Initial Trials to Extend the Storage-Life of Cod and Mackerel using Sodium Hypochlorite or Ozone to Treat Ice and Refrigerated Seawater

Seafish Report No.498

July 1996

SEAFISH

The Sea Fish Industry Authority

Seafish Technology

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Author: R. Watson
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Acknowledgements

The author wishes to thank and acknowledge Branagh Cherry and Dr C. Alaslavar from the University of Humberside for their hard work and valuable contribution to this work.

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Summary

Despite diminishing fish stocks, increasing restrictions on the quantities of fish landed, the demands for consistent quality by the corporate food industry and the higher prices which can be attained for top quality fish; poor quality fish is still being landed into UK ports.

As well as addressing the more traditional handling problems, Seafish has identified the use of techniques such as ozonation and chlorination which may enable improvement in fish quality by reducing bacterial spoilage through the use of treated ice or of treated refrigerated seawater (RSW).

A series of laboratory trials were carried out to determine if using treated ice containing a chlorine or ozone residual would reduce the rate of spoilage and extend the storage life of cod. Similar work was carried out to determine if chlorine or ozone dosing would similarly effect cod and mackerel in a pilot scale RSW system.

Neither treatment when incorporated into ice or RSW gave any improvement in the quality of cod. However, holding mackerel in RSW which received a 40 mg/l chlorine dose every 48 hours reduced the rate of bacterial spoilage by over 50%, resulting in fish which remained acceptable for over nine days. The work will continue.

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1. Introduction

Despite diminishing fish stocks, increasing restrictions on the quantities of fish landed, the demands for consistent quality by the corporate food industry and the higher prices which can be attained for top quality fish; it is apparent that poor quality fish is still being landed into UK ports.

After capture and icing, the initial spoilage of fish is predominantly caused by natural enzymatic processes. A few days after capture, the bacterial population on the skin and in the gut cavity of the fish rapidly multiplies. From this point onwards bacterial spoilage becomes the most important mechanism in the deterioration of the fish. Bacterial action on the low molecular weight components of the fish muscle causes the familiar odours and flavours associated with spoiled fish.

Previous Seafish work (Ref 1) has shown that a low chlorine dose (supplied by sodium hypochlorite) can effectively kill some types of bacteria in seawater. A preliminary trial with ionization (low levels of copper and silver ions) also showed that the shelf life of cod could be marginally improved by storage in treated Refrigerated Sea Water (RSW).

It was thought that chlorine or ozone used to treat the water used to make ice, or periodically dosed into RSW systems, may extend storage life by reducing the rate of bacterial spoilage. If a disinfectant residual could be incorporated into ice it would not only produce sterile ice but the melt water may kill spoilage bacteria on the skin of the fish. In RSW it was thought that a dose of disinfectant every couple of days may keep the spoilage bacteria in the water and on the fish down to low levels.

The report describes a series of laboratory trials that were carried out to determine the effect of sodium hypochlorite and ozone on the sensory and microbiological quality of cod stored in treated ice; and cod and mackerel stored in a treated RSW system. The work was carried out by Seafish in conjunction with Hull Public Health Laboratory Service (PHLS) but duplicate fish and water samples were also analysed by the University of Humberside (School of Food, Fisheries and Environmental Studies) as part of an under-graduate research project.

Due to poor weather restricting the supply of fish and the limited access to University laboratory facilities during holidays, the University received an incomplete set of samples. Although the results mirrored the equivalent results obtained from the PHLS, they are not included in this report but are reported separately (Ref 2).

2. Trials Sequence

The following trials were carried out in the Seafish Laboratory at Hull during the period November 1995 to January 1996:

Trial I **Determination of the effect of both chlorine and ozone treated ice on the storage of cod**

This trial was carried out to determine if ice containing a chlorine or ozone residual could extend the storage life of boxed and iced cod.

Trial II **Determination of the effect of chlorine concentration on the storage of cod and mackerel held in treated RSW**

This trial was carried out to determine the effect of a range of chlorine dose levels on the sensory quality of cod and mackerel held in treated RSW. The results of the trial could then be used to determine the optimum chlorine dose for a pilot scale RSW Trial.

Trial III **Determination of the effect of chlorine dosing on the storage of cod and mackerel held in a pilot scale RSW tank**

This trial was carried out to determine the effect of two chlorine dose levels on the microbiological and sensory quality of cod and mackerel held in a pilot scale RSW system.

Trial IV **Determination of the effect of ozone dosing on the storage of cod and mackerel held in a pilot scale RSW tank**

This trial was carried out to determine the effect of ozone alone and of ozone in conjunction with UV treatment, on the microbiological and sensory quality of cod and mackerel held in a pilot scale RSW system. UV was used after ozonation to destroy any remaining ozone residual which should improve the bactericidal action and reduce the risk of causing oxidative rancidity in the fish.

3. Equipment

3.1 Pilot Scale RSW Tanks

A diagram of the pilot scale RSW tanks used in Trials III and IV is shown in Figure 1.

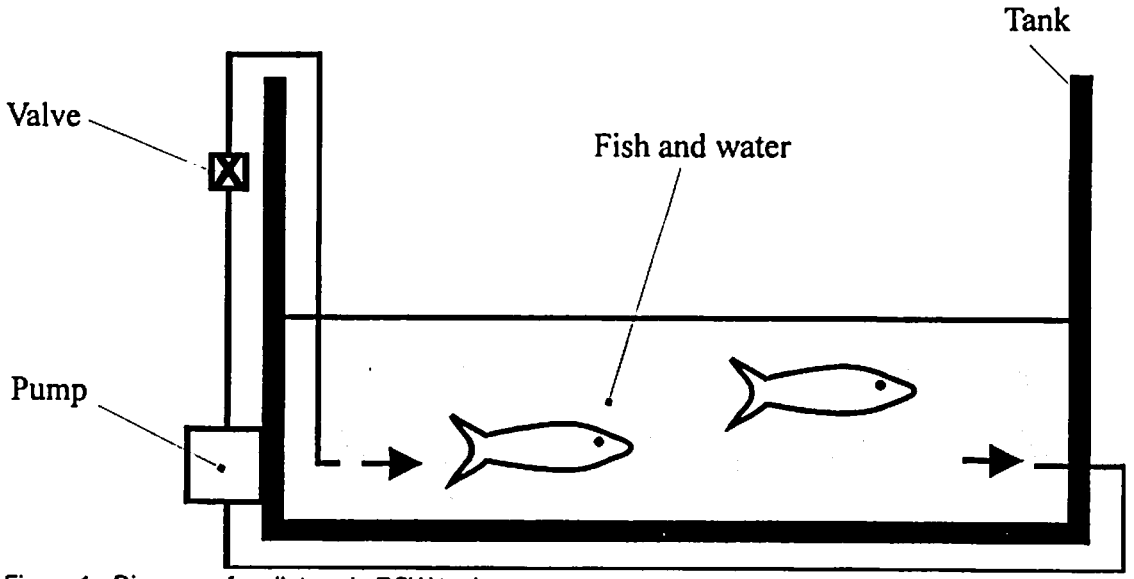

Figure 1 - Diagram of a pilot scale RSW tank

The 1000 mm x 600 mm x 660 mm tanks were constructed from an Allibert No. 21250 jumbox. The water in the tank was circulated using an Eheim 1060 pump with the flow rate regulated by an ABS ball valve. Pipework consisted of 25 mm reinforced flexible PVC hose.

