defined as coming to an end when the average sensory scores have reached 5.5 on the Torry scheme. The March and May results showed an exponential increase in TMA in the haddock during storage time which is in agreement with many other experiments. However the sensory results for the December and the May haddock behaved in a very similar way as can been seen in figure 11.

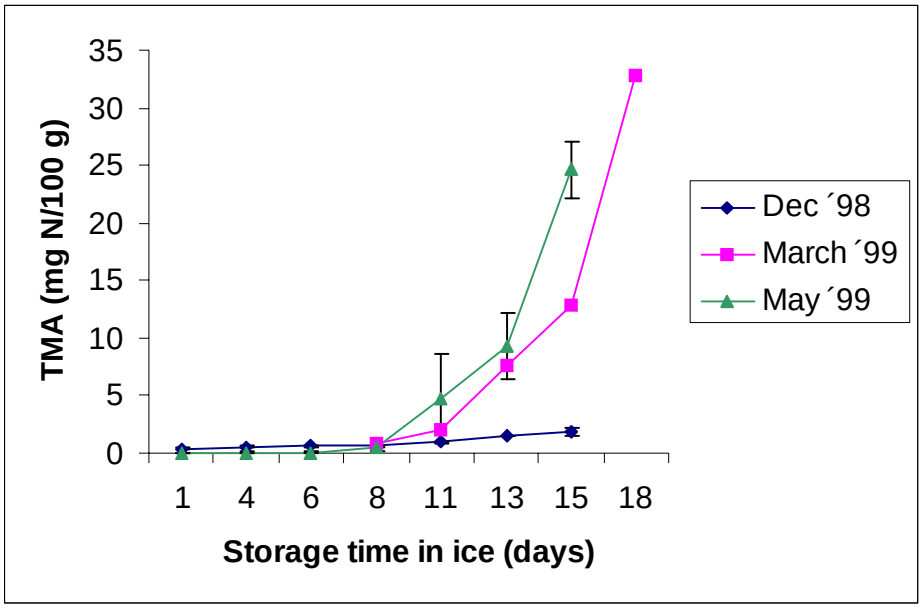


Fig. 10. *Comparison of TMA values for haddock, caught in December ´98, March ´99 and May ´99, during storage of the whole, gutted fish.*

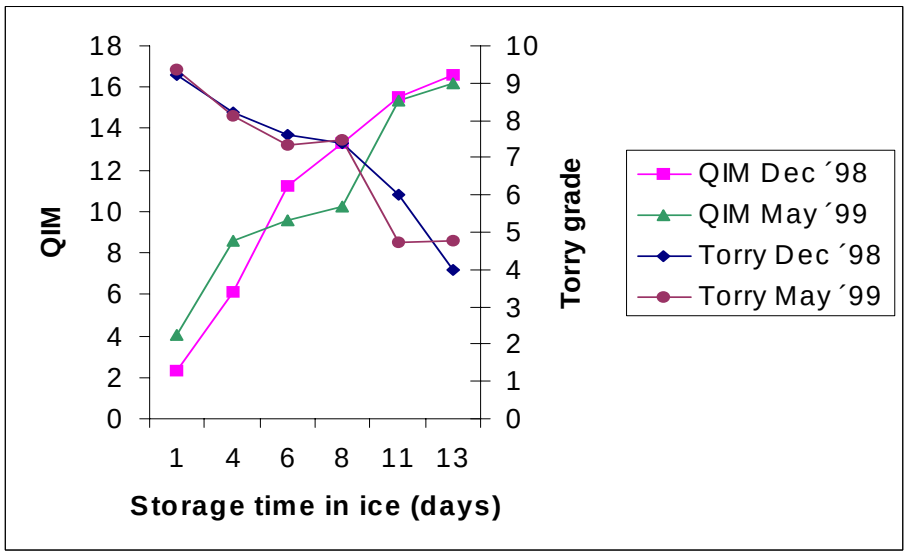


Fig. 11. *Sensory evaluation of haddock, by QI method and Torry grades, during storage of the whole, gutted fish.*

Figure 12 shows the results from the microbial counts. A similar experiment was done on haddock in May '99 and the comparison is well demonstrated on the graph. Total viable

counts (TVC, 15°C) of the skin was found to be lower for the species during the winter period when compared to the spring trial. This could be due to lower initial bacterial load on the fish at that time of the year and little temperature abuse during handling on board because of low environmental temperatures compared to the spring period. Nevertheless, TVC of the skin was close to log 7 CFU/cm^2 at sensory rejection during all trials. Interestingly, the *Pseudomonas* counts were higher than H_2S-producer counts during the winter trials while the contrary was observed during the spring experiments. H_2S-producers and *Pseudomonas* spp. are generally considered to be fish spoilage bacteria, but were found to occur at rather low levels in the flesh of the spoiled fish assessed, i.e. making up 0.5 to 19% of the final bacterial load. The low production of TMA in the December experiment, compared to the March and May experiment, might be explained by the low proportion of H_2S- producing bacteria in the flesh as these bacteria are known to be TMA producers.

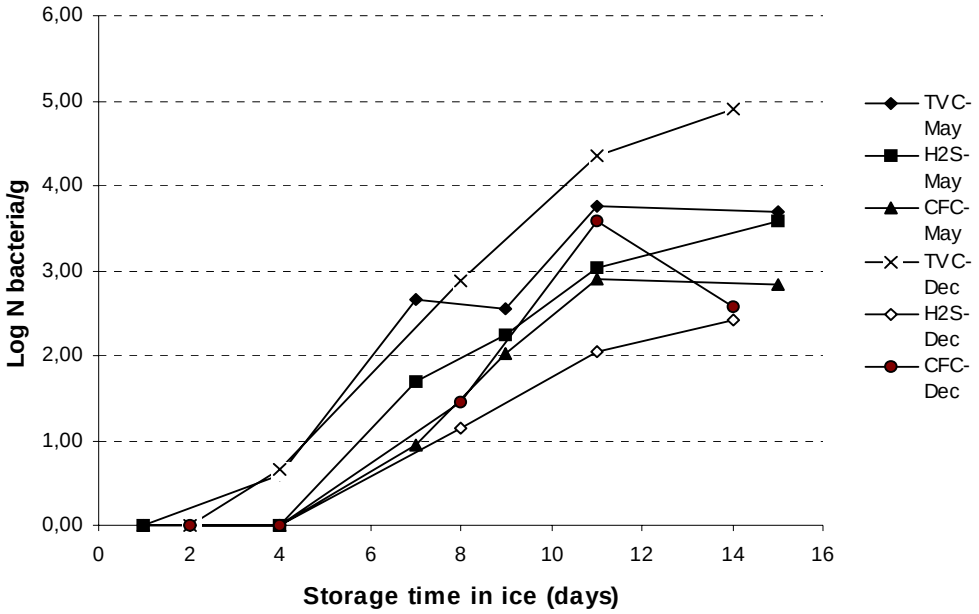


Fig. 12. *Growth of bacteria from skin on plate count agar (TVC), iron agar (H_2S) and CFC medium during iced storage of haddock.*

Figure 13 show that the TMA and TVB in the haddock eyes increased in similar way as the TMA and TVB in the haddock fillets. The main difference was though that the values were much lower in the eyes for the same sampling day, just as was observed for the cod eyes in figure 7.

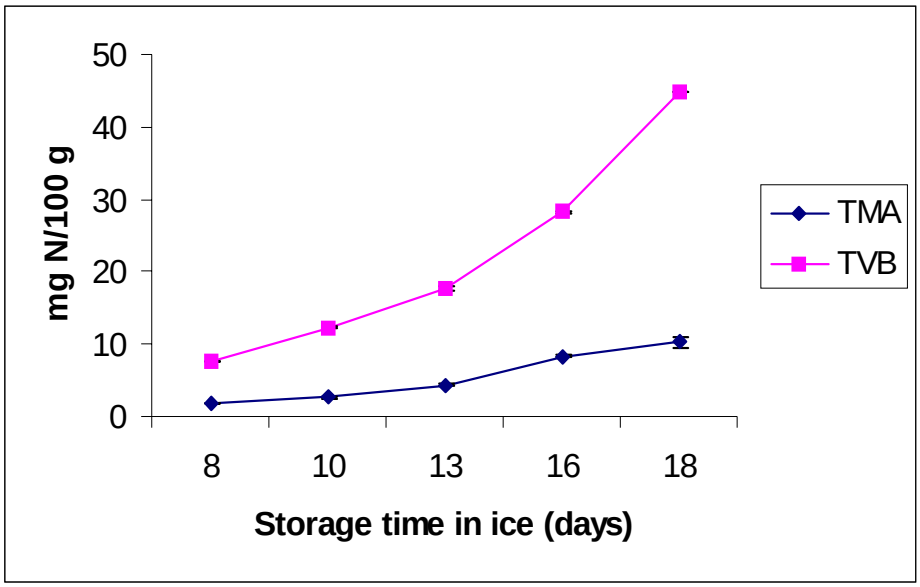


Fig. 13. *Changes in TMA and TVB values in haddock eyes, during storage in ice of the whole, gutted fish, caught in March 1999.*

3.3. Herring (*Clupea harengus harengus*)

The TMA and TVB results for herring stored in ice at 0°C is shown in figure 14. Figures 15 and 16 are extracted from the tables and they show the best linear fit between the different methods to determine TMA and TVB. The correlation between the WEFTA Codex method and FIGD to determine TVB at is rather poor (R^2=0,931). The sensory limits for ungutted herring stored in ice is normally concidered 7-9 days. As figure 14 demonstrates the TVB has only reached 17 mgN/100g at day 7, when determined by FIGD, but 38 mg/100g when measured by WEFTA Codex method but the correlation is better after that. The results of TMA in herring measured by the FIGD technique were very similar to the results of the other methods.

