

## FISHING INDUSTRY RESEARCH TRUST ACCOUNT

## FINAL REPORT

1. Title of project: Development of fish handling, processing and packaging systems, and their influence on the quality of Australian seafood products.
2. Organization: CSIRO, Division of Food Research.
3. Section: Tasmanian Food Research Unit (TFRU), Hobart.
4. Persons responsible for programme:  
  
Dr June Olley, D.Sc., Leader, TFRU.  
Mr H. Allan Bremner, M.Sc., Experimental Officer.
5. Location of operation: The work was based at the Tasmanian Food Research Unit but some experiments were carried out aboard the New South Wales FRV 'Kapala'.
6. Work schedule:  
  
Commencement date: 1.7.80  
Completion date: 30.6.83
7. Final Report: Ten papers have been published as a result of the work (Appendices 1-10). Two Tasmanian Regional Laboratory Occasional Papers Nos. 8 and 9 have also been prepared. These are not included with the final report as they are too detailed, but are available on request.

The unpublished work has been given some precedence in this final report as the published data is available in the Appendices. However, our overall findings are discussed in the light of overseas experience and in the case of vacuum packaging by comparison with the meat literature.

The final report has four major facets:

- a) Engineering.
- b) Vacuum packaging of fish with and without potassium sorbate.
- c) Chilling of fish in R.S.W.
- d) Evaluation of fish quality.

## ENGINEERING

Fishing and handling practices are often the result of evolution rather than planning and thus the types of boat and the chilling systems aboard vary considerably. The employment of an engineer on the grant was envisaged as a method of training personnel in the rational design of chilling systems for vessels and also for shore based installations. The engineer (S.J. Sykes) has been assisted in his training by visits of two overseas engineers specialising in the practical and theoretical aspects of chilling. Mr. J. Graham, Head of the Engineering Section of Torry Research Station, visited the Tasmanian Food Research Unit from January to April, 1982. Graham and Sykes surveyed Tasmanian and Victorian fishing boats and also freezing and frozen storage facilities. Their findings have been published in Australian Fisheries (Appendices 1 and 2).

Professor R.L. Earle was the second visitor arranged and he spent two weeks at the Tasmanian Food Research Unit with Sykes in January, 1983. As a result of Earle's visit, Sykes was familiarised with the methods of determining freezing times for a variety of products using a modified version of Planck's equation (Cleland and Earle 1982). The accuracy of this method depends on the estimation of the number of equivalent heat transfer dimensions, EHTD, which takes account of the geometry of the product to be frozen. In the case of a regularly-shaped product, such as a 10 kg carton of scallops, the value of EHTD can be easily calculated (Fig. 1). This example was worked out as the direct result of an industrial request.

The surveys by Graham and Sykes showed a lack of understanding of the principles of heat transfer on the part of some fishermen and refrigeration contractors. It would seem that independent engineering surveys of the chilling systems both aboard fishing vessels and in land based installations are to be recommended. Advice is now freely available from the TFRU before installations are built. It should be noted that a survey of installations in Adelaide and Port Lincoln in July 1983 indicated a more developed industry.

In their land based surveys of Victoria and Tasmania, Graham and Sykes found deficiencies in certain key areas of design and operation. Freezing and storage of frozen products in the same chamber and poor distribution of cold air over the material to be frozen were common problems.

The fans used in many air-blast freezers were found to be unsuitable. A fan operating in a properly ducted freezing tunnel should be able to maintain the required air flow against a substantial pressure drop. This pressure drop is caused by friction between the air and the surfaces over which it is passing, and is approximately proportional to the square of the air speed over the surface. If the required air speed is to be maintained, the fan must be capable of operating against the consequent pressure drop. Fans of this type usually have blades with aerofoil sections. Many of the fans observed were capable of delivering an adequate air flow

only when discharging into free air. This type of fan is suitable for use in cold stores but not in freezers.

The survey revealed that some designers and operators lack knowledge in estimating the product heat load and in how that heat is to be transferred from the product to the secondary refrigerant which is, in most cases, air. Graham and Sykes recommend that a good design of freezer is more likely to result if a single contractor were responsible for the whole layout and subcontracted for the elements he could not supply himself.

Dissemination of knowledge is obviously required through such bodies as the Australian Maritime College and the National and Victorian Mobile Training Units. A set of suitable course notes will be prepared at the commencement of the next grant.

#### VACUUM PACKAGING OF FISH WITH AND WITHOUT POTASSIUM SORBATE

There is considerable interest being shown throughout the world in achieving extension of shelf life of packaged fish for the supermarket and retail trade. The ability of a package system to extend shelf life is as much dependent on the temperature of storage as on providing an environment in the package to retard spoilage. Temperatures of 0°C to 1°C are recommended in the cold chain (Regenstein and Regenstein 1981, Anon. 1982) but few operations put this recommendation into practice and all the present experimentation has been done at 4°C.

Many workers have examined vacuum and modified atmosphere packaging of fish but there are many variables to consider. Rather than adopt an *ad hoc* approach, those factors which allow successful packaging of meats were first reviewed. The shelf life of meat can be successfully extended by vacuum packaging because the respiring tissue uses the available oxygen replacing it with CO<sub>2</sub> (which has a strong inhibitory effect on spoilage organisms); the post mortem pH drops below 6, a pH below which *Alteromonas putrefaciens*, a major spoilage organism, does not grow; and *Lactobacilli*, which are initially only present in low concentrations, grow to dominate the flora and actively inhibit other organisms as well as maintaining the low pH (Husband 1982). Initial attempts to lower fish pH by dipping it in citrate buffers were not successful. When the dip pH was low enough to affect a permanent flesh pH change, the result was precipitation of sarcoplasmic proteins to give an unsightly, unacceptable product (McMeekin, Hulse and Bremner 1982 : Appendix 3). Addition of glucose as a source of fermentable carbohydrate to encourage *Lactobacilli* was not successful either since sweet, sickly odours were produced in the packs (McMeekin, Hulse and Bremner 1982). Scallop meat was therefore chosen as a suitable material which because of a higher glycogen content than fish was likely to provide conditions of low post mortem pH and fermentable carbohydrate; and scallops with and without the addition of *Lactobacilli* were vacuum packaged. It was found that *Vibrio* spp. which formed a high proportion (near 100%) of the initial flora

before packaging could proliferate under these conditions and cause spoilage just as rapidly as in aerobically packaged scallops. Neither did the *Lactobacilli* exert any inhibitory effect on the *Vibrios* (Bremner and Statham 1983a : Appendix 4). It seems from these experiments that it is unlikely that conditions can be established in packaged fish that will lead to extension of shelf life analagous to the situation with meat and that other factors are required.

One such factor is the use of additives to inhibit spoilage and the most promising safe additive appears to be potassium sorbate. This substance has an LD<sub>50</sub> similar to sodium chloride and there are no suspicions of chronic toxicity, mutagenic or teratogenic problems since it is a salt of a fatty acid. Its use at 0.1% in conjunction with vacuum packaging resulted in a short extension of storage life of blue grenadier fillets (Statham and Bremner 1983 : Appendix 5). Again sorbate is more active at a lower pH than that normally found in fish and striking results have been obtained using 0.1% K-sorbate on vacuum packaged scallops to give a product stable for one month at 4°C (Bremner and Statham 1983b : Appendix 6). This is seen as opening up potential export and domestic markets for chilled rather than frozen scallops but sorbate has yet to be approved as an additive for fish although its use in other foods is widespread.

With the exception of one small initial experiment on Queensland bream, a study of modified atmosphere storage has been reserved for the next grant. The vacuum packaging results are summarised in Table 1.

#### CHILLING OF FISH IN REFRIGERATED SEA WATER

It was thought initially that the mobile RSW unit available at the Tasmanian Food Research Unit would be invaluable in comparing the relative merits of ice and RSW. The recent trend towards quality assurance rather than quality control (Gorga and Ronsivalli 1983) contraindicates holding of fish for more than two days at sea. Traditionally in comparative experiments fish have been held for two weeks and more. When land based experiments are carried out the fish are often two days dead and have already been held in ice or RSW. These early days are the key to quality and can obviously only be studied by extensive sea-going. The exchange of salt and water are poorly understood (Rankin and Davenport 1981; Tomlinson and Geiger 1965), but in the living state, sodium is continuously pumped out of fish tissues by a Na<sup>+</sup>-K<sup>+</sup> ATPase. This enzyme maintains its function after death so long as energy is available. The amount of energy is determined by the amount of struggling which fish have undergone in the trawl. The rapid changes in net handling and design as a result of flume tank experiments means that each catch must be considered on its own merit. However, our experiences with the mobile unit and two experiments with morwong and anchovy are worth reporting as useful lessons were learned. More definite results were obtained aboard the FRV 'Kapala' on techniques for

handling gemfish. However, on a research vessel, commercial catches are not taken and the ratios of RSW to fish are unrealistic.

*The mobile land-based RSW unit:* The mobile unit which can be run off a domestic power supply has been described by Thrower and Stafford (1981). It uses a commercially available vertical plate chiller as a heat exchanger. This was chosen at the time for the following reasons: 1. superior heat transfer performance; 2. flow rates and hence cooling rates can be easily varied for experimental purposes; 3. during the period in which the unit was being designed, plate chillers of this type were being produced for use in shipboard RSW systems (Merryful 1978) and an objective assessment of their performance was considered desirable.

The equipment is capable of chilling 1,000 litres of water from 16°C to -1.5°C in 5 h and has a separate tank for iced storage of fish. After two preliminary experiments rapid corrosion of the stainless steel prevented further work for several months. This was caused by the presence of panels made of material other than the specified type 316 stainless steel. Sykes (1982 : Appendix 7) has drawn attention to the reagent DAMAC DL-12 marketed in Australia by Selby's Scientific Ltd., for checking all stainless steel components for the correct molybdenum content. While this reagent and its applications are well known to large engineering firms, managers and foremen of small companies may not be aware of them.

#### Operational problems with the system and their commercial implications

*Performance of the plate chiller:* The seawater is distributed over the plate surfaces by plastic runnels which are quickly blocked with scum from the stored fish causing reduced and uneven flow over the plate surfaces. This causes ice to form in localised areas where the flow has been reduced. As a layer of ice forms, the surface it covers is effectively prevented from contributing to the heat transfer thereby reducing the efficiency of the chiller. Problems associated with poor distribution of seawater in the land-based unit are likely to be considerably exacerbated in shipboard installations where the motion of the vessel will further disturb the flow pattern across the plate surfaces. A chiller of this type installed on a commercial fishing vessel was found to be unsatisfactory for this reason. The unit was replaced with coils attached to the walls of the tank, but a shell-and-tube chiller would have been preferable. The shell and tube chiller described by Roach, Harrison and Tarr (1961), still appears to offer the best combination of performance, simplicity and ease of cleaning.

*Cleanliness of seawater:* Seawater taken from the Derwent Estuary or adjacent bays with a large tidal demand has confirmed earlier observations of Brown and McMeekin (1977) that these waters and muds are heavily contaminated with *Vibrio*, *Pseudomonas* and *Alteromonas putrefaciens* which are often dominant in the later stages of fish spoilage. Lee and Kolbe (1982) found that the microbial counts and composition of the microbial populations were quite different in four different RSW systems for shrimp and noted that the quality of

the RSW would ultimately determine the quality of the RSW-held shrimp. CODEX Alimentarius Commission (1976) recommends that tanks, pumps and heat exchangers should be cleaned immediately after discharge of the catch with potable water or clean sea water to prevent deposition and drying of debris. Bacterial counts may be high in harbours or estuaries and Hewitt, Kelman and McDonald (1978) have pointed out that tanks on RSW/CSW boats must also be cleaned on the way to the fishing grounds, where the supply of clean water is not limiting.

*Design of RSW units:* The mobile RSW Unit is fitted with horizontal sparge pipes for delivery of chilled water and gases. These had dead ends, which made cleaning difficult. The unit is routinely cleaned with a general purpose detergent, washed with fresh water and sanitised with a commercial chlorine based sanitiser. Despite this, clean ocean water (with a bacterial load near 40 c.f.u./mL) when circulated at  $-0.5^{\circ}\text{C}$  in the mobile unit for 24 h showed a bacterial load of pseudomonads of  $1.0 \times 10^4$  c.f.u./mL. Lee and Kolbe (1982) pointed out that inadequately cleaned surfaces in fish holds, debris in pipes, and wooden pound boards contributed to the problem of contamination of the fish by the circulating RSW itself even after a good cleaning regime. They recommend a greater emphasis on construction materials, a piping system having a minimum of dead-ends and the installation of a cleaning loop. Drilling holes through the dead-ends of the sparge pipes in the TFRU unit reduced the subsequent contamination of seawater five fold. Our own experiences indicate that an investigation of the sanitation of RSW tanks on fishing boats might be worthwhile.

RSW and icing systems cannot be compared experimentally unless both ice and RSW and its environs are equally clean, and the systems are used in their most effective manner.

*Experiments with morwong and anchovy:* Whole jackass morwong appeared to have an excellent storage life in RSW. The large scales on the fish remained adherent and there is no problem with blocking of the RSW pumping systems by fish scales or froth and slime. The fish were frozen for subsequent taste testing. The trained panel did not distinguish between the fish held for 7 days and 22 days and trimethylamine values were remarkably low. With the southern anchovy, on the other hand, the RSW foamed excessively. Large quantities of scum were formed from oil and feed released from the fish, which had been feeding on the local crustacean *Nyctiphanes australis*. This foaming problem is well known in Scandinavian waters. The sardine (*Clupea sprattus*) feeds on highly indigestible plankton and the intestines of the fish secrete large quantities of potent enzymes which autolyse the stomach walls giving rise to the condition known as belly burst. If the fish are "feedy" they are left alive in the net for 3 days to evacuate the gut (Stenstrom 1965). Roach, Harrison and Tarr (1961) favoured RSW rather than ice for sardines, to obtain lower temperatures, to avoid crushing and to reduce labour. The Tasmanian anchovy suffered from the crushing and spillage of gut contents caused by being packed in ice for transport to the RSW Unit at the TFRU. However, storage in RSW would appear

to present other problems. The fish held in RSW for 75 h passed through rigor mortis more rapidly than those fish which were subsequently re-iced and had higher hypoxanthine values (despite the lower temperature  $-1.3^{\circ}\text{C}$  to  $-1.5^{\circ}\text{C}$ ). Smith *et al.* (1980) have noted in experiments with herring that "on the whole the rate of development of hypoxanthine was lower in the iced storage experiments than the RSW".

*Salt and water exchange in RSW:* Our work with water and subsequent gains together with salt uptake of fish held in RSW are too complex to be described here but are to be found in Tasmanian Regional Laboratory Occasional Paper No. 8. The response of tasters to salt in fish muscle has been published (Appendix 8).

#### On board experiments with gemfish

Collaboration with scientists aboard the New South Wales FRV 'Kapala' enabled the study of on-board handling of gemfish (Appendices 9 & 10). The conclusions on the initial cold-storage properties of the species after five different on-board handling treatments are contained in Appendix 9. The final paper on the results of a full years cold storage are yet to be prepared, but the overall conclusions are listed in Table 2.

TABLE 2: *Quality of frozen gemfish after different on-board handling procedures.*

- *Prompt chilling on-board is the key to maintaining gemfish quality.*
- *Gutting of gemfish before freezing is essential if chilling of the fish has been delayed or ineffective.*
- *Many of the deteriorative changes are not obvious by visual inspection of whole fish or fillets.*
- *Gutting prior to chilling in RSW for 24h markedly enhance the salt uptake of fillets but not to an undesirable level.*
- *Salt concentrations between 3-5g/kg in fillets does not appear to induce increased rancidity during cold storage at  $-18^{\circ}\text{C}$  for one year.*
- *Marked textural differences between treatments were evident in frozen gemfish after six weeks cold storage at  $-18^{\circ}\text{C}$ .*
- *Gemfish left at ambient temperatures for 24h and frozen ungutted show a decrease in fibre structure as well as dryness, firmness and toughness.*
- *Frozen, and subsequent chilled storage magnifies damage caused by poor on-board handling.*

## CHEMICAL, PHYSICAL AND SENSORY EVALUATION OF FISH

When any process or treatment is tested, the fish must be evaluated by objective or subjective methods. We have had considerable success with sensory evaluation of fish and shellfish and some of the methods are described in the Appendices. Further details are available in Tasmanian Food Research Unit Occasional Paper No. 9. The traditional and newer chemical and physical tests have been less successful in our hands but again detailed information may be obtained from the Occasional Paper.

The gist of this report was given at the 16th AIFST Convention, Melbourne, May 1983 and the following general observations were made.

