FAIR CT-98-3833

Implementation on board of systems of atmospheres with variable composition applied to fresh fish. Continuation on shore of the modified atmosphere chain.

Individual Progress Report for the period
from 01-01-00 to 31-12-00

Type of contract:	Demonstration project		
Total cost:	1402,283 kECU	***EC contribution:***	700,331 kECU or 50%
Participant n°6 total cost:	251,511 kECU	***EC contribution to partner n°6:***	110,1 kECU or 44%
Commencement date:	01-01-99	***Duration:***	2 years
Completion date:	31-12-00		
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Actions in the project during the 24-month period:

Task 1.	Participated in the task	total contribution:	1 man-month
Task 2.	Participated in the task	total contribution:	0.5 man-month
Task 3.	Did not participate	total contribution:	0 man-month
Task 5.	Participated in the task	total contribution:	7 man-months
Task 6.	Participated in the task	total contribution:	8 man-months
Task 8.	Participated in the task	total contribution:	0.8 man-month
Task 9.	Did not participate	total contribution:	0 man-month
Task 10.	Did not participate	total contribution:	0 man-month
Task 11.	Final report	total contribution:	2 man-month
		Total contribution:	**19.3 man-months**

Research activities during the second reporting period

IFL has participated in tasks 1, 2, 5, 6, 8 and 11 during the second year of the project. Tasks 3, 9 and 10 were omitted.

Task 1. Safety rules for using MA on board

Subtasks 1.2.-1.4. Definition of safety system for carrying out the project/ Proposal for Safety Regulations for fishing vessels

Regarding storage and use of N_2, O_2 and CO_2 gases on board fishing vessels: The regulations should cover gas pipes and plugs, storage of gases and all electronic components. All gas accessories should fulfil EU regulation no. 15. Every electronic accessory should be explosive free. All pipes must be fastened in order to prevent vibration. Gas tanks should be kept in upright position in a well ventilated area, outside the engine room and crews' cabin. The tanks must be protected from sea and weather if they are up on deck in such a manner that they can be directly approached from open deck. If gas tanks are kept under deck, they must be kept in a special room and ventilated out of the ship's hull. Gas tanks must always be properly fastened. O_2 concentration used in the gas mixture should never be higher than 40%. Gas sensors should be found where the storing tanks are kept to detect any leak.

Task 2. Study of the European vessels potentially interested in MA

Subtask 2.2. Medium round trip vessels.

IFL carried out a survey on the potential users of MA systems on boards. A total of thirteen persons (shipowners, captains and head engineers) on five medium round trip vessels/ boats from a small island to the South of Iceland, Vestmannaeyjar (which is one of the biggest fishery ports in Iceland), were interviewed regarding their interest in utilising MA to affect the storage life of fresh iced fish. The following questions were asked:

Q1) Are you interested in knowing more about modified atmosphere (MA) bulk storage and its use on board?

Q2) Using MA bulk storage requires the utilisation and storage of gas (CO_2, N_2 and O_2) on board vessels/boats. What is your opinion on keeping gas on board your vessel/boat?

Q3) Would you be ready to redesign your vessel/boat with the intention of keeping fish in MA container if this storage method would give the following advantages:
a) improve fish quality and extending its shelf life?
b) allow you to extend the fishing trip?
c) increase the value of the catch?

Q4) What factors would be the most prevailing for changing from conventional bulk storage to MA bulk storage of fish on board?

All answers were considered and each answer/comment given the score 1. Finally, the sum of all scores for each factor were put into Table 1.

Table 1. Answers and scores

Question number	One	Two	Three	Four
No interest / comment	6	4		
Yes / ok.	2	2	2	
Maybe	5		10	
Danger		6		
Better quality			5	2
Higher price			10	10
Longer trips			1	
Low investment cost				3
Safety				1
No extra work				2

For the first question, it may be said that there appears to be little interest in using MA on board. Eleven out of thirteen persons answered the question by saying no or maybe. Only two persons were affirmative. The results from question two indicate that the participants are afraid of using gas on board their fishing vessels or boats or do not comment on it. From the results of question three, it is obvious that higher price and better fish quality are the most important factors in deciding whether MA bulk storage should be used on board. It is interesting that only one participant considers it as an advantage to extend the length of the fishing trip. Similarly, question four indicates that the participants consider the most prevailing factor to be higher fish prices, but also low investment cost.

Task 3. Implementation on board of bulk storage with MA

Subtask 3.2. Solutions for the hold of old vessels

The results of the cod (*Gadus morhua*) trials conducted at the IFL and reported during the first year of the project put in doubt the effectiveness/use of MA bulk storage technology on cod for the Icelandic fleet (medium round trip vessels). It should be mentioned that Shewan (1950) once concluded that gas storage on board fishing vessels was only worthwile for fish which should be more than 14 days in ice before landing. Also based on the comments described in subtask 2.2, the research group at the IFL decided to continue simulating on board trials while assessing the effect of MA bulk storage on another fish species, redfish (*Sebastes marinus*). This third task was therefore omitted.

Tasks 5 and 6. Trials on board and on shore; vessels with medium long round trips

Subtask 5.1. Trials on board (simulated) - medium trip vessels

Subtask 6.1. Definition of fish handling methods prior to packaging

Subtask 6.2. Packaging of commercial samples

During this reporting period, IFL carried out three trials where on board conditions were simulated comparing the storage of conventionally iced redfish (*Sebastes marinus*) with that of MA-stored iced redfish (using an airtight container). The fish was caught in March, May and November 2000, obtained 2 days postcatch and still in *rigor mortis*. The gas mixture used was made up of 60% CO_2 and 40% N_2. In the first two trials the fish was stored whole, ungutted for 10 days either in MA or air (on ice) in 70L plastic boxes placed in a cooling room (0.9 and 1.8°C for respective trials), then processed as skinless fillets and stored in air (MA-ICE and ICE-ICE treatments) or in nylon bags with MA (MA-MAP and ICE-MAP treatments) till sensory rejection or longer. The gas mixture used for the MAP fillets was the same as for whole fish.

During the third trial, the fish was kept whole and ungutted during the entire storage period in a cooler (1.9°C). The MA container was opened at 3 occasions (on days 7, 16 and 22) for sampling purposes, closed and reflushed. On day 7, 33 iced fish were placed into the container (ICE-MA treatment) prior to reflushing while 33 MA-stored fish were removed from the container and further stored aerobically on ice (treatment A). Removal of MA-stored fish from the container was repeated on day 16 (treatment B, i.e. 14 days in MA, then air-stored). Overall, 5 treatments were tested: ICE, MA, ICE-MA, A and B. These various treatments were meant to simulate different storage alternatives/situations relating to short/long fishing trips (treatments A and B; 5 and 14 days under MA) and overseas MA bulk shipping of initially ice-stored (7 days) redfish (ICE-MA). Conventional ice-storage and MA bulk storage of whole fish in the container were the control treatments.

The fish was frequently sampled (in duplicate) and evaluated by sensory evaluation, microbiological and chemical tests. A texture profile analysis was performed during the March trial. Detailed description of the methodology and the results can be found in Appendix 1.

Subtask 5.2.-5.3.-5.5. Biochemical, microbiological & sensory analyses
Subtask 6.4.-6.5. Analysis and consumer test - Market test
Comparison of the ICE- and MA-stored whole redfish showed that similar sensory quality was obtained among these two treatments after 5, 10 and 14 days of storage, as determined by the modified Torry scale. However, the sensory quality of MA-stored redfish, as determined by the Quality Index Method (QIM) was slightly lesser, mainly due to apparent eye defects. Moreover, MA-stored redfish was significantly tougher than traditionally iced fish, when assessed cooked. In fact, very little shelf life extension (2 days or 10.5%) was obtained by storing whole redfish under MA in bulk, despite lower levels of volatile bases and total viable counts (TVC) at sensory rejection (Table 2).

Interestingly, similar sensory shelf life was obtained whether whole redfish was air-stored on ice for 7 days prior to MA bulk storage (ICE-MA), MA-stored for 5 or 14 days prior to aerobic storage (treatments A and B), or MA-stored for the whole period. The ICE-MA treatment caused little eye defects, while leading to a significantly tougher muscle. Apart from the textural problems caused by most of the differently MA-treated fish, it is believed that their eye defects would be obvious to consumers/buyers and therefore contribute to a lower price product.

The best alternative was found to be treatment A, as it provided a shelf life extension of 2 days while maintaining a similar texture to that of traditionally iced-stored fish. These findings could have a great significance for the Icelandic export of whole redfish, as it would allow for traditional fishing, followed by early landing (2 days old catch) and overseas MA bulk shipping (allowing for up to 5 days) to other European markets.

Table 2. Data at sensory rejection of ungutted redfish stored whole under different conditions (November trial)

	Various treatments tested				
Information/ Analyses performed	**ICE**	**MA**	**ICE-MA**	**A**	**B**
End of sensory shelf life at day[1]	**19**	**21**	**21**	**21**	**22**
End of sensory shelf life (QIM)[2]	20.5	22	20	19	20

Estimated TVC (log $_{10}$ CFU/ g)	**5.9**	**2.9**	**3.2**	**6.3**	**3.7**
Estimated H_2S-producer counts	4.3	1.95	2.4	5.5	2.9
% H_2S-producers	**2.5%**	**11.2%**	**15.9%**	**15.9%**	**15.9%**
Estimated *Pseudomonas* counts	4.9	1.9	2.2	4.8	2.5
% *Pseudomonas* spp.	**8.9%**	**10.0%**	**10.0%**	**3.4%**	**6.3%**
TVB-N content (mg N / 100 g)	**24.2**	12.8	13.7	**16.2**	14.2
TMA content (mg N / 100 g)	**5.3**	0.5	0.8	**4.0**	0.7
pH	7.0	6.6	6.7	6.9	6.9
TBA values (mg mal/ kg)	0.2	0.3	0.4	0.6	0.3

1: total shelf life including days from catch, based on the evaluation of cooked fillets using the Torry scale

2: total shelf life including days from catch, based on the evaluation of whole raw fish using QIM

Table 3. Data at sensory rejection of redfish fillets stored under different conditions (March and May trials)