Commercial RSW vessels run tanks with a fish to water ratio varying between 1:1 to 4:1. For the purpose of these preliminary trials a ratio of 1:1 was chosen as practical difficulties were encountered treating the water and keeping the fish fully immersed. If the trials prove successful a 4:1 ratio could be used in further trials on a larger scale.

3.2 Ozone Generation and Water Treatment Equipment

The equipment used in Trials I and III is shown schematically in Figure 2 overleaf.

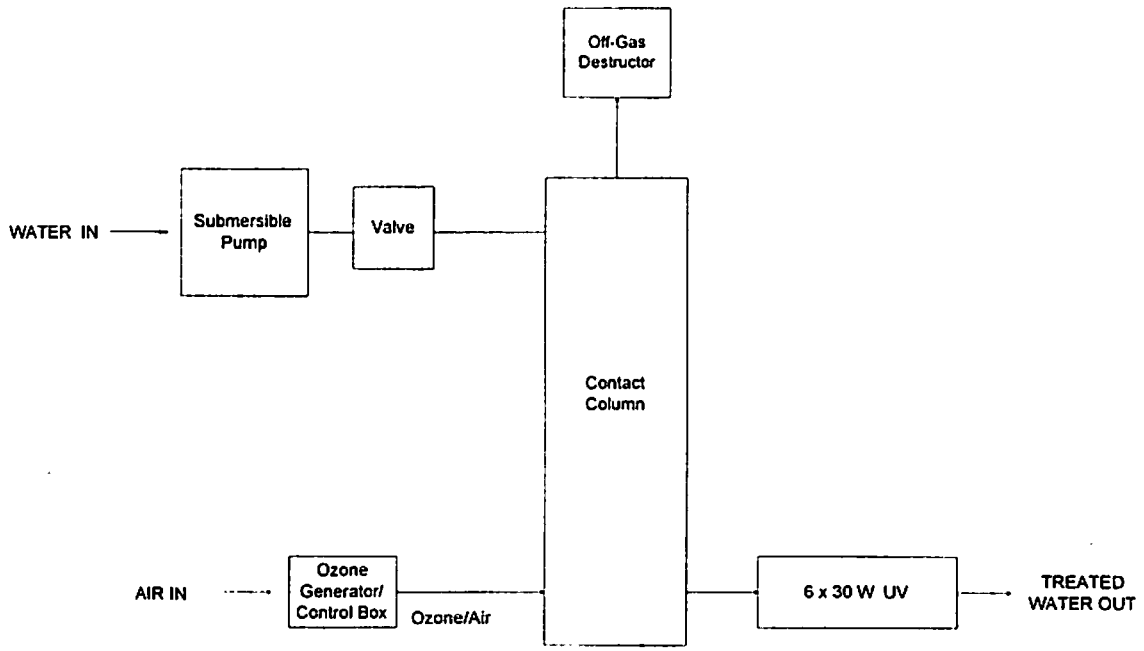

Figure 2 - Ozone generation equipment

The equipment consisted of an RK 2™ 2.5 g/h ozone generator, with twin internal air dryers and an Octopus ™ programmable controller/data logger, and a contact column to treat the water, both supplied by Dryden Aquaculture. The controller had two probe inputs which were used to measure the oxidation-reduction potential (ORP) (redox) and pH of the treated water. The 1500 mm x 500 mm diameter seawater contact column was constructed in 316 stainless steel. The air flow into the ozone generator was set to 5 cf/hr.

The water flow into the contact column was regulated using an Eheim 1060 submersible pump and an ABS ball valve. A 6 x 30 W UVAQ™ UV unit was connected to the water outlet from the contact column. The UV unit could be switched on or off independently of the ozone generator. Ozone not entering solution in the contact column was destroyed by an activated carbon filter in the off-gas destructor.

All pipework carrying ozonated air consisted of PTFE tubing. Pipework for the water consisted of 25 mm reinforced flexible PVC hose.

4. Common Experimental Methods

4.1 Ozonation of Water

To ozonate water for treated ice production, the submersible pump connected to the water inlet hose of the contact column was placed into a tank containing 200 litres of fresh water. The water was pumped into the contact column at 10 l/min and the ozonated water was ducted back into the tank. The ozone output dial on the ozone generator was set to 80% (maximum). The pH and ORP probes were placed in the reservoir. The water was then recycled round the system until the desired redox potential was achieved. RSW water was ozonated by draining each RSW tank into a large container and repeating the above process. The treated water was immediately returned to the RSW tank.

4.2 Chlorination of Water

The amount of free and total chlorine in a nominal 5% w/w available chlorine solution was determined using a calibrated Hach DR2000 Spectrophotometer and DPD reagents. The water to be treated was manually dosed with the sodium hypochlorite solution to give the specified chlorine dose.

4.3 Sensory Assessment

Raw and cooked sensory assessment was carried out on the fish using the Torry freshness scoring scheme (Appendix I). After filleting, the fillets were rinsed in fresh water before being steamed for 20 minutes prior to assessment by a minimum of three sensory assessment experts.

4.4 Microbiological Analysis

Fish and water samples were analysed by the Namas accredited PHLS department of Hull Royal Infirmary. Samples were analysed for Total Viable Count (TVC) at 37° C and *Pseudonas sp.* Organisms were enumerated by using either a spiral plate count or membrane filtration according to the standard methods detailed in HMSO document No.71.

When possible the remaining duplicate samples were analysed by research students of the University of Humberside (School of Food, Fisheries and Environmental Studies). These samples were analysed for TVC, *Pseudomnas sp*, Total Coliform bacteria (TC), histamine forming bacteria and histamine content.

4.5 Fish Supply

The cod used in the trials were small to medium sized gutted fish obtained from a single haul of a local Bridlington day boat. The mackerel used in the trials were whole fish from the Engish Channel fishery, landed into Portsmouth and transported to Hull boxed and iced in refrigerated transport.