### SOME OVERALL CONCLUSIONS

- \* Independent engineering surveys of the chilling systems both aboard fishing vessels and in land based installations are to be recommended and the information should be incorporated into training courses.
- \* Salt uptake in RSW is often slower than indicated in the literature as pre-rigor fish do not take up salt rapidly. Salt uptake is inversely related to fat content, but is enhanced by gutting and exposure of skinless surfaces.
- \* Shore based experimental RSW units have some disadvantages but could enable trials of equipment such as heat exchangers before placement on commercial fishing vessels. The importance of cleaning routines was established and surveys of bacterial loads in commercial vessels using RSW could be of value.
- \* Studies of chilling of fish in RSW, CSW, or ice should be done on commercial fishing vessels with personnel employed specifically to work at sea.
- \* Vacuum packaging of fish to extend shelf life would appear to offer few advantages unless the addition of sorbate to low pH species is considered. Scallops which fulfilled these requirements showed marked extension of shelf life which could have valuable commercial implications.
- \* Assessment of the quality of fish after storage in ice, RSW, freezing or vacuum packaging requires a level of expertise not readily available to only a small part of the fishing industry. Temperature control could easily be monitored and would provide a firm base from which quality assurance programmes could be started.



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Table 1. Description and results for packaged fish held at 4°C.

Expt. No.	Material	Month caught	Treatments	Initial pH	Minimum pH	Initial bacterial count (c.f.u./cm <sup>2</sup> or c.f.u./g and dominant genus)	Maximum bacterial count (c.f.u./cm <sup>2</sup> or c.f.u./g and dominant genus)	Time to unacceptability (days)
1	Morwong	April	Vac.packed polythene*	6.55	6.55		3.4x10 <sup>8</sup>	7
			Vac.packed barrier bag*	6.12	6.12	3.0x10 <sup>3</sup>	6.3x10 <sup>7</sup>	7
			Vac.packed E. bag*	6.14	6.14		4.2x10 <sup>8</sup>	7
2	Morwong	May	Vac.packed polythene	6.41	6.36	5.1x10 <sup>5</sup>	1.1x10 <sup>9</sup>	7
			Vac.packed barrier bag	6.61	6.15	6.4x10 <sup>5</sup> <i>Alteromonas</i>	4.0x10 <sup>8</sup> <i>Alteromonas</i>	7
			Vac.packed E. bag	6.52	6.47	5.2x10 <sup>5</sup>	7.9x10 <sup>8</sup>	4
			Aerobic packed polythene	6.62	6.60	4.2x10 <sup>5</sup>	1.2x10 <sup>9</sup>	4
3	Scallop	July	Vac.packed	7.06	5.52 <sup>±</sup>	2.8x10 <sup>5</sup>	3.4x10 <sup>8</sup> lactobacilli	7
			Vac.packed + <i>L.plantarum</i>	7.07	5.39 <sup>±</sup>	6.3x10 <sup>4</sup> <i>Vibrio</i>	3.2x10 <sup>7</sup> <i>Vibrio</i>	7
			Aerobic packed polythene	7.14	5.99	3.2x10 <sup>5</sup>	2.4x10 <sup>8</sup> lactobacilli	7
							4.0x10 <sup>7</sup> <i>Vibrio</i>	7
4	Scallop	November	Vac. packed	6.23	5.90	3.7x10 <sup>4</sup>	7.8x10 <sup>6</sup> <i>Vibrio</i>	6
			Vac.packed + sorbate <sup>†</sup>	6.56	6.00	1.5x10 <sup>4</sup> <i>Vibrio</i>	1.9x10 <sup>5</sup> <i>Pseudomonas</i>	48
			Aerobic packed polythene	6.23	5.90	6.5x10 <sup>4</sup>	4.5x10 <sup>7</sup> <i>Vibrio</i>	6
5	Blue grenadier	November	Vac. packed	6.70	6.70	1.6x10 <sup>3</sup> <i>Moraxella</i> /	2.1x10 <sup>8</sup> <i>Vibrio</i>	11
			Vac.packed + sorbate	6.71	6.65	2.3x10 <sup>3</sup> <i>Moraxella</i> /	1.1x10 <sup>7</sup> <i>Pseudomonas</i>	14
			Aerobic packed polythene	6.69	6.69	<i>Pseudomonas</i> /	1.4x10 <sup>9</sup> <i>Moraxella</i>	7
						<i>Moraxella</i>		
6	Scallop	August	Vac. packed + sorbate		5.18	2.4x10 <sup>4</sup> <i>Vibrio</i>	1.4x10 <sup>8</sup> <i>Pseudomonas</i>	7
7	Bream, Queensland	September	Vac. packed	6.30	6.30		1.3x10 <sup>7</sup> <i>Vibrio/Aeromonas</i>	28
			100% CO <sub>2</sub>	6.30	6.28	5.3x10 <sup>3</sup> <i>Micrococcus</i>	5.7x10 <sup>8</sup> lactobacilli <sup>†</sup>	
			Initial gas analysis: CO <sub>2</sub> 97%; O <sub>2</sub> 0.18%; N <sub>2</sub> 0.83%				4.4x10 <sup>7</sup> <i>Alteromonas</i>	8
							1.9x10 <sup>7</sup> <i>Vibrio/Aeromonas</i> & <i>Alteromonas</i>	12

\* Oxygen transmission rates 25°C, 75% R.H. (mL.m<sup>-2</sup>.24h<sup>-1</sup>.atm<sup>-1</sup>)

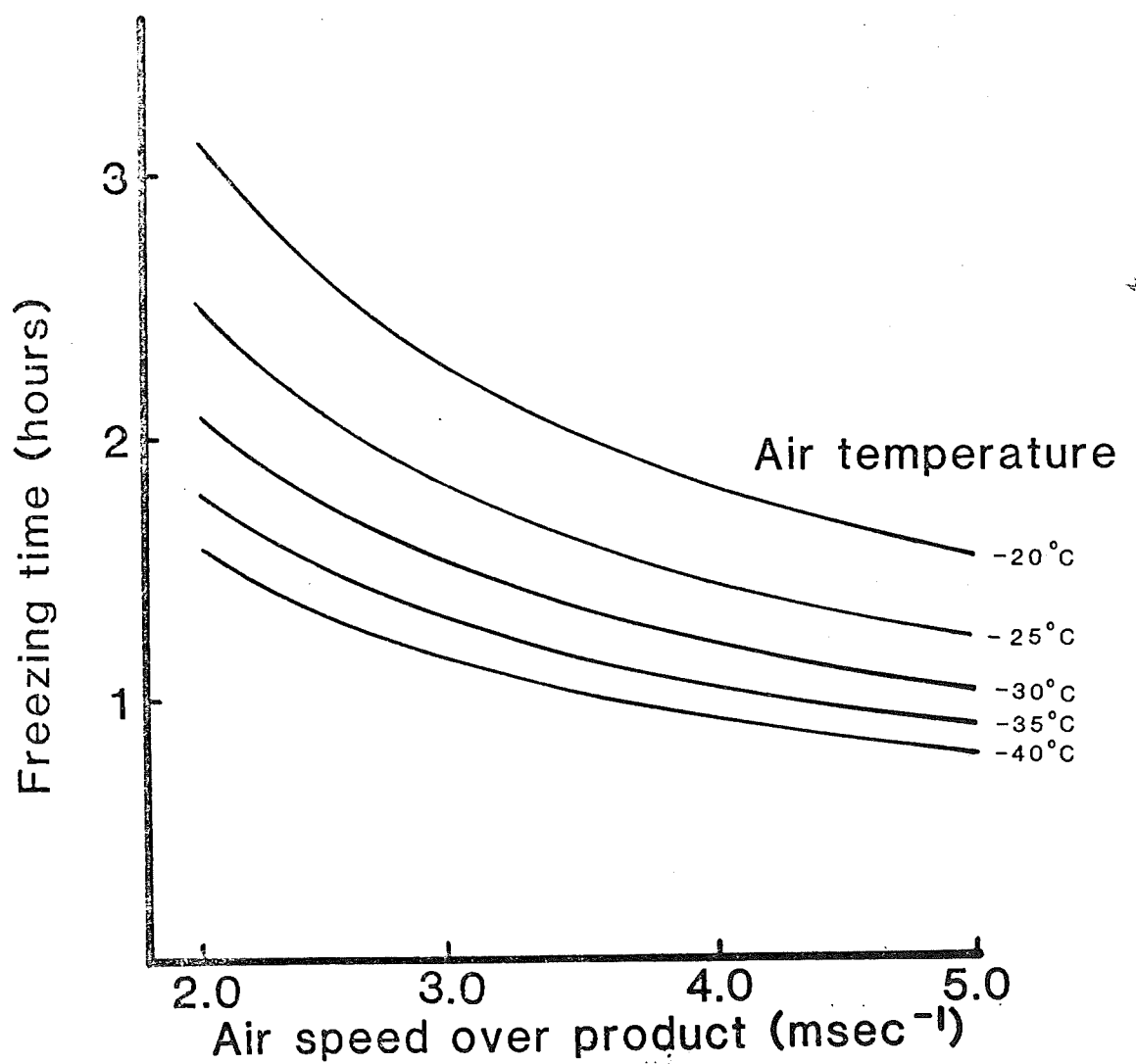
Polythene &gt; 2400

E. bag ~ 2400

Barrier bag ~ 45

<sup>†</sup> 1g/kg K-sorbate (w/w)<sup>±</sup> low pH due to lactic acid production by lactobacilli.<sup>†</sup> Lactobacilli formed visible colonies on scallops after 21 days.

Relationship between freezing time and air speed for 25mm thick scallop packs.



# Refrigerated sea water — its use in Australia

THE chilling of fish in refrigerated sea water (RSW) is an alternative to using ice, but only under special circumstances does it have a clear advantage.

The main benefit in using RSW is that less effort is needed to handle the fish on board fishing vessels. Other advantages are that:

- there is less chance of the fish being crushed or losing weight during storage;
- a slightly lower temperature of about  $-1^{\circ}\text{C}$  is achieved without partially freezing the fish; and
- washing and bleeding of gutted fish is more effective.

The results achieved depend on the species. With some species RSW has an advantage over ice for only two or three days but thereafter the fish spoil more quickly. Other species are reported to keep better in ice, even during the first few days. Storage limits are also curtailed in some cases due to the take up of excessive amounts of water and salt. Salt uptake accelerates rapidly after the fish have passed through rigor mortis.

These limitations prevent the wider use of RSW storage and it is therefore mainly confined to the short-term storage of particular species that are caught in large quantities within a short time. This avoids delays at ambient temperature that would result when sorting and icing a large catch.

Many of the situations where RSW is used on board Australian fishing vessels are better suited to the use of ice for chilling and some of the practices that have evolved do not fully achieve the benefits possible from RSW storage.

by J. Graham and S. J. Sykes

John Graham is Head of the Engineering Section at Torry Research Station, Aberdeen, and Stephen Sykes is an experimental officer in CSIRO's Tasmanian Food Research Unit. John Graham recently spent three months with the Food Research Unit. He and Stephen Sykes looked at the chilling systems aboard some Victorian and Tasmanian fishing vessels and their observations on current practices in the use of refrigerated sea water may provide food for thought for those operating or installing such systems.

## Current practice

The following comments and observations on Australian RSW practice are not based on a full survey but what was seen is considered to be representative of current practice.

1. With few exceptions, agitation or circulation of the water is not used to achieve effective cooling and avoid temperature stratification. Cooling rates therefore will be slow and storage conditions variable.

2. Catches are usually small compared with the holding capacity of the tanks and, to avoid difficulties during unloading, the fish are contained in net bags before being lowered into the water. Circulation of water within the nets will be poor and cooling times therefore will be longer. Also small quantities of fish contained in relatively large quantities of water increase the chance of an unacceptable uptake of salt since the salt availability is relatively greater.

3. When storage times are long (exceeding three to four days), it is often the practice to change the sea water, and in many cases this need may only have arisen when optimum storage times have been exceeded. This will improve the appearance of the fish and reduce the bacterial load in the water, but it also results in a fresh supply of salt at a time when fish are more susceptible to salt uptake, which may result in unacceptable levels.

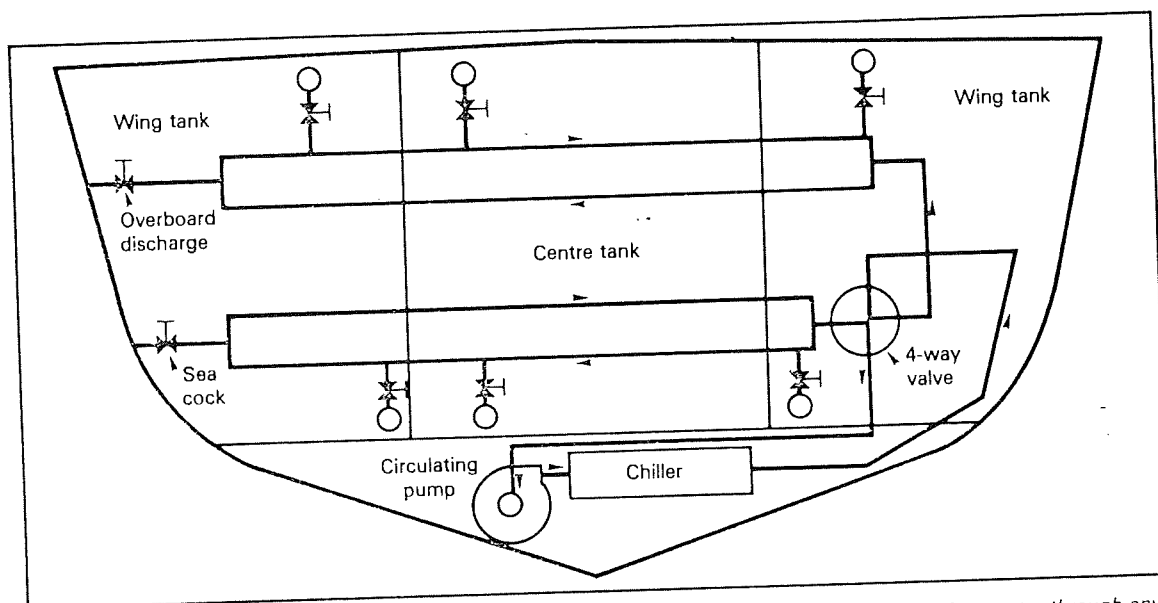
4. In many installations the RSW is cooled by banks of cooling coils within the storage tanks. Fish in contact with the coils therefore may be partially frozen, especially if there is no circulation or agitation of the water. This partial freezing adversely affects the quality of the fish and also makes unloading difficult. External cooling by means of a heat exchanger and circulating of the water by pumping avoids this problem.

5. When internal cooling coils are used, it is frequently the practice to deliberately allow ice to build up on the coils to provide a buffer against breakdown of the refrigeration plant and, mistakenly, to increase cooling rates when fish are added.

However the ice frozen out will be almost pure freshwater ice and the remaining unfrozen water therefore has a higher salt content. This will increase the rate of salt uptake and may result in an unacceptable level in the fish.

Also the higher salt concentration allows the water to be cooled to a lower temperature without freezing and when this happens slow, partial freezing of the fish will follow.

6. Brine spray systems are us-



Layout of a typical three-tank RSW system. Note that use of a four-way valve allows circulation of seawater through any or all of the tanks, and from top to bottom (as shown) or vice versa. (Reproduced from Roach, Harrison and Tarr, 1961, with permission of Govt. of Canada, Department of Fisheries and Oceans.)

ed in some vessels but, unlike systems which use total immersion, they do not provide two of the advantages associated with the use of RSW. The fish are loaded into boxes and stacked in the hold where they are sprayed with the water. Under these circumstances cooling rates will be much slower and more uneven than they would be by total immersion. Also the potential for crushing the fish by overfilling the boxes still exists.

An added disadvantage of spraying is the occurrence of frothing, which results in an unattractive scum on the surface of the fish. This can be washed off at the time of unloading but unless clean chilled water is used, washing may have a further detrimental effect on quality.

7. Only one and two-tank systems are used, whereas a three-tank system is easier to operate. A three-tank system (Fig. 1) allows the centre tank to be filled and pre-cooled, and the water is then used in both wing tanks as required. This three-tank system has the following advantages:

- the minimum quantity of water is pre-cooled for full storage;
- the ship's stability can be more easily maintained at all times; and

- water can be added to the fish in the tanks and not vice versa, and this makes it easier to achieve maximum fish holding capacity.

8. Most systems are operated without temperature control other than by natural starting and stopping of the refrigeration compressor. This type of control is inadequate. Also there is a danger of excessive cooling resulting in ice and fish being frozen to the cooling coils, fish being partially frozen in the ice tank and a high concentration of salt being present.

### Quality of fish

Opinions expressed about the quality of fish chilled in RSW are confusing and often contradictory, with widely held views that it is both good and indifferent.