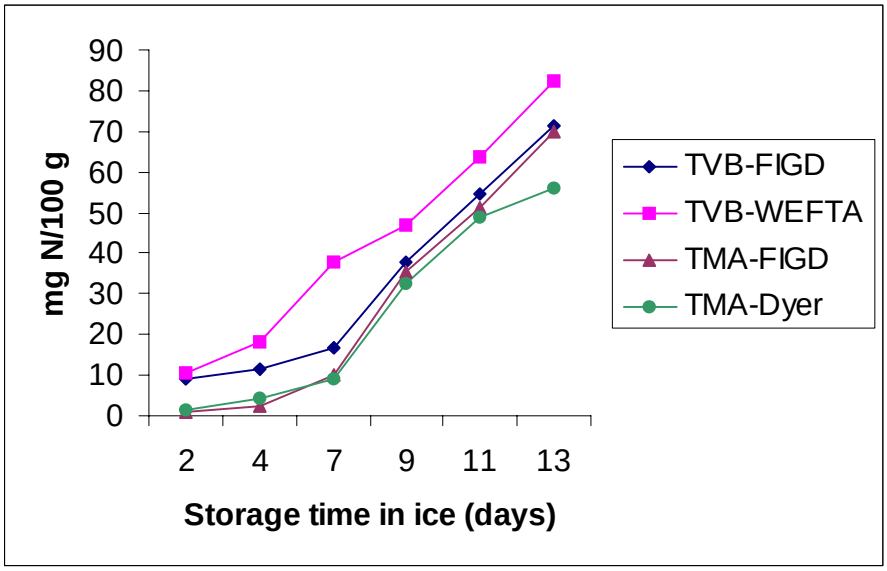


Fig. 14. *Changes in TMA and TVB in herring during storage in ice, using different analysis methods.*

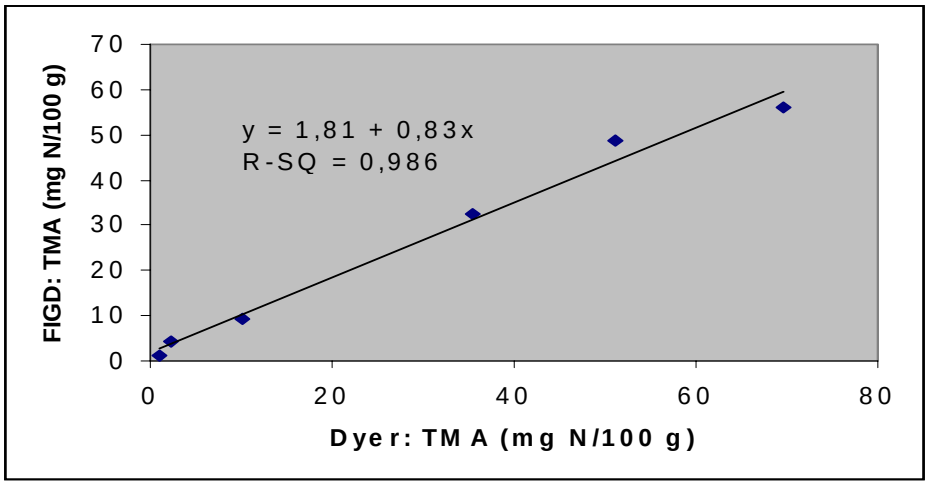


Fig. 15. *Regression analysis for TMA in herring during storage in ice: FIGD against Dyer.*

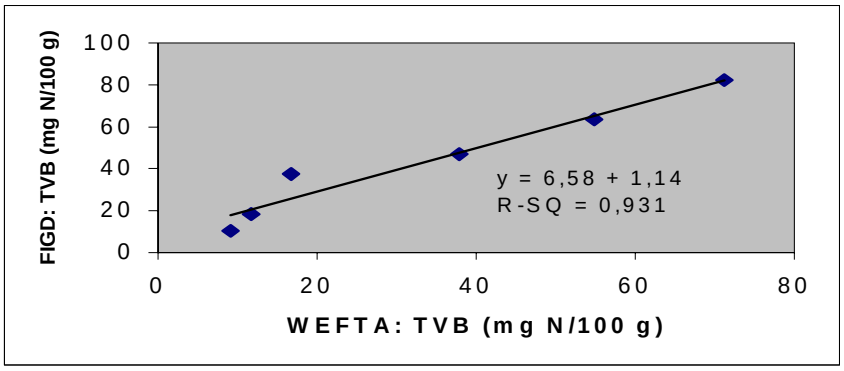


Fig. 16. *Regression analysis for TVB in herring during storage in ice: FIGD against WEFTA Codex.*

3.4. Northern shrimp (*Pandalus borealis*)

Figures 17, 18 and 19 show the TMA and TVB results from storage experiments of shrimp that was caught NW of Iceland in August and September 1999. The Quality Index scores go from 0 for the freshest shrimp, up to 11 for the most spoiled one. It was observed that at the 5^{th} storage day the shrimp had lost all of its freshness characteristics and some spoilage indicators, such as faint ammonia odor had begun to appear. The TMA value at that time was however still low (1,3 mgN/100 g) but TVB was at the rejection limit for seafood or 35 mg N/100g. There was a good correlation between the QI and storage time (R^2 = 0,987) but the TMA Index in the September experiment gave also a satisfactory results (R^2 = 0,956).

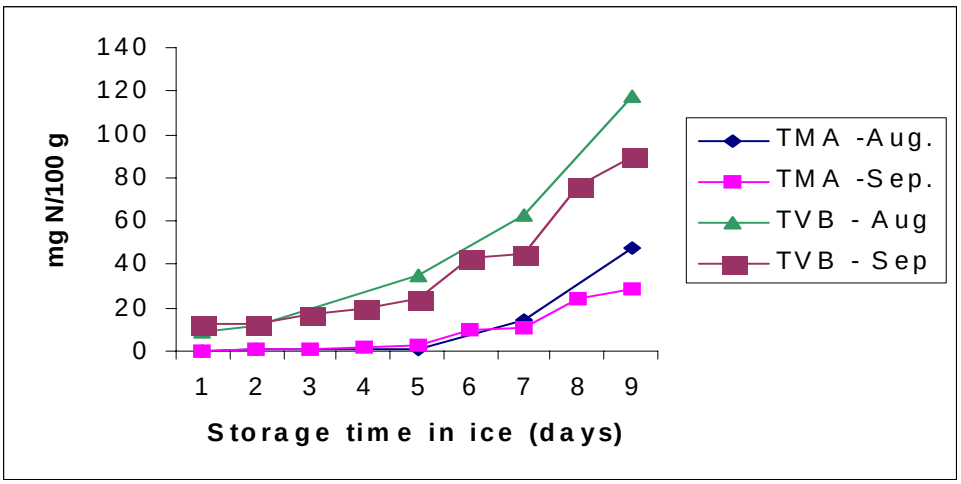


Fig. 17. *Changes in TMA and TVB during storage of shrimp in ice, caught in August and September 1999.*

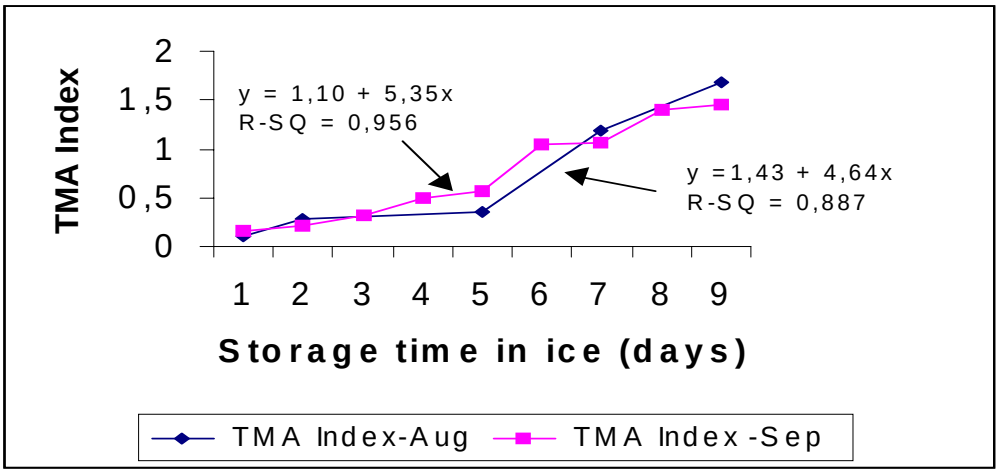


Fig. 18. *TMA Index ($Log_{10}(1+TMA)$) for shrimp, caught in August and September 1999, during storage in ice.*

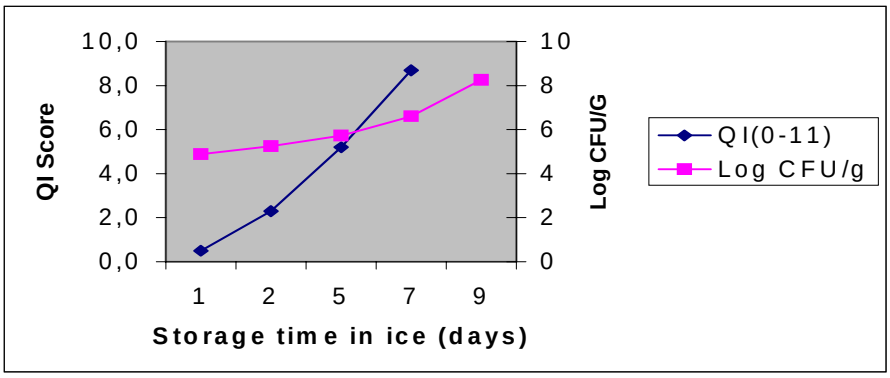


Fig. 19. *Quality Index (0-11) and Total Viable Counts (as Log_{10} CFU/g) during storage of shrimp in ice that was caught in August 1999.*

Sub-task 2.i.

A few attempts were done to evaluate the possibility to use exudates from fish samples to determine TMA and TVB instead of using the extraction procedure to extract the volatile compounds out of the fish muscle into the trichloroacetic acid (TCA) extraction medium.

The procedure to get a transparent and clean exudate from the fish muscle has not yet been investigated enough. The method to obtained the exudate by pressure was tested but that method was not very successful because the exudate was rather opaque and seemed to contain some tissue particles that had to be removed in order to be able to inject the sample. This was especially the case when the fish was of high post-mortem The sample had to be centrifuged at a high speed in order to get it transparent but that was of course an additional step in the procedure. The clear sample of c.a. 0,5 ml that was obtained was injected in the injection valve but the TMA and TVB results calculated from the peaks on the chart recorder were not representative to the same TCA extraction samples nor the decompose indices (QIM and Torry score). This was tried for a few samples of haddock at a different stages of spoilage but without success. However, more tests must be done to find out if some other methods, to get the exudates from the fish, can be used and to find out if other fish species give better and more representative results than the haddock.

Sub-task 3.i.