5. Trial I - Determination of the Effect of Both Chlorine and Ozone Treated Ice on the Storage of Cod

5.1 Purpose

This trial was carried out to determine if the quality and storage-life of gutted cod could be improved by using chlorine or ozone treated ice. The trial compared the microbiological and sensory quality of gutted cod kept in ice made from water dosed with 20 mg/l and 80 mg/l of chlorine, freshly ozonated water and untreated water.

5.2 Method

A 200 litre sample of fresh water was ozonated for 10 minutes to raise the ORP from 250 mV to 900 mV which corresponds to an ozone concentration of approximately 2.0 mg/l. The ozone concentration in the water was measured using a Hach DR 2000 spectrophotometer and indigo trisulphonate reagents. The ozonated water was immediately fed into a Ziegra ice machine to produce ozonated flake ice.

A fish box was filled with 30 kg of cod and alternate layers of ozonated ice in a 3:1 fish to ice ratio. The boxed fish was held in a chill store running at 2° C. Melted ice was replaced as necessary.

Prior to storage, raw and cooked sensory assessment was carried out on four fish. Four 250 g samples of fish flesh along with 4 x 250 g samples of ice were sent for microbiological analysis.

The above procedure was repeated with ice made from water treated with a solution of sodium hypochlorite to give a chlorine dose of 20 mg/l and 80 mg/l and with ice from the local UK™ ice plant which was used as a control.

After 16 hours, 250 g of ice made with ozonated water was melted and the ozone concentration and ORP was re-measured to determine if an ozone residual remained in the ice.

The boxes of fish were re-iced every 4 days to keep a sufficient amount of ice around each fish.

After 6 and 12 days storage the sampling procedure was repeated with fish taken from each treatment.

5.3 Results

The results of the trial are shown in Table 1 overleaf.

Table 1 - The microbiological and sensory quality of cod stored in treated ice

Time on Ice (Days)	Treatment	Average Bacterial Count Fish Flesh		Average Torry Sensory Score		
		TVC (cfu/g)	Pseudomonas (cfu/g)	Gill Odour	Cooked Odour	Cooked Flavour
0	Control	1.1×10^5	2.2×10^5	9.50	9.50	9.50
6	Control	9.2×10^5	5.0×10^5	8.00	7.50	7.50
	Hypochlorite 20 mg/l	7.6×10^5	4.0×10^5	8.00	7.50	7.50
	Hypochlorite 80 mg/l	7.0×10^5	2.3×10^5	8.00	7.50	7.00
	Ozone	2.5×10^5	9.7×10^4	8.00	7.50	7.25
12	Control	1.5×10^7	1.5×10^8	5.50	5.00	5.50
	Hypochlorite 20 mg/l	2.2×10^7	2.2×10^8	4.50	5.00	5.75
	Hypochlorite 80 mg/l	3.0×10^7	2.4×10^6	5.50	5.00	5.50
	Ozone	8.4×10^6	1.2×10^6	5.00	5.00	6.00

Key: TVC = Total Viable Count

The microbiological results for the samples of melted ice showed no TVC or *Pseudomenas sp* bacteria in any sample.

A sensible and stable direct reading of the amount of ozone in the water could not be achieved with the spectrophotometer.

The redox potential of the melted ozone treated ice was 540 mV after 16 hours.

No significant difference in sensory or microbiological quality was observed between fish kept in the treated ice and fish kept in untreated ice over the duration of the trial.

5.4 Discussion

At the concentrations used, neither ozone or sodium hypochlorite treated ice had a significant effect on the microbiological or sensory quality of gutted cod.

The trial could be repeated using higher chlorine doses but at 80 mg/l the treated ice smelled quite strongly of chlorine. At higher doses it is possible that tainting of the fish could occur.

Ozone measurement in water and especially seawater is notoriously difficult. It proved impossible to measure the ozone residual in water or melted ice using the indigo

trisulphonate method due to a widely fluctuating reading. After 16 hours the redox potential of the melted ozonated ice was over double that of untreated fresh water. However, due to the highly reactive nature of ozone, it is likely that no ozone residual would remain in the ice after a few hours. The elevated ORP observed is likely to have been caused by residual chemical products formed as a result of ozonation.

6. Trial II - Determination of the Effect of Chlorine Concentration on the Storage of Cod and Mackerel Held in Treated RSW

6.1 Purpose

This bench scale trial was carried out to determine the effect of a range of chlorine dose levels on the sensory quality of cod and mackerel held in treated RSW. The results of the trial could be used to determine the optimum chlorine dose required for a larger scale RSW trial to avoid tainting the fish.

The trial compared the sensory quality of gutted cod and whole mackerel kept in RSW water treated with sodium hypochlorite to give a chlorine dose of 0 mg/l, 5 mg/l, 20 mg/l and 50 mg/l. The sensory quality of the fish was determined after 0, 4 and 9 days on ice.

6.2 Method

Four 20 l PVC buckets were each filled with 10 litres of artificial seawater (ASW). Sodium hypochlorite solution was added to each bucket to give a chlorine dose of 0 mg/l (as a control), 5 mg/l, 20 mg/l and 50 mg/l. Four cod were placed into each bucket.

All buckets were stored at – 1 °C in a chill store.

Prior to storage, raw and cooked sensory assessment was carried out on four fish.

The water in each bucket was replaced every 48 hours.This procedure was repeated using a further four buckets each containing 8 mackerel (approximately 10 kg).

After 4 and 9 days storage, sensory assessment was repeated on two fish from each bucket.

6.3 Results

The results of the trial are shown in Table 2 overleaf.

Table 2 - The sensory quality of cod and mackerel stored in chlorinated RSW

Time in RSW (days)	Chlorine Level (mg/l)	Average Cod Sensory Assessment Score			Average Mackerel Sensory Assessment Score		
		Gill Odour	Cooked Odour	Cooked Flavour	Gill Odour	Cooked Odour	Cooked Flavour
0	0	9.00	8.50	9.00	7.50	7.00	7.50
4	0	8.00	8.50	8.50	7.00	6.50	7.00
	5	7.50	7.00	7.00	7.00	7.00	7.50
	20	8.00	7.00	7.00	7.00	6.00	6.75
	50	7.5	8.00 Deodorized/ neutral odour	8.00	7.00	7.25	7.25
9	0	5.50	5.50	5.50	3.00 Sour smell	3.50	3.50
	5	6.00	5.50	5.00	3.50 Faint sour	3.50	4.50
	20	6.00	5.75	6.00	5.00 Neutral/ fresh odour	4.00	5.00
	50	5.50 Low gill odour	7.00 Faint neutral odour	5.50	6.00 Neutral/ fresh odour	4.50	6.00

No difference in the raw appearance of the cod held in the different treatments was observed. However, cod held in 50 mg/l of sodium hypochlorite had a neutral/deodorised gill odour after 9 days and the cooked fish had an unusual deodorised, neutral/fresh smell. No differences in the cooked flavour between the treatments was observed. All cod samples developed a strong salty flavour.