In some cases the fish chilled in RSW (however badly the chilling is done) are compared with unchilled fish and invariably the former are favoured.

In other cases no account is taken of the storage time and when this is excessive, RSW storage is criticised, often because of a high water or salt uptake. At other times a favourable judge-

ment made immediately after removal from the RSW is different from one made some time later due to changes in quality resulting from drip loss from fish that may have taken up an excessive amount of water during a long storage period.

### Conclusion

It would seem that indiscriminate use of RSW and, possibly, the lack of detail about the requirements for operation under Australian conditions have resulted in an uncertain overall view about the benefits of RSW and there is therefore a need for a rational program of research and development to resolve this situation.

### Suggested reading

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2. J. H. Kelman, 1977, *Stowage of Fish in Chilled Sea Water*, Torrey Advisory Note No. 73.
3. S. W. Roach, J. S. M. Harrison, H. L. A. Tarr, 1961, *Storage and Transport of Fish in Refrigerated Sea Water*, Fisheries Research Board of Canada, Bulletin No. 126.

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## Room for improvement in fish freezing procedures

AIR blast freezers are widely used in the fishing industry because they are versatile and allow products of varied shapes and sizes to be frozen. They are built to suit individual requirements and are seldom supplied from a standard range of units. This versatility and variety, however, all too often result in poor designs and misuse in operation.

The state of air blast freezing of fish in Australia leaves considerable scope for improvement and this article reviews current practice and suggests some changes that will result in better freezer designs and improved freezing standards.

### Current designs

One type of air blast freezer that is still constructed in Australia, but is considered obsolete in other countries, is the 'sharp freezer'. This consists of an elongated room with a central passageway; the produce is loaded on shelves formed by the cooling coils on each side. In some freezers, fans in the passageway assist air circulation.

In this type of freezer loading and unloading times are long, and often take up the entire working day, leaving freezing to be completed overnight. Also, heat transfer is poor since there is little contact between the product and the pipes, and air circulation is inadequate. Freezing times are therefore long and, depending on the product, freezing may not be accomplished within the 24-hour cycle normally operated.

Another type of freezer widely used has the same basic layout as the sharp freezer but the cooling coils are replaced by racks of

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shelves, and refrigeration is achieved by means of a unit cooler at the far end of the passageway.

This type of freezer is less effective than the sharp freezer because there is no heat transfer by contact with the cooling coils and, again, no attempt is made to duct the air over the surface of the product.

Few tests have been made of current freezer performance but one test of the second type of freezer described showed that for 60-mm-deep cartons of trout, freezing was not completed after a period of 20 hours (Fig. 1).

Some recently built freezers have some of the elements of good design but significant omissions in the ducting of the air will result in long and variable freezing times (Fig. 2).

Air takes the path of least resistance. Therefore much of the air is recirculated at the cooler or bypasses the produce. This type of freezer can, with little added cost,

be modified to give better freezing performance.

### Product dimensions, packaging

A number of requirements dictate the choice of dimensions and type of packaging used to prepare fish before freezing. The one that should be given highest priority is the need to freeze the product in a given time to meet requirements set out in freezing standards and regulations. In this respect, some current Australian practice makes it difficult to achieve this aim even in a well-designed freezer.

To give an example, one popular package has 10 kg of fish in plastic bags within a corrugated cardboard carton. The carton is 520 mm x 320 mm x 60 mm deep. Some freezing times for this product are given in the following table and these clearly show that the depth of package and the type of packaging result in long freezing times under poor heat-transfer conditions. When the lid is left off, the freezing time is reduced by about 25 per cent, but to achieve freezing times of less than 10 hours (as has been specified in Department of Primary Industry export regulations) freezer operating conditions would need to be improved greatly.

### Requirements of air blast freezers

An essential requirement, absent in most air blast freezers, is a high air velocity over the surface of produce in all parts of the freezer. In some badly-designed freezers air velocity over some of the produce is little better than that resulting from natural convection. As can be seen in the

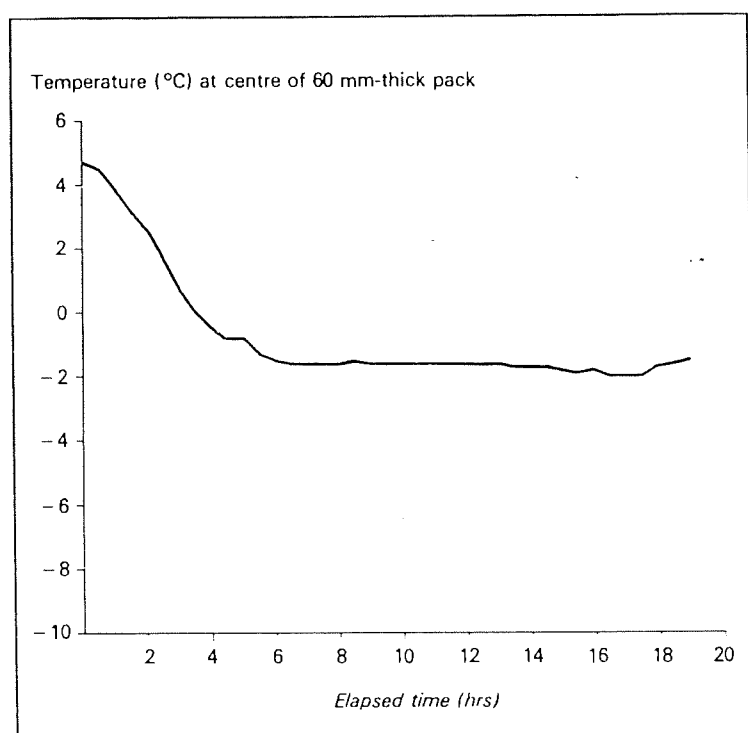


Figure 1. Example of slow freezing in an un-ducted room freezer.

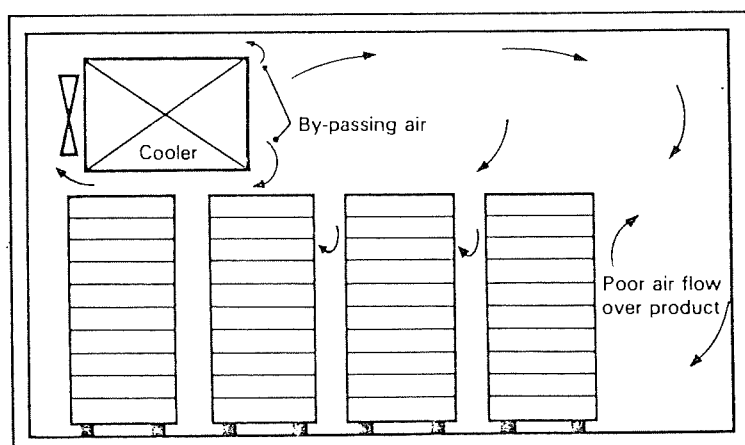


Figure 2. Poor air circulation in a freezer due to absence of ducting.

Freezing time of 60 mm-deep corrugated cardboard cartons of fish

Air temperature (°C)	Air velocity (m/s)	Freezing time* (hours)	Remarks
-35	5	8	
-35	Natural convection	45	
-15	5	6	Lid off
-15	Natural convection	33	Lid off

\*Fish reduced to -12°C at centre of pack

table, if this is combined with high air temperature freezing times can be extremely long.

A compromise has to be made between air velocity and high fan power (Fig. 3), and experience has shown that in a batch air blast freezer a design air velocity of five metres a second is suitable.

To achieve uniform air velocity the freezer should be designed in the form of a tunnel and ducted to ensure there is no bypassing or short circuiting of air (Fig. 4). Uniform air distribution also means that produce should be loaded so that it is distributed across the working section of the freezer, especially when partial loads are frozen.

Care should be taken to leave acceptable spacing between the shelves and produce: too much causes short-circuiting of the air, while too little reduces the air flow; both result in long freezing times.

There is no hard and fast rule for shelf spacing but, as a guide, a spacing equal to two-thirds of the thickness of the product, up to a maximum of 50 mm, is adequate and the same spacing is also appropriate between the walls and roof of the tunnel and the pallets or trolleys of fish.

Another essential loading practice that does not seem to be widely observed is that warm, newly-loaded fish should never be placed upstream of partially-frozen fish. Air flow and loading arrangements should therefore ensure that when freezers are loaded intermittently, the air flows from the coldest to the warmest produce.

Another important requirement in an air blast freezer is that the design air temperature should be suitable for reducing the temperature of the product to the intended temperature of cold storage. Australian practice is to store frozen fish at a temperature of -18°C; therefore the temperature in the air blast freezer should be at least 5°C lower at -23°C.

This temperature is not always reached in present freezers. It may



be that to achieve desirable freezing times for some products even lower temperatures will be required. However air temperatures below  $-35^{\circ}\text{C}$  are not practical and consideration would then have to be given to changing the product dimensions and packaging to achieve acceptable freezing times.

It should be noted that low air temperatures and long freezing times are sometimes compatible and this often confuses the operator. This happens when heat transfer is poor (invariably due to poor air flow over the product) and freezing is slow. The refrigeration equipment under these conditions will operate under light load and reduce the air temperature below the design value.

### Special-purpose freezers

Although air blast freezers are widely used they are relatively inefficient since air is not a good heat transfer medium. If the quantities of a given product to be frozen justify the purchase of a special-purpose freezer, it will certainly give improved freezing performance.

For instance, regular-shaped packages like the 10-kg cartons previously described can be frozen in a horizontal plate freezer and freezing times of about four hours are likely. Apart from improved freezing performance this type of freezer also is less susceptible to misuse by the operator.

### Purchasing

It seems to be common in Australia for the purchaser to have individual contracts with suppliers of the various components of a freezer, such as refrigeration machinery and the insulated cabinet. This does not guarantee a well-designed freezer unless the purchaser himself has a good deal of expertise. It is more likely that a good design will result if a single contract is placed with a main contractor who will then sub-contract for the components he cannot supply himself.

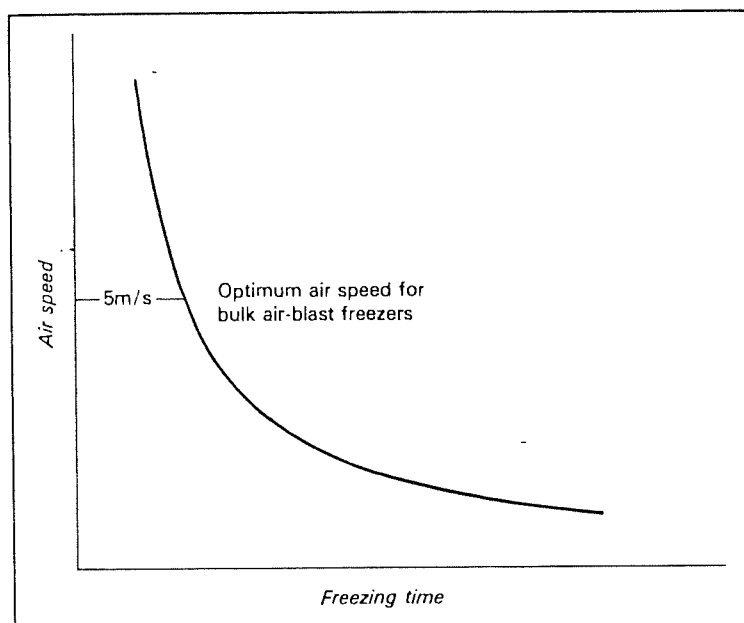


Figure 3. A typical relationship between air speed and freezing time.

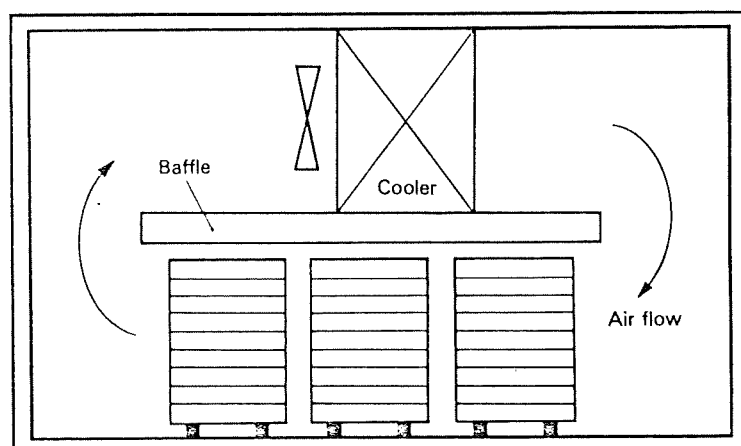


Figure 4. Ideal layout of an air blast freezer. (Compare with Fig. 2.)

Reputable suppliers of refrigeration equipment can provide reliable freezers which they will guarantee to meet a specification that is mutually acceptable to both the supplier and purchaser.

### Advice

In many ways fish freezing is more advanced and more demanding than for other foods and specialist knowledge is required for the design and operation of equipment. As in other countries, few people in Australia have the necessary breadth of experience in

both refrigeration and fish processing technology.

It was principally to fill this gap that the Tasmanian Food Research Unit of the CSIRO Division of Food Research recently recruited an engineer. It is hoped this move will provide some of the impetus required to improve present freezer designs and upgrade freezing standards.

### Suggested reading

*Freezing in Fisheries*, FAO Fisheries Technical Paper No. 167, Food and Agricultural Organisation of the United Nations, Rome, May 1977.

# Spoilage association of vacuum packed sand flathead (*Platycephalus bassensis*) fillets

T. A. McMEEKIN\*, L. HULSE and H. A. BREMNER

The spoilage association of vacuum packed sand flathead fillets was dominated by *Alteromonas* spp., and shelf life was not significantly greater than that of aerobic controls. Attempts to induce development of lactic acid bacteria by reduction in pH or addition of glucose were unsuccessful. The treatments reduced the contribution of *Alteromonas* spp. to the spoilage association with a proportionate increase in *Enterobacteriaceae*.

There is currently considerable interest in extending the shelf life of chilled, packed fish for the supermarket trade. In this trade the fish may be fresh but is more likely to have previously been frozen before thawing and packing. The process of vacuum packing has received little attention with fish but is a well established means of extending the shelf life of red meats. With meats of normal pH ( $\approx 5.5$ ) the Gram negative aerobic flora is inhibited by low oxygen tension, and increased carbon dioxide levels and lactobacilli are dominant. These organisms do not produce sulphides, amines and other compounds associated with putrefaction, and a shelf life of at least 10 weeks at 0°C can be obtained (Newton & Gill 1980). On high pH meat ( $>6.0$ ), facultatively anaerobic Gram negative bacteria, particularly *Alteromonas putrefaciens*, predominate and a marked extension in shelf life is not achieved (Gill & Newton 1979).

A similar situation might be inferred for most marine fish where the pH is normally greater than 6.0 and the presence of trimethylamine oxide provides an electron acceptor which can be used by *A. putrefaciens* with resultant formation of trimethylamine (Castell & Snow 1951). Furthermore, the incidence of lactobacilli in marine situations is uncertain although Schröder *et al.* (1980) described the development of *Lactobacillus plantarum* in chill stored herring following injection of sterile glucose into the peritoneum. Nevertheless, Huss (1972), Banks, Nickelson and Finne (1980) and Jensen *et al.* (1980) reported reduced bacterial growth on vacuum packed fish and a change in the spoilage association compared to that in aerobic controls. To extend the shelf life of vacuum packed high pH meat, Gill and Newton (1979) and Newton and Gill (1980) suggested reduction in pH to inhibit *A. putrefaciens* and addition of a readily utilisable substrate such as glucose to delay metabolism of amino acids and encourage growth of lactic acid bacteria.

This communication reports the effect of vacuum packing coupled with these strategies on the spoilage association of vacuum packed sand flathead (*Platycephalus bassensis*) fillets. Flathead were used since they are common table fish available all year round in most Australian states. Moreover Ball (1980) has recently described the aerobic spoilage association in samples of this species obtained from the same supplier.

## Materials and methods

### Packing procedure

Frozen fillets of sand flathead (*P. bassensis*) were obtained from a wholesale outlet. The experiments were carried out serially, and the fillets were thawed when required and subjected to one of the following treatments before packaging: untreated; dipped in citrate buffer pH 4.8 (Newton & Gill 1980); dipped in citrate buffer pH 5.4 (Cruikshank 1965); dipped in glucose (50 g/L); and dipped in citrate buffer pH 5.4 containing glucose (50 g/L). Fillets were packed under vacuum in Cryovac W gauge barrier bags using a Rheem Tipper Clipper Vacuum packing machine (Union, NJ). The packing film was a flexible laminate of ethylene vinyl acetate and polyvinylidene chloride (nominal thickness 0.09 mm, oxygen permeability (unshrunk) at 3.5°C,  $2.1 \text{ mL m}^{-2} \cdot 24 \text{ h}^{-1} \cdot \text{atm}^{-1}$ ). Immediately after packing, the fillets were briefly dipped in hot water (75–80°C) to shrink the film. Also individual untreated fillets were wrapped loosely in low-density polyethylene film. All samples were stored at 4°C for up to 14 days.