Information/ Analyses performed	Various treatments tested			
	ICE-ICE	ICE-MAP	MA-ICE	MA-MAP
	Trial 1 / Trial 2	Trial 1 / Trial 2	Trial 1 / Trial 2	Trial 1 / Trial 2
End of sensory shelf life at day[1]	**17 / 16**	**20 / 20**	**17.5 / 16**	**23 / 26**
Estimated TVC (log $_{10}$ CFU/ g)	**8.2 / 7.9**	**7.0 / 7.1**	**5.8 / 6.1**	**6.9 / 7.1**
Estimated H_2S-producer counts	7.0 / 6.6	6.5 / 6.1	4.6 / 4.8	4.6 / 5.2
% H_2S-producers	**6.6% / 5.1%**	**31.6% / 10.7%**	**7.4% / 5.1%**	**0.6% / 1.1%**
Estimated *Pseudomonas* counts	6.4 / 7.1	4.9 / 6.7	4.6 / 5.1	3.8 / 5.5
% *Pseudomonas* spp.	**1.5% / 17.8%**	**0.8% / 47.9%**	**6.4% / 10.0%**	**0.1% / 2.8%**
TVB-N content (mg N / 100 g)	23.3 / 14.8	19.8 / 49.7	12.5 / 18.2	33.0 / 44.8
TMA content (mg N / 100 g)	2.5 / 1.7	1.8 / 4.3	0.2 / 0.3	6.6 / 14.8
pH	7.0 / 7.2	6.5 / 6.6	6.8 / 7.1	6.5 / 6.5
TBA values (mg mal/ kg)	0.3 / 1.0	0.21 / 0.33	0.50 / 0.65	1.01 / 0.35

1: total shelf life including days from catch

Table 3 summarises the results obtained at sensory rejection of redfish fillets during two trials assessing the effect of MA bulk storage of whole, ungutted redfish simulating on board conditions (medium round trip of 10 days), followed by on shore processing and MA packaging (MAP) or traditional ice storage. A much greater shelf life extension was obtained by filleting/deskinning MA-stored redfish followed by MAP storage (8-10 days or 47-63%) than by storing fillets, that were processed from MA- or air-stored fish, on ice (air). Similarly, a shelf life extension (3-4 days or 18-25%) was obtained for MAP fillets processed from traditionally air-stored redfish. It should be mentioned that the air- and MA-stored fish were of similar overall quality prior to processing, despite the fact that MA-stored fish was significantly tougher. It follows that some of the sensory attributes (toughness mainly) of the MAP fillets were significantly different than those for traditionally iced fillets. However, lesser differences were observed as storage progressed.

Overall, it can be said that the microbiological analyses showed the hindering effect of the gas mixture on the proliferation of the microflora in the fish and fillets, as reflected by the lower counts in these treatments. However, higher TVB-N content was found in MAP fillets, evidencing the establishment of a more potentially spoiling microflora in these treatments.

Finally, it should be pointed out that even though the MAP storage of the fillets provided a certain shelf life extension when compared to traditional ice storage, this achievement is questionable as it only contributed to the shelf life extension of low quality fillets (Torry score of 6-7 out of 10) at the cost of a drier and/or tougher muscle.

No in-depth consumer/market tests were conducted, but our results are based on thorough sensory analyses conducted by a trained panel, as described in Appendices 1 and 2.

Subtask 5.6. Technical assessment

An assessment of the effectiveness of the various treatments evaluated brings us to conclude that the following alternatives may be promising under the present Icelandic conditions:

a) **treatment A**, involving traditional fishing of redfish on board short round trip vessels, followed by landing of 2 days old catch and overseas MA bulk shipping of whole redfish to other European markets. The maximum length of the MA bulk shipping/storage should be 5 days. This treatment should provide a shelf life extension of at least 2 days over traditional ice storage (19 days), without causing textural defects but possibly some eye defects. This could be overcome by filleting the fish prior to its marketing.

b) **MA-MAP fillets**, starting with either MA bulk storage on board medium round trip vessels or landing of 2 days old catch from short round trip vessels, followed by MA bulk storage (maximum 10 days) on shore until market demand for MAP fillets is required. The maximum shelf life of MAP fillets from these conditions is expected to be 14 days. Of course, adequate temperature control is required. It is not to be forgotten that low quality fillets should result from this treatment, maintaining a low Torry score (7-6 out of 10) for about 8-10 days, as opposed to one day for fast deteriorating air-stored fillets.

Task 8. Quality criteria to be used in MA

Many methods have been proposed or tested for measuring fish quality. Sensory methods are still the most satisfactorily way of assessing the freshness of fish (Connell, 1975; Howgate, 1982; Ólafsdóttir et al., 1997). Quality deterioration of fish is first characterised by the initial loss of the fresh fish flavour (sweet, seaweedy) which is followed by the development of a neutral odour/flavour, leading to the detection of off-odours/flavours and the rejection of the fish. The deteriorative changes occurring in fish result in the gradual accumulation of certain compounds in the flesh. Quantification of these compounds can provide a measure of the progress of deterioration (Connell, 1975).

Methods involving sensory, chemical and microbiological evaluation of redfish were used during the course of the three trials conducted over the reporting period. It was found that flavour was the decisive parameter of the Torry scheme to determine sensory shelf life. Correlation of this sensory parameter to various chemical and microbiological parameters measured was done to determine proper indices of fish spoilage. Rehbein *et al.* (1994) previously reported that measurement of TVB-N and TMA, obtained from ice-stored redfish, gave no information about quality changes during the first 12 days of storage. However, quality loss could be estimated by these tests only on later stages of storage. Also, it has been found (Burt *et al.*, 1975, 1976a, b) that a logarithmic transformation of TMA [TMA index = $\log_{10}$ (TMA+1)] provides better information during the early days of ice storage. Therefore, correlation of transformed TMA and TVB-N values with the sensory parameter was also evaluated.

Table 4 gives the correlation coefficients obtained for the three trials conducted, independently of the treatments assessed. It should be mentioned that negative correlation coefficients were expected for all parameters, resulting from the correlation of increasing values against decreasing values for the sensory score as spoilage progressed, with the exception of the positive TMAO correlation coefficients. No common parameters correlated well (r>0.9) for all three trials (Table 4). This is believed to be due to the different spoilage patterns observed in the various treatments assessed. This means that none of the parameters measured can be used as criteria/indices of spoilage for redfish per se, i.e. no one value of limit of acceptability can be used for fish stored under different conditions. However, correlation coefficients for the microbiological data were higher during the first two trials involving processing/filleting of whole redfish, mainly due to the proportional proliferation of the microflora compared to the proportional decrease of redfish sensory quality. Lower correlation coefficients were seen for the third trial, where redfish was kept whole and ungutted during the entire storage period, probably due to the larger differences in counts observed for the different treatments assessed. Nevertheless, it can be expected that higher correlation coefficients will be obtained while comparing data within treatments.

Table 4. Correlation coefficients (r) relating chemical and microbiological parameters with sensory parameter (flavour)

CORRELATION OF DATA: Flavour vs. other parameter	**Trial 1** (February)	**Trial 2** (May)	**Trial 3** (November)
pH	-0.483	-0.346	-0.287

TMA	-0.517	-0.270	-0.452
Log (TMA+1)	-0.633	-0.383	-0.584
TMAO	0.791	NA	0.466
TVB-N	-0.399	-0.269	-0.022
Log TVB-N	-0.350	-0.248	-0.082
P ratio (TMA/TVB-N)	-0.618	-0.381	-0.487
TVC (LH)	**-0.820**	**-0.922**	-0.354
TVC (IA)	**-0.819**	**-0.929**	-0.400
H_2S producers	**-0.839**	**-0.901**	-0.429
***Pseudomonas* counts**	**-0.804**	**-0.925**	-0.460

Consequently, correlation of the sensory parameter to the various parameters measured for each treatment evaluated was verified for the three trials (Tables 5 to 7). Usually the transformed TMA values correlated better with the sensory parameter than the TMA values did, and was even the best correlating parameter among the chemical parameters evaluated. Higher correlation was obtained for iced fish while decreasing coefficients were seen with an increasing importance of MA in the treatments. Even though such transformed TMA values appear to be a possible chemical index of spoilage, it would not be a practical one as it is highly dependent to the storage method used as shown by Figure 1. This means that such a spoilage index would have to be determined for each different storage method, which does not suit today's reality of fish inspection.

Interestingly, most of the microbiological data correlated well with the sensory parameter, especially for the first two trials (Tables 5 and 6). Figures 2 and 3 illustrate how combined microbiological data of the first two trials for each respective treatment correlated with the sensory parameter (flavour). Generally, it can be said that a better correlation was seen for H_2S-producer counts than TVC. From Figure 3, it is shown that 2 main lines can be drawn for the 4 different treatments, grouping ICE-ICE and ICE-MAP, and MA-ICE and MA-MAP data together. In other words, 2 equations can be obtained by combining the data based on their initial bulk storage treatment, either traditional ice or MA. Figure 4 illustrates these findings where 2 equations are obtained. The following linear equations are for:

traditional ice bulk storage **y = -0.5548x + 9.2356** (R^2 = 0.9204) and

MA bulk storage **y = -0.6895x + 8.8867** (R^2 = 0.9389)

of whole, ungutted redfish prior to fish filleting (Figure 4).

Based on these equations, it can be expected that redfish fillets will have reached sensory rejection at a H_2S-producer count of **6.7 log/g or 4.9 log/g**, **based on the initial bulk storage method using traditional ice or MA, respectively.** It follows that the microbiological spoilage index for redfish initially stored under MA may be dependent of the gas mixture as proliferation of H_2S-producers is known to be influenced by the composition of the gas mixture. Therefore, this spoilage index should be used with caution. Also, it should be specified that H_2S-producer counts are only used as an indicator of spoilage as this parameter appeared to be the only one measured in this study that correlated well with the sensory parameter. *Photobacterium phosphoreum* has been reported to be the main spoiler of MAP fish (Dalgaard *et al*., 1997), but could not be measured selectively in this study. The methodology defined by Dalgaard *et al*. (1996) was supposed to be tested during the course of this project but due to technical problems and defects of the MALTHUS apparatus available at the IFL, it was not possible.