Mackerel held in 50 mg/l treated RSW had significantly less bloodshot eyes compared to mackerel held in lower hypochlorite concentrations and the control. Raw mackerel held in untreated RSW had a faint sour odour which was not detected in fish from the other treatments, this difference became more exaggerated after 9 days.The cooked odour and flavour of mackerel kept in 50 mg/l treated RSW was slightly better than the control after 4 days storage. After 9 days storage the difference in quality was considerable.

The treated RSW water was considerably clearer than untreated water. This was particularly apparent for water containing mackerel (see Figure 3 overleaf).

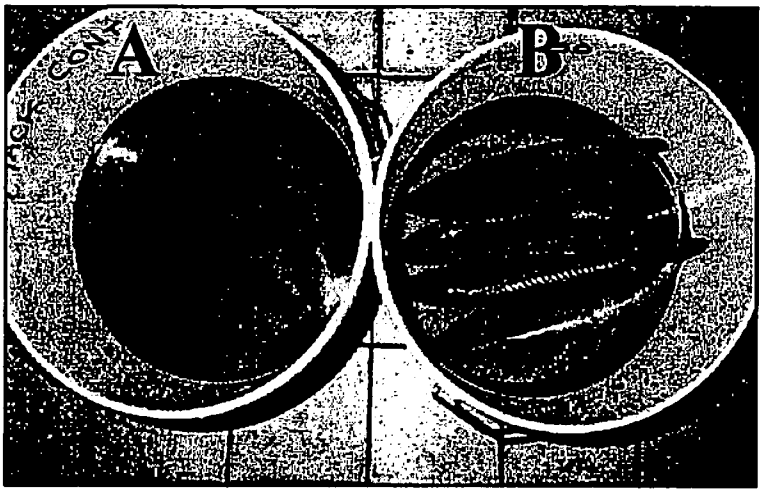

Figure 3 - A comparison of the turbidity of RSW water containing mackerel after 48 hours (A) Untreated (B) 50 mg/l sodium hypochlorite treated water

6.4 Discussion

The sensory quality of gutted cod was not improved by storage in sodium hypochlorite treated RSW.

However, a dramatic improvement in the quality of whole mackerel was achieved by storage in 50 mg/l treated RSW. The treatment slowed the rate of spoilage by almost 50%, and the fish remained acceptable after 9 days storage although the treatment had little effect during the first four days of storage. This would be expected as bacterial spoilage would normally only become significant a few days after capture.

The ineffectiveness of chlorine to extend the shelf life of cod may be due to the amount or nature of dissolved and suspended organic material in the cod RSW water, possibly as a result of the cut flesh. The sodium hypochlorite could be quickly bound by this material which would reduce the amount of disinfectant available to kill bacteria in the water and on the fish.

Untreated mackerel RSW water although red with blood was clearer than untreated cod RSW which suggests less organic material was present. The addition of sodium hypochlorite produced a clearer RSW water for both mackerel and cod which suggests that the disinfectant reduced bacterial action in the water.

It is quite possible that dosing an RSW system with over 50 mg/l chlorine every 48 hours would cause tainting of the fish. Further treated RSW trials should be carried out using a chlorine dose of 40 mg/l or less.

7. Trial III - Determination of the Effect of Chlorine Dosing on the Storage of Cod and Mackerel Held in a Pilot Scale RSW Tank

7.1 Purpose

The previous trial showed that the addition of a small amount of sodium hypochlorite could significantly slow the spoilage of mackerel in chilled seawater. This trial was carried out to repeat the work on a larger scale, using pilot scale tanks to simulate commercial RSW conditions as closely as possible within a laboratory environment.

Six RSW tanks were built with a simple water recirculation system. Refrigeration was achieved by keeping the tanks in a chill store. Two levels of sodium hypochlorite were used to give a chlorine dose of 20 mg/l and 40 mg/l about every 48 hours. Sensory and microbiological assessment was carried out to compare the quality of both cod and mackerel kept in treated and untreated RSW over an extended storage period.

7.2 Method

Three of the pilot scale RSW tanks were each filled with 30 litres of sea water taken from inside Bridlington Harbour. The water in each was treated with sodium hypochlorite solution to give a chlorine dose of 0 mg/l, 20 mg/l and 40 mg/l. Each tank was filled with 30 kg of cod. The water recirculation rate of each tank was set to 0.5 l/min (one change per hour) before each tank was placed in a chill store set to maintain the RSW temperature at –1 °C.

The above procedure was repeated with a further three tanks and fresh mackerel.

Prior to storage, raw and cooked sensory assessment was carried out on four fish. Four 250 ml samples of seawater were taken from each tank before and after sodium hypochlorite dosing along with 4 x 250 g samples of fish flesh (taken from 4 fish) and were sent for microbiological analysis.

The treated tanks containing cod were re-dosed with sodium hypochlorite after 2,5,7,9 and 12 days. (Keeping as close to 48 hour intervals as the sampling periods, dictated by access to the University laboratory facilities, would allow). For the cod the sensory and microbiological assessment was repeated after 5 days and 12 days. (Fish were removed for assessment before sodium hypochlorite dosing was carried out).

The treated tanks containing mackerel were re-dosed after 2,4,6 and 9 days. Mackerel assessment was repeated after 4 and 9 days.

7.3 Results

The results of the trial are shown in Tables 3 and 4 and Figure 4 overleaf.

Table 3 - The microbiological and sensory quality of cod stored in chlorinated RSW

Time in RSW (days)	Chlorine dose (mg/l)	Average Microbiological Count					Average Torry Sensory Score		
		Fish Flesh		Seawater					
		TVC (cfu/g)	*Pseu-domonas* (cfu/g)		TVC (cfu/ml)	*Pseu-domonas* (cfu/ml)	Gill Odour	Cooked Odour	Cooked flavour
0	0	6.0×10^5	1.0×10^4		5.0×10^4	7.0×10^4	9.00	9.00	9.00
	20	N/A	N/A		9.0×10^2	ND	N/A	N/A	N/A
	40	N/A	N/A		ND	ND	N/A	N/A	N/A
5	0	4.0×10^4	4.0×10^4		2.3×10^3	9.5×10^2	7.50	8.00	7.25
	20	4.5×10^4	4.5×10^4	A	1.8×10^6	1.0×10^3	7.50	8.00	7.25
				B	9.0×10^5	1.0×10^3			
	40	2.5×10^4	2.0×10^4	A	6.5×10^3	1.0×10^3	7.50	8.25	7.75
				B	1.6×10^2	9.0×10^2			
12	0	2.5×10^5	2.2×10^5		2.5×10^3	1.0×10^3	3.50	3.75	4.00
	20	1.8×10^5	8.0×10^4	A	2.0×10^4	1.0×10^3	Faint neutral odour	4.25	4.50
				B	3.0×10^4	9.5×10^2			
	40	2.0×10^5	1.7×10^5	A	1.0×10^3	9.5×10^2	Faint neutral odour	4.75	5.25
				B	5.0×10^2	9.0×10^2			