### Sampling procedure

Individual fillets were removed at random for microbiological analysis and organoleptic assessment during each storage trial with the exception of those wrapped in polyethylene (aerobic storage) which were examined after 14 days only. Fish muscle (16 cm<sup>2</sup>) was aseptically removed and homogenised in 100 mL saline using a Colworth Stomacher. Serial decimal dilutions were prepared for the homogenate and spread on the surface of salt nutrient agar (SNA) and the medium of de Man, Rogosa and Sharpe (1960) (MRS). Plates were incubated at 22°C for 3–5 days.

### Identification of the spoilage flora

A total of 320 colonies of SNA were selected for identification. These were streaked on SNA to check purity and subsequently maintained on SNA slopes. Sixteen colonies from MRS were also identified. The Gram reaction and cell morphology of all isolates were noted; Gram positive isolates were identified further on the basis of the catalase tests using hydrogen peroxide (3 mL/L) and reaction in the medium of Hugh and Leifson (1953). Gram negative isolates were identified by the following characteristics: motility; flagella arrangement; oxidase reaction; oxidative or fermentative attack on glucose; DNAase production and sensitivity to Vibriostat (0/129). The methods used were those recommended by Hendrie and Shewan (1979) and Lee, Hendrie and Shewan (1979). *Enterobacteriaceae* were further identified by using the Microbact 12E System (Disposable Products, Adelaide, SA).

### Trimethylamine content on fillets

The trimethylamine (TMA) content of freshly thawed

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Table 1. Estimated numbers ( $\log_{10}$ ) of bacteria on SNA\* during storage of vacuum packed fillets at 4°C

Time (days)	Untreated	Citrate buffer (pH 4.8)	Citrate buffer (pH 5.4)	Glucose (50 g/L)	Glucose in citrate buffer (pH 5.4; 50 g/L)
0	4.46†	4.54‡	4.39‡	4.55‡	4.61‡
2	5.78	4.76	5.14		
4	7.48	5.68	6.32	6.83	6.21
7	7.93	6.29	7.21		
10	8.02	6.70	7.77	7.84	6.92
14§	8.11	7.40	8.15	8.38	7.68

\*Salt Nutrient Agar; †Values represent mean of 7 estimates; ‡Values represent mean of 4 estimates; §Mean value for 8 aerobically stored fillets was 9.32

Table 2. Spoilage associations of vacuum-packed fillets after 14 days at 4°C (% of genera)

Genera	Untreated	Citrate buffer (pH 4.8)	Citrate buffer (pH 5.4)	Glucose (50 g/L)	Glucose in citrate buffer (pH 5.4; 50 g/L)
<i>Alteromonas</i>	65	0	35	20	35
<i>Vibrio/Aeromonas</i>	20	35	0	5	0
<i>Enterobacteriaceae</i>	15	65	65	75	65
<i>Enterobacter</i> sp.	10	30	25	20	15
<i>Serratia</i> sp.		35	30	50	20
<i>Klebsiella</i> sp.				5	30
<i>Escherichia</i> sp.	5		10		

Table 3. Surface pH values of vacuum-packed fillets during storage at 4°C

Time (days)	Untreated	Citrate buffer (pH 4.8)	Citrate buffer (pH 5.4)	Glucose (50 g/L)	Glucose in citrate buffer (pH 5.4; 50 g/L)
0	6.23*	5.38†	5.76†		
2	6.33				
4	6.21	5.57	6.11	6.14†	6.12†
7	6.50				
10	6.67	5.37	6.23	5.88	5.80
14‡	6.59	5.58	6.32	6.07	6.02

\*Mean of 10 determinations on 10 fillets; †Mean of 10 determinations on 4 fillets; ‡Mean value for 8 aerobically stored fillets was 7.19

Table 4. Trimethylamine content of muscle after 14 days at 4°C

Treatment	Fillet No.	TMA-N (mg/100 g)
Aerobic	1	33.7*
	2	36.5
	3	34.1
Vacuum-packed untreated	1	42.0
	2	39.4
	3	35.4
Citrate buffer, pH 4.8	1	6.8
	2	9.0
	3	4.0
Citrate buffer, pH 4.8	1	32.3
	2	33.8
	3	31.9

\*Each value represents a mean of 3 replicate determinations

Table 5. Estimated shelf life and spoilage characteristics of fillets stored at 4°C

Treatment	Shelf life (days)	Spoilage characteristics
Aerobic	8-9	Slime, putrid fish odour
Vacuum-packed		
	8-9	Slime, sulphide and off-odour
	8-9	Milky exudate, bleached flesh
Citrate buffer (pH 4.8)	10	Sulphide and off-odour, milky exudate
Citrate buffer (pH 5.4)	10	Slime, sweet odour
Glucose (50 g/L)	9	Sour, sulphide and off-odour, bleached flesh
Glucose in citrate buffer (pH 5.4; 50 g/L)		

Table 6. Estimated numbers ( $\log_{10}$ ) of bacteria on MRS\* during storage of vacuum packed fillets at 4°C

Time (days)	Untreated	Citrate buffer (pH 4.8)	Citrate buffer (pH 5.4)	Glucose (50 g/L)	Glucose in citrate buffer (pH 5.4; 50 g/L)
0	2.26†				1.72‡
2	3.69	3.10‡	3.17‡		
4	3.74	3.29	3.85	4.00‡	3.52
7	4.16	4.10	4.32		
10	4.33	4.60	4.78	4.31	4.55
14	4.55	4.86	4.85	5.33	5.57

\*The medium of de Man, Rogosa and Sharpe (1960); †Values represent mean of 7 estimates; ‡Values represent mean of 4 estimates

fillets, spoiled vacuum packed fillets and aerobically spoiled fillets was determined spectrophotometrically following extraction in 0.6M perchloric acid (Tozawa, Enokihara & Amano 1971).

#### Surface pH during storage

The surface pH of fresh, aerobically stored and vacuum packed fillets was determined using a surface electrode (Radiometer, Copenhagen). Ten measurements taken at random over the total area of exposed fish muscle were recorded for each fillet.

#### Evaluation of spoilage

At each sampling time fillets were examined by the authors for off-odours, changes in colour or appearance of muscle, and changes in the appearance of the exudate surrounding the fillet. Shelf life was estimated subjectively using these characteristics.

### Results and discussion

The estimated numbers of bacteria recovered on SNA throughout storage of the fish in each treatment are shown in Table 1. Estimated numbers of bacteria initially present were  $\approx 10^4$ /cm<sup>2</sup> muscle which rose to  $10^7$ – $10^8$ /cm<sup>2</sup> after 14 days at 4°C. In comparison, the numbers recovered from aerobically stored fillets were  $\approx 10^9$ /cm<sup>2</sup> muscle (mean of 8 determinations), which were significantly higher ( $p < 0.01$ ) (analysis of variance) than those for vacuum packed treatments. There were no significant differences (analysis of variance) between viable counts on SNA for untreated vacuum packed fillets and any of the treated vacuum packed fillets. The initial flora of sand flathead fillet muscle comprised mainly Gram positive types, particularly coryneform bacteria (35%) and *Micrococcus* spp. (40%). During storage at 4°C these were rapidly replaced by Gram negative bacteria. On untreated vacuum packed fillets, *Alteromonas* spp. had emerged as the dominant bacteria (50% of flora) after only 4 days and comprised 65% after 14 days at 4°C (Table 2). This result was expected as Ball (1980) demonstrated that *Alteromonas* spp., including *A. putrefaciens*, were the major spoilage organisms of aerobically stored sand flathead. Thus vacuum packing did not increase the shelf life or inhibit *Alteromonas* spp.

Dipping fillets in citrate buffer (pH 4.8) resulted in a reduction of the surface pH to 5.3–5.5 throughout storage (Table 3) and the complete inhibition of *Alteromonas* spp. with consequent non-appearance of the sulphide-like odours present in packs of undipped fillets. Similarly, TMA levels were significantly reduced (Table 4), but unfortunately the treatment caused undesirable changes in the appearance of the fillets. The flesh became bleached and opaque and exuded a milky white fluid (Table 5). Moreover, the treatment merely selected another group of Gram negative facultatively anaerobic bacteria identified as members of the *Enterobacteriaceae*. *Enterobacter* spp. and *Serratia* spp. predominated after 14 days (Table 2).

Dipping the fillets in glucose (50 g/L) alone so as to provide a readily utilisable substrate to encourage lactobacilli or in glucose (50 g/L) in citrate buffer (pH 5.4) to modify pH as well did not result in selection of lactobacilli. Although the proportion of *Alteromonas* spp. was reduced these were replaced by Gram negative facultative anaerobes (Table 2). The surface pH values were lower than in the undipped fillets, but shelf life was not increased and the fillets were rejected because of sickly sweet odours and bleached flesh. A treatment combining citrate buffer (pH 4.8) and glucose (50 g/L) was not included because of the undesirable changes in appearance which occurred at this pH level. The initial incidence of lactobacilli was always low, in some cases not detectable, and after 14 days counts obtained on MRS were less than 1% of those on SNA (Table 6). This situation might have been expected from the paucity of data reporting lactic bacteria in marine situations; in fact only a few reports indicate that lactobacilli develop rapidly on stored marine products (Fieger & Novak 1951, Schröder *et al.* 1980). Fieger and Novak (1951) dealt with oysters which contained a large amount of glycogen while Schröder *et al.* (1980) added glucose to their fish.

Furthermore, in the present study the count obtained on MRS over-estimated the lactic acid bacteria present. Of 16 representative colonies identified from MRS, 4 were *Lactobacillus* spp., 5 were enterics, 2 were coryneforms and 5 were yeasts.

The results presented here suggested that unless they are deliberately added it is unlikely that lactic acid bacteria will proliferate on vacuum-packed fillets sufficiently to compete effectively with *A. putrefaciens* and other Gram negative facultative anaerobes. Even with deliberate addition, the establishment of a predominantly lactic flora is by no means certain and Alexander (1977) cited numerous instances where deliberate addition of selected organisms to natural ecosystems has failed due to homeostatic mechanisms. Alternatively the gaseous atmosphere might be modified by addition of carbon dioxide which would have a deleterious effect on the Gram negative component of the flora. Sutherland *et al.* (1977) and Gill and Tan (1980) have demonstrated that *A. putrefaciens* is particularly susceptible to added carbon dioxide. Flushing of commercial bulk packs with carbon dioxide extended the shelf life of poultry carcasses to 27 days at 1.1°C (Sander & Soo 1978). A similar extension might be expected with fish in view of the fact that chicken tissues also have pH values >6.0 and support rapid growth of *A. putrefaciens* when wrapped in impermeable film without added carbon dioxide (Barnes & Melton 1971).

Another approach is to add an antibacterial compound effective against the major component of the spoilage flora. Chung and Lee (1981), in an examination of aerobically stored English sole (*Parophrys retulus*), noted the absence of *A. putrefaciens* on fillets treated with potassium sorbate. Preliminary studies in our laboratory have indicated marked extension of shelf life at 4-5°C for fillets treated with potassium sorbate. *Alteromonas putrefaciens* was not recovered as part of the flora at any sampling time up to 28 days.

In summary, while it was possible to manipulate the spoilage association of vacuum packed sand flathead fillets by reduction in pH or addition of glucose, none of the treatments significantly increased shelf life. Treatment with a pH 4.8 citrate buffer completely eliminated *Alteromonas* spp. from the spoilage association with a corresponding reduction in levels of sulphides and trimethylamine, but caused undesirable changes in the appearance of the packed product. Further work will examine the effect of adding lactic acid bacteria, controlled atmosphere storage of fish and in particular the effect of sorbate treatment on the spoilage association and shelf life of vacuum packed fish.

#### Acknowledgements

The authors are indebted to Drs C. J. Thomas and J. Olley for advice and constructive criticism. Mr P. Kearney employed under a Fishing Industry Research Trust Account Grant assisted with the trimethylamine analyses.

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# Spoilage of vacuum-packed chill-stored scallops with added lactobacilli

H. A. BREMNER and J. A. STATHAM

The effects of different packing regimes on the spoilage characteristics of chill-stored scallops (*Pecten alba*) were investigated. Scallops were packed aerobically in vacuum packs and in vacuum packs with an inoculum of lactobacilli and the microbial flora examined after several days storage at 4°C. *Vibrio* spp. dominated the initial flora ( $\sim 10^5$  cfu/g) and were still present in the vacuum-packed scallops after 13 days' storage ( $\sim 10^7$  cfu/g), while in the aerobic pack microbial counts were higher ( $\sim 10^9$  cfu/g), 95% of which were *Alteromonas*. While the inoculated lactobacilli grew slowly in the vacuum pack, other organisms were not suppressed and spoilage proceeded at a rate similar to that in the aerobic and vacuum packs as judged by microbiological and raw odour assessment.

Scallops along with many other shellfish command high prices when marketed fresh. Comparatively little work has been reported on the spoilage flora of scallops. Thomson *et al.* (1974) found the normal spoilage flora of queen scallops (*Chlamys opercularis*) to be similar to that occurring on fish except that *Moraxella/Acinetobacter* spp. outgrew pseudomonads. The pattern of spoilage of the species common to south eastern Australia (*Pecten alba*) has never been described. As with other seafoods chilled storage life is limited and alternative methods of packaging and preservation need investigation.

Scallops differ from fish in that post-mortem glycogen levels are relatively high, being in the order of 3% (Groninger & Brandt 1970). Low final pH would therefore be expected, even though octopine produced by the reductive condensation of arginine and pyruvate (Hiltz & Dyer 1971, Sakaguchi, Hiltz & Dyer 1975) and succinate (O'Doherty & Feltham 1971) are the major end products of post-mortem glycolysis in the muscle of the scallop species *Placopecten magellanicus*, rather than lactate as occurs in other muscle types. High glucose levels derived from glycogen may also support a population of lactic acid bacteria, resulting in inhibition or suppression of the Gram negative spoilage flora. Gilliland and Speck (1975) found an inoculum of  $10^6$  cfu/g of *Lactobacillus bulgaricus* to inhibit the psychrotrophic bacteria on fresh crab meat, while Moon *et al.* (1982) showed no such inhibition by either *L. casei* or *Streptococcus lactis* in shrimp. The presence of lactobacilli has been shown also to inhibit the growth of *Clostridium botulinum* type E in fish products (Johannsen 1965, Raa & Gildberg 1982). Lowered pH and production of antibacterial agents, possibly organic peroxides, are thought to inhibit growth and toxin production.

There have been several reports of lactobacilli being present in marine products, including crab meat (Lee & Pfeifer 1975, Ward, Pierson & Van Tassell 1977), fish muscle (Banks, Nickelson & Finne 1980, Knøchel 1981) and fish intestines (Schroeder *et al.* 1980); however, lactobacilli do not appear consistently.

The initial flora of fresh shellfish determines, to some extent, the nature of the subsequent spoilage association and in turn this can be influenced by geographic and seasonal differences. This investigation aimed to determine the spoilage association of vacuum-packed scallops and establish the effect of a lactobacillus inoculum on this association.

## Materials and methods

### Packing procedure

Scallops were caught at night (August 1981) in Banks Strait, NE Tasmania, and taken by truck the next day to the processing factory. The following morning they were alive when shucked by the normal commercial process. The scallop meat complete with roes was taken to the laboratory where it was sorted randomly into four groups each of 224 scallops which in turn were packed into 14 bags. The treatments are summarised in Table 1. Lactobacilli were added to all the scallops in treatment L by mixing with them 40 mL of a fresh culture of *Lactobacillus plantarum* (NCDO 343) to give an inoculum of approximately  $8.7 \times 10^8$  cfu/g. The scallops were then randomly allocated 16 to each pack.

Two packs from each treatment were opened initially and after storage for 3, 7, 10 and 15 days; single packs from each treatment were opened after 23, 27 and 38 days in store. At each sampling time the appearance of the scallops was noted and after opening the packs the intensity and nature of the odour was recorded.