Table 5. Correlation coefficients (r) relating chemical and microbiological parameters with sensory parameter (flavour) measured during the March trial for each different treatment assessed

CORRELATION OF DATA:				
Flavour vs. other parameter	**ICE-ICE**	**ICE-MAP**	**MA-ICE**	**MA-MAP**
pH	**-0.894**	-0.325	-0.656	0.211
TMA	**-0.988**	-0.810	**-0.977**	-0.657
Log (TMA+1)	**-0.999**	**-0.932**	**-0.972**	-0.718
TMAO	0.825	0.850	0.844	0.879
TVB-N	-0.855	-0.590	0.999	-0.488
Log TVB-N	-0.828	-0.606	1.000	-0.438
P ratio (TMA/TVB-N)	**-0.996**	-0.838	**-1.000**	-0.702
TVC (LH)	**-0.933**	**-0.915**	**-0.968**	**-0.909**
TVC (IA)	**-0.929**	**-0.923**	**-0.973**	**-0.922**
H_2S producers	**-0.939**	**-0.951**	**-0.973**	**-0.972**
***Pseudomonas* counts**	**-0.958**	**-0.911**	**-0.956**	**-0.966**

Table 6. Correlation coefficients (r) relating chemical and microbiological parameters with sensory parameter (flavour) measured during the May trial for each different treatment assessed

CORRELATION OF DATA:				
Flavour vs. other parameter	**ICE-ICE**	**ICE-MAP**	**MA-ICE**	**MA-MAP**
pH	**-1.000**	0.041	-0.845	0.505
TMA	**-0.960**	**-0.919**	**-0.999**	-0.839
Log (TMA+1)	**-0.982**	**-0.941**	**-0.995**	**-0.890**
TMAO	**0.957**	0.252	0.882	0.396
TVB-N	0.996	-0.644	0.032	-0.772
Log TVB-N	0.999	-0.613	-0.017	-0.742
P ratio (TMA/TVB-N)	**-0.953**	**-0.919**	**-0.903**	-0.692
TVC (LH)	**-1.000**	**-0.970**	**-0.997**	**-0.957**
TVC (IA)	**-1.000**	**-0.972**	**-0.997**	**-0.951**
H_2S producers	**-0.996**	**-0.981**	**-0.997**	**-0.940**
***Pseudomonas* counts**	**-1.000**	**-0.970**	**-0.997**	**-0.968**

Table 7. Correlation coefficients (r) relating chemical and microbiological parameters with sensory parameter (flavour) measured during the November trial for each different treatment assessed

CORRELATION OF DATA: Flavour vs. other parameter	ICE	MA	ICE-MA	A: MA(5)-ICE	B: MA(14)-ICE
pH	-0.879	0.675	0.610	-0.502	-0.330
TMA	**-0.984**	-0.866	**-0.972**	-0.739	**-0.971**
Log (TMA+1)	**-0.952**	-0.869	**-0.964**	-0.784	**-0.977**
TMAO	0.745	0.459	0.620	**0.930**	0.565
TVB-N	**-0.892**	0.997	0.961	0.720	0.792
Log TVB-N	-0.886	0.993	0.973	0.693	0.775
P ratio (TMA/TVB-N)	**-0.944**	**-0.926**	**-0.975**	-0.748	**-1.000**
TVC (LH)	**-0.922**	-0.830	-0.732	**-0.893**	-0.878
TVC (IA)	**-0.915**	**-0.907**	-0.818	**-0.929**	**-0.914**
H_2S producers	**-0.942**	-0.856	**-0.989**	**-0.909**	**-0.912**
***Pseudomonas* counts**	**-0.945**	**-0.951**	-0.654	**-0.944**	**-0.940**

Definition of a spoilage index for the differently treated MA fish kept whole during the entire storage period was not as evident. Based on Table 7, only the P ratio and H_2S-producer counts gave higher correlation coefficients for most treatments. Figures 5 and 6 demonstrate the correlation of these parameters to the sensory parameter (flavour) for the treatments evaluated. H_2S-producer counts were not found to be an appropriate spoilage index as little coherence was seen among the treatments. On the other hand, P ratio was found to be a possible spoilage index for the treatments with a longer storage period under MA, such as ICE-MA, B and MA (Figure 6). Nevertheless, some deviation from the line is apparent and does not make it an index of choice. Other parameters should be evaluated to determine a better spoilage index under these conditions.

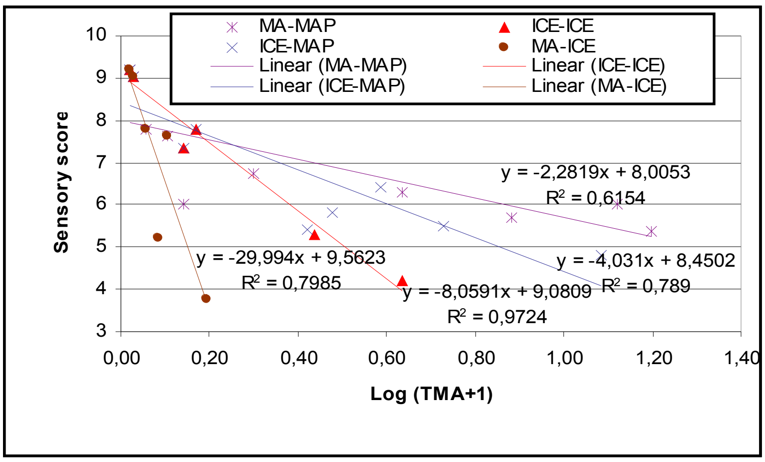

Figure 1. Correlation of sensory parameter (flavour) to logarithmic transformed TMA values obtained during the first two trials for each different treatment

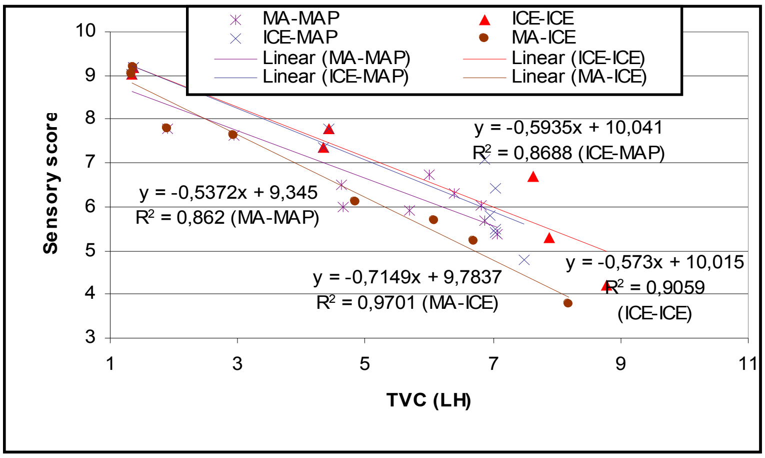

Figure 2. Correlation of sensory parameter (flavour) to total viable counts (LH, 15°C) obtained during the first two trials for each different treatment

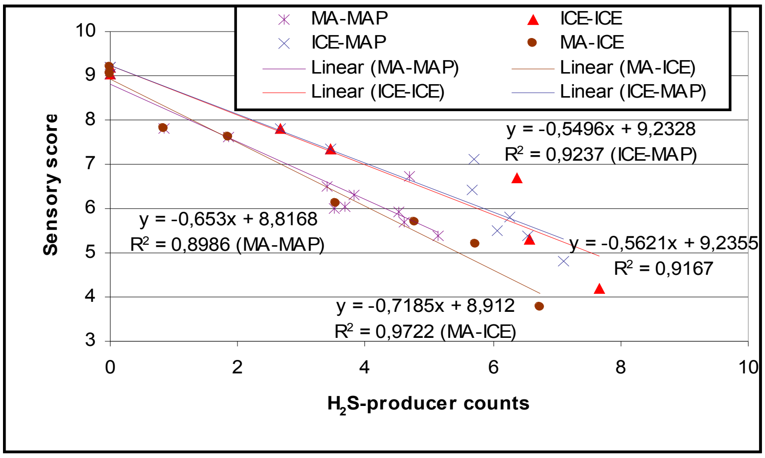

Figure 3. Correlation of sensory parameter (flavour) to H_2S-producer counts obtained during the first two trials for each different treatment

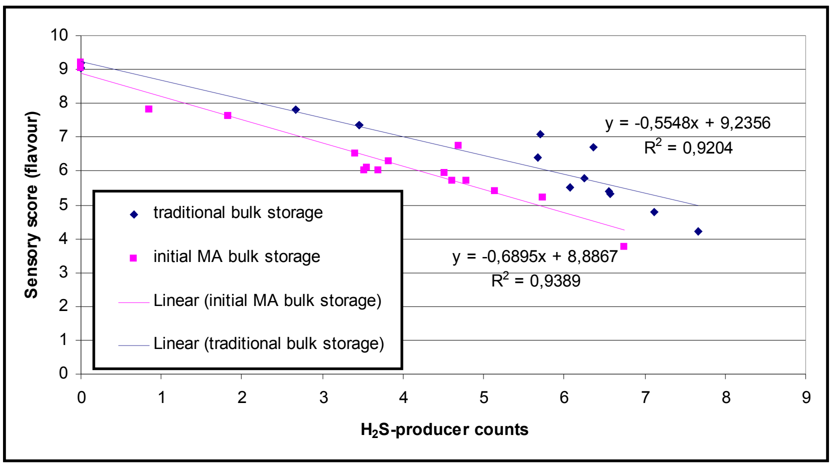

Figure 4. Correlation of sensory parameter (flavour) to H_2S-producer counts

obtained during the first two trials according to the initial bulk storage method used

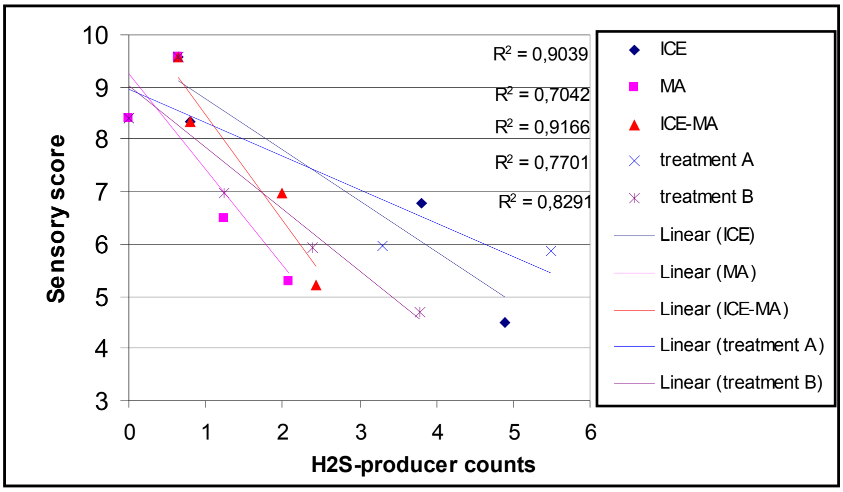

Figure 5. Correlation of sensory parameter (flavour) to H_2S-producer counts obtained during storage of whole, ungutted redfish (November trial)