Key:
- A - Before hypochlorite dosing
- B - After further hypochlorite dosing
- ND - Not detected
- N/A - Not applicable
- TVC - Total Viable Count

Table 4 - The microbiological and sensory quality of mackerel stored in chlorinated RSW

Time in RSW (days)	Chlorine (mg/l)	Average Microbiological Count					Average Torry Sensory Score		
		Fish Flesh		Seawater				Cooked	
		TVC (cfu/g)	*Pseu-domonas* (cfu/g)		TVC (cfu/ml)	*Pseu-domonas* (cfu/ml)	Gill Odour	Cooked Odour	Cooked Flavour
0	0	1.5×10^4	1.0×10^4		5.0×10^4	7.5×10^4	7.50	7.00	7.25
	20	N/A	N/A		9.0×10^2	ND	N/A	N/A	N/A
	40	N/A	N/A		ND	ND	N/A	N/A	N/A
4	0	7.6×10^4	5.5×10^3		2.4×10^3	3×10^3	3.00	5.50	5.75
	20	4.5×10^4	5.5×10^3	A	2.6×10^4	7.5×10^3	6.00	6.00	6.50
				B	7.9×10^4	8.3×10^3			
	40	3.5×10^4	2.5×10^3	A	6.5×10^3	1.0×10^3	6.50	6.00	6.00
				B	1.6×10^2	9.0×10^2			
9	0	3.2×10^6	1.9×10^5		6.2×10^3	9.0×10^2	1.50	1.50	2.00
	20	1.7×10^6	7.5×10^4	A	1.8×10^4	9.5×10^2	5.00 Faint neutral odour	5.75	5.25
				B	1.5×10^4	1.0×10^3			
	40	4×10^5	5.5×10^4	A	4.0×10^3	9.5×10^2	5.00 Faint neutral odour	5.50	5.75
				B	7.0×10^1	9.5×10^2			

Key:

A	- Before further hypochlorite dosing
B	- After further hypochlorite dosing
ND	- Not detected
N/A	- Not applicable
TVC	- Total Viable Count

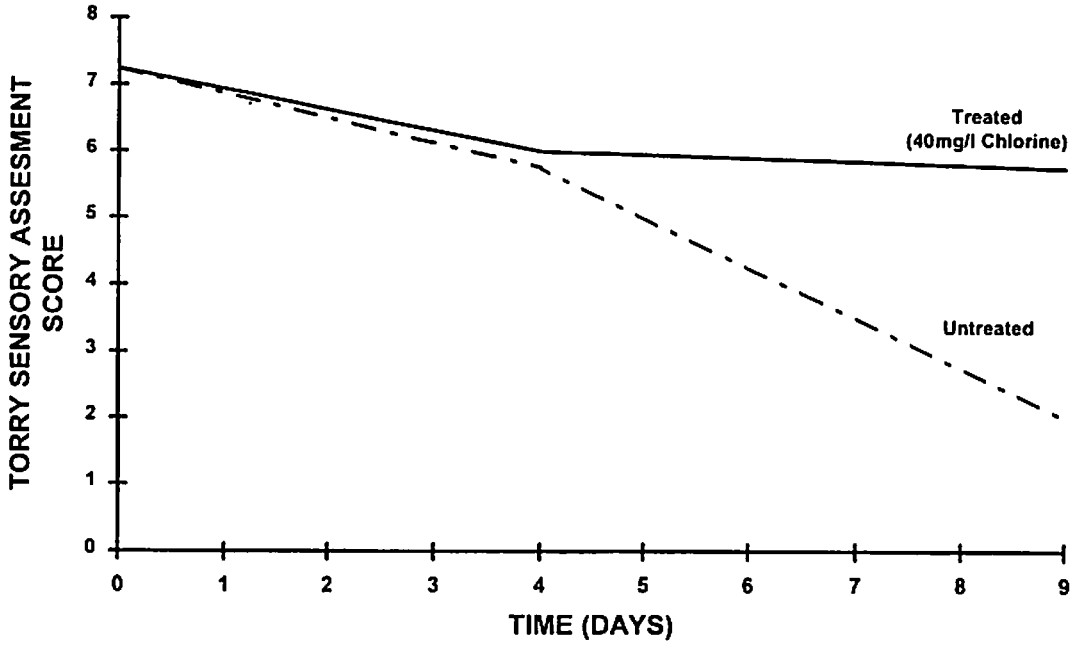

Figure 4 - The quality of mackerel in treated and untreated RSW

No difference in the raw appearance of cod held in the different RSW treatments was observed during the trial. After 12 days, fish stored in 20 mg/l and 40 mg/l had a very faint or neutralised gill odour. The cooked odour and flavour of cod kept in the 40 mg/l treated RSW was slightly better than the untreated control after 5 days. This difference became more pronounced after 12 days. All samples had a strong salty flavour. Hypochlorite treatment at either level did not significantly reduce the bacterial loading of the cod flesh. A slight reduction in the bacterial loading of the RSW water was achieved after each 40 mg/l chlorine dose.

Mackerel held in treated RSW showed less reddening of the eyes and significantly fresher gill odour than the control fish after 4 days. This difference became more pronounced after 9 days. The cooked odour and flavour of the mackerel kept in treated RSW was slightly better than fish from the untreated control after 4 days. After 9 days this difference in quality was considerable. After 9 days the flesh of mackerel kept in 40 mg/l treated RSW had a significantly lower TVC bacterial loading than the control. A large reduction in bacterial loading of the RSW water was achieved after each 40 mg/l chlorine dose.

7.4 Discussion

Only a small improvement in the quality of cod could be achieved on extended storage in sodium hypochlorite treated RSW. The same treatment used for mackerel dramatically

slowed the rate of microbiological spoilage by over 50% resulting in the fish remaining acceptable after 9 days.

As expected, the treatment had little effect on the fish over the first few days of storage when the spoilage is predominantly enzymatic.

The microbiological results confirmed that sodium hypochlorite dosing reduced the rate of bacterial spoilage. The higher bacterial kill observed when sodium hypochlorite was added to mackerel RSW suggests that either the cod RSW contains more organic material which binds up the disinfectant or the organisms which spoil cod are more resistant to chlorine.