### Analytical

A perchloric acid extract (Mackie & Thomson 1974) of two scallops from each pack was used to determine hypoxanthine (Jones *et al.* 1964). Perchloric acid extracts were also used to determine trimethylamine according to Tozawa, Enokihara and Amano (1971), trimethylamine oxide by the same method after reduction with  $\text{TiCl}_3$  (Yamagata, Horimoto & Nagaoka 1969) and glycogen according to Carroll, Longley and Roe (1956). Octopine and arginine were determined as described by Hiltz and Dyer (1971). Samples and standards were run on PEI-cellulose F pre-coated 0.1 mm thick plates and developed with Sakaguchi's reagent. Arginine gives an orange spot which is removed by shaking with cation exchange resin. Octopine gives a pink spot which at higher concentrations (10  $\mu\text{L}$ ) tends to run spread both before and behind the salt in the extract but in more dilute extracts which contain less salt gives a single spot when a 5  $\mu\text{L}$  aliquot of a 1:1 dilution of the sample is used.  $R_f$  values obtained were 0.40 for arginine and 0.24 for octopine.

Scallop flesh pH was determined using an Orion pH meter equipped with a spear electrode; moisture contents were determined by oven drying at 105°C to constant weight.

### Microbiology

#### Sampling

One scallop was removed at random from each pack, weighed and homogenised in sterile saline for 3 min by Colworth Stomacher. Serial decimal dilutions were spread plated onto nutrient agar containing 2.5% NaCl (SNA). The medium of de

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Man, Rogosa and Sharpe (1960) adjusted to pH 5.5 with lactic acid (MRS<sub>1</sub>) and the medium of Gardner (1966) were used to estimate numbers of presumptive lactic acid bacteria and *Brochothrix thermosphacta* respectively. All plates were incubated at 22°C for 3–5 days. MRS<sub>1</sub> plates were flooded with 3% H<sub>2</sub>O<sub>2</sub> and only catalase negative colonies were counted. Gardner's medium was flooded with Kovacs' oxidase solution and only oxidase negative colonies were counted. Plates were flooded only when isolates were not required for further identification.

A sulphur-rich iron-containing medium (BCA) was used for enumeration of hydrogen sulphide-producing bacteria. The medium was composed of: Bacto beef extract (Difco) 0.3%, yeast extract (Sigma) 0.3%, Bacto peptone (Difco) 0.5%, Bacto tryptone (Difco) 1.5%, ferrie citrate 0.3%, Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> 0.05%, cysteine HCl 0.04%, NaCl 2.5%, Bacto agar (Difco) 1.5%; pH was adjusted to 7.0 using KOH. The plates were spread with inoculum, allowed to dry and overlaid with the same medium. Hydrogen sulphide-producers appear as black colonies within the agar (E. Gorczyca, pers. comm.). Total bacterial estimates were also obtained from this medium to allow a comparison of such counts with those from SNA. All plates were incubated at 22°C for 3–5 days.

#### Identification of isolates

Colonies were isolated randomly from SNA plates inoculated with samples taken at 0 and 15 days. In all cases 20 isolates were taken from each duplicate sample, giving a total of 40 isolates from each treatment at each sampling time. Gram negative isolates were identified according to Shewan, Hobbs and Hodgkiss (1960). Gram positive isolates were identified according to *Bergey's manual of determinative bacteriology* (Buccanan & Gibbons 1974).

#### Statistical methods

Analysis of variance followed by determination of least significant differences was used to differentiate treatment effects.

#### Geographical survey of bacterial flora of scallops

In this investigation scallops from Perth (WA), Sydney (NSW), Lakes Entrance and Port Phillip Bay (Vic) were obtained during January–February 1982. They were sampled in duplicate as previously described. Twenty isolates from each sample were identified, giving a total of 40 isolates from each location.

## Results and discussion

#### Chemical composition

Table 2 gives the results of the chemical analyses performed on the fresh scallops.

#### Hypoxanthine

Hypoxanthine is the ultimate post-mortem breakdown product of ATP. Changes in hypoxanthine levels in the chill-stored scallops are demonstrated in Figure 1. Hiltz and Dyer (1973), Thomson *et al.* (1974) and Nakamura *et al.* (1976) all considered hypoxanthine a useful indicator of scallop freshness. It is necessary, however, to know the thermal history of the scallops before interpreting the significance of a particular hypoxanthine level. For example, Hiltz and Dyer (1973) found that the ultimate levels of hypoxanthine in chilled scallop meats rose to approximately 3 µmole/g, while for frozen scallops which were then thawed and stored the ultimate level rose to 6 µmole/g due to freeze-thaw stimulation of the 5'-nucleotidase, AMP phosphohydrolase. Thomson *et al.* (1975) found similar levels in queen scallops, but for prepacked meats stored at 4°C intermediate levels near 4.5 µmole/g were obtained. They considered the limits of edibility corresponded to hypoxanthine concentrations of 2.5–2.8 µmole/g in whole iced queen scallops, 3.5–4.0 µmole/g in prepacked meats and 4.0–5.0 µmole/g in prepacked meats obtained from thawed, whole queen scallops which had been frozen.

In the present experiment, levels of 3.5–4.0 µmole/g in the scallops were found after storage for about 15 days at 4°C (Fig. 1). Scallops in all treatments were well spoilt by this time although the raw odours (see below) typical of each treatment

Table 1. Summary of treatments

Code	Treatment	Storage temp. (°C)
V — vacuum	Vacuum packed in barrier bags*	4
L — lactobacilli added	Vacuum packed in barrier bags after addition of $8.7 \times 10^5$ cells/g <i>Lactobacillus plantarum</i>	4
A — aerobic	Packed in polyethylene bags, tied at the neck	4
F — frozen	Vacuum packed in barrier bags	–18

\*Cryovac L gauge barrier bags — W.R. Grace Pty Ltd, Melbourne, nominal OTR (unshrunk) 3.5 mL m<sup>2</sup> 24 h<sup>–1</sup> atm<sup>–1</sup> at 3.5°C and 75% RH.

Table 2. Chemical composition of fresh scallops

Moisture (g/100g)	78.8 (SE 0.18)
Glycogen (g/100g)	0.8–1.0
TMAO (mg TMAO-N/100g) — muscle	44.8
— roe	10.7
TMA (mg TMA-N/100g) — muscle	1.2
— roe	0.4
Roe (g/100g)	38.1

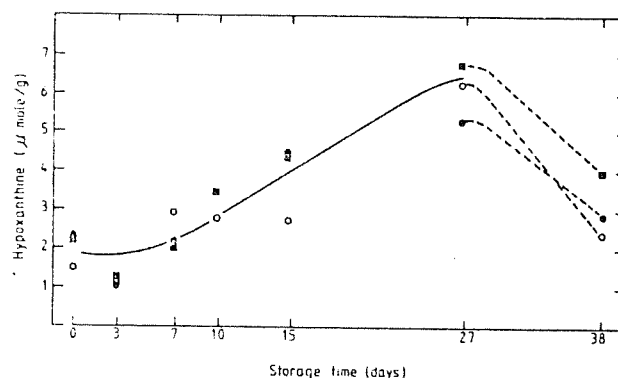


Figure 1. Changes in hypoxanthine levels in scallops stored at 4°C: ●, vacuum packed; ○, vacuum packed plus *L. plantarum*; ■, aerobic.

were different. The odours are a reflection on the different microbial populations (see below), whereas hypoxanthine production is a biochemical change in the muscle itself. Only at advanced stages of spoilage after 27 days were the effects of bacterial breakdown of hypoxanthine evident (Burt 1977). The hypoxanthine levels in the frozen samples did not change during the storage period of 38 days.

#### pH Changes

The change in pH of the scallop muscle surface during storage is shown in Figure 2. The initial drop of approximately 1 pH unit in all treatments is interpreted as being due to post-mortem glycolysis. Surface pH values showed an overall decrease throughout the remainder of the storage period. After storage for 10 days, surface pH of the scallop muscle was noted to be significantly higher ( $p < 0.001$ ) than that of the interior of the muscle. It was not until after a further 28 days that surface pH was found to have fallen sufficiently to become significantly lower ( $p < 0.001$ ) than that of the interior of the muscle. No differences between surface and internal pH were noted in frozen samples until after 27 days, when surface pH became significantly lower ( $p < 0.001$ ) than internal pH. Although bacterial activity is confined to the surface of proteinaceous material this difference may have significance when recording pH values from homogenates.

#### Products of post-mortem glycolysis

The detection of arginine and octopine by chromatography showed that *Pecten alba* follows the same metabolic pathway as *Placopecten magellanicus*.

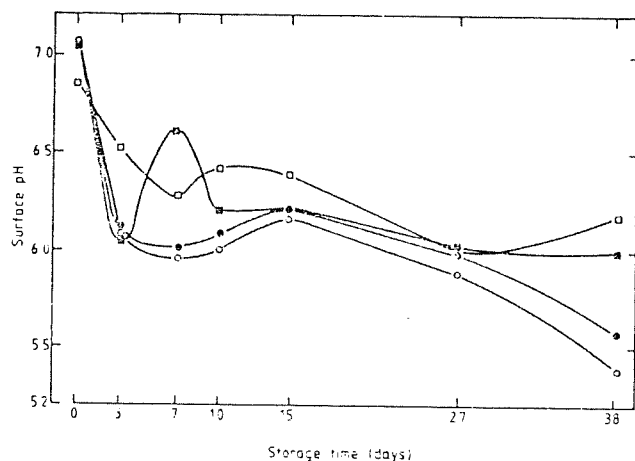


Figure 2. Surface pH of stored scallops: ●, vacuum packed; ○, vacuum packed plus *L. plantarum*; ■, aerobic; □, frozen.

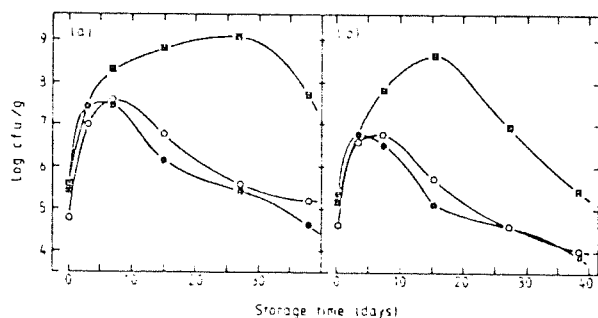


Figure 3. Bacterial numbers on scallops stored at 4°C: (a), estimated on salt nutrient agar; (b), estimated on black colony agar; ●, vacuum packed; ○, vacuum packed plus *L. plantarum*; ■, aerobic; □, frozen.

#### Bacterial numbers

Estimates of bacterial numbers obtained from SNA and BCA are shown in Figure 3. Cell numbers in the two vacuum-packed treatments, estimated on SNA, reached a maximum of  $10^7$  cfu/g at 7 days. The counts obtained from aerobically-stored scallops reached a maximum of  $10^8$  cfu/g after storage for 28 days, the numbers being significantly different ( $p < 0.001$ ) from those of vacuum-packed scallops. No lag phase was exhibited in any treatment.

Bacterial numbers estimated on BCA (Fig. 3) showed a trend similar to those of SNA during the first 20 days in storage, but were significantly lower ( $p < 0.05$ ) at each sampling time. This reduction in total counts can be attributed to the molten agar overlays ( $52^\circ\text{C}$ ) reducing psychrotroph numbers and can be overcome by using solid overlays (Hobbs & Hodgkiss 1982).

The numbers of  $\text{H}_2\text{S}$ -producing colonies detected on BCA (Fig. 4a) showed a trend similar to that of the total bacterial estimates and represented approximately 1% of the total population throughout storage. This proportion is variable, however, with no black colonies occurring on some plates. In general, fewer definite black colonies appeared on crowded plates.

Presumptive lactic acid bacteria determined by growth on MRS<sub>2</sub>, and the absence of catalase occurred in both chill-stored vacuum-packed treatments with and without added lactobacilli, reaching a maximum of  $10^8$  cfu/g after 38 days (Fig. 4b). Isolates taken from each treatment differed considerably; those from treatment L were homofermentative rods biochemically similar to the *L. plantarum* used as inoculum, while those from treatment V were difficult to subculture and could not be grown in the biochemical test media which were used for further

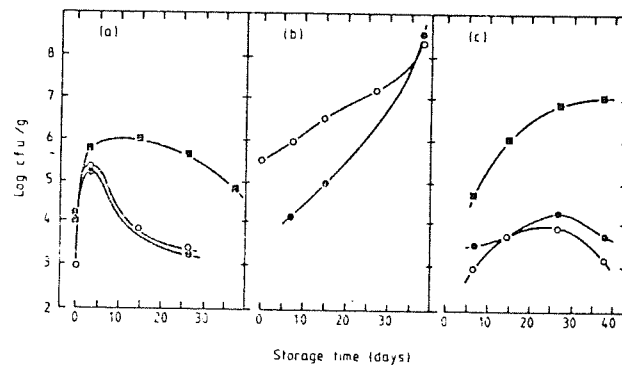


Figure 4. Bacterial numbers on scallops stored at 4°C: (a), estimated numbers of  $\text{H}_2\text{S}$ -producing colonies on black colony agar; (b), numbers of presumptive lactobacilli estimated on MRS agar adjusted to pH 5.5; (c), numbers of *Brochothrix thermosphacta* estimated on Gardner's medium; ●, vacuum packed; ○, vacuum packed plus *L. plantarum*; ■, aerobic; □, frozen.

Table 3. Changes in bacterial flora of scallops stored at 4°C

Storage time (days)	Treatment	Genera present (%)			
		<i>Vibrio</i>	<i>Alteromonas</i>	<i>Staphylococcus</i>	<i>Aeromonas</i>
0	V — vacuum-packed	100	—	—	—
	L — vacuum-packed + <i>L. plantarum</i>	92.5	5	2.5	—
	A — aerobic	95	5	—	—
15	V — vacuum-packed	95	—	—	5
	L — vacuum-packed + <i>L. plantarum</i>	97.5	—	—	2.5
	A — aerobic	7.5	92.5	—	—

identification or in scallop broths. These isolates from treatment V were possibly a more fastidious strain of marine origin. *Brochothrix thermosphacta* was found in all treatments after 3–7 days in storage; it became a significant proportion of the total flora, however, only in the aerobic treatment, reaching approximately  $10^7$  cells/g after 38 days (Fig. 4c). Reports on the occurrence of *B. thermosphacta* in seafoods are rare, it being generally considered as a spoilage organism on meats. It has, however, been shown to represent a significant proportion of the bacterial flora of black drum (*Pogonias cromis*) (Nickelson *et al.* 1980). Inhibition of anaerobic growth of *B. thermosphacta* on beef occurs at pH values below 5.8 (Campbell *et al.* 1979) and is brought about by increasing levels of undissociated lactic acid (Grau 1980). A similar type of inhibition may occur in vacuum-packed scallops if a population of lactic acid bacteria is established. No lactic acid bacteria or *B. thermosphacta* were detected on SNA plates due to the lack of a carbohydrate source and the necessary vitamins. This medium therefore tends to represent a Gram negative count rather than a 'total' count.

#### Bacterial flora

The composition of the bacterial flora isolated from SNA is shown in Table 3. *Vibrio* spp. were the predominant types in the initial flora; they persisted throughout storage in the vacuum-packed treatments. *Alteromonas* spp. proliferated in the aerobic packs becoming the predominant type at spoilage. In all chill-stored treatments spoilage as indicated by the raw odour was well advanced by 15 days, suggesting that *Vibrio* spp. are active spoilers of scallops, producing undesirable odours and flavours at rates similar to *Alteromonas* spp. However, the experiment was continued to monitor the bacterial interactions. The spoilage role of the *Vibrio* spp. was confirmed by the



Table 4. Raw scallop odours compared with frozen samples

Treatment	Storage time at 4°C (days)					
	3	7	10	15	27	38
V — vacuum packed	Definitely H <sub>2</sub> S, SO <sub>2</sub>	Slightly off, seafood, oyster, sulphide	Very definite H <sub>2</sub> S, sweet, fruity, rotten apple, seaweed	Sulphide, fruity, H <sub>2</sub> S, mussels, sweaty socks	Oysters, mussels, vinegar, dimethyl sulphide	Hot rock pools, vinegar, yeasty, old seaweed, sulphide, gumboots, fruity
L — inoculated with lactobacilli then vacuum packed	H <sub>2</sub> S, eggs, curried eggs	Sharp, HCl, pungent, meaty	Fruity, metallic, slight H <sub>2</sub> S, fruit, vinegar, seafood	Shellfish, mussels, oysters, pungent	Acid, oyster, shellfish, mussel, sour milk, slight old socks	Pungent, old cabbage, decaying vegetables, fruity (but sharp, different from treatment V)
A — aerobic pack	Old seaweedy, not scallop	Ammonia, scallop, shellfish, sweaty feet, old socks	Sour pastry, sour milk, yeasty, sour dough	Cheesy, musty, H <sub>2</sub> S, acid, vinegar, sour, ammonia	Sour dough, yeasty, NH <sub>3</sub> , vinegar	Fruity, yeasty, old puff pastry, sour

unpleasant off-odours produced when isolates were inoculated into sterile scallop extract.