Even though this sub-task has not been the on the work-plan during this period the first steps have been taken to produce a new FIGD equipment that will be ready for the industrial partner in due time. The new FIGD will be ready by the end of November 1999 and will have a few advantages over the currently used FIGD.

1. The new FIGD is equipped with a micro-processor (micro-computer) that has the ability to calculate the signals from the injected standards and the samples and display the values on a small screen within the unit.
2. It can easily be connected to a computer with a common program such an Excel if there will be a need for further processing of the results.
3. The use of chart recorder is not essential.
4. It is more compacted than the old one and it can easily be transferred between places because it is fit up in a sturdy but a handy briefcase.

4. CONCLUSIONS

TMA measured by the FIGD method and the WEFTA Codex method gave similar result for all species (R^2 > 0,96). TVB measured by the FIGD method gave at average 60% lower results than if measured by the Dyer method for all species.

TMA and TVB values in the cod and haddock eyes were lower than the in the fillets but showed a similar exponential pattern.

The detection limit for TMA and TVB by the FIGD method is 0,1 mg N/100 g and spiked samples gave at average 99,9 % recovery.

The advantages of the FIGD method:

- ✓ It was faster and easier to use than comparable methods.
- ✓ It only required 0,1 ml of extractable sample.
- ✓ The FIGD equipment is inexpensive (<3000 EURO).

The disadvantages of the FIGD method:

- ✓ The extraction process and the dilutions of "strong" extracts were limiting factors.
- ✓ The TMA baseline was not as stable as the TVB baseline.
- ✓ The FIGD- TVB method gave about 60% lower values than the WEFTA Codex method.

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APPENDIX

Fresshness score sheet for iced cod and haddock

cooked fish (Torry score)

score	Odour	Flavour
10	Initially weak odour of sweet, boiled milk, starchy followed by strenghthening of these odours.	Watery, metallic, starchy. Initially no sweetness but meaty flavours with slight sweetness may develop.
9	Shellfish, seaweed, boiled meat,	Sweet, meaty characteristic.
8	Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity.
7	Woodshavings, woodsap, vanillin	Neutral
6	Condensed milk, boiled potato	Insipid
5	Milk jug odours boiled clothes- like	Slight sourness, trace of "off"-flavours
4	Lactic acid, sour milk TMA	Slight bitterness, sour, "off"-flavours, TMA
3	Lower fatty acids (eg acetic or butyric acids) composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide

Quality (QIM) parameter for <u>cod</u> and <u>haddock</u>		**Description**	**Score**
Appearance	Skin	Bright, iridiscent pigmentation	0
		Rather dull, becoming discoloured	1
		Dull	2
	Stiffness	In rigor	0
		Firm, elastic	1
		Soft,	2
		Very soft	3
Eyes	Cornea	Clear	0
		Opalescent	1
		Milky	2
	Form	Conwex	0
		Flat, slightly sunken	1
		Sunken, concave	2
	Colour of pupil	Black	0
		Opaque	1
		Gray	2
Gills	Colour	Bright	0
		Less coloured, becoming discoloured	1
		Discoloured, brown spots	2
		Brown, discoloured	3
	Smell	Fresh. seeaweedy, metallic	0
		Neutral, grassy, musty	1

		Yeast, bread, beer, sour milk	2
		Acetic acid, sulphuric, very sour	3
	Mucus	Clear	0
		Milky	1
		Milky, dark, opaque	2
Fillets	Colour	Translucent, bluish	0
		Waxy, milky	1
		Opaque, yellow,brown spots	2
Blood	Colour	Red	0
		Dark red	1
		Brown	2
Quality index 0 - 23			**0 - 23**

Quality Index Method (QIM) schemes for whole shrimp

Quality param.	**Description**	**Score**
Dark in the head	None	0
	Some (25%)	1
	Many (50-75%)	2
	All (75-100%)	3
Color	Pink / red	0
	Light Pink	1
	Yellowish	2
	Yellow , greenish, grayish discolouration	3
Roe color	Copper green	0
	Discoloured, faded	1
	Dark	2
Odor	Fresh, seaweady	0
	Faint odor, reminding of tar,	1
	Faint ammonia odor	2
	Obvious ammonia odor,sour, putrid	3
Quality index		**0- 11**

PROGRESS REPORT FROM THE ICELANDIC FISHERIES LABORATORIES
Progress Report 3: 01. 10 1999 – 31. 12. 2000.

SUMMARY

In the 3rd and final period of this project, Qualpoiss 2 (FAIR PL. 97.3253), two comprehensive storage experiments were conducted on cod in co-operation with another FAIR project called Mustec (FAIR CT98-4076). The experiments took place in Reykjavík in November 1999 and in Tromsö in May 2000. TMA and TVB were measured with the FIGD technique and the freshness was also determined using other non-destructive methods such as measuring the electronic properties by RT-Meter. The TMA/TVB results were similar in both experiments where the values remained very low up to day 11 of post mortem age. The RT-meter readings showed better linearity with storage time. Storage experiments of iced whole gutted haddock and haddock fillets were also done in order to see if there was a difference in TMA and TVB, measured by FIGD, between the fillets and the whole haddock. The fillets spoiled faster and showed much higher TMA and TVB values at the end of the experiments (15-18 days) due to better access of bacteria to the fish muscle in the fillets. Major part of this period was reserved for development of improved version of FIGD. The production of the first IFL-FIGD was finished in September 2000 and it was introduced to the other laboratories participating in Qualpoiss 2 and Musctec and to a few fish processing plants in Iceland. The IFL-FIGD version contains microprocessor that automatically calculates the signal from the detector and dispay results in mgN/100g on a small screen. The chart recorder was therefore not necessary and the FIGD is compacted in a small easy-to-carry case. Even though the IFL-FIGD is easier to use than the original FIGD it is still not convenient enough for the industry and can not distinguish between the freshness stage of the fish for the first week or more of storage of whole gutted cod or haddock in ice. The FIGD technique is though beneficial to the fish research laboratories and can replace more inconvenient techniques to determine TMA and TVB, as has taken place at IFL.

1. INTRODUCTION

The IFL tasks for the final year, that covered the period from October 1999 through December 2000, were divided into 4 periods, A, B, C and D.

A. In the first quarter of the period (October – December 1999) the objectives were:

I. To do comprehensive experiment, in November 1999 in Reykjavik, on cod during storage in ice by determining TMA, TVB, using the FIGD-technique and other more commonly used methods. The pH of the fish tissue was also determined and the P-ratio was calculated. Parallel to these determinations co-operation with another EU-project called by short name "Mustec"(FAIR CT98-407) was carried out by using non-destructive methods to evaluate the freshness of the cod during storage. These measurements involved for example determination of the texture, odour by an electronic nose, electrical properties of the skin by Torry-meter, Fish-tester and RT-Meter and different properties of the fish -tissue against light. Results from all these non-destructive methods and the simple and rapid FIGD-method would give a very comprehensive indication of the freshness stage of the fish. The usefulness of the FIGD method could also be evaluated when it was compared with so many different methods to determine freshness of cod.

II. To develop a simpler and more reliable FIGD-equipment in order to have portable and more automatic equipment that will be adequate for the industry.

B. The IFL task for the second quarterly report (January to March 2000) period of this project was divided into three sub-tasks:

I. To observe if there was any difference between two different FIGD detectors. One was supplied by the co-ordinator (IFHQ-Hull) at the beginning of the project. The other was handed out by the co-ordinator at the meeting in Portugal in November 1999.

II. To compare the recovery of TMA and TVB standards TMA-HCl and NH_4Cl respectively) using the FIGD technique and steam distillation of TCA extract.

III. To carry on with the development of a new and improved type of FIGD equipment.

C. The IFL task for the third quarterly report (April to June 2000) period of this project was divided into three sub-tasks:

I. To host and organize a plenary meeting in May (10 and 11th) at IFL in Reykjavik, Iceland, which included a half day work-in where the different FIGD detectors were compared and the procedure to determine TMA and TVB was exercised.

II. To carry out two storage experiments on haddock fillets and whole haddock stored in ice in order to find out the difference in TMA and TVB using FIGD technology.

III. To participate in a storage experiment in Tromsö Norway in May 23-31st by using FIGD to determine TMA, TMAO and TVB in fresh and thawed cod and fresh-farmed salmon in collaboration with the "Mustec" FAIR CT98-4076 project.

IV. To carry on with the development of a new and improved type of FIGD equipment.

D. The IFL task for the forth-quarterly report (July to December 2000) period of this project was divided into three sub-tasks:

I. To finish the development of the IFL version of FIGD equipment.

II. To participate in the final plenary and results collation meeting in UB-Barcelona.

III. To introduce the FIGD technique, with emphasis on the IFL version, to other IFL laboratories, fish processing plants in Iceland, the members of the Mustec project participants and members of Firskeri Forskning in Tromsö Norway.

2. MATERIALS AND METHODS

2.1 Experiments

A I. Experiment. The IFL participant, in collaboration with the Mustec group, carried out a storage experiment in Reykjavik on cod stored in ice. TMA and TVB determinations were done on the fillets tissue of the cod with the Flow Injection Gas Diffusion Method (FIGD). Parallel to this the IFL participant determined pH and the freshness score according to the electronic properties of the skin with the RT-meter.

The cod was caught in November 1999 in fishing grounds SW of Iceland. It was brought bled, gutted and iced into 90L boxes on board and brought ashore a few hours after catch. The fish was kept for storage trial at 0 to 1°C. Samples of the fillets and were taken at 2-3 days interval during the storage period. On each day of sampling 8 fillets of 8 fish were examined. Pooled sample of the eyes was taken.

CII. Experiment. Fresh haddock fillets, of 2 days post mortem age, were stored in ice in insulated plastic boxes for up to 14 days at 2°C in May 2000. For comparison, a storage experiment was done on whole gutted haddock that was stored in ice. Thawed cod fillets, that were frozen one year earlier, were stored un-iced at 2°C for up to 12 days. Samples were taken with 2-3 days interval and the TMA and TVB were measured.

CIII. Experiment. Fresh cod was caught alive in N-Norway fishing ground and kept alive but starved in a cage for 3 weeks before it was slaughtered for the experiment. The thawed cod got same treatment but it was slaughtered and frozen on April 6th 2000 and was thus stored frozen for 6 weeks. It was thawed in air for two days before the different freshness (spoilage) indicators were determined. The fish was frozen at – 40°C for 1 day and kept in freezer at –30°C after that. Fresh-farmed salmon was slaughtered after 3 weeks starving period.