8. Trial IV - Determination of the Effect of Ozone Dosing on the Storage of Cod and Mackerel Held in a Pilot Scale RSW Tank

8.1 Purpose

The previous trial showed that sodium hypochlorite can dramatically slow the rate of microbiological spoilage of mackerel but has little effect on cod. It was thought that ozone may have several advantages over sodium hypochlorite for treating an RSW system. Ozone could enable a greater biocidal effect which may slow the spoilage of cod as well as mackerel without the risk of chemical taints and chlorine compounds being transferred to the fish.

The previous trial was repeated using ozone treatment about every 48 hrs instead of sodium hypochlorite. As highly reactive free radicals produced by the ozone may accelerate oxidative rancidity in oil rich fish such as mackerel, a further RSW system was set up which passed the ozonated water through a UV system to remove any ozone residual prior to returning it to the tank.

8.2 Method

The trial was carried out according to the method detailed in the previous trial. The seawater in the ozonated tanks was treated by recirculation through the ozone generating equipment for 10 minutes with the UV switched off (Section 4.2). The procedure was then repeated to treat the water in the ozone/UV tanks with the UV switched on.

All the treated tanks were re-ozonated after 2,5,7,9 and 12 days. Thawed mackerel was used in the trial, as fresh was unobtainable.

8.3 Results

Results of the trial are shown in Tables 5 and 6 overleaf.

Table 5 - The microbiological and sensory quality of cod stored in ozone treated RSW

Time in RSW (days)	Treatment	Average Microbiological Count					Average Torry Sensory Score		
		Fish Flesh		Seawater					
		TVC (cfu/g)	*Pseudomonas* (cfu/g)		TVC (cfu/ml)	*Pseudomonas* (cfu/ml)	Gill Odour	Cooked Odour	Cooked Flavour
0	Control	1.5×10^4	1.0×10^4		5.0×10^4	<100	9.50	9.50	9.25
	Ozone	N/A	N/A		<100	<100	N/A	N/A	N/A
	Ozone + UV	N/A	N/A		<100	<100	N/A	N/A	N/A
5	Control	8.0×10^5	3.5×10^3		2.4×10^3	3×10^3	7.50	6.00	7.00
	Ozone	4.7×10^4	2.3×10^6	A	---	---	6.50	6.75	6.50
				B	7.9×10^4	8.3×10^3			
	Ozone + UV	4.0×10^5	3.1×10^5	A	6.5×10^3	1.0×10^3	6.50	7.00	7.00
				B	1.6×10^2	9.0×10^2			
12	Control	6.7×10^4	5×10^4		1.2×10^4	1.0×10^3	3.50	3.50	4.00
	Ozone	1.1×10^6	9.7×10^4	A	1.7×10^4	9.5×10^3	4.00	4.00	4.50
				B	1.8×10^4	1.0×10^3			
	Ozone + UV	1.0×10^5	8.0×10^4	A	6.1×10^3	9.5×10^2	4.00	4.00	4.50
				B	1.0×10^3	5.5×10^3			

Key:
- A - Before further ozonation
- B - After further ozonation
- TVC - Total Viable Count
- --- - Samples lost
- N/A - Not applicable

Table 6 - The microbiological and sensory quality of mackerel stored in treated ozone treated RSW

Time in RSW (days)	Treatment	Average Microbiological Count					Average Torry Sensory Score		
		Fish Flesh		Seawater					
		TVC (cfu/g)	*Pseudomonas* (cfu/g)		TVC (cfu/ml)	*Pseudomonas* (cfu/ml)	Gill Odour	Cooked Odour	Cooked Flavour
0	Control	2.5×10^4	9×10^2		5.1×10^4	<100	7.00	7.00	7.00
	Ozone	N/A	N/A		<100	<100	N/A	N/A	N/A
	Ozone + UV	N/A	N/A		<100	<100	N/A	N/A	N/A
5	Control	2.1×10^5	1.6×10^3		2.0×10^3	9.5×10^3	6.00	6.50	6.75
	Ozone	2.0×10^4	2.0×10^6	A	---	---	6.00	5.50	6.50
				B	1.3×10^4	9.0×10^3			
	Ozone + UV	2.0×10^5	2.1×10^5	A	1.2×10^3	1.0×10^3	6.00	6.00	6.50
				B	9.0×10^2	9.0×10^2			
12	Control	5.0×10^4	3.9×10^4		2.0×10^4	9.5×10^3	2.00	2.00	3.00
	Ozone	5.0×10^6	3.5×10^4	A	1.5×10^4	9.5×10^3	1.50	2.00	3.00
				B	1.0×10^4	9.5×10^3			
	Ozone + UV	1.1×10^5	1.6×10^4	A	2.1×10^3	2.1×10^2	2.00	2.00	3.00
				B	1.8×10^3	1.0×10^3			

Key: A - Before further ozonation; B - After further ozonation; TVC - Total Viable Count; --- - Samples lost; N/A - Not applicable

The initial ozonation of the clean seawater raised the ORP from 267 mV to 800 mV.

Ozonation of the RSW which had contained fish raised the ORP from approximately 115 mV to 200 mV. Ozonation/UV treatment raised the ORP from approximately 125 mV to 136 mV.

No difference in either the raw appearance, cooked odour/flavour, microbiological quality of the fish flesh or RSW water was observed for either cod or mackerel by either of the two treatments.

8.4 Discussion

The ozone dose was too low to have an effect on the quality of cod or mackerel stored in treated RSW.

As both treatments gave a good bacterial kill in clean seawater before fish were added, it is likely that the biological material (blood, slime, dissolved and suspended pieces of fish etc) which accumulated in the RSW, neutralised the ozone before disinfection could take place. The trial should be repeated with the water receiving a much higher dose of ozone.

9. Overall Discussion and Conclusions

It is considered unlikely that sodium hypochlorite or ozone incorporated into ice could be used to significantly improve the quality of boxed and iced fish. Higher concentrations of sodium hypochlorite could be used but tainting may then occur. It is thought that ozone is too reactive for a residual to remain in the ice long enough to affect the quality of fish. However such treatments could be recommended, as part of good hygiene practice, to disinfect non-potable water prior to ice production to ensure clean ice and to reduce any risk of product contamination.

Sodium hypochlorite dosing can dramatically slow the rate of microbiological spoilage of mackerel in an RSW system. A chlorine dose of 40 mg/l every 48 hours reduced the rate of microbiological spoilage by over 50% resulting in the fish remaining acceptable after 9 days storage. As expected, the treatment had little effect over the first few days after capture when the spoilage is predominantly enzymatic.