#### Raw odour and colour

The odours detected during chill storage of the scallops in treatments V, L and A are listed in Table 4. The frozen scallops maintained their fresh seaweedy, scallop odours throughout and were used as a reference.

There were no detectable colour changes of the packs in chill storage until 15 days when it was obvious that the roe colour had faded and the natural fawn/creamy colour of the flesh was fading to grey. After 15 days in storage, gas production and frothy liquors were evident in packs in treatments V and L and by 27 days all the chill-stored bags had become inflated.

The inhibitory effect of carbon dioxide on bacterial growth is well documented and it may be that the slow build-up of the gas in these packs resulted in the decline in bacterial numbers after storage for 10 days (Fig 3) and the eventual inflation of the bags.

#### Geographical survey of bacterial flora of scallops

*Vibrio* spp. were common to the bacterial flora of scallops from various locations around Australia but comprised a larger proportion of the total flora in scallops from the cooler regions such as Tasmania, Port Phillip Bay and Lakes Entrance. It would appear from this cursory investigation that scallops generally have a limited native flora composed of Gram negative, facultatively anaerobic rods.

#### Conclusions

In summary, *Vibrio* spp. are the predominant bacterial types occurring in fresh scallops and are capable of causing spoilage of both aerobically-stored and vacuum-packed scallops. Inoculation of scallops with lactobacilli did not alter the spoilage pattern; it appears that *Vibrio* spp., unlike *Alteromonas*, are not susceptible to antibacterial effects exerted by lactobacilli.

#### Acknowledgement

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## Effect of Potassium Sorbate on Spoilage of Blue Grenadier (*Macruronus novaezelandiae*) as Assessed by Microbiology and Sensory Profiles

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### ABSTRACT

Blue grenadier fillets (*Macruronus novaezelandiae*), pH 6.7, which had been stored frozen for 3 wk were thawed and repacked under vacuum and in air with and without the addition of 0.1% potassium sorbate. The effects of these treatments on the microbial flora were noted after subsequent storage of the fillets at 4°C. Pseudomonads comprised >90% of the total flora of sorbate-treated fish, whereas *Vibrio* spp. (85%) and *Moraxella* spp. (70%) predominated in vacuum packed and aerobically stored fillets, respectively. Sensory profiles of odor and flavor of the stored material were constructed. The acceptability of the aerobically stored fillets had significantly decreased after 7 d of storage. Vacuum packaging in conjunction with 0.1% potassium sorbate results in a minimal extension of shelf-life.

Satisfactory methods for considerably improving the shelf-life of chill-stored fish products are the subject of much recent research (3,4,6,8,9,13,16,19). Attempts have been made to simulate conditions similar to those existing in vacuum-packaged red meats, by the addition of utilizable carbohydrates and adjustment of pH (14) and addition of lactobacilli (3); however, none has proved successful. Low post-mortem glycogen levels make fish products analogous to high pH red meats, and similar problems occur when vacuum packaging these products. It appears that, apart from irradiation, the addition of antimicrobial agents in conjunction with packaging under vacuum, or in some modified atmosphere, may be necessary if extension of chilled shelf-life is to be gained.

The antimicrobial effects of potassium sorbate have been used for many years to preserve a wide variety of foodstuffs (23) and more recently its potential application to seafood preservation is being investigated (4,6,8). Sorbic acid has a very low acute toxicity and is metabolized by normal physiological pathways (10). It is listed by the FDA as a GRAS substance in the USA. It has been shown to have the ability to inhibit pathogens including *Vibrio parahaemolyticus* (19), *Staphylococcus aureus* (20), *Clostridium botulinum* (1,12) and the food spoilage bacteria *Pseudomonas fluorescens* (17) and *Pseudomonas* (now *Al-*

*teromonas putrefaciens* (18), although the effectiveness depends on the pH which controls the proportion of undissociated acid that is the active form (1). By interpolation of the figures given by Blocher et al. (1), the proportion of undissociated acid in aqueous solution is halved by changing the pH from 6.5 to 6.8, i.e., from 2.5 and 1.2% undissociated acid, respectively, although the proportion present when the substance is applied to a complex substrate like fish flesh at these pH values is not predictable. The inhibitory action of sorbate is reported to be quite effective in the range of pH 6.0 to 6.5 (23), but at the point of processing fish may often have a slightly higher pH than this. This can be due to its inherent properties, to stress during trawling, to seasonal poor condition or to the normal delays that occur between catching and processing.

Debevere and Voets (8) found that sorbate selectively inhibited trimethylamine (TMA) producing bacteria on cod (pH not reported), whereas Chung and Lee (6) showed that addition of potassium sorbate to an homogenate of English sole (pH not reported) resulted in an extension of the lag phase of bacterial growth with inhibition of H<sub>2</sub>S – producing *P. putrefaciens*. Regenstein et al. (16) used sorbate ice (pH 6.5) to extend the shelf-life of red hake. Storage of red hake in 1% sorbate ice in a modified atmosphere of CO<sub>2</sub>:O<sub>2</sub>:N<sub>2</sub> (60:21:19) was more effective than sorbate ice alone (9). A considerable extension of shelf-life for vacuum-packaged scallops (initial pH 6.2) treated with 0.1% potassium sorbate was found by Bremner and Statham (4).

Blue grenadier is related to the merluccid hakes (2) and is an increasingly important trawl fish caught in New Zealand and south eastern Australian waters. It is inherently soft and often has a high pH (5). This communication reports the effects of potassium sorbate on the spoilage association and the nature of the spoilage of vacuum packaged and aerobically stored blue grenadier stored at 4°C.

### MATERIALS AND METHODS

Blue grenadier were caught by trawling (Nov. 81) and were stored in the headed and gutted form in ice for 3 d until they reached the laboratory. The fish had a moisture content of 81.4% and a fat content of 1.8% (means of 4 samples). They were in reasonably good condition with an

initial bacterial flora of *Moraxella*, *Pseudomonas* and *flavobacteria* containing  $7 \times 10^3$  cells/cm<sup>2</sup>. The fish were inspected, filleted, trimmed and individually packed (skin on) in barrier bags (Cryovac U gauge O.T.R. approx. 3.5 ml/m<sup>2</sup>/24 h/atm, W. R. Grace (Aust.) Pty). Some fillets showed signs of deterioration in the belly area. After 21 d of frozen storage at -18°C, the fillets were thawed for 17 h at 4°C. Each fillet was cut into pieces weighing approx. 50 g. These were randomly allocated to five treatments (Table 1).

Each treatment consisted of 14 bags, each containing three pieces of fillet. Potassium sorbate was applied in treatments VS and AS by adding sufficient 10% solution of potassium sorbate (food grade) to all fillets in a large plastic bag in an effort to ensure addition of a known amount. The solution was gently mixed over the fillets by kneading the bag to give an overall concentration of 0.1% potassium sorbate (wt/wt). No attempt was made to adjust the pH of the flesh. Fish flesh is an effective buffer and dipping the flesh in citric acid or citrate buffers has proven unsatisfactory due to precipitation of the proteins on the flesh surface (14) and slight bleaching effects (25). The barrier bags containing the vacuum-packaged samples (treatments V, VS and F) were heat shrunk by dipping for a few seconds in hot water (85°C), then cooling immediately in ice water.

Packs were examined in duplicate at each of the sampling times. Immediately each pack was opened, the contents were sniffed by the experimenters and the intensities of fish odor and off-odor noted as a consensus score on a 0 to 9 scale (0, no fish odor to 9, extreme fish odor; 0, no off-odor to 9, extremely strong off-odor). Descriptions of off-odor were also recorded.

#### Analytical

Perchloric acid extracts were used to determine trimethylamine by the method of Tozawa et al. (24) on samples taken adjacent to the areas used for microbiological counts. The pH of fish flesh was determined using an Orion pH meter equipped with a spear electrode.

#### Microbiology

**Sampling.** A 16-cm<sup>2</sup> skin sample was removed from one fillet piece from each duplicate pack. The skin sample was homogenized in sterile saline for 3 min using a Colworth Stomacher 400 and the homogenate then used to prepare serial decimal dilutions for spread plating. Nutrient agar containing 2.5% NaCl (SNA) was used for the estimation of bacterial numbers throughout the experiment. This medium was employed as an alternative to seawater agar, which has been used extensively for culturing bacteria from fish (21). All plates were incubated at 22°C for 3 to 5 d.

**Identification of isolates.** Twenty colonies were randomly selected from SNA plates of the initial samples and from those taken after 11 d of storage. Gram-negative isolates were placed into genera according to the scheme of Shewan et al. (22) and were further identified using the scheme of Hendrie and Shewan (11).

#### Profile panel

The six pieces of fillet from each treatment were broiled simultaneously to a center temperature of 72°C. To minimize variability, the cooked pieces were shredded with a fork and well-mixed. The mixed flesh was then distributed between two bowls and reheated in a microwave oven to a temperature of 70 to 80°C immediately before being presented to the panel.

The panel consisted of 10 to 15 trained people grouped around a table. Each panelist was provided with two score sheets listing words which had been used previously to describe odor and flavor attributes of seafoods in terms of other familiar foods and sensations. The odor profile consisted of 67 descriptive terms, the flavor profile of 52 terms. Panelists sniffed the cooked fish as often as they liked and recorded their impressions on the odor profile sheets using scores ranging from 0 (absent) through 1 (slight), 2 (moderate), 3 (strong), 4 (very strong) to 5 (extremely strong). Fish odor intensity and off-odor intensity were marked on the same scale. The findings were discussed briefly and panelists were allowed to amend their sheets if they wished. Each panelist then tasted the fish and recorded his or her impressions on the same 0 to 5 scoring system on the flavor profile sheets, which also listed flavor intensity and off-flavor intensity. Overall acceptability was marked independently without discussion on a 'Smiley' scale. Panelists were asked to use this scale in the following manner: "See which face most closely conveys your impression of the product, then write down the score corresponding to this face". A score of 1 corresponded to the lowest acceptability and 7 to the highest acceptability (Table 2). A discussion session on flavor was held, then the next sample was assessed for odor and flavor. Ten tasters were common to the assessments of all treatments after 0, 4, 7 and 11 d of storage and nine of these tasters were common to assessment of treatments V, F and VS after 14 d, and treatments F and VS after 18 d of storage.

## RESULTS AND DISCUSSION

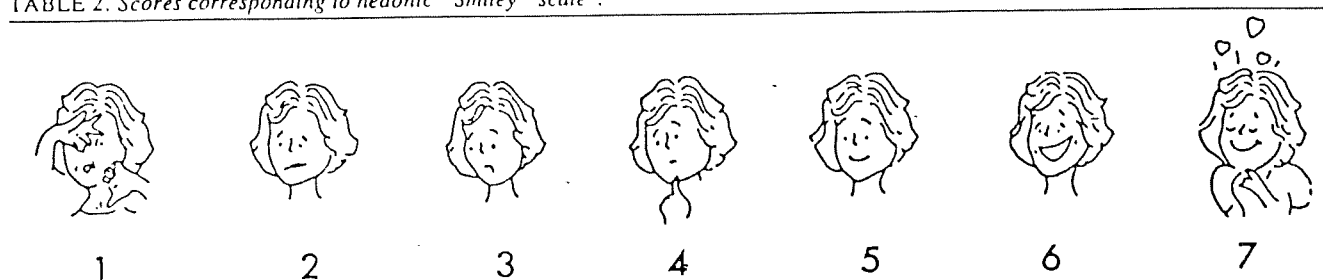
#### Trimethylamine

Treatment with potassium sorbate suppressed TMA-forming organisms, particularly in the vacuum packs (treatment VS, Table 3). This is in agreement with the observation of Debevere and Voets (8) that potassium sorbate selectively inhibited TMA-producing bacteria. Vacuum packaging alone (treatment V) suppressed TMA formation in comparison with the aerobic treatment. At day 0 the mean for the five treatments, (0.3 mg TMA-N/100 g), was low, indicating fish of good initial quality (7).

TABLE 1. Summary of treatments.

Treatment	Packaging	Storage temperature (°C)	Sampling times (d)
V	Vacuum packaged in barrier bags	4	0,4,7,11,14
VS	Treated with 0.1% potassium sorbate; vacuum packaged in barrier bags	4	0,4,7,11,14,18
A	Packaged in polyethylene bags tied at the neck	4	0,4,7,11
AS	Treated with 0.1% potassium sorbate; packaged in polyethylene bags tied at the neck	4	0,4,7,11
F	Vacuum packaged in barrier bags	-18	0,4,7,11,14,18

TABLE 2. Scores corresponding to hedonic "Smiley" scale<sup>a</sup>.



<sup>a</sup>Smiley scale courtesy of General Food Corporation.

TABLE 3. Levels of trimethylamine (TMA-N mg/100 g)<sup>a</sup> stored in fillets.

Treatment <sup>b</sup>	Storage time (d)					
	0	4	7	11	14	18
V	0.3	0.4	0.5	2.9	10.7	78
VS	0.4	0.2	0.4	0.7	0.6	1
A	0.3	0.3	2.5	14.6	42	83
AS	0.2	0.4	0.3	1.1	1.6	3.6
F	0.3	N.D. <sup>c</sup>	0.3	0.4	0.2	0.2

<sup>a</sup>Values shown are means from two bags per treatment.

<sup>b</sup>Treatments are described in Table 1.

<sup>c</sup>N.D., not determined.

#### pH

The mean initial pH was 6.7. Changes in external pH values within each treatment are shown in Fig. 1. Significant increases ( $P < 0.05$ ) occurred after 7 d of storage in fillets stored aerobically, with and without added potassium sorbate. Between 11 and 18 d of storage, additional significant increases ( $P < 0.001$ ) occurred in treatments A, AS and V. No changes occurred in the pH of fillets in treatments VS and F. The external pH value became significantly higher ( $P < 0.001$ ) than that of the interior of the muscle as spoilage progressed, except in treatment VS and F where external pH remained constant.

#### Microbiology

**Viable counts.** Bacterial numbers obtained on SNA are shown in Fig. 2. Analysis of variance showed bacterial numbers in treatment VS to be significantly lower ( $P < 0.001$ ) than for the other three treatments. No lag phase occurred in either of the aerobically stored treatments, and bacterial counts reached  $10^9$  colony-forming units (CFU)/cm<sup>2</sup> and  $10^8$  CFU/cm<sup>2</sup> in treatments A and AS, respectively. Treatment V exhibited a short lag phase of about 4 d, after which bacterial numbers increased rapidly to a maximum of  $10^8$  CFU/cm<sup>2</sup>. An extended lag phase occurred in treatment VS, lasting for at least 7 d. This suggests the effect of potassium sorbate on the bacterial flora in vacuum-packaged fish was not bactericidal, as found with vacuum-packaged scallops (4), but was bacteriostatic, maintaining low bacterial numbers over the first few days of storage.

**Bacterial flora.** The bacterial flora present initially and after 11 d of storage are shown in Table 4. The differences in the composition of the initial flora are likely to be due

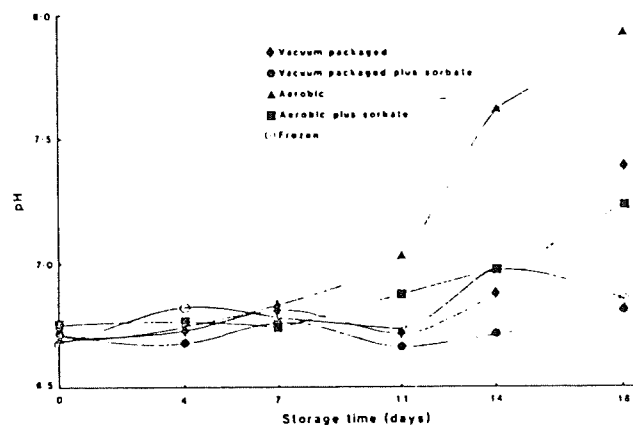


Figure 1. Changes in surface pH of blue grenadier fillets during storage at 4°C.

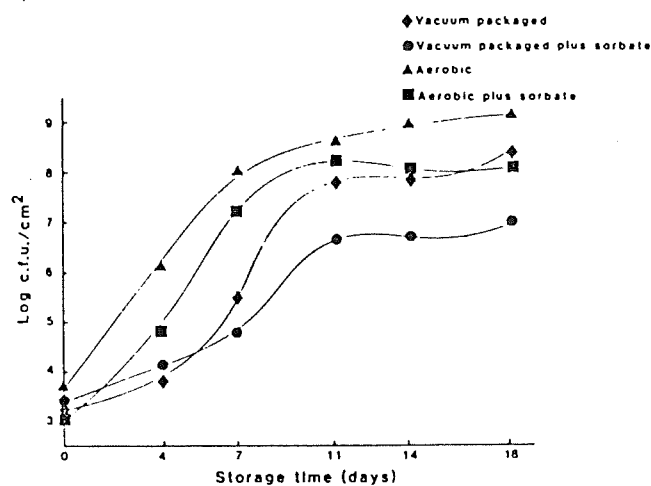


Figure 2. Bacterial numbers on blue grenadier fillets during storage at 4°C.

between ~~the~~ <sup>the</sup> fillet variation, caused in part by freezing and thawing. *Moraxella*, *Pseudomonas* and flavobacteria predominated in the flora at day 0. After storage for 11 d, the fish with added sorbate supported flora composed almost exclusively of *Pseudomonas* spp. The majority of those in treatment AS belonged to the pseudomonad group which produce diffusible fluorescent pigment (11).