2.2. Chemical analysis

TVB-N was measured with two different methods:

a) Flow Injection Gas Diffusion (FIGD) as descried in the technical annex.

b) Steam distillation of TCA extract as descried in the technical annex with some modifications that was agreed on during discussion on the Peterhead meeting in July. One hundred grams of fish were mixed with 200 ml of trichloroacetic acid (TCA). Twenty-five ml of the TCA extract, 6 ml of Noah (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 distillatory) 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.

TMA-N was also measured using two methods:

c) Flow Injection Gas Diffusion (FIGD) as descried in the technical annex.

FIGD technique:

Reagents: All Chemicals were p.a. Sodium Hydroxide (M): 1,0; Bromothymol blue pH 6,50 (g/L): 0,3; Formaldehyde: 20%; Trichloroacetic acid (Merck), Ammonium chloride (NH_4Cl) for TVB determination and TMA crystalline HCl standard for TMA determination.

Flow rate (ml/min): 1,0

Mixing coil (cm): 15

d) Dyer method except KOH was used instead of K_2CO_3 because it blocked better the primary and secondary amines. (AOAC, 15th edit.1990).

2.3. Sensory evaluation

On each sampling day 10-12 members of trained panel evaluated the fish. Each panel member evaluated 5 fish according to the Quality Index Method.

2.4. Physical measurements

The RT-Fresh meter measures the electrical resistance of the fish-skin. The values will decrease with time of storage of post-mortem fish. The RT-meter was used by the IFL participant but the Torrymeter and the Fish-Testers were used by the Scottish and The German participants of the Mustec project respectively.

3. RESULTS AND DISCUSSION

AI.

Experiment

Figures 1 shows the changes in TMA and TVB during storage of cod fillets in ice. The TVB values were determined by the FIGD and the WEFTA Codex method and as has been demonstrated earlier (Periodic reports 1-3 1999) the TVB values measured by the FIGD method gives considerable lower values than obtained when TVB was measured by the WEFTA Codex method. This is especially the case when the "white fish" species, cod and haddock, were measured but the difference between methods does not seem to be as much when TVB was measured on shrimp and haddock.

The evolution of pH and the calculated P-ratio is shown in figure 2. The pH is under 7 for the first 4 days but over the neutral 7 at day 7 and levels off after that. In the case of the pH it was an indicator of the very fresh fish for the first 4 days but there were no measurements done on the fillets between day 4 and 7. However it could be stated that at day 7 the fish has lost its major freshness characteristics and the rise in pH was probably due to breakdown products from *Pseudomanas* bacteria. The P-ratio acts in a similar way as the TMA curve, stays very low from day 1 until day 11 but there is a gap between day 11 and 15 so it can not been confirmed if the low values would have continued up to day

14 but when the TMA and P-ratio were measured at day 15 the values had risen significantly.

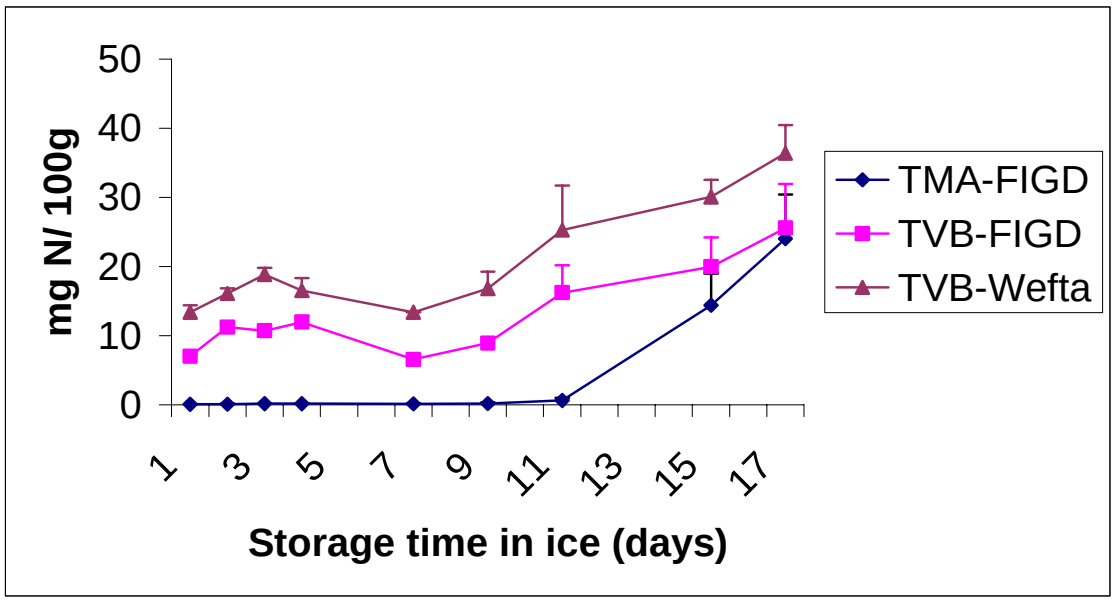

Fig. 1. *Determination of TMA and TVB (using two different methods) during storage of cod in ice.*

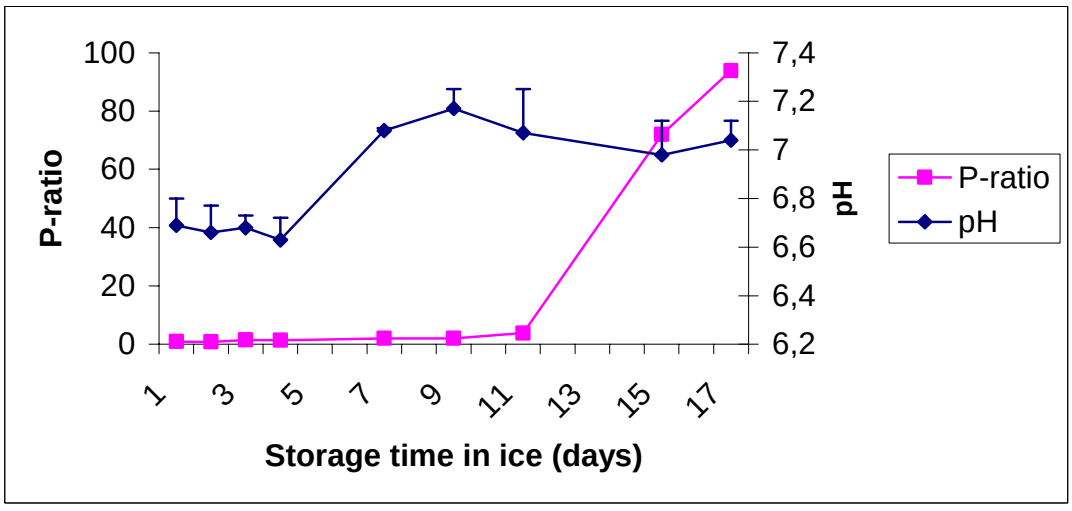

Fig. 2. *Determination of P-ratio ((TMA/TVB) 100)) during storage of cod in ice.*

Sensory analysis and RT-meter

The results from the sensory analysis that was done on the cod by the members of the Mustec group are shown in figure 3. There was a good correlation between storage time and QIM scores where R^2 was 0,982 and QIM score = 0,707 + 1,00(days in ice) and the standard deviation was low. The score range was from 0 (for the freshest fish to 20 (for the most decomposed fish).

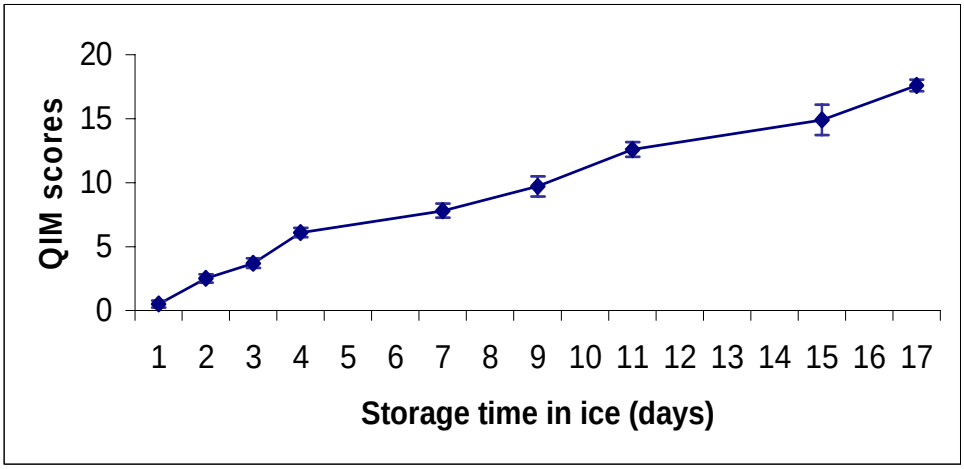

Fig. 3. *Sensory analysis of cod evaluated by the QIM method (0 is best, 20 is worst)*

Comparison of three different instruments to measure electrical properties with the RT-Freshness Grader, the Torrymeter and the Intelectron Fishtester was done on the cod skin during storage in ice. The results are shown in figure 4. It was observed that all the results showed a similar correlation with time. The Icelandic RT-Grader had
R^2 = 0,971, the Torrymeter had R^2 = 0,965 and the Fishtester had R^2 = 0,969. The standard deviation was lowest in the RT-Grader but highest in the Fishtester. All these instrument are very fast and reliable but only for whole fresh fish but they were could not be used after the fish was filleted.