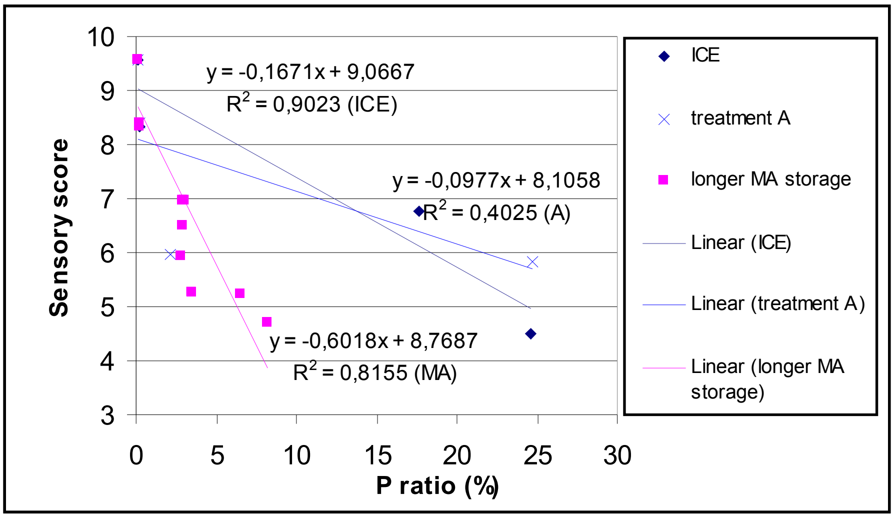

Figure 6. Correlation of sensory parameter (flavour) to P ratio (TMA/TVB-N) obtained during storage of whole, ungutted redfish (November trial)

Tasks 9 & 10. Implementation of HACCP and ISO Standards / Professional training

Similarly to task 3, both tasks were omitted as implementation on board of MA bulk storage technology was not performed.

Task 11. Final report

The final report is under progress and will summarise the work conducted at the IFL during the 24-month period of the project.

Dissemination

A presentation summarizing the first 15 months of the project was given at WEFTA 2000, the 30th Anniversary meeting held in Faroe Islands last June 2000 (19-22). It was untitled: "Shelf life of MAP fillets as affected by prior storage of whole/gutted fish in modified atmosphere", and presented by Dr. Gudmundur Stefánsson.

Two to three scientific papers are planned to be sent for publication during the year of 2001, following completion of the final report. Also, a summary paper discussing the work performed at the IFL during the 24-month period of the project is intended to be published in an Icelandic fish processing journal (Fiskvinnslan).

The results of the second year of the project should be presented at the 31st WEFTA meeting to be held next May 2001 in Espoo (Finland).

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APPENDIX 1: SHELF LIFE STUDIES OF FRESH REDFISH (*SEBASTES MARINUS*) STORED ON ICE UNDER DIFFERENT ATMOSPHERIC CONDITIONS

Materials & Methods

Experimental set-up

Redfish was caught in March, May and November 2000 to the South of Iceland and handled on board according to good practices. The fish was received to the laboratory 2 days postcatch, ungutted and still in *rigor mortis*. The air-stored fish was immediately placed into regular 70L plastic boxes between layers of ice (about 20 fish per box) and stored in a cooling room with re-icing as required. A previously built container was used for MA bulk storage. The inner part measured 130 x 130 x 130 cm and was made of 3 mm stainless steel plates welded together. The outer part was made of plastic coated steel. The container had a 40 mm insulation. The plastic boxes to be placed into the gas container had been perforated (22 holes on the walls) to facilitate the access of gas to the fish. Two piles of plastic boxes (2 x 4) were set up in the container. The boxes at the bottom were filled with ice, but contained no fish (trials 1 and 2), while 2-3 shovels of ice were added to the other boxes. Temperature in the container was recorded during storage using two temperature loggers (Optic StowAway®, Onset Computer corporation, USA), one placed on top of the iced fish while the other was situated in an opposite lower box. The container was closed and flushed to reach a gas mixture of about 60% CO_2 / 40% N_2 (food grade, ÍSAGA, Iceland), as measured from a valve situated on top of the container with a gas measuring device (Dansensor, Combi Check 9800-2, PBI Dansensor, Denmark). The container was stored in a cooling room. The average room temperature recorded in each trial was 0.9°C (March), 1.8°C (May), and 1.9°C (November).

Sampling and further processing

Upon receipt (day 2), 13 fish were sampled for sensory, microbiological and chemical evaluation. Texture analyses were also performed during the first trial. On day twelfth, the composition of the gas mixture in the container was evaluated and the container opened (trials 1 and 2). Fifteen fish from each treatment (ICE and MA) were used for sensory, microbiological and chemical analyses, while the rest was filleted, deskinned and either placed directly between layers of ice (ICE-ICE and MA-ICE treatments) or gas packaged (ICE-MAP and MA-MAP) into plastic bags (Riloten, 55PA/ 60LDPE, 25 x 45 cm, 115 µm; Plastprent, Iceland) by flushing CO_2 and N_2 to reach a gas mixture of 60% CO_2 / 40% N_2 and stored on ice in the cooling room (0-2°C) till sensory rejection. Sampling was carried out regularly (in duplicate).

For the third trial, the fish was kept whole and ungutted during the entire storage period. The container was opened at 3 occasions (on days 7, 16 and 22) for sampling purposes, closed and reflushed. On day 7, 33 iced fish were placed into the container (ICE-MA treatment) prior to reflushing while 33 MA-fish were removed from the container and further stored aerobically (treatment A). Removal of MA-fish from the container was repeated on day 16 (treatment B, i.e. 14 days in MA, then air-stored).

Overall, 5 treatments were tested and sampled regularly in duplicate: ICE, MA, ICE-MA, A and B.

Sensory evaluation
Freshness of the fish was evaluated by two methods: Quality Index Method (QIM) for whole, raw fish and the modified Torry scale for cooked fillets (Shewan *et al*., 1953), rating from 10 = very fresh to 3 = very spoiled, with a rejection level of 5.5. Three whole fish were assessed initially (day 2) for sensory quality using QIM (see Appendix 2), while 3-4 fish were filleted and assessed cooked (steam oven: 98°C for 5 min.) using the modified Torry scale This evaluation was done by the Icelandic Fisheries Laboratories panel (9-12 trained members) and was decisive in defining the end of shelf life of the fish. As the Torry scaling system has been developed for iced air-stored fish, the panellists were asked to score flavour and odour of the cooked fillets separately to verify the plausibility of this scale for MAP fish.

Also, a quantitative descriptive analysis (QDA) of texture was performed, assessing the dryness-juiciness and toughness-tenderness of the cooked fillets. The results were scored on a 0-100% scale using an uncalibrated line. On day twelfth (trials 1 and 2), samples from both treatments were evaluated whole (raw) and cooked as initially. Following filleting of the fish, 4 new treatments were created: ICE-ICE, ICE-MAP, MA-ICE, MA-MAP. During the third trial, both raw and cooked fish were assessed at every sampling day. Duplicate cooked samples were provided to the panellists for evaluation throughout the storage trials.

Microbiological analyses
Upon receipt of the fish, total viable counts (TVC) of the flesh were evaluated using 4 whole fish (2 samples). Each fish was aseptically skinned, pieces of flesh removed and comminuted in a mixer. Twenty-five grams of minced flesh were mixed with 225 ml of cooled Maximum recovery diluent (MRD, Oxoid) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required. Spread-plating of aliquots was done on modified Long & Hammer's medium (Van Spreekens, 1974) and Iron Agar (Gram *et al*., 1987), both containing 1% NaCl, with aerobic incubation at 15°C for 4-5 days. After incubation, evaluation of total viable counts was done in a Darkfield Quebec Colony Counter (Spencer). The detection limit was 20 colony forming units (CFU)/g. H_2S-producers (black colonies on Iron Agar) were also counted. Presumptive *Pseudomonas* counts (15°C, 4-5 days) were obtained using the modified CFC medium (Stanbridge & Board, 1994).

Chemical analyses
Chemical analyses were done with the rest of the flesh mince prepared for microbiological analyses within 1 h of preparation. The pH was measured in 5 grams of mince moistened with 5 ml of deionised water. The pH meter was calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25°C) (Radiometer Analytical A/S, Bagsvaerd, Denmark). TVB-N, TMA and TMAO contents of fish were evaluated by flow injection gas diffusion (FIGD) as described by Ruiz-Capillas & Horner (1999). TMAO was converted to TMA (Bystedt *et al*., 1959) prior to its measurement. As previous research work carried out at IFL using FIGD methodology has shown that on average 60% lower TVB-N values are obtained than by steam distillation, the results were multiplied by a factor of 1.67 to make up for this difference and get a better estimate of the TVB-N content. Thiobarbituric acid (TBA) was measured by

spectrophotometry (538 nm) as described by Tarladgis *et al.* (1960). All these compounds were assessed throughout storage.

Texture measurement
The texture analyser used during the first trial was the Stable Micro Systems (TA.XT2i; Godalming, UK). The test done was the Texture Profile Analysis (TPA). TPA is a test with the diameter of the compression plate much larger than the diameter of the sample (aluminium compression plate, 100 diameter (P/100); pre test speed 2.0 mm/s; speed in sample 0.8 mm/s; strain (distance) 80%).

Sample preparation for the TPA required the removal of 2-3 cm of the neck (top part) of the deskinned fillet. Three 2.5-cm slices were then cut across the fillet. Each slice was then again cut into two 2.5-cm cubes (sample size 2.5 x 2.5 cm). All samples were stored on plastic film on ice until analysed. The samples were compressed to 20% of their original height (two compression cycles). Hardness and cohesiveness were determined. From the resulting force time-curve, hardness is defined as the peak force during the first compression cycle ("first bite") and cohesiveness is defined as the ratio of the positive force area during the second compression to that during the first compression. Hardness is the force needed to compress the sample to a certain strain (%) or distance (mm), while cohesiveness is a criterion of the inner strength of a sample, i.e. how much can the sample be deformed before it breaks.