It is thought that increasing the concentration of sodium hypochlorite dosing above 40 mg/l every 48 hours for RSW systems could result in odour and flavour tainting.

This type of treatment may be beneficial for boats fishing for longer than 3-4 days. In addition, as fish supplies become more scarce and catching and landing areas change, overland transport may become more common. This technology could be incorporated into an overland transport system or processor holding facilities. Further development of this technology is recommended.

Chlorine dosed RSW (40 mg/l) only slightly slowed the bacterial spoilage of cod and is unlikely to be commercially useful. This may be due to the type or amount of protein released into the water by cod binding up the disinfectant, or to the resistance of the type of bacteria which spoil cod.

Ozonation of RSW had no significant effect at the levels of ozonation investigated. Further laboratory scale work is required to investigate higher levels of ozonation.

10. Further Work

Laboratory scale chlorine dosed RSW trials will be repeated with mackerel, using the higher commercially used fish to water ratio of 4:1. If successful, the use of chlorine dosing will be investigated in commercial vessel, transport and storage applications. The work will involve following the quality of the treated fish from catching through to consumption.

If this work demonstrates practical fish quality advantages then additional work must be carried out to determine the safety and the amount of chlorine compounds taken up by the fish.

Pilot scale ozone treated RSW trials will be repeated in the laboratory on cod and mackerel using a higher ozone dose and will also be followed up commercially if successful.

11. References

1. WATSON R.B., (1996) Trials to Assess the Effectiveness of Ionization, Chlorination and UV Irradiation for the Disinfection of Seawater. Seafish Report No. 473.

2. CHERRY B., (1996) An Investigation into the Possibility of Using Ozonated RSW Onboard Ship Processing Plants to Enhance the Quality of Fish. Bsc (Hons) Food Studies, Final Year Report, University of Humberside

Appendix 1
(Torry Sensory Assessment Scoring Sheets)

Definitions of Some Terms Used in Sensory Assessment of Fish

Odour/Flavour	
Seaweedy	Fresh, sharp, clean odour associated with the seaside. May be qualitified by 'stale' which is the odour of slightly rotten seaweed.
Shellfish	The odour of fresh bivalve shellfish when freshly gathered. Similar to seaweedy and marine but a more rounded and richer odour.
Neutral	A week indefinite odour or flavour without specific fresh or stale character.
Caprylic	The odour of goats or horses.
Milky	The odour or flavour of fresh milk. Sweetish and slightly creamy. Not the flavour of sterilised or tinned milk.
Peppery	The odour or flavour of freshly-ground pepper. Not the irritant action.
Metallic	'Coin in the mouth' sensation.
'Old Boots'	The odour of old leather and sweat.
Marine	An odour pertaining to the seaside.
Mealy	The odour or flavour of raw potatoes and flour.
Nutty	The full flavour of nuts, eg walnuts, hazelnuts.
Rancid	The unpleasant odour or flavour of stale oil, stale fat.
Aromatic	A fragrant, sweet smelling odour.
Acrid	A sharp, pungent, prickling sensation.
Blown Oil	The odour or flavour of stale fish oil.
Green Plant	The odour or flavour of young, tender shoots.
Citric	The odour or flavour of oranges, limes, etc.
Curry	A spicy, fragrant odour or flavour.
Butterscotch	The odour or flavour of toffee.
Butyric Acid	The odour or flavour of stale butter.
Acetic Acid	The odour or flavour of vinegar.
Lactic Acid	The odour or flavour of sour milk.
Tallowy	The odour or flavour of animal fat, suet.
Garlic	The pungent odour or flavour of freshly crushed garlic.
Chloroform	An anaesthetic-like odour.
Musty	An odour vaguely suggesting damp cellars, mildew or hay, or ground oatmeal
Bready	The yeasty odour of fresh bread.
Beery	A brewery odour.
Grassy	The odour of composted grass.
Fresh-cut Grass	The pronounced, slightly sharp, odour of freshly cut grass.
Fruity	A sweet-acid odour, suggesting over-ripe fruit.

Freshness Score Sheet for Iced Cod - Raw Fish

Score	Eyes	Skin	Texture and Effect of Rigor Mortis	Flesh and Belly Flaps	Kidney and Blood	Gills		Score
						Appearance	Odour	
10	Bulging, convex lens, black pupil, crystal-clear cornea.	Bright, well-differentiated colours, glossy, transparent slime.	Flesh firm and elastic. Body pre-rigor or in rigor.	Cut surface stained with blood. Bluish translucency around backbone. Fillet may have rough appearance due to rigor mortis contraction.	Bright red, blood flows readily.	Glossy, bright red or pink clear mucus.	Initially very little odour increasing to sharp, iodine, starchy, metallic odours changing to less shart seaweedy, shellfish odours.	10
9	Convex lens, black pupil with loss of initial clarity.		Flesh firm and elastic. Muscle blocks apparent. In or just pasing through rigor.	White with bluish translucency, may be corrugated dur to rigor mortis effect.	Bright red, blood does not flow.			9
8	Slight flattening or plane, loss of brilliance.	Loss of brillance of colour.	Firm, elastic to the touch.	White flesh with some loss of bluish transluency. Slight yellowing of cut surfaces of belly flaps.	Slight loss of brightness of blood.	Loss of gloss and brightness, slight loss of colour	Fresh cut grass. Seaweedy and shellfish odours just detectable.	8
7							Slight mousy, musty, milky or caprylic	7
6	Slightly sunken, slightly grey pupil, slight opalescence of cornea.	Loss of differentiation and general fading of colours; overall greyness. Opaque and somewhat milky slime.	Softening of the flesh, finger indentations retained, some grittiness near tail.	Waxy appearance of the flesh, reddening around the kidney region. Cut surfaces of the belly flaps brown and discoloured.	Loss of brighness, some browning.	Some discolouration of the gills and cloudiness of the mucus.	Bready, malty, beery, yeasty.	6
5							Lactic acid, sour milk or oily.	5
4	Sunken, milky white pupil, opaque cornea.	Further loss of skin colour. Thick yellow knotted slime with bacterial discolouration. Wrinkling of skin on nose.	Softer flesh, definite grittiness.	Some opacity reddening along backbone and brown discolouration of the belly flaps.	Brownish kidney blood.	Slight bleaching and brown discolouration with some yellow bacterial mucus.	Lower fatty acid odours (eg acetic or butyric acids), composted grass, 'old boots', slightly sweet, fruity or chloroform-like.	4
3							Stale cabbage water, stale turnips, 'sour sink', wet matches.	3