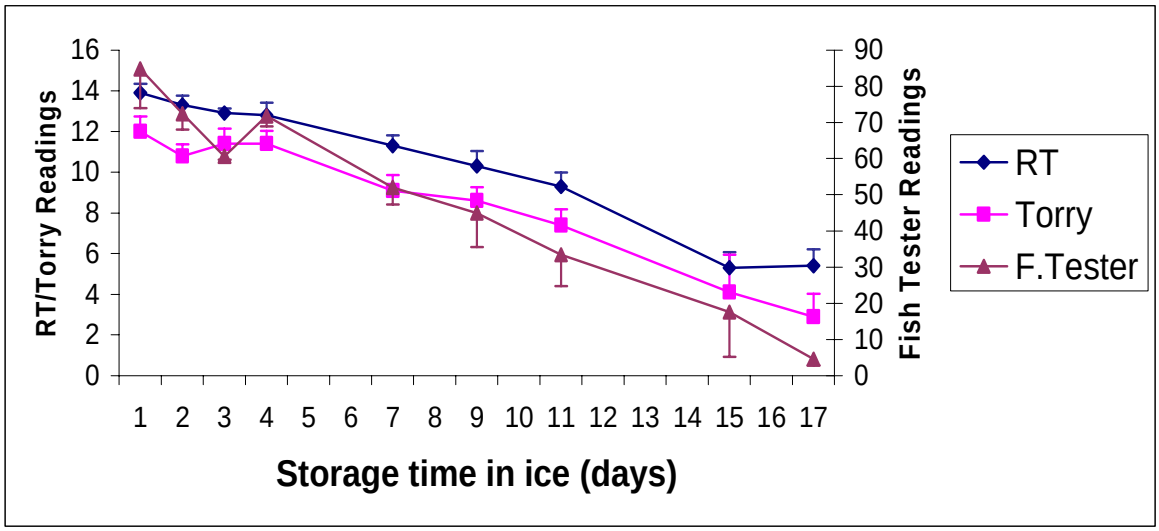

Fig. 4. *Comparison of three physical instuments, RT Meter, Torry Meter and Fish-Tester, to determine the freshness score of "Reykjavik" cod stored in ice by measuring the electrical properties of the skin*

BI.

Comparison of two FIGD boxes

Table 1 shows the TMA (TMA-HCl) and TVB (NH_4Cl) standard solutions using the two FIGD light diodes boxes (equipments). The voltage sensitivity used for TVB determinations was the same for both boxes and the peak heights were very similar. For TMA the sensitivity for the box 1 (old box) was more than for box 2 (new box) in order to get similar peak heights for both of the boxes. That caused more disturbances in the baseline on the chart recorder by using box 1 rather than box 2 and therefore it was more difficult to determine low values of TMA using box 1 due to more fluctuation of the baseline. However the results from the storage experiment on fresh haddock and frozen cod fillets did not reveal any significant difference between the two FIGD boxes, as is demonstrated in table 2. It is clearly demonstrated in figures 5 and 6 where the regression lines and the correlation coefficients are excellent.

It was interesting to see the great jump in TMA and TVB values between post mortem age 7 and 8, where TMA and TVB values are close to 0,3 and 8 mg N/100g at day 7 respectively compared to 25 and 30 mg N/100g fro TMA and TVB. A similar rise was observed in fresh, whole, gutted haddock stored in ice but that shift took place between day 11 and 13 (se periodic report 3-99. According to this the shelf life of haddock fillets stored in ice are 4-5 days shorter than for haddock stored gutted but unfilleted.

Table 1. Comparison of two FIGD boxes on the TVB and TMA standard solutions

TVB	**NH_4Cl std.**	**Voltages**	**NH_4Cl std.**	**Voltages**
	Box 1 (old)	**0,2x1/10 V**	Box 2 (new)	**0,2x1/10 V**
Std. (uM/L)	**Average (mm)**	**mm/uM l^{-1}**	**Average (mm)**	**mm/uM l^{-1}**
500	**313**	0,626	**290**	0,580
200	**124**	0,62	**118**	0,590
100	**62,5**	0,625	**59**	0,590
50	**31**	0,62	**30**	0,600
0	**0**	0	**0**	0,000
Average (mm/uM)		0,62275		0,590
TMA	**TMA-HCl std.**	**Voltages**	**TMA-HCl std.**	**Voltages**
	Box 1 (old)	**50mVx1/10 V**	Box 2 (new)	**0,1x1/10 V**
Std. (uM/L)	**Average (mm)**	(mm/uM)	**Average (mm)**	(mm/uM)
200	**115**	0,575	**153**	0,765
150	**84,5**	0,563	**112**	0,747
100	**60**	0,600	**68**	0,68
50	**30**	0,600	**29**	0,58
0	**0**	0	**0**	0
Average (mm/uM)		0,585		0,74

Table 2. TMA and TVB in fresh haddock fillets and thawed cod fillets during storage

Haddock Cod	Box 1 (old) TMA mgN/100g	Box2 (new) TMA mgN/100g	Box 1 (old) TVB mgN/100g	Box2 (new) TVB mgN/100g
Day 2a	0,04	0,03	4,96	4,91
Day 2b	0,03	0,02	5,69	4,79
Day 5c	0,16	0,17	6,24	5,79
Day 5d	0,06	0,06	5,65	5,07
Day 7e	0,21	0,25	6,78	6,48
Day 7f	0,33	0,32	8,4	8,25
Day 8g	26,4	25,74	30,1	29,56
Day 9h	27,3	28,26	33,28	32,89
Day 9i	22,09	23,14	30,37	30,14
Day 12j	40,82	39,85	50,72	50,18
Day 12k	38,54	36,75	47,49	47,65
Day 14l	39,55	40,24	56,5	66,85
Day 14m	40,56	40,25	56,5	54,28
Day 2 Frozen	0,12	0,14	8,78	7,84
Day 5 Frozen	0,21	0,14	8,85	7,74
Day 8 Frozen	14,7	14,81	28,25	27,56
Day 12 Frozen	59,77	57,14	79,46	77,14

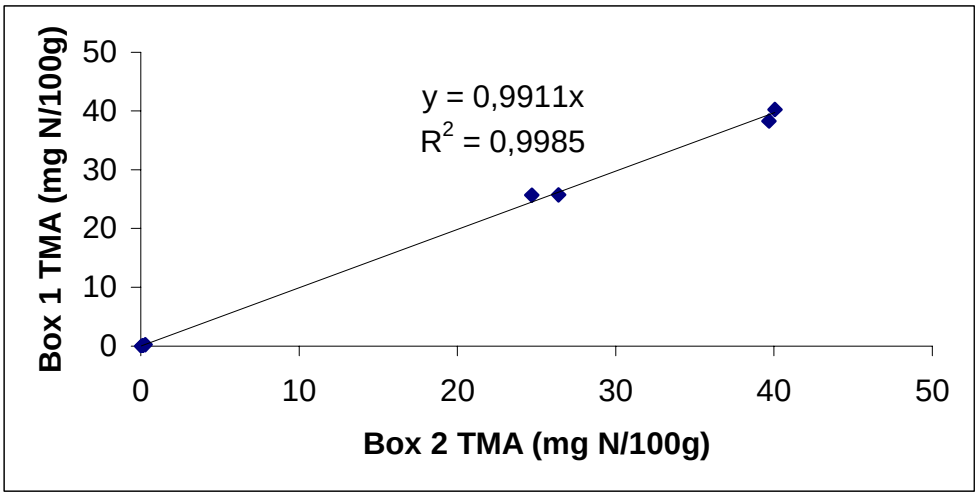

Fig. 5. *The least squares regression line and the correlation coefficient for the two FIGD detectors (boxes) for TMA in haddock fillets given in table 2.*

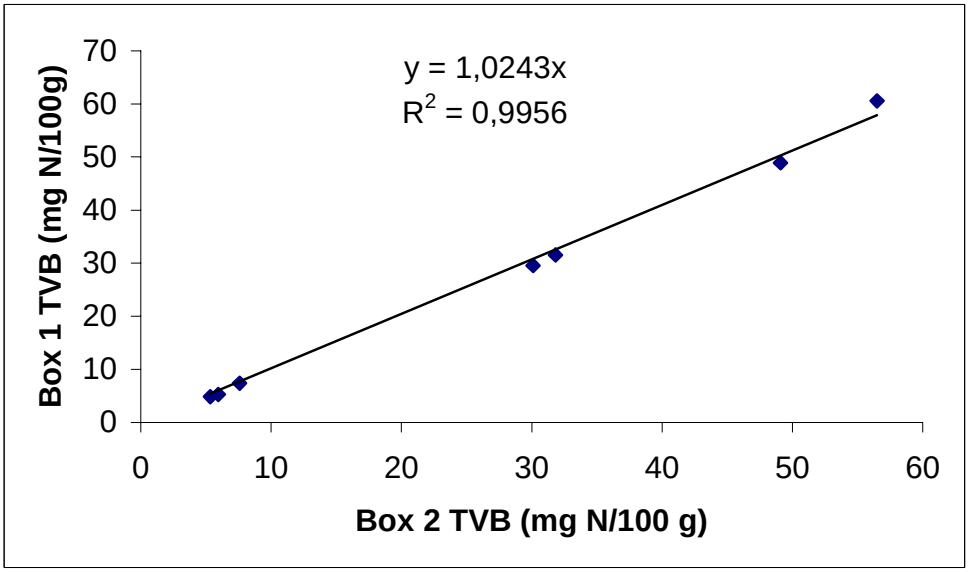

Fig. 6. *The least squares regression line and the correlation coefficient for the two FIGD detectors (boxes) for TVB in haddock fillets given in table 2.*

BII.

Recovery tests

As table 3 shows the recovery of TMA-HCl standard is very different from one sample to the other, ranging from 64% to 98% in different samples of fish, using the two FIGD boxes. The TMA standard used was high in concentration or about 5000 uM/l, which was probably too concentrated to be recovered fully through the extraction process. Similar results are observed when the NH_4Cl standard, which represent the TVB, was tested for recovery, as can be observed in table 4, and there was no statistical difference between the FIGD and steam distillation in regards to the recovery. Therefore it will be the next task on recovery experiments to use weaker standard solutions and to check and standardize the extraction procedure.