All pseudomonads isolated from sorbate-treated fish produced fruity odors and were not capable of reducing trimethylamine oxidase (TMAO) either in broth cultures or

on fish. The occurrence of pseudomonads in sorbate-treated vacuum-packaged fish suggests an ability to utilize a compounds, other than TMAO, as an electron acceptor for anaerobic respiration, while at the same time being resistant to the inhibitory effect of sorbate.

The composition of the flora of aerobically stored fish with no added sorbate remained similar to that found on fresh fish. However, *Moraxella* became more significant

TABLE 4. *Microflora of blue grenadier stored at 4°C.*

Genera	Treatments <sup>a</sup>				Treatments			
	V	VS	A	AS	V	VS	A	AS
	Initial flora				Flora after 11 d			
<i>Moraxella</i>	85 <sup>b</sup>	55	35	50	-	-	70	-
<i>Flavobacteria</i>	5	10	15	35	-	-	-	-
<i>Pseudomonas</i>	10	30	50	10	-	95	25	90
<i>Alteromonas</i>	-	-	-	-	10	5	-	-
<i>Vibrio</i>	-	5	-	5	85	-	5	-
<i>Aeromonas</i>	-	-	-	-	5	-	-	10

<sup>a</sup>Treatments are described in Table 1.

<sup>b</sup>Percent of total.

TABLE 5. *Odor scores<sup>a</sup> for raw chill stored (4°C) blue grenadier<sup>b</sup>.*

Treatment <sup>c</sup>	Storage time (d)					
	0	4	7	11	14	18
V	6	4	3	3	2	1
VS	5	4	3	3	2	3
A	5	3	2	2	1	0
AS	5	3	3	1	1	1

<sup>a</sup>Consensus scoring on an unstructured integer scale from 0 (no typical fish odor) to 9 (extremely strong fish odor).

<sup>b</sup>Frozen blue grenadier was scored 5 throughout the trial.

<sup>c</sup>Treatments are described in Table 1.

TABLE 6. *Off-odor scores<sup>a</sup> and comments for raw chill stored (4°C) blue grenadier<sup>b</sup>.*

Treatment <sup>c</sup>	Storage time (d)				
	4	7	11	4	18
V	1 <sup>a</sup> Floury Baby powder	2 Pungent (like HCL)	2 Sweet Metallic Musty Pasty	4 Ammoniacal Sharp Fruity Cheesey	6 Sour Sulfides
VS	1 Sweet Slightly pungent	1 Bland	2 Whey-like Sour	2 Sharp Floury	1 Pasty Slightly pungent
A	2 Pastry Doughy Sour	3 Sour milk Old pastry Yeast	5 Sour Tropical fruit Old vegetables Unacceptable	7 Old whey Old pastry Ammonia Fruity	9 Blocked drains Sweet floral Fruit Sour old grease-traps
AS	1 Bland Plastic bag	1 Bland Slightly sweet	4 Sour tropical fruit Pineapple cans Sulfides	6 Floury Doughy Pineapple cans Fruity Cheesey	7 Sour sinks Fruity Sweet Sour Drains

<sup>a</sup>Consensus scores on an unstructured integer scale from 0 (no off-odor) to 9 (extremely strong off-odor).

<sup>b</sup>Frozen blue grenadier odor described as salty, fishy, floury paste.

<sup>c</sup>Treatments are described in Table 1.

than *Pseudomonas* after 11 d. The flora of vacuum-packaged fillets without sorbate was comprised of a high proportion of *Vibrio* spp., with lower numbers of *Alteromonas* and *Aeromonas*. *Vibrio* spp. were shown to be active spoilers in vacuum-packaged scallops (3,4), where low oxygen tension inhibits strict aerobes.

#### Raw odor scores

The blue grenadier did not have an intense fish odor, rating approximately 5 on a 0 to 9 scale. This rating decreased at each sampling time, particularly for fish in treatments A and AS (Table 5). The off-odor scores and comments (Table 6) clearly indicate the rapid development of objectionable odors in fish in treatments A and AS. Off-odors developed more slowly and were less objectionable in fish in treatment V, whereas fish in treatment VS did not develop objectionable odors, although pack to pack variations within samplings were evident.

#### Profile panel

Although the procedure used for this panel was similar to that reported by Quarmby et al. (15), the present results are listed in tabular, rather than the previous graphic, form. Attributes which were erratically or sporadically scored were deleted from the results. There were no substantial differences in the odor and flavor profiles for the fish assessed at the start of the experiment or for those assessed after frozen storage for 4, 7 and 11 d. Hence the eight sets of results for both odor and flavor were combined to form a profile against which the results of storage within and between treatments could be compared. For convenience, panel total scores have been displayed (N.B. 10 tasters). The profiles for odor (Table 7) and flavor (Table 8) list the

TABLE 7. Odor profile of stored blue grenadier.

Attributes	Treatments <sup>a</sup>													
	Fresh/Frozen		V			VS			A			AS		
	Storage time (d) 4°C		Storage time (d) 4°C			Storage time (d) 4°C			Storage time (d) 4°C			Storage time (d) 4°C		
	Mean (n) <sup>b</sup>	2 S.D.	4	7	11	4	7	11	4	7	11	4	7	11
Boiled fish	18.0(8.9)	6.5	22(10) <sup>c</sup>	25(10)	20(9)	26 <sup>d</sup> (10)	13(9)	11 <sup>d</sup> (7)	15(9)	13(8)	3 <sup>d</sup> (3)	18(9)	16(10)	9 <sup>d</sup> (6)
Milk (boiled)	1.5(1.3)	1.5	3(3)		1(1)	6 <sup>d</sup> (4)	1(1)	1(1)				2(2)		1(1)
Milk (sour)				1(1)	4(3)					2(2)				
Burnt	5.1(4.1)	5.3	5(3)	4(3)	6(5)	3(3)	12 <sup>d</sup> (8)	4(4)	10(5)	1(1)	1(1)	4(3)	4(3)	3(3)
Sweet	1.4(1.1)	2.6	1(1)	4(3)		3(3)	3(2)		1(1)	1(1)	2(1)	1(1)	3(2)	1(1)
Tropical fruit esters								1(1)			15(7)	4(4)	1(1)	14(7)
Boiled meat (beef)	1.6(1.4)	2.8	1(1)	3(2)	1(1)		2(2)	2(2)	1(1)	2(2)		7 <sup>d</sup> (5)	1(1)	
Potatoes (boiled)	3.8(2.5)	5.3	3(2)	4(3)	6(5)	8(5)	1(1)	2(1)				3(2)		
Cardboard	2.1(1.6)	4.7		2(1)	2(2)		7(5)	2(2)	1(1)				6(6)	
Musty (dry)	1.6(1.4)	2.8	6(3)	4(3)	3(3)		2(2)	3(2)	1(1)		1(1)	3(2)	1(1)	4(3)
Potatoes (baked)	2.5(1.8)	6.8	2(2)	2(2)	3(2)		4(3)	1(1)	4(3)	1(1)	1(1)	2(2)	2(1)	5(3)
Cheesey					1(1)			1(1)		2(1)	22(10)			
Ammonia (amine)	1.1(0.9)	3.6			2(1)		1(1)	3(2)	6 <sup>d</sup> (5)	3(2)	11 <sup>d</sup> (5)	6 <sup>d</sup> (5)	1(1)	
Sulfide					4(2)			1(1)	1(1)	2(1)	9(4)	2(2)		9(5)
Sour cloths			4(3)	1(1)	3(3)	2(2)	3(3)	10(4)	5(3)	17(9)	2(1)	1(1)	6(4)	2(1)
Body odor					1(1)					7(4)	1(1)	1(1)	3(3)	1(1)
Dirty socks								2(1)	3(2)	16(6)	10(4)	1(1)	7(5)	
Urinal					8(6)			3(1)			3(2)			
Pungent					3(3)			2(1)	1(1)	9(4)	5(3)	1(1)		1(1)
Wet straw	5.5(4.0)	6.3	7(5)	6(5)		7(5)	7(3)	3(2)	1(1)	5(2)	2(1)	1(1)	4(4)	
Odor (intensity)	22.8(9.4)	8.4	21(10)	27(10)	24(9)	29(10)	20(9)	17(9)	19(9)	19(9)	9 <sup>d</sup> (7)	18(8)	17(9)	19(9)
Off-odor	8.3(5.4)	4.8	13(7)	8(6)	17 <sup>d</sup> (10)	9(6)	17 <sup>d</sup> (10)	20 <sup>d</sup> (9)	17 <sup>d</sup> (8)	29 <sup>d</sup> (10)	39 <sup>d</sup> (9)	16 <sup>d</sup> (9)	16 <sup>d</sup> (8)	25 <sup>d</sup> (9)

<sup>a</sup>Treatments are described in Table 1.<sup>b</sup>Mean of 5 fresh and 3 frozen samples; (n) average number of panelists contributing to that mean.<sup>c</sup>Total panel score and in brackets number of panelists contributing to that score.<sup>d</sup>Significant changes, values higher or lower than the mean for the fresh frozen samples by standard deviations.

TABLE 8. Flavor profile of stored blue grenadier.

Attributes	Treatments <sup>a</sup>													
	Fresh/Frozen		V			VS			A			AS		
			Storage time (d) 4°C			Storage time (d) 4°C			Storage time (d) 4°C			Storage time (d) 4°C		
	Mean (n) <sup>b</sup>	2 S.D.	4	7	11	4	7	11	4	7	11	4	7	11
Boiled fish	17.9(8.9)	4.6	18 <sup>c</sup> (10)	19(9)	16(9)	21(10)	10 <sup>d</sup> (6)	11 <sup>d</sup> (7)	24 <sup>d</sup> (10)	7 <sup>d</sup> (6)	2 <sup>d</sup> (2)	21(9)	12 <sup>d</sup> (7)	5 <sup>d</sup> (5)
Sweet	6.8(4.3)	6.2	12(7)	4(3)	1(1)	7(6)	9(6)	2(2)	4(4)	1(1)	0 <sup>d</sup> (0)	12(8)	2(2)	1(1)
Salty	0.8(0.8)	1.8	1(1)	2(2)	1(1)				5 <sup>d</sup> (5)	2(2)		3(3)	1(1)	
Bitter	1.5(1.5)	2.6	4(3)	1(1)	6 <sup>d</sup> (4)		9 <sup>d</sup> (6)	8 <sup>d</sup> (5)	6 <sup>d</sup> (5)	8 <sup>d</sup> (6)	6 <sup>d</sup> (3)	5 <sup>d</sup> (5)	10 <sup>d</sup> (6)	11 <sup>d</sup> (5)
Metallic				1(1)	1(1)					1(1)	2(1)	4(4)	1(1)	
Astringent	0.9(0.8)	1.7	1(1)		1(1)	1(1)	2(1)		2(2)	4 <sup>d</sup> (2)	2(1)	2(2)	8 <sup>d</sup> (6)	1(1)
Sour				2(2)	3(2)		3(2)		3(3)	11(5)	6(2)	4(4)	10(6)	10(4)
Burnt	0.9(1.0)	1.8	3(3)		1(1)	2(2)	1(1)	1(1)	2(2)			2(1)	1(1)	
Milk (fresh)	1.3(1.3)	2.8	1(1)	3(3)	1(1)	2(2)		1(1)					3(3)	
Rancid									4(3)	3(2)	10(4)	7(7)	2(2)	1(1)
Sardines (canned)	1.8(1.3)	4.6							8 <sup>d</sup> (6)	2(2)	2(1)	1(1)	1(1)	
Greasy	2.4(2.1)	4.0		1(1)	2(2)		2(1)	2(1)		2(1)	3(1)		1(1)	
Waxy	4.5(3.8)	4.8	6(4)	7(5)	11 <sup>d</sup> (7)	2(2)	3(3)	10 <sup>d</sup> (6)	8(6)	7(4)	5(4)	10 <sup>d</sup> (6)	4(3)	8(6)
Soapy	2.3(2.0)	3.6	3(3)	4(4)	7 <sup>d</sup> (5)	4(3)	7 <sup>d</sup> (4)	7 <sup>d</sup> (5)	3(2)	11 <sup>d</sup> (6)	13 <sup>d</sup> (7)	4(4)	9 <sup>d</sup> (5)	17 <sup>d</sup> (9)
Potatoes (boiled)	5.8(3.9)	5.2	7(4)	9(6)	4(3)	8(5)	3(2)	4(3)	4(3)	3(2)		2(2)	2(1)	0 <sup>d</sup> (0)
Meaty (roast)	3.0(2.0)	5.0	4(3)	2(2)		5(3)	3(2)		1(1)	2(1)			1(1)	
Cardboard	7.0(5.0)	8.8	5(3)	10(7)	9(7)	7(4)	12(8)	10(6)	11(8)	13(7)	1(1)	9(5)	7(5)	6(3)
Musty (dry)	3.5(2.5)	4.2	3(3)	2(2)	10 <sup>d</sup> (6)	6(4)	2(2)	10 <sup>d</sup> (6)	2(2)	10 <sup>d</sup> (4)	2(2)	3(3)	3(2)	17 <sup>d</sup> (9)
Grassy			1(1)	1(1)	2(1)		1(1)	1(1)		3(2)	3(2)	4(3)	1(1)	4(3)
Flavor (intensity)	24.8(10.0)	4.8	21(10)	25(10)	20(10)	21(10)	22(10)	21(10)	24(10)	15 <sup>d</sup> (9)	4 <sup>d</sup> (4)	26(10)	20(9)	13 <sup>d</sup> (10)
Off-flavor	8.8(6.4)	3.8	11(9)	9(7)	17 <sup>d</sup> (9)	10(8)	13(7)	16 <sup>d</sup> (9)	17 <sup>d</sup> (10)	22 <sup>d</sup> (9)	40 <sup>d</sup> (10)	16 <sup>d</sup> (9)	19 <sup>d</sup> (9)	33 <sup>d</sup> (10)

<sup>a</sup>Treatments are described in Table 1.<sup>b</sup>Mean of 5 fresh and 3 frozen samples; (n) average number of panelists contributing to that mean.<sup>c</sup>Total panel score and in brackets the number of panelists contributing to that score.<sup>d</sup>Significant changes, values higher or lower than the mean for the fresh/frozen samples by 2 standard deviations.

mean panel total score for the eight fresh/frozen samples, the average number of panelists contributing to that mean, the value of two standard deviations (S.D.) of the mean, the panel total scores for each treatment on each of the sampling days, and, in brackets, the number of panelists contributing to that total. Panel scores which were greater than two S.D. from the mean for the fresh/frozen fish were considered to be significant changes from that mean and are marked by an asterisk. Note also that where scores for an attribute increase or decrease during storage, this is generally reflected in the number of tasters who scored that attribute.

The panel described the main odor attributes of blue grenadier as boiled fish, wet straw, burnt and potatoes (both boiled and baked), with an off-odor intensity equivalent to slight. The main flavor components of blue grenadier as described by the panel were boiled fish, cardboard, sweet, boiled potatoes, waxy, dry musty and meaty. There was no loss in boiled fish odor or flavor in treatment V during storage, but significant losses occurred in the other three treatments. Bitter, soapy and musty dry flavor notes were detected in the fresh/frozen fish and increased significantly in all treatments. An ammoniacal odor was detected in both aerobic treatments after 4 d of storage.

Descriptors which were not present initially could not be assigned S.D. values. A summary of the appearance of these undesirable attributes is listed in Table 9. With the exception of an odor of sour cloths, these descriptors were limited to the aerobic treatments or only appeared on the eleventh day of storage in the vacuum-packaged treatment. This was probably due to the differences in bacterial numbers (Fig. 2) rather than to differences in spoilage organisms.

#### Overall acceptability (Smiley)

Overall acceptability results for fish in all treatments assessed after 0, 4, 7 and 11 d of storage (10 tasters), in

TABLE 9. Summary of undesirable attributes appearing during storage.