Results & Discussion

IFL carried out three trials where on board conditions were simulated comparing the storage of conventionally iced redfish (*Sebastes marinus*) with that of MA-stored iced redfish (using an airtight container). The fish was caught in March, May and November 2000, and obtained 2 days postcatch. The composition of the gas mixture ($CO_2/O_2/N_2$) flushed into the container at the beginning of each trial was 60.3/0.2/39.5 (%), 59.9/0.3/39.8 (%) and 60.0/0.1/39.9 (%), respectively. The composition of the gas mixture ($CO_2/O_2/N_2$) upon opening of the container on day 12 was found to be 47.7/4.2/48.1 (%) and 39.8/6.3/53.9 (%) for the first and second trials. The greater drop in CO_2 during the second trial could be due to the larger quantity of fish loaded into the container at the beginning of the trial.

Sensory evaluation and shelf life of the fish/ fillets

Redfish initially ice-stored whole and ungutted for 10 days, then filleted/ deskinned and stored on ice had a (total) sensory shelf life of 16-17 days (from catch). Figure 1 illustrates the results of the sensory evaluation carried out during the second trial (May). As the results of the first two trials are in good agreement, mainly those relating to the second trial are presented graphically. When the fillets were MA-packaged, they had a longer shelf life (3-4 days) than ice-stored ones. On the other hand, a reversed set-up, starting with a 10-day MA storage of whole fish which were then processed and stored on ice, did not provide any shelf life extension. This is probably due to the fact that the ICE-MAP storage has the advantage of retarding further development of the aerobic microflora that become established during the first 10 days of storage, as opposed to the rapid proliferation of the previously hindered aerobic microflora under the MA-ICE storage. In other words, ICE-MAP storage is expected to have a greater hindering effect on the spoilage microflora than MA-ICE storage, hence leading to a shelf life extension. Similarly, MA-MAP storage had an even greater hindering effect, which led to a shelf life extension of 6-10 days.

Even though the use of MA bulk/ MAP storage generally contributed to the extension of shelf life of redfish fillets, the use of this gas mixture had an obvious effect on the sensory quality of the fish/ fillets. At day 12, upon the opening of the MA container, the results of the sensory evaluation of whole fish (QIM) indicated that the overall quality of MA-stored fish was similar to that of iced fish (data not shown). Sensory evaluation of cooked fillets (modified Torry scale) revealed the similar flavour and odour of the differently treated fish samples. However, the MA-stored fish was significantly tougher (Figure 1). Even though the shelf life of the fish stored as MA-MAP and ICE-MAP treatments was prolonged, some of their sensory attributes (dryness and toughness) were significantly different to those of the fish stored conventionally on ice. For instance, cooked fillets from both MA-MAP and ICE-MAP treatments were significantly drier than those from the 2 other treatments on day 15 (first trial, data not shown), while MA-MAP fillets were also tougher. However, lesser differences were observed upon further storage.

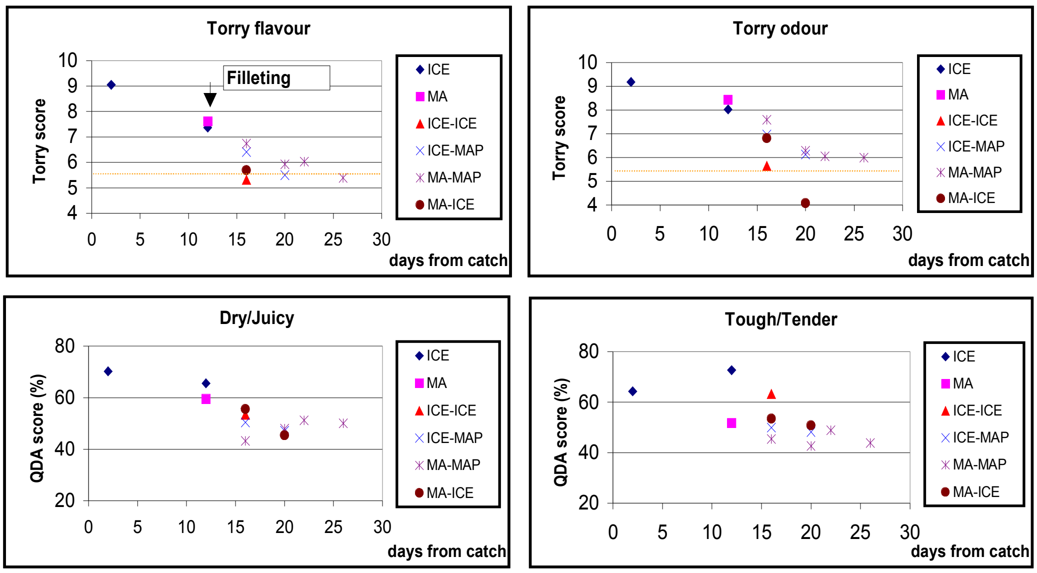

Figure 1. Sensory evaluation of redfish differently processed and stored (May trial)

Similar findings were observed during the second trial (Figure 1). However, the fillets of the ICE-MAP treatment were not significantly drier than the ice-treated ones, but were as tough as MA-MAP fillets on day 16. Interestingly, the MA-MAP fillets had slightly higher flavour scores than the ICE-MAP fillets throughout the storage period of the second trial. Also, a longer shelf life (26 days) was obtained for MA-MAP fillets than during the previous trial (23 days). It is interesting to point out that the texture profile analysis (first trial) did not show much differences among ICE and MA fish on day 12, as opposed to the increasing hardness of the fillets measured among the groups (MA-MAP > MA-ICE > ICE-MA > ICE-ICE) later on during storage (see below).

Comparison of the sensory attributes evaluated indicates that flavour is the attribute weighing the most in the determination of shelf life. Finally, it should be said that even though the MA storage provided a certain shelf life extension when compared to traditional ice storage, this achievement is questionable as it only contributed to the shelf life extension of low quality fillets (Torry score of 6-7 out of 10) at the cost of a drier and/or tougher muscle.

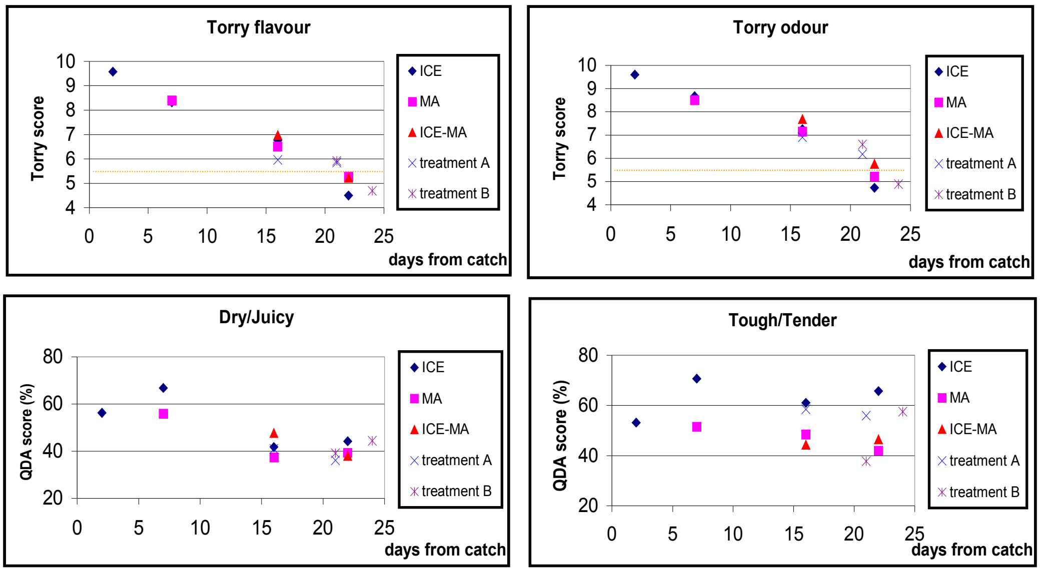

Figure 2. Sensory evaluation of redfish stored whole under different conditions (November trial)

The last trial conducted assessed the effect of different storage conditions on the shelf life of whole, ungutted redfish. The various treatments evaluated were meant to simulate different storage alternatives/situations relating to short/long fishing trips (treatments A and B; 5 and 14 days under MA) and overseas MA bulk shipping of initially ice-stored (7 days) redfish (ICE-MA). Conventional ice-storage and MA bulk storage of whole fish in the container were the control treatments. Figure 2 illustrates the changes in sensory quality that took place in these treatments. Based on the flavour score, the sensory shelf life was 19 days for iced fish, 21 days for fish of MA, ICE-MA and A treatments, and 22 days for treatment B. Overall, very little shelf life extension was obtained with MA storage of whole fish as opposed to processed fish in previous trials. Constant renewal of the gas mixture in the container (MA) following its opening (on days 7 and 16) may have affected the sensory quality of the fish more dramatically than expected. The best alternative was treatment B (14 days of MA storage followed by conventional ice storage of 6 days) as judged by the Torry method.

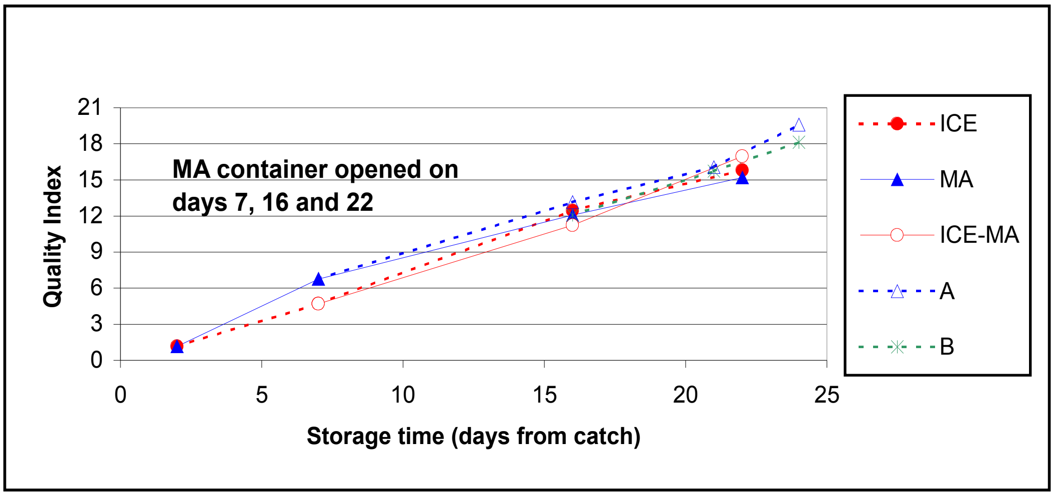

Figure 3. Freshness evaluation of whole redfish stored under different conditions (November trial) by the Quality Index Method (QIM; sensory rejection at score 15)