Table 3. Recovery of TMA-HCl standard through the extraction procedure with different fish samples

Recovery	**TMA (uM TMA-HCl /L)**				
	Box 1 Catfish	**Box 1 Herring A**	**Box 1 Shrimp A**	**Box 1 Herring B**	**Box 1 Shrimp B**
Sample (uM TMA/L)	2636	17724	767	84	55
Spiked sample (uM TMA/L)	6873	22618	5178	3262	3171
Standard (uM TMA/L)	5000	5000	5000	4420	4420
Recovery (%)	**84,7**	**97,9**	**88,2**	**71,9**	**70,5**
	Box 2 Haddock A	**Box 2 Haddock B**	**Box 2 Herring B**	**Box 2 Shrimp B**	**Box 2 Haddock C**
Sample (uM TMA/L)	13	53	789	335	25
Spiked sample (uM TMA/L)	3621	3539	3684	4015	3511
Standard (uM TMA/L)	4420	4785	4785	4785	4420
Recovery (%)	**81,64**	**69,7**	**57,9**	**73,6**	**69,7**

Table 4. Recovery of NH_4Cl standard through the extraction procedure with different fish samples

TVB	NH_4Cl (5000uM/L)				
		FIGD	Recovery (%)	Distillation	Recovery (%)
Haddock	Unspiked	3179	62,7	5600	67,2
	Spiked	6333		8960	
Herring	Unspiked	3941	65,9	6048	123,2
	Spiked	7238		12208	
Shrimp	Unspiked	3177	94,1	3752	69,4
	Spiked	7881		7224	

CI. Plenary meeting in Reykjavik – comparison of FIGD detectors

Comparison was done on FIGD detectors from different participants, where every other step was identical, e.g. the same extraction procedure, the same standard, and tubings length, mixing coils, pump, pumping speed and chart recorder. Table 5 shows the difference of FIGD detectors from each participant. The highest peak was from the original IFL-Icelandic detector (box 1) and the lowest peak were from EB-Spain. The IFL detector gave 110 times higher peaks than the EB detector but the next lowest peaked under the same conditions came from IPIMAR in Portugal, where the peaks were 6,5 times lower than the IFL peaks. In spite of this difference there was no significant difference between the TVB results in 8 days post mortem old haddock fillets, even though the higher peaks standard FIGD detectors had tendency to give a little higher results than the lower FIGD detector peaks. There seems to be difference in the brightness of light diodes that results in difference sensitivity of the detectors, giving different peak sizes on the chart recorder

Table 5. **Comparison of TVB results in 8 days post mortem haddock and in NH_4Cl by using different FIGD detectors form each project partner**

TVB (mg N/100g)	IFL	IPIMAR	TEI	EB	AB
Voltage	200mVx1/10V	200mVx1/10V	200mVx1/10V	50mVx1/10V	200mVx1/10V
500 uM NH_4Cl (mm)	163	25	135	59	94
8 days haddock	30,1	28,8	29,0	27,8	29,2

CII. Storage experiments on Icelandic headed and gutted haddock and haddock fillets

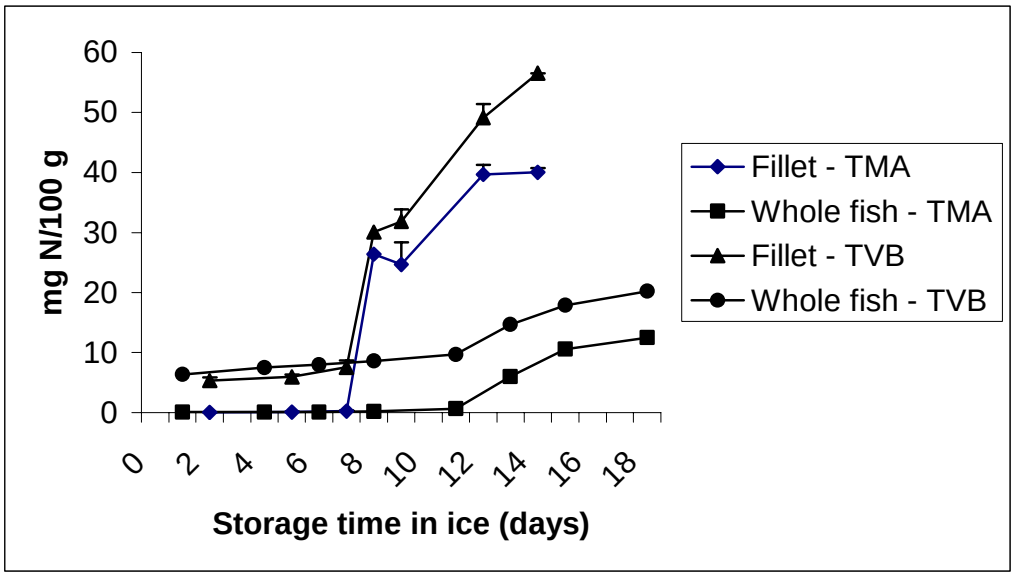

Fig. 7. *TMA and TVB in headed and gutted haddock (whole fish) and haddock fillets during storage in ice.*

Figure 7 shows that there was a vast difference between haddock stored headed and gutted (whole fish) and when the haddock muscle was stored as fillets with skin on. For both experiments the fish were stored in ice at 1°C. The whole fish does not show any increase in TMA or TVB until after day 11 post-mortem age. The TVB was still under 20 mgN/100 g and TMA under 10 at day 18 when the experiment ended. The fillets, on the other hand, demonstrate much higher values much sooner than the whole fish. TVB and TMA have reached 29 and 25 mg N/100 g respectively at day 8 and the TVB gets close to 60 mg N/100g at day 18 whereas the TMA almost touches the 40 mg N/100 g value. That great difference could be explained by the great surface area of the fillets, allowing the bacteria to penetrate the muscle much easily the in the whole fish where the muscle is much better protected from the environment.

CIII. Storage experiment on "Tromsö" cod and salmon

Table 6 and figure 8 show the TMA, TMAO, TVB and pH results for fresh cod during ice storage. The TMA and TVB patterns are similar as was shown in previous storage experiments and TMAO decreases during storage time as the TMAO is reduced to TMA when the bacteria population increases, but the *Shewanella putrefacience* responsible bacteria produce the enzyme that catalyse the reduction. The pH is about the same throughout the storage time and does not give any indication of the freshness quality of the fish. Figure 9 demonstrates that there is no significant difference between the FIGD and Dyer method to determine TMA.

Table 6. Development of TMA, TMAO and TVB during storage of fresh cod

Days	TMA	s.d.	TVB	s.d.	TMAO	s.d.	pH	s.d.
0	0,03	0	7,25	0,26	78,8	10,6	6,61	0,15
1	0,03	0	10,5	0,5	88,3	8,4	6,62	0,17
3	0,03	0	9,94	0	84,8	14,6	6,69	0,21
5	0,08	0	9,39	0,01	82,5	17,8	6,67	0,15
8	0,94	0,05	10,27	0,19	88,0	11,5	6,66	0,21
11	3,53	0,1	14,17	2,46	64,4	6,23	6,57	0,15
14	11,9	0,2	19,75	0,99	68,9	12,3	6,68	0,2
17	26,74	0,46	42,33	0,38	45,7	18	6,89	0,06

s.d. : standard deviation

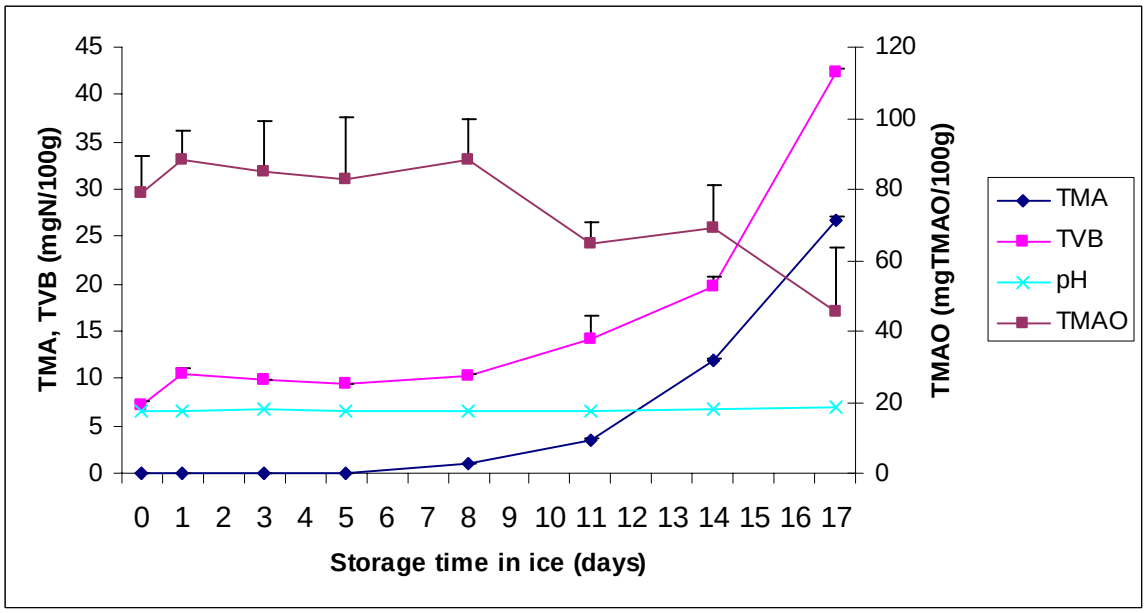

Fig. 8. *Development of TMA, TMAO and TVB during storage of fresh "Tromsö" cod*

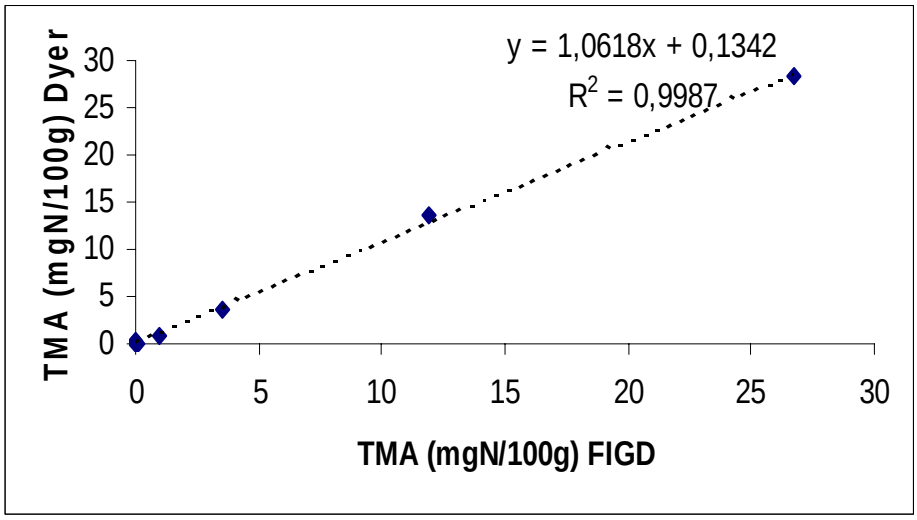

Fig. 9. *The least square regression line for different analytical methods (Dyer and FIGD) to determine TMA in "Tromsö" Cod.*

Table 7 shows the TMAO values in salmon is very low compared to fresh or frozen cod and most other seafood species. However it was surprising to see that the TMAO values for the fresh 1 day old post mortem salmon was lower than in the 13 day old and spoiled salmon. TMAO is though present in salmon since it lives in salt-water environments part of its life.