Freshness Score Sheet for Iced Cod - Cooked Fish

Score	Odour	Flavour	Texture, Mouth Feel and Appearance	Score
10	Initially weak odour of seet, boiled milk, starchy followed by strengthening of these odours	Watery, metallic, starchy. Initially no sweetness but meaty flavours with slight sweetness may develop	Dry, crumbly with short tough fibres	10
9	Shellfish, seaweed, boiled meat, raw green plant	Sweet, meaty, creamy, green plant, characteristic	Succulent, fibrous. Initially firm going softer with storage. Appearance originally white and opaque going yellowish and waxy on storage	9
8	Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity		8
7	Woodshavings, woodsap, vanillin	Neutral		7
6	Condensed milk, caramel, toffee-like	Insipid		6
5	Milk jug odours, boiled potato, boiled clothes-like	Slight sourness, trace of 'off' flavours		5
4	Lactic acid, sour milk, 'byre-like'	Slight bitterness, sour, 'off' flavours		4
3	Lower fatty acids (e.g. acetic or butyric acids), composted grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide		3

Mackerel Scoring Scales (Revised April 1986)

Raw Fish

Appearance of Skin and Body	
Score	**Description**
8	Firm body with silky smooth skin, lateral line and reticulations on upper surface well-defined. Body colours iridescent with strong royal blue and turquoise colours on upper surface and blue and violet colours on ventral surface with a silvery sheen. Passing into rigor mortis.
7	Loss of colour definition, some blood stains apparent. Passing out, or out of rigor mortis.
6	Colours of dorsal surface paler, reticulations grey, ventral surface white with golden tinge. Patchy iridescence.
5	Washed out colours, definite golden tinge to skin, patchy iridescence. Body soft with blood red/brown slime oozing from gill-covers. Skin wrinkles on flexing.
4	Fish limp and floppy with distinct ice marks. Washed out colours with mottling or golden tinge.
3	Little distinction between upper and lower surfaces. Skin very wrinkled with distinct ice marks. Body very soft.
2	Yellow slime apparent with belly burst of ungutted fish.
1	Thick knotted yellow slime, gritty skin with wrinkling on the nose.

Appearance of the Eyes	
Score	**Description**
8	Bulging convex eye with protruding lens. Shiny jet black-blue pupil with metallic brown iris. Eye-cap water clear.
7	Convex eye, lens plane with cornea. Pupil less shiny, irish green/blue. Slight clouding of eye-cap.
5	Flattening of eye but still convex, wrinkled pupil with slight cloudying of the lens. Silvery iris, starting to wrinkle. Yellowing of eye-cap.
4	Eye-ball plane with eye socket. Cloudy lens with silvery iris showing black specks. Golden eye eye-cap.
3	Concave or flattened eye with cloudy pupil. Yellow eye-cap.
2	Concave eye covered in film of yellow slime. Reddening around eye-socket.
1	Sunken eye covered in thick yellow slim, bleached eye-cap with peppered appearance.

Mackerel Scoring Scales

Raw Fish

Appearance of Gills	
Score	**Description**
8	Uniformly dark red/purple in colour with free blood and water-clear slime present.
7	Dark purple/maroon in colour with paler edge. Congealed blood present with opaque slime.
5	Loss of colour with red/brown slime.
4	Browning of gills with patchy bleaching, increase in quantity of slime resulting in blood red/brown slime oozing from gill cover.
3	Marked bleaching and browning of gills, covered in thick slime.
2	Further bleaching of gills with pink/brown viscous slime.
1	Gills completely bleached or washed-out pink colour and starting to disintegrate.

Odour of Gills	
Score	**Description**
8	Weak, delicate odours, cloying sweet, sharp, pepper, halogens, seaweed, blood.
7	More definite odours as above, also fragant, fresh grass, fruity, metallic, shellfish.
6	Dull muddy odours, musty, mousy, malty, cardboard, linseed oil, cod liver oil, discuits, blood.
5	Stale odours as above, also butterscotch, wet cardboard, wet dogs.
4	Mixture of 5 and 3 odours.
3	Sweet-rotten odours, oil, sweet rotten fruit (gratefruit), old grass cuttings, sickly sour.
2	Sour and sweaty odours, rancid, slight sulphury, yeast, slight ammonia.
1	Sulphury odours, rotten turnips, sour sink, wet matches, compost heap, sour cheese.
0	Strong faecal and ammoniacal odours.

Mackerel Scoring Scales

Cooked Fish Odours

Score	Description
8	Shellfish, fresh seawee, halogens (iodine), fresh blood, fresh lemons/chicken, sweet oil, muddy.
7	Fresh lamb stew, boiled potatoes, washing soda solution, onions, biscuits, sweet oil.
6	Earthy, slight spicy, cury, white chicken meat, fresh mushrooms, cardboard.
5	Waxy, new leather, wet paper, cardboard, dried meat extract, curry, just detectable rancidity.
4	Slight rancidity, cold mutton stew, woody, old mushrooms, KOH fish digest, wet paper, leather.
3	Slight rancidity, caramel, yeasty, stales, musty, malty, greasy stale chicken fat/chicken skins.
2	Sweaty, sour, rancid, stale cheese, sulphides, pickled herring, rotten fruit (sweet odour), charred.
1	Sulphides, burnt/acrid, rotten meat, vomit.
0	Strong ammoniacal, faecal, nauseating.

Cooked Fish Flavours

Score	Description
8	Sweet, starch, astringent, metallic, blood, meaty (cold lean beef), green-plant, spicy lemons, muddy, strong sweet oil. Red meat: strong meaty, sweet.
7	Sweet, oily chicken (white meat), blood, herbs (eg parsley), roast meat (cold lamb, pork), starch, astringent, insipid , earthy, mushrooms, onions/lemons. Red meat: strong meaty
6	Sweet, earthy, cardboard, slight curry, bland sweet oil, onions/lemons. Red meat: strong meaty
5	Slightly sweet, weak meaty, just detectable rancidity, musty, wet paper, cardboard, neutral bland oil, new leather. Red meat: strong meaty, slightly rancid.
4	Neutral bland oil, greasy cold chicken, slight rancidity, sweet/sour, caramel, acidic after-taste. Red meat: strong meaty, rancid, sulphury.
3	Slightly sour, rancid, stale roast meat, cold mutton stew, yeast, burning senstationon side of tongue, 'coin-in-mouth' sensation, acrid. Red meat: strong rancidity, sulphury.
2	Sour, rancid, rotten fruit (sweet sensation), chicken skins, charred paper, sulphides Red meat: strong rancidity, sulphury, tased with difficulty.
1	Strong rancidity, bitter, burnt/acrid, strong sulphides, rotten cabbages, rotten meat. Red meat: nauseating rancidity and sulphury.
0	Nauseating, ammoniacal, very strong sulphides, tased with difficulty.