However, quality evaluation using QIM (Figure 3) gave slightly different shelf life values, a shortened shelf life (19-20 days) for MA-ICE (A and B) and ICE-MA treatments while the ICE and MA treatments had a lenghtened shelf life (20.5 and 22 days, respectively). These results are based on the rejection of samples upon reaching a score of 15. These differences are mainly due to the different parameters evaluated by the 2 sensory methods, overall appearance and texture of raw fish for QIM as opposed to flavour and odour of cooked fillets for the Torry method. The main defect reported by the panellists for the differently treated MA fish (combinations of ICE and MA) was faster eye discolouration which was first mentioned on day 7 (Figure 4). Some panellists commented on the skin discolouration of some MA treated fish samples but the average scores did not indicate these differences, as shown in Figure 4. Photographs of the whole, ungutted redfish were taken at 3 sampling days (7, 16 and 22) for the ICE and MA treatments and are found in Appendix 3. It is shown that from day 7, MA bulk storage caused earlier eye defects. On the other hand, MA bulk storage contributed to maintaining the freshness appearance of the gills, as observed on day 16 where a typical red colour was seen. It should be mentioned that the panellists reported lesser off-odours from the gills of MA-treated fish (data not shown). Based on the average QIM score given by the panellists for gills' colour and odour on days 16 and 22, it appears that the MA bulk storage retarded the deterioration of the gills by 6 days when compared to the ice bulk storage. Bacterial proliferation is known to occur rapidly on gills of iced fish. MA bulk storage therefore contributed to slowing down bacterial growth on the fish.

The MA treated fish with the least defects was that sampled from the ICE-MA treatment. It should be pointed out that because of the other parameters evaluated by QIM, the resulting shelf life for the various MA treatments was generally higher than that of the ICE treatment. However, the defects mentioned in most of the differently MA-treated fish would be obvious to consumers/buyers and may contribute to a lower price product if the product is sold whole.

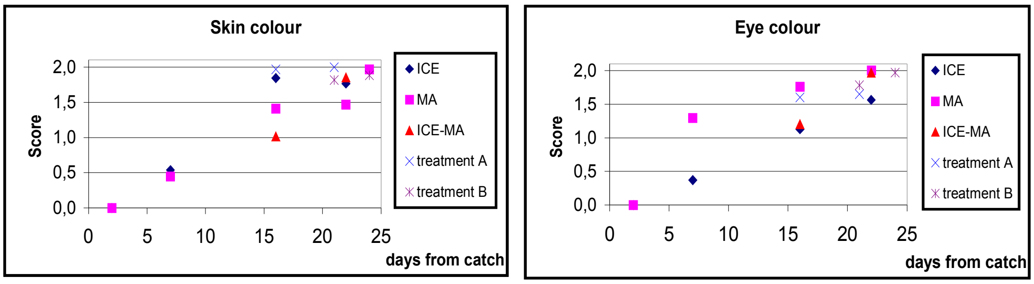

Figure 4. Evaluation of skin and eye colour of redfish stored under different atmospheric conditions (score 0 = very shiny skin/black eyes; 2 = mat, discoloured skin/gray eyes)

The quantitative descriptive analysis (QDA) of the cooked fish, as illustrated in Figure 2, provided some additional information concerning its sensory quality. The different treatments tested did not affect the juiciness of the fish significantly, but significant toughness was reported at several occasions, mainly for treatments MA (throughout storage), ICE-MA (days 16 and 22) and B (day 21). On the other hand, treatment A did not bring any significant textural changes to the fish as it was comparable to the ICE treatment. One may conclude that treatment A should be a better alternative than treatment MA or B, by extending the sensory shelf life by 2 days while maintaining a similar texture to that of conventionally ice-stored fish.

Microbiological analyses

Microbiological quality of the fish at the beginning (day 2) of the storage trials was generally good with total viable counts (TVC) of the flesh just at the limit of detection (20 CFU/g or log 1.3/g), while H_2S-producers and presumptive *Pseudomonas* counts were below the detection level. As expected, bacterial growth occurred at a faster rate in fish/fillets stored under aerobic (ICE) environment than MA/MAP storage (Figure 5). When microbiological data of the March and May trials are compared at sensory rejection, it is found that TVC (LH, 15°C) were comparable within treatments but the levels reached were different among the treatments. The iced fish was about log 8/g, while the MA-MAP and ICE-MAP treatments were one log lower as opposed to two logs lower for MA-ICE. Such differences obviously exclude the use of TVC as a spoilage index of fresh fish.

However, these results may bring some light to the understanding of the microbial spoilage process taking place in fish stored under different environmental conditions. The fact that the aerobic microflora proliferating in traditionally iced fish reached higher levels before sensory rejection occurred than in MA-stored fish, may indicate the development of a more potentially spoiling microflora in fish stored in a MA environment, i.e. a microflora producing higher levels of metabolites per bacterial cell (Dalgaard, 1995). It was previously suggested that ICE-MAP and MA-MAP storage would have a greater hindering effect on the spoilage microflora than MA-ICE storage. However, higher counts were obtained in ICE-MAP and MA-MAP fillets. Again, the development of a more potentially spoiling microflora in MA-ICE fillets may explain their lower counts at sensory rejection.

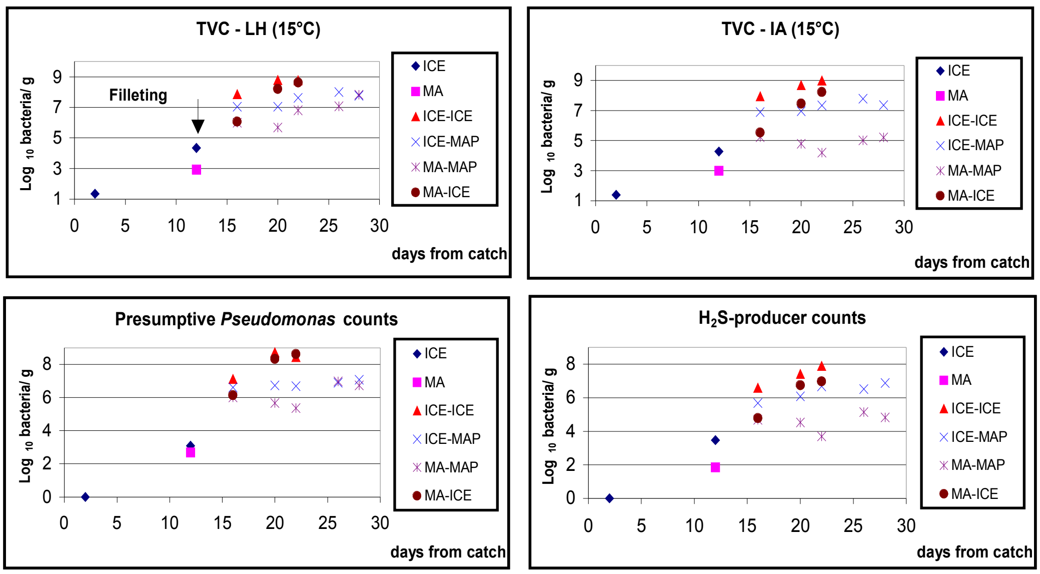

Figure 5. Microbiological evaluation of redfish differently processed and stored (May trial)

Comparison of the Long & Hammer's medium (LH) and Iron Agar (IA) for the evaluation of TVC indicated that higher counts were generally obtained with LH. This is well evidenced when comparing the counts of the MA-MAP treatment (Figure 5).

The differences were noticeable from day 20 and were amplified with further storage, with LH counts of 2 logs higher. Similarly, Dalgaard *et al.* (1996) reported higher counts of MAP fish fillets using LH than IA.

The ratio of H_2S-producers and presumptive *Pseudomonas* spp. in the established microflora (Figure 5) at sensory rejection was calculated based on the counts obtained using Iron agar and modified CFC medium, respectively, and compared to TVC (LH) (see Table 1). The lowest levels of H_2S-producers were seen in MA-MAP fillets (1%), while higher levels (5-7%) were found in MA-ICE and ICE-ICE fillets. The ICE-MAP environment seemed to be suitable for these bacteria as their ratio was found to range from 11 to 32%. The advantage of the ICE-MAP treatment to the H_2S-producers may be explained by their higher levels found in iced fish than in MA fish on day 12, as well as by their slightly faster growth rate than that observed in MA-MAP fillets. It has been shown that a slower growth rate is observed for *Shewanella putrefaciens*, H_2S-producing bacteria, with an increasing CO_2 concentration in MA (Dalgaard, 1993). Also, it has been found that aerobic growth of *S. putrefaciens* can be reduced due to the presence of competitive/hindering *Pseudomonas* spp. (Gram & Melchiorsen, 1992; Lauzon, 1997). Therefore, the possibly better physiological state of air-grown *S. putrefaciens* cells than MA-grown cells, as well as the reduced bacterial interaction/competition due to the lower expected levels of *Pseudomonas* spp. in MA environment could explain the higher levels of H_2S-producers in the ICE-MAP treatment as compared to the other treatments.

As expected, a low ratio of presumptive *Pseudomonas* spp. (Figure 5) was seen in MA-MAP fillets (0.1-3%), while higher levels were found in MA-ICE (6-10%) and ICE-ICE (2-18%) fillets. Contradictory results were obtained for the ICE-MAP fillets when both trials are compared. A low level (0.8%) was found during the first trial, as opposed to a ratio of 48% during the second trial. Interestingly, a lower level of H_2S-producing bacteria were found during the second trial coinciding with the high level of *Pseudomonas* spp. These differences in the level of *Pseudomonas* are not believed to be due to the leakage of the gas from the MAP fillets, as in that case there should have been a similar level to that seen with the ICE-ICE treatment (18%). It is more likely that the differences lie in the proliferation of *Pseudomonas* spp. tolerating MA environment better.

The last trial conducted assessed the effect of different storage conditions on the quality of whole, ungutted redfish. Figure 6 illustrates the microbiological differences observed throughout the storage period. The results are somehow similar to those obtained for the differently treated fillets during the first two trials. The flesh microflora of conventionally iced fish proliferated at a faster rate, reaching higher cell counts (log 6) at sensory rejection, while a slowly developing microflora was seen in

MA-stored fish (log 3 at rejection). The effect of MA on the microflora establishing in iced fish was clearly demonstrated by the ICE-MA treatment, where a lag phase of about 2 weeks was observed upon the MA storage of the iced fish. However, proliferation of H_2S-producers was detected and these bacteria represented almost 16% of the spoilage microflora at sensory rejection.