Table 7. Development of TMA, TMAO and TVB during storage of thawed cod and fresh salmon

SALMON

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

THAWED COD

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

Similar measurements as were done in the "Tromsö" cod as were done on the "Reykjavik" cod (see figure 4) using different hand-held instruments to measure the electrical properties of the fish skin or tissue. As figure 10 shows the electrical tester readings are almost linear function with days in ice as was the case for the "Reykjavik" cod the RT-Meter gives slightly better linearity than the other two testers. The electrical testers give much better linearity with days in ice than TMA and TVB, independent of which method is used, and the testers give immediate response. However the testers can only operate properly on fresh, unfrozen and undamaged fish but not on skinless fillets or thawed fish, whereas FIGD would be more reliable.

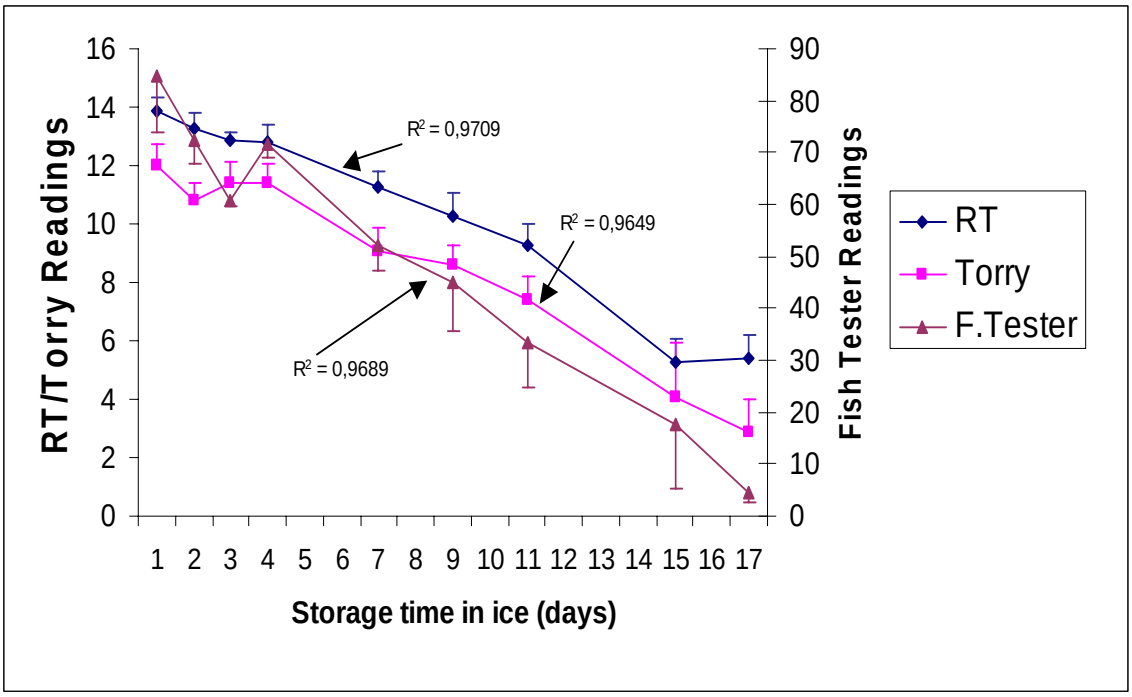

Fig. 10. *Comparison of three physical instuments, RT Meter, Torry Meter and Fish-Tester, to determine the freshness score of "Tromsö" cod stored in ice by measuring the electrical properties of the skin*

A II, BIII, CIV, DI. Development of new and compacted FIGD equipment

The design and production of a new FIGD device started in 1999. The aim was to make more user-friendly FIGD equipment where the use of chart recorder was unnecessary and it would be easy to carry it from one place to another. Early at the developing time it was decided to use an efficient microprocessor or microcomputer that would be able to store up to 100 data and calculate the signals from the standards and the samples immediately with a special Analogue-Digital program. The production of the first new FIGD is completed (figure 11). The values appear in the units of a choice (e.g. mg N/100g) on an easy-to-read digital screen. The results are very similar to the results from the old FIGD but the sensitivity of low TMA results could be determined more reliable where the detection limit is 0,03 mg N/100 g but not more then 0,1 mg N/100 g in the original FIGD equipment. The hardware is basically such that the original FIGD measuring technique with two optic-sensors. The signals are greatly magnified compared with special comparators. The start of each measurement the light intensity from the sensors-

sensors is stabilised and compared in the comparator until an exact 0 value or baseline appears. The light-intensity in the reference sensor is locked and it becomes therefore the reference value. At that stage the instrument is ready for analysis of samples (or standards) and measures the difference from the sensors and browse the measuring trail but at the end the top value of the measurement is displayed on the screen. That value is then used for further calculations in the program. The chart recorder can be excluded and the use of Excel program is possible for further calculations and storage of results.

The electronic part of the work with the new FIGD equipment was done by Stýring ehf., Háteigsvegur 7, 105 Reykjavik, c/o Gunnar Gudvardsson but otherwise the design and production was done at Icelandic Fisheries Laboratories by Sigurdur
Einarsson and Ovind Glömme. The case is made of PVC and stainless steel and the total weight of the case filled with all chemicals is 11,5 kg and the size is 23 x 39 x 44 cm (height x length x depth).

Fig. 11. *Photograph of the new compacted Icelandic version of FIGD (IFL-FIGD)*

DII. To participate in the final plenary and results collation meeting in UB-Barcelona.

The meeting was held in University of Barcelona (Departament de Nutrició i Bromatologia) September 14 and 15th 2000. It was decided to get a 3 months extention of the project in order to be able to introduce the technique to the industry in each country. It was also decided that the UB participant would prepare a blind samples of fish extracts sample in 7,5% Trichloroacetic acid solutions and hand it out to each participant at the end of the meeting. The IFL participant measured these samples in Iceland using both the original FIGD (HIFI-FIGD) and the new Icelandic FIGD (IFL-FIGD). The results (table 8 and figures 12 and 13) indicate no significant difference ($p<.05$) between the IFL and the HIFI FIGDs. Similar results were obtained when the samples from Morag (table 9 and figures 14 and 15) examined.

Table 8. TMA and TVB in unknown sample extracts, prepared by Sofia and Sonia in EB-Barcelona, using two different FIGD equipments, builds by HIFI in Hull and IFL in Reykjavik

Sample	*HIFI-FIGD* TMA mgN/100g	s.d.*	*IFL-FIGD* TMA mgN/100g	s.d.	*HIFI-FIGD* TVB mgN/100g	s.d.	*IFL-FIGD* TVB mgN/100g	s.d.
4	0,54	0,01	0,7	0,01	25,7	1,45	26,3	0,42
12	0,1	0,01	0,15	0,01	12,4	0,048	13,4	0,22
27	39,2	0,23	41,25	1,25	152,4	5,56	154,3	2,24
33	40,3	1,25	41,3	2,24	99,0	2,85	97,4	2,12
45	38,8	1,14	39,9	0,045	111,9	4,25	110,1	3,56

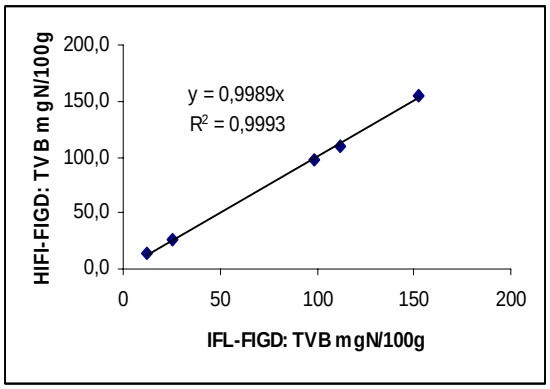

Fig. 12. *The least square regression line for TVB in EB-Barcelona samples using HIFI-FIGD vs. IFL-FIGD.*

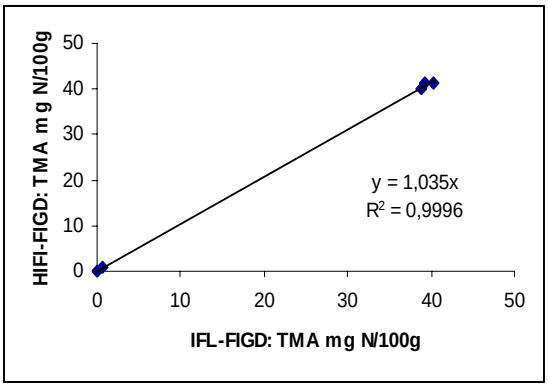

Fig. 13. *The least regression line for TMA in EB-Barcelona samples using HIFI-FIGD vs. IFL-FIGD*

Table 9. TMA and TVB in unknown sample extracts, prepared by Morag in AB-Peterhead, using two different FIGD equipments, build by HIFI in Hull and IFL in Reykjavik

Sample	HIFI-FIGD TMA mgN/100g	s.d.*	IFL-FIGD TMA mgN/100g	s.d.	HIFI-FIGD TVB mgN/100g	s.d.	IFL-FIGD TVB mgN/100g	s.d.
1	0,51	0,25	0,65	0,31	25,7	2,42	24,8	1,52
6	3,31	0,12	3,85	0,08	24,8	1,25	26,6	1,88
15	0,3	0,05	0,45	0,01	16,6	1,62	17,5	1,1
41	0,25	0,06	0,52	0,01	0,29	0,01	0,19	0,05
45	0,07	0,01	0,12	0,01	0,29	0,01	0,33	0,06
60	0,07	0,01	0,2	0,01	1,09	0,05	1,56	0,85
69	0,43	0,2	0,65	0,22	0,67	0,02	0,78	0,2
71	40,5	2,25	42,65	2,25	45,7	4,42	46,66	4,42

* s.d.: standard deviation

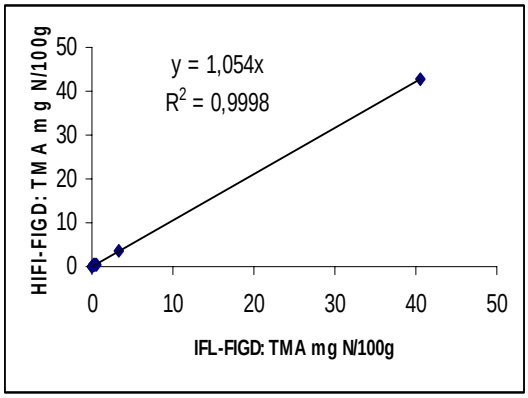

Fig. 14. *The least square regression line for TMA in AB-Peterhead samples using HIFI-FIGD vs. IFL-FIGD*

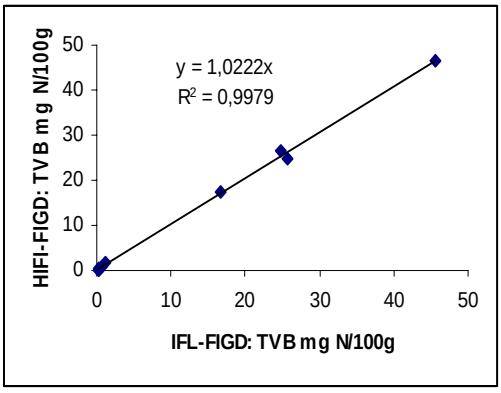

Fig. 15. *The least square regression line for TVB in AB-Peterhead samples using HIFI-FIGD vs. IFL-FIGD*

DIII. Introduction of the FIGD technique and the IFL version of FIGD

As has been said earlier in this report it was decided to co-operate with other EC FAIR project no: CT98-4076 called Development of Multi-Sensor Techniques for Monitoring the Quality of Fish ("Mustec). The main objective of that project is "To devise a multi-sensor method for rapid monitoring of fish quality". The methods used are different but mainly physical methods for monitoring the quality of fish but the methods are all non-destructive to the measured fish. The FIGD method used to measure TMA and TVB is destructive since it is not possible to measure the volatiles in the exudate of the fish. However it was vital for the Mustec project to have a conventional chemical method to determine volatiles in the fish muscle for comparative reasons. It was also good for this project to have comparison with non-destructive methods such as RT-Meter and Torrymeter. Therefore the IFL-participant used the FIGD technique to measure TMA, TVB and in some instances TMAO is storage experiment of cod in November 1999 in Reykjavik and in cod and salmon in May 2000 in Tromsö. The opportunity was used to introduce the technique to the Mustec participants and the TMA and TVB results were used in the Mustec Progress Report 2001. The Mustec participants are: Dr. Paul Nesvadba coordinator from the Food Science and Technology Research Centre in

Aberdeen, Mrs Guðrún Ólafsdóttir and Mrs Soffía Vala Tryggvadóttir from Icelandic Fisheries Laboratories in Reykjavik, Dr. Karsten Heia from Fiskeri forskning in Tromsö, Dr. Mercedes Careche from CSIC-Instituto del Frio in Madrid, Dr. Jörg Oehenschlager form Federal Research Centre for Fisheries in Hamburg, Dr. Corrado Di Natale from University of Rome and Dr. Bo Jörgensen from Danish Institute for Fisheries Research in Lyngby.

Fiskeri Forskning in Tromsö Norway was especially interested in the FIGD technique and wanted to try to use it in their lab. I sent Björn Gundersen from FF a FIGD detector and injection valve and they are now trying for TMA/TVB determinations for fish storage experiments.

Here in Iceland the new IFL-version of FIGD (fugure 11) has been introduced to other IFL-branches, in Akureyri and Neskaupsstaður and to members of the larges fish processing plants in Iceland, Útgerðarfélag Akureyrar (ÚA) and Síldarvinnslan in Neskaupsstað (SVN). The members of ÚA and SVN were interested for technique and admitted the it was faster and easier to use than other methods that they were familiar to, such as Dyer method and the WEFTA steam distillation method of fish extracts. However the problem of spoiled or decomposed fish is not a major problem today because the boats (trawlers) do not stay out on sea for more than a week compared to 14 to 20 days as was common 10-15 years ago. As has been shown in this project and many more, TMA and TVB values will not increase significantly in most sea foods until after 8-10 days of iced storage, when the signs of spoilage become more obvious. Determination of TMA and TVB, either by the FIGD technique or the conventional techniques, gives therefore little information about the freshness of the fish or prediction about remaining storage life of fish processed in Iceland, where the owner of the boat and the processing plant is the same. The fish markets in Iceland is different because the buyer often does not know the post-mortem age of the fish and how well it was iced and treated onboard the boat. However it is well known that when there is a great demand for fish, the emphasis on fish freshness and quality becomes less important.

The limitation of the FIGD technique is still too time consuming and requires some skills to operate, even though the IFL-FIGD is much easier to use and transfer more users-friendly. It is therefore unlikely that the Icelandic fish markets would be interested to use this technique as a routine check of the freshness of spoilage state of the fish. They would probably use FIGD once in a while when there is a disagreement between seller and buyer judged by sensory analysis of the fish. The use of faster equipment to determine freshness where the correlation between freshness values and post-mortem age of the fish is better as well, such as RT-Meter, Torrymeter and Fish-Tester, would be preferred over FIGD.

The application of FIGD is however very beneficial in a laboratory such as IFL where the technique can replace more time- chemical and energy consuming techniques such as steam distillation and Dyer method where toluene and picric acid are used. The FIGD has already almost replaced other techniques to determine TMA and TVB at IFL in Reykjavik and it has been taken into the account that the TVB values from FIGD are 60% lower than TVB values from steam distillation as was explained in previous reports. The IFL-FIGD could also be useful for fish processing chains where the use of one easy-to-carry FIGD would be enough instead of having one in every plant.

4. FINALIZATION OF TASKS

The IFL initial tasks for the final period's progress report of this project are described as tasks 2 and 3 in the proposal.

The aim of task 2 was adaptation of the sampling procedure to make the FIGD methodology more suitable for commercial, on-line operation as a freshness monitoring procedure. This included to investigate the adaptation of the sample extract preparation methodology for a more convenient "on-line" usage methodology where the goal was to yield about 0,5 ml exudate which would be sufficient for P-ratio determination by the FIGD method. The results obtained by this method would be compared and validated against the conventional extract (TCA or PCA) on a whole fish fillet.

It was not possible to finalize all the original tasks that were set in the proposal. Therefore it was decided at the second plenary meeting in Athens and at the mid-term progress review meeting in Reykjavik to reorganize the tasks for the final year of the project.

According to the proposal, task 1 and its subtasks were generally fulfilled even though the P-ratio was not as good indicator of spoilage as was expected it would be in the proposal (sub-task 1 vii and viii).

Different methodologies to obtain an exudate form fish sample sufficient to measure both TMA and TVB were tested. About 100 g of fish tissues was vacuum packed under different pressure setting in order to squeeze out enough water/liquid of the fish to measure that could be measured. It was not possible to get a clear liquid, which is essential for injection into the FIGD. In spite of the milky exudate, due to small peptides and other small insoluble particles, the exudate was injected into the FIGD injection valve but it caused clogging problems in the tubings and very random results for the same sample. Therefore it was agreed at the Reykjavik meeting to use TCA extract instead of exudate for further work.

According to task 3 of the proposal an “on-line” factory trials would be performed using FIGD methodology to determine TMA and TVB in the fish flesh through handling, processing, storage, distribution and retail display. The aim was to assess its appropriateness as an objective test of fish freshness for routine industrial monitoring schemes. Instead of performing these experiments inside a fish processing plant it was decided that the IFL participant would take part in an other EC project called “Mustec” FAIR CT98-4076, where the aim was to use non-destructive methods to determine freshness of fish. It was very beneficial for both projects to cooperate in such a way. The two experiments, in Reykjavik and Tromsö, are described in the result chapter and a very useful comparison was done on different equipments and methods to determine freshness/spoilage in fresh and thawed cod and salmon.

APPENDICES

Appendix 1. Trimethylamine (TMA mgN/100 g), total volatile nitrogen (TVB mgN/100 g) P-ratio (TMA/TVB * 100) and pH in "Reykjavik" cod (May 2000) during storage of whole, gutted cod in ice

Day	TMA-FIGD	s.d.	TVB-FIGD	s.d.	TVB-Wefta	s.d.	pH	s.d.	P-ratio
1	0,1	0,02	7,0	0,24	13,4	1,03	6,69	0,11	1,00
2	0,1	0,06	11,2	0,45	16,1	0,73	6,66	0,11	0,80
3	0,2	0,02	10,7	0,25	18,9	0,92	6,68	0,05	1,49
4	0,2	0,04	12,0	0,91	16,5	1,82	6,63	0,09	1,34
7	0,1	0,06	6,6	0,56	13,4	0,01	7,08	0,01	1,98
9	0,2	0,19	8,9	0,86	16,8	2,43	7,17	0,08	2,02
11	0,6	0,4	16,2	3,98	25,3	6,43	7,07	0,18	3,82
15	14,4	4,59	20,0	4,25	30,1	2,49	6,98	0,14	72,0
17	24,0	6,4	25,6	6,38	36,4	4,09	7,04	0,08	93,9

Appendix 2. Trimethylamine (TMA), total volatile basic nitrogen (TVB) and pH in haddock fillets, caught in May 2000 in Iceland, during storage in ice

Day	**TMA**	**s.d.**	**TVB**	**s.d.**	**pH**	**s.d.**
2	0,03	0,01	5,1	0,41	6,7	0,06
5	0,11	0,06	5,7	0,48	6,7	0,09
7	0,28	0,06	7,5	0,99	6,8	0,06
8	26,1	0,47	29,8	0,38	6,9	
9	25,2	3,04	31,7	1,64	7,0	0,07
12	39,0	1,76	49,0	1,68	7,1	0,00
14	40,2	0,43	58,5	5,64	7,1	0,14
16					7,6	0,05

Appendix 3. Log_{10} H_2S producing bacteria and log_{10} total viable counts in haddock fillets, caught in May 2000 in Iceland, during storage in ice

Day	**log H2S**	**s.d.**	**log TVC**	**s.d.**
2	1,54	0,34	3,26	0,00
5	3,30	0,26	4,215	0,39
7	4,17	0,30	5,665	0,18
8	3,85	0,25	6,74	0,20
9	3,30	0,42	7,38	0,11
12	6,62	0,59	8,08	0,11
14	6,91	0,46	8,19	0,37
16	7,34	0,27	8,11	0,35

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