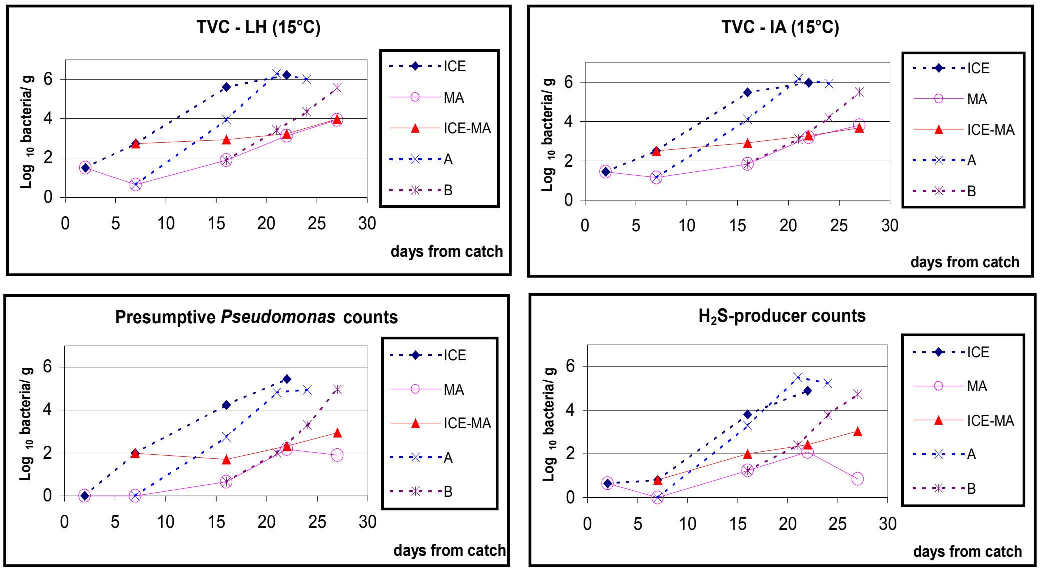

Figure 6. Microbiological evaluation of redfish stored whole under different conditions (November trial; treatments A and B = MA storage of whole fish (5 and 14 days) followed by air/ice storage)

Table 1. Levels (%) of H_2S-producers and presumptive *Pseudomonas* spp. in the flesh of redfish (at sensory rejection) stored under different atmospheric conditions

Treatments	Type of product	Levels (%) of H_2S-producers	Levels (%) of Presumptive *Pseudomonas*
ICE-ICE	fillets (March trial)	6.6	1.5
ICE-MAP		31.6	0.8
MA-ICE		7.4	6.4
MA-MAP		0.6	0.1
ICE	whole fish (November trial)	2.5	8.9
MA		11.2	10.0
ICE-MA		15.9	10.0
A (5 days in MA + air storage)		15.9	3.4
B (14 days in MA + air storage)		15.9	6.3

On the other hand, aerobic storage of previously MA-stored fish (treatments A and B) led to the rapid proliferation of the MA-hindered microflora. The microflora of treatment A reached similar levels to that of the iced fish, but with a higher level (16%) of H_2S-producers at sensory rejection (as opposed to 2.5% for iced fish, see Table 1). H_2S-producers were found to grow to the same proportion (16%) of the total microflora for treatment B which reached a lower bacterial level at spoilage. This could be expected as the fish of treatment B was kept 9 days longer under MA storage prior to its aerobic storage. It should be pointed out that higher levels of H_2S-producers and presumptive *Pseudomonas* spp. than expected were found in the flesh of whole fish stored under MA storage. A probable explanation is the lesser effect of CO_2 on the flesh of whole fish as opposed to singly packaged MA fillets, more easily accessible.

Chemical analyses

The effect of the different MA treatments on the production of various chemical compounds in the fish flesh was evaluated during the three trials conducted and compared to traditional ice storage. Trimethylamine (TMA) content has been used as a spoilage index for traditional ice-stored fish and usually reaches 10-15 mg N/100 g of flesh upon sensory rejection (Magnússon & Martinsdóttir, 1995). However, lower TMA values (about 6 mg N/100 g) have been reported at sensory rejection of whole redfish or fillets (Martinsdóttir & Magnússon, 1993). TMA is mainly derived from bacterial breakdown of trimethylamine oxide (TMAO) which is naturally found in marine fish (Pedraso-Menabrito & Regenstein, 1990). Total volatile bases (TVB-N) content is an alternative to measuring TMA, and includes ammonia, dimethylamine (DMA) and TMA. Nieper & Stockemer (1986) proposed a content of 25 mg/100 g TVB-N in redfish as an acceptable limit of consumption. P ratio (TMA/TVB-N) can be calculated to show the importance of the proportion of TMA in the overall production of volatile bases. Thiobarbituric acid (TBA) test is a means of evaluating the extent of lipid oxidation.

Figure 7 demonstrates the results of the various chemical measurements performed during the second trial (May). Similar findings were observed during the first trial (data not shown). At incipient spoilage (sensory rejection), TMA production was generally low, being highest (14.8 mg N/100 g) in the MA-MAP fillets, and was found in decreasing amounts in the following treatments: ICE-MAP (4.3 mg), ICE-ICE (1.7 mg) and MA-ICE (0.3 mg). The higher TMA content found in MA-MAP fillets could be foreseen as TMA production is expected to proceed at a faster rate at conditions of low oxygen tension (Huss, 1992). It should be pointed out that very rapid TMA production was noticed during overt spoilage (> day 20, passed sensory rejection) for the ICE-MAP treatment. Late TMA production may be explained by the sudden proliferation/spoilage potential of spoilers, such as *Shewanella putrefaciens* (H_2S-producers), at overt spoilage (Lauzon, 1997). Nevertheless, the low production of TMA indicate the lesser importance of this compound in the spoilage profile of redfish from these treatments.

It follows that the production of other compounds must have accounted for the sensory deterioration of redfish. In fact, the low P ratio (2-30%) observed at sensory rejection of the different treatments (between days 16-26) indicated that the production of volatile bases, other than TMA, should have contributed to quality loss. TVB-N content of the air-stored fillets (ICE-ICE and MA-ICE treatments: 14.8 and 18.2 mg N/100g, respectively) at sensory rejection was found to be below the proposed acceptable limit of consumption (Nieper & Stockemer, 1986), while MA-treated fillets (ICE-MAP and MA-MAP) contained almost twice as much as TVB-N as the proposed limit. On the other hand, the low TBA values found for all treatments demonstrated the little importance of lipid oxidation in the spoilage profile of redfish.

The pH measurements (Figure 7) illustrated the effect of CO_2 dissolving into the fillets during storage. The pH of the fish/fillets increased as the time period under which the fish/fillets were stored under MA decreased: MA-MAP fillets with pH 6.6 to 6.5, ICE-MAP with 6.8-6.6, MA-ICE with 7.0 and ICE-ICE reaching 7.2 at sensory rejection. The pH drop usually observed in MAP fish is believed to be sufficient to disrupt enzymatic reactions essential for bacterial proliferation (Banks *et al*., 1980)

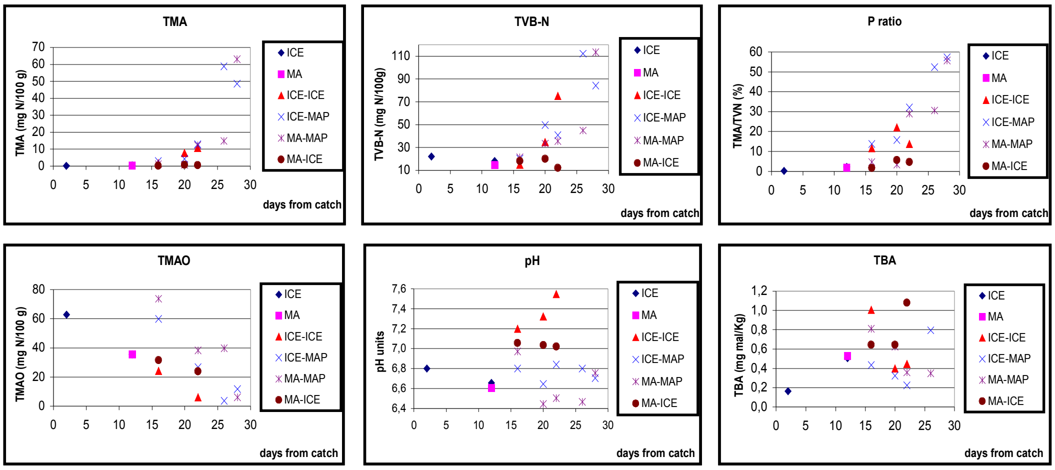

Figure 7. Chemical evaluation of redfish differently processed and stored (May trial)

Figure 8 demonstrates the results of the various chemical measurements performed during the third trial (November) where whole, ungutted redfish was stored under different environmental conditions. Similarly to the 2 previous trials, TMA content was generally low in the differently treated fish. Interestingly, the highest TMA content was found in fish stored in air for most or all of the storage period (ICE = 5.3 mg N/100 g, and treatment A = 4.0 mg) as opposed to very low TMA levels (0.5 to 0.8 mg) in MA-treated fish at sensory rejection. These findings are contradictory to those obtained for the fillets (Figure 7), where the MAP fillets had a higher TMA content. These differences probably reflect the variability of the spoilage microflora establishing in processed (fillets) and non-processed (whole) fish. In other words, the bacterial contamination occurring on the fillets while being processed does not appear to be similar to that occurring in whole fish, especially under MA storage.

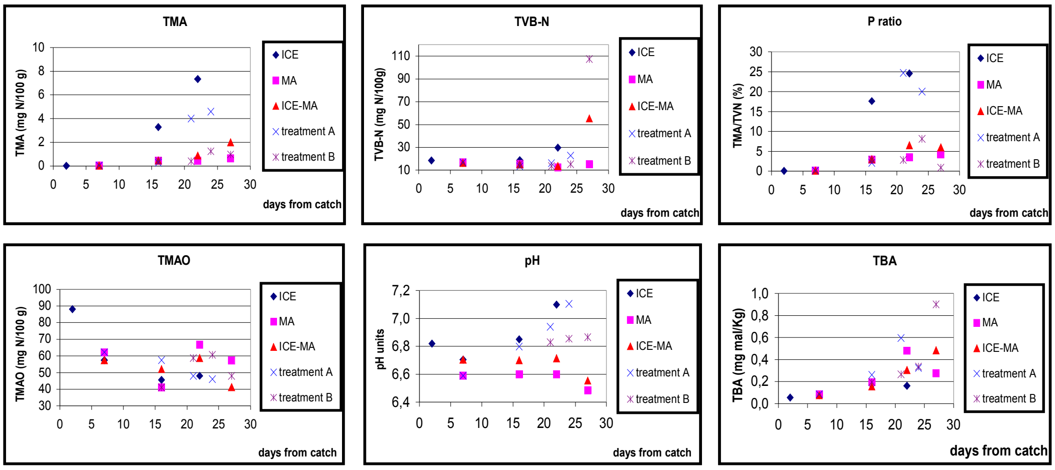

Figure 8. Chemical evaluation of redfish stored whole under different conditions (November trial; treatments A and B = MA storage of whole fish (5 and 14 days) followed by air/ice storage)

It was previously pointed out that higher levels of H_2S-producers and presumptive *Pseudomonas* spp. than expected were found in the flesh of whole fish stored under MA storage. The explanation given was the probably lesser effect of CO_2 on the flesh of whole fish as opposed to singly packaged MA fillets. The presence of these bacterial groups may therefore contribute to some bacterial interaction leading to the slower proliferation of otherwise dominating bacterial groups under MA storage of fish. Such bacterial interaction could be responsible for the low TMA content of MA-stored fish.

Again, TVB-N content was found to be higher in fish stored in air for most or all of the storage period (ICE = 24.2 mg N/100 g, and treatment A = 16.2 mg) and close to the proposed limit of acceptability of 25 mg N/100 g. Consequently, the P ratio for the fish stored mostly under MA (MA, ICE-MA, B) was below 10%, while that of treatments ICE and A was 22 and 25%, respectively. Similarly to the previous trials, low TBA values were found and pH was lower for MA-treated fish than air-stored fish.

Texture measurement

Figure 9 presents the results of the texture profile analysis performed on the raw fillets during the March trial. The initial measurement shows a harder muscle at the beginning of the trial than after ice storage for 10 days, which evidences the *rigor mortis* state of the muscle on day 2. Based on the findings of the sensory evaluation, the MA-stored fish was found to be tougher than iced fish on day 12. However, the texture profile analysis (TPA) showed that little differences were seen among the fish of these two treatments on day 12. One should be careful while comparing the assessment of a cooked muscle by the sensory panel to the results of the TPA of a raw muscle. On the other hand, obvious differences were noticed on day 19, considering both hardness and cohesiveness. The largest differences were found between the ICE-ICE and MA-MAP fillets. Interestingly, the sensory panel found the MA-MAP fillets to be significantly tougher than the MA-ICE ones on that sampling day, while little differences were reported among the MA-ICE and ICE-MAP fillets (data not shown). Decreased hardness was measured on day 23, probably due to the softening of the muscle taking place during overt spoilage.

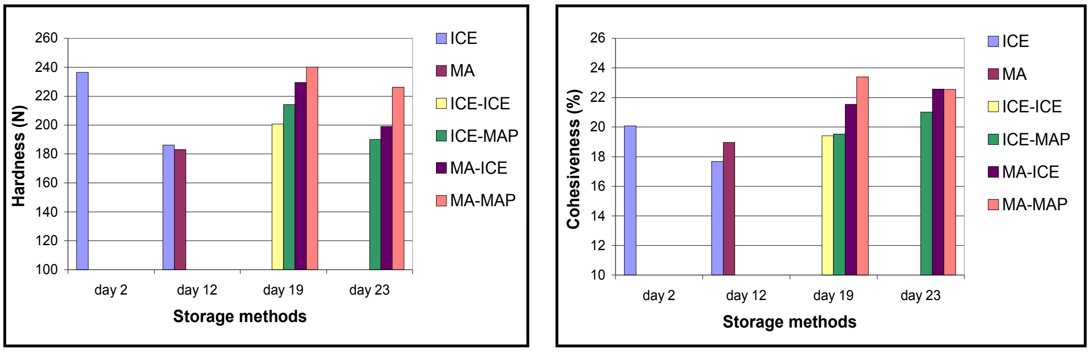

Figure 9. Texture evaluation of redfish differently processed and stored (May trial)

Conclusion

Comparison of the ICE- and MA-stored whole redfish showed that similar sensory quality was obtained among these two treatments after 5, 10 and 14 days of storage, as determined by the modified Torry scale. However, the sensory quality of MA-stored redfish, as determined by the Quality Index Method (QIM) was slightly lesser, mainly due to apparent eye defects. Moreover, MA-stored redfish was significantly tougher than traditionally iced fish, when assessed cooked. In fact, very little shelf life extension (2 days or about 10%) was obtained by storing whole redfish under MA in bulk, despite lower levels of volatile bases and total viable counts (TVC) at sensory rejection.

Interestingly, similar sensory shelf life was obtained for whole, ungutted redfish irrespective of the storage method: (a) in air (on ice) for 7 days prior to MA bulk storage (ICE-MA), (b) MA-stored for 5 or 14 days prior to aerobic storage (treatments A and B, respectively), or (c) MA-stored for the whole period. The ICE-MA treatment caused little eye defects, while leading to a significantly tougher muscle. Apart from the textural problems of most of the differently MA-treated fish, it is believed that their eye defects would be obvious to consumers/buyers and therefore contribute to a lower price product.

The best alternative for MA bulk storage of whole redfish was found to be treatment A, as it provided a shelf life extension of 2 days while maintaining a similar texture to that of traditionally iced-stored fish. These findings could have a great significance for the Icelandic export of whole redfish, as it would allow for traditional fishing, followed by early landing (2 days old catch) and overseas MA bulk shipping (allowing for up to 5 days) to other European markets.

MA bulk storage of whole, ungutted redfish simulating on board conditions (medium round trip of 10 days), followed by on shore processing and MA packaging (MAP) of the fillets (MA-MAP) contributed to a great shelf life extension (8-10 days or 47-63%) when compared to the shelf life of 16-17 days for traditionally air-stored fish processed as skinless fillets (ICE-ICE) on day 12. Similarly, MA packaging of fillets processed from 12-day old air-stored fish led to a shelf life extension of 3-4 days (18-25%) when compared to traditionally air-stored fish/fillets.

It should be mentioned that the air- and MA-stored fish were of similar overall quality prior to processing, despite the fact that MA-stored fish was significantly tougher. It follows that some of the sensory attributes (toughness mainly) of the MAP fillets were significantly different than those for traditionally iced fillets. However, lesser differences were observed as storage progressed. Overall, it can be said that the microbiological analyses showed the hindering effect of the gas mixture on the proliferation of the microflora in the fish and fillets, as reflected by the lower counts in these treatments. However, higher TVB-N content was found in MAP fillets, evidencing the establishment of a more potentially spoiling microflora in these treatments.

Finally, it should be pointed out that even though the MAP storage of the fillets provided a certain shelf life extension when compared to traditional ice storage, this achievement is questionable as it only contributed to the shelf life extension of low quality fillets (Torry score of 6-7 out of 10) at the cost of a drier and/or tougher muscle.

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APPENDIX 2. METHODS USED FOR EVALUATION OF QUALITY/SHELF LIFE

A - Quality Index Method (QIM) scheme for whole redfish *(Sebastes marinus)*

Quality parameter		Description	Score	Scores given					
				Sample codes:					
Appearance:	**Skin**	Bright, iridescent 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	Convex	0						
		Flat, slightly sunken	1						
		Sunken, concave	2						
	Colour of pupil	Black	0						
		Opaque	1						
		Gray	2						
Gills:	**Colour**	Blood red	0						
		Reminds of beef	1						
		Reddish areas	2						
		Rusty, dark brown	3						
	Smell	Fresh, seaweedy, metallic	0						
		Neutral, grassy, musty	1						
		Yeast, bread, beer, sour milk	2						
		Acetic acid, sulphuric, very sour	3						
	Mucus	Clear	0						
		Milky	1						
		Discoloured, rusty, brown, clotted	2						
Viscera:	**Solution**	Whole	0						
		Beginning to dissolve	1						
		Viscera dissolved	2						
Fillets:	**Colour**	Translucent, bluish	0						
		Waxy, milky	1						
		Opaque, yellow, brown spots	2						
Quality Index (0-23)		**Sum:**							

B - Sheet for comments (for the development of QIM for MA-stored ungutted redfish)

Date: ____________ Name: _______________

If a sample is in some way different from the descriptions given in the QIM sheet for ice stored redfish, specify the difference here:

Sample no __________		**Description:**
Appearance, texture	Skin	
	Texture	
Eyes	Cornea	
	Form	
	Colour of pupil	
Gills	Colour	
	Smell	
	Mucus	
Viscera	Solution	
Fillets	Colour	
Other?		

C - Torry scheme for medium fat fish (redfish) - freshness score sheet for cooked fish

score	Odour	Flavour
10	Initially weak odour of boiled cod liver, fresh oil, starchy	Boiled cod liver Watery, metallic
9	Shellfish, seaweed, boiled meat, oil, cod liver	Oily, boiled cod liver 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, rancid
4	Lactic acid, sour milk, TMA	Slight bitterness, sour, "off"-flavours, TMA, rancid
3	Lower fatty acids (e.g. acetic or butyric acids) composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide, rancid

D - Sensory evaluation of steam-heated redfish

Date ____________________

Name ____________________

Give the proper score for sour odour and off-odour

Score	Sour odour description	Off-odour description
0	no sour odour	no off-odour
1/2	hardly detectable	hardly detectable
1	trace	trace
2	little	little
3	obvious	obvious
4	very sour odour	strong off-odour

sample no	score for sour odour	score for off-odour

Comments: ____

____

Give the proper score for sour flavour and off-flavour

Score	Sour flavour description	Off-flavour description
0	no sour flavour	no off-flavour
1/2	hardly detectable	hardly detectable
1	trace	trace
2	little	little
3	obvious	obvious
4	very sour flavour	strong off-flavour

sample no	score for sour flavour	score for off-flavour

Comments: ____

____

APPENDIX 3. PHOTOGRAPHS OF WHOLE, UNGUTTED REDFISH (*SEBASTES MARINUS*) STORED IN BULK IN AIR (ICE) OR UNDER MODIFIED ATMOSPHERE (MA: 60% CO_2/ 40% N_2) FOR 22 DAYS