Attribute	Day of first appearance <sup>a</sup>			
	V <sup>b</sup>	VS	A	AS
<b>Odor</b>				
Sour cloths	4	7	4	7
Urinal	11		7	7
Pungent	11		7	
Sour milk	11			
Tropical fruity esters			11	4
Cheesey			11	
Sulfide			11	11
Body odor			7	7
Dirty socks			7	7
<b>Flavor</b>				
Metallic				4
Sour			4	4
Rancid			4	4
Grassy				4

<sup>a</sup>Only those attributes detected by over 33% of the panel are listed.

<sup>b</sup>Treatments are described in Table 1.

treatments V, VS and F after 14 d (9 tasters) and VS and F after 18 d of storage (9 tasters) are shown in Fig. 3. The results up to 11 d were subjected to analysis of variance. Fish in treatment A had a significantly lower score ( $P < 0.05$ ) after 4 d than fish in treatments F, V and VS. After 11 d of storage, fish in treatments V and VS had significantly lower scores ( $P < 0.05$ ) than the fish in frozen treatment F, but were not significantly different from each other. Five of nine panelists rejected treatment V after 14 d, the remaining four giving a mean overall acceptability score of 1.5. After 18 d of storage, fish in treatment VS was rated 0.8 scale units lower than fish in treatment F. The fish at the start of the experiment and the frozen samples were never rated highly, a result typical of this type of panel. There were several reasons for this: (a) the fish were prepared and presented in an unprepossessing manner and (b) the panel was trained to be highly critical. More importantly, the ratings are a valid judgment of the fish and a reflection of its current low market value, even when it has been well-handled and is in quite good condition (low bacterial counts, low TMA).

#### CONCLUSIONS

Potassium sorbate at a level as low as 0.1% clearly has no effect on the shelf-life of aerobically stored fish (initial pH 6.7), but it did have a noticeable preservative effect on

Smiley scale

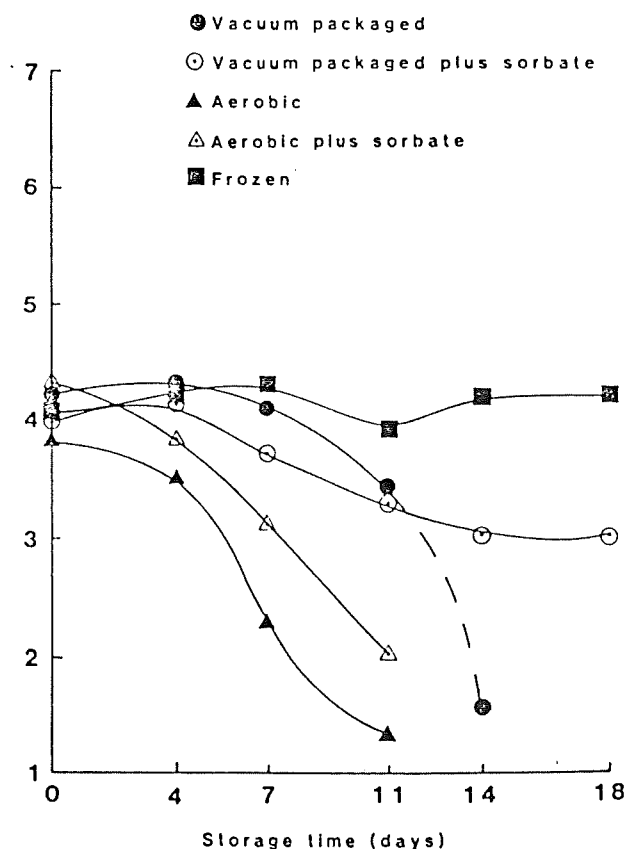


Figure 3. General acceptability of blue grenadier stored at 4°C.



vacuum-packaged fish at this pH, as judged by bacterial numbers, TMA levels, acceptability and raw odor scores. Treatment with sorbate did not maintain the initial quality of the product and this may have been due, in part, to variation in pH between fish pieces or to uneven application of the solution which in industrial practice would more likely be applied more efficiently as a spray or by dipping.

Inhibition of the normal spoilage flora by additives such as sorbate, which results in a product of lingering low quality, may not be desirable. Such conditions may give *Clostridium botulinum* the opportunity to successfully compete with the reduced bacterial load. This then places more emphasis on the need for stringent temperature control below 4°C and reliance on adequate cooking of the product before consumption.

#### ACKNOWLEDGMENTS

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## Effect of Potassium Sorbate on Refrigerated Storage of Vacuum Packed Scallops

H. ALLAN BREMNER and JO A. STATHAM

### ABSTRACT

Scallops (*Pecten alba*) were packaged aerobically, vacuum packaged, treated with 0.1% K-sorbate then packaged, or vacuum packaged and frozen. The initial flora was predominantly *Vibrio* which grew well in the aerobic packs ( $\sim 10^8$  c.f.u./g after 6 days at 4°C) and in the vacuum packs ( $\sim 10^7$  c.f.u./g after 6 days 4°C). The microbial counts in the sorbate-treated scallops were low ( $\sim 10^3$  c.f.u./g) after 6 days at 4°C and rose to  $10^5$  c.f.u./g by 22 days, remaining at this level until the experiment ended after 48 days. Extensive use of taste panels as an evaluative tool enabled construction of odor and flavor profiles which showed clearly the nature of the changes that occurred during storage. The sorbate-treated scallops stored for up to 28 days at 4°C remained as acceptable as the frozen controls.

### INTRODUCTION

VACUUM PACKAGING of raw fish products for chilled storage has not resulted in the substantial shelf life extension that can be gained with red meats. The principle differences in spoilage patterns are due to: the presence in seafoods of trimethylamine oxide (TMAO) an osmoregulatory compound, which is reduced by bacteria to trimethylamine (TMA) during anaerobic respiration; relatively low postmortem glycogen levels, resulting in a relatively high pH; and the apparent scarcity of indigenous marine lactobacilli which would maintain a pH low enough to inhibit the normal spoilage organisms, in particular *Alteromonas*.

Adjustment of pH with citrate buffers in conjunction with addition of glucose was shown to alter the spoilage association of vacuum packaged sand flathead fillets (McMeekin et al., 1982). There was no improvement in shelf life due to changes in appearance and odor. Vacuum packaging alone, and in the presence of added lactobacilli, did not increase the shelf life of scallops, since the indigenous flora of *Vibrio* spp. was capable of growth and spoilage at a rate similar to that occurring in aerobically packed scallops (Bremner and Statham, 1983).

Potassium sorbate may effectively extend the shelf life of packaged fish. Devere and Voets (1972) showed 0.135% K-sorbate almost completely inhibited the spoilage of cod fillets for 6 days by slowing the growth of bacteria capable of producing TMA. Chung and Lee (1981) found the presence of 1.0% K-sorbate extended the lag phase to over 6 days at 0°C but did not alter the spoilage flora of flounder homogenate stored aerobically.

This paper reports the results of the effects of vacuum packaging, with and without added 0.1% K-sorbate, on the shelf life of chill-stored scallops as assessed by microbiology and sensory profile methods.

### MATERIALS & METHODS

THE SCALLOPS were caught off the South East Coast of Tasmania in November, 1981, and were taken to a local factory for the normal washing and shucking process. Immediately after shucking, the

scallop meat, complete with roes, was packed in polythene bags in ice and taken to the laboratory where it was stored overnight.

For allocation to treatments the scallops were weighed into four 4 kg lots. Each treatment (Table 1) consisted of 14 bags each containing approximately 16 scallops. Forty ml of 10% K-sorbate (food grade, Mauri Tasmania Co.) were added to the 4 kg of scallops in treatment S (0.1% K-sorbate w/w) contained in a plastic bag. The solution was mixed immediately through the scallops by gentle kneading of the bag. For the vacuum packed scallops a vacuum of 25 mm Hg was drawn using a Boss packaging machine, equipped with a heat sealer.

Two packs from each treatment were opened initially and after storage for 2, 6, 9 and 13 days. Single packs from all treatments were opened after storage for 22 and 29 days and from treatments S and F only after 48 days. Immediately after sampling the experimenters recorded their impressions of raw odor, using the thawed frozen samples as a reference.

### Analytical

Crude protein was measured by the method of Rexroad and Cathey (1976) and glycogen by that of Carroll et al. (1956). The pH of the scallop flesh was determined using an Orion pH meter equipped with a spear electrode. Moisture contents were determined by oven drying at 105°C to constant weight.

### Microbiology

Sampling. One scallop was removed from each pack, weighed and homogenized for 3 min using a Colworth Stomacher. Serial decimal dilutions were spread plated onto Nutrient Agar containing 2.5% NaCl (SNA). The medium of de Man et al. (1960) adjusted to pH 5.5 with lactic acid (MRS<sub>5.5</sub>) and the medium of Gardner (1966) were used to estimate numbers of presumptive lactobacilli and *Brochothrix thermosphacta* respectively. All plates were incubated at 22°C for 3–5 days. MRS<sub>5.5</sub> plates were flooded with 3% H<sub>2</sub>O<sub>2</sub> and only catalase negative colonies were counted. Gardner's medium was flooded with Kovacs oxidase solution and only oxidase negative colonies were counted. Plates were flooded only when isolates were not required for further identification.

Identification of isolates. Colonies were isolated randomly from SNA plates inoculated with samples taken after 0, 6 and 13 days storage. In all cases 20 isolates were taken from each duplicate sample, giving a total of 40 isolates from each treatment at each sampling time. Gram negative isolates were identified by the scheme of Shewan et al. (1960). Gram positive isolates were identified according to *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974).

Statistical methods. Analysis of variance followed by determination of least significant differences was used to differentiate treatment effects.

### Profile panel

The profile panel consisted of 10–15 staff members experienced in assessing seafoods and in the profile technique. Training sessions on fresh and chill-stored scallops were held in the weeks before the start of the experiment. Scallops from each treatment were cooked sequentially to a center temperature of 70–75°C in stainless steel bowls in a water bath set at 85°C.

The sequence was randomized at each assessment time and the samples identified by a two-digit code. Panelists were not aware that one sample was a frozen control (treatment F). All four treatments V, S, F and A were assessed after 0, 2, 6, 9, and 13 days storage, while treatments S and F only were assessed after 22, 29 and 48 days storage. Two bowls containing the cooked scallops were presented to the panelists who were grouped around the table. Panelists sniffed the cooked scallops and independently marked their impres-

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Table 1—Summary of treatments

Code	Treatment	Storage temp (°C)	Sampling times (Days)
V — Vacuum	Vacuum packaged in barrier bags <sup>a</sup>	4	0,2,6,9,13,22,29
S — Sorbate	Treated with 0.1% K-sorbate vacuum packaged in barrier bags	4	0,2,6,9,13,22,29,48
F — Frozen	Vacuum packaged in barrier bags	-18	0,2,6,9,13,22,29,48
A — Aerobic	Packaged in polyethylene bags tied at the neck	4	0,2,6,9,13,22,29

<sup>a</sup> Cryovac U gauge, W.R. Grace Pty. Ltd., Melbourne. Nominal O.T.R. (unshrunk)  $3.5 \text{ ml m}^{-2} 24 \text{ h}^{-1} \text{ atm}^{-1}$  at  $3.5^\circ\text{C}$  and 75% R.H.

Table 2—Chemical composition of fresh scallops

Protein (g/100g)	19.8 (S.E. 0.32)
Moisture (g/100g) — muscle	78.8 (S.E. 0.21)
— roe	78.8 (S.E. 0.22)
Glycogen — muscle	1.7%
— roe	0.4%
% roe	30.9%

sions on the odor profile sheets. These sheets listed nearly 70 attributes which had been found, from experience, to be useful in describing the odor of seafoods in general and scallops in particular. Each attribute selected was scored on a scale from 0 (absent), 1 (slight), 2 (moderate), 3 (strong), 4 (very strong), to 5 (extremely strong) (Anon., 1977). Scallop odor intensity and off odor intensity were marked on the same scale. The panel leader then conducted a short session of exchange of impressions at which panelists were free to amend their sheets if they wished. Each panelist then tasted a scallop and recorded his or her flavor impressions on the same 0 to 5 scale on a flavor profile which listed 70 attributes (similar, but not necessarily the same as those listed in the odor profile) including intensity of scallop flavor and off flavor. General acceptability was marked independently on the General Foods "Smiley Scale." Panelists were asked to use this scale in the following manner. "See which face most clearly conveys your impression of the product than write down the score corresponding to this face." The scale of seven faces was used with a score of 1 corresponding to the lowest acceptability and 7 corresponding to the highest acceptability. A discussion session followed, then the next sample was assessed for odor and flavor. For the scallops assessed after 0, 2, 6, 9 and 13 days of storage ten panelists were common to all sessions while for the assessment after 22, 29 and 48 days five of these panelists were present on each occasion.

## RESULTS & DISCUSSION

### Chemical composition

Table 2 shows the results of the chemical analyses performed on the fresh scallops.

**pH changes.** Initial surface pH values of scallops decreased rapidly during the first 2 days of storage (Fig. 1) to below pH 6.0, in the aerobic and vacuum packaged treatments. Surface pH values of chill stored scallops became significantly higher ( $p < 0.01$ ) than flesh values during storage, while there was no such difference in the frozen scallops.

**Bacterial numbers.** Bacterial numbers in treatments A and V increased rapidly during the first 6 days of storage with no evident lag phase (Fig. 2). Counts from scallops to which K-sorbate had been added decreased from  $1 \times 10^4$  c.f.u./g to below  $1 \times 10^3$  c.f.u./g after 2 days, suggesting a bactericidal effect on the initial flora. An extended lag phase lasted for 13 days, after which time bacterial numbers increased to a maximum of  $2.5 \times 10^5$  c.f.u./g.

No lactobacilli or *B. thermosphacta* were detected on selective media used only after 13 days in storage. The presence of these genera found previously on scallops (Bremner and Statham, 1983) and their apparent absence in this case

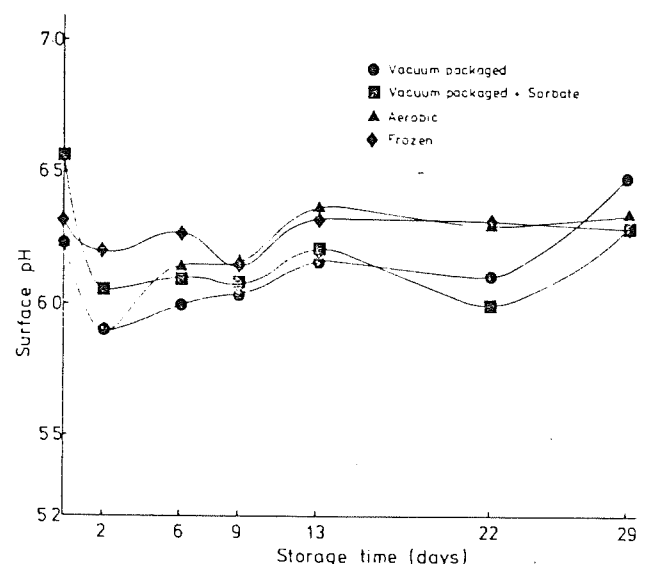


Fig. 1—Surface pH readings for stored scallops. (Freehand curves drawn through data).

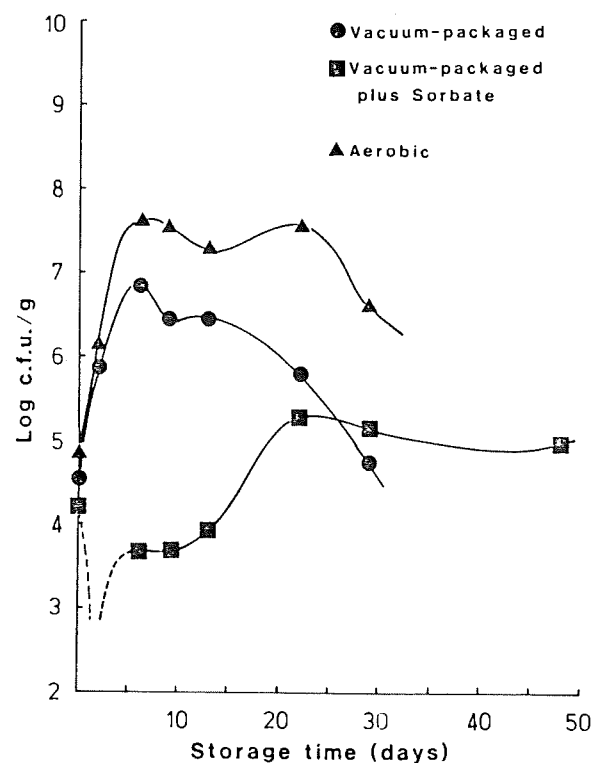


Fig. 2—Bacterial numbers on scallops stored at  $4^\circ\text{C}$  estimated on salt nutrient agar. (Freehand curves drawn through data).

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may be due to seasonal variation, such as stage of spawning cycle, or different handling practices which affect contamination during processing.

Table 3—Changes in bacterial flora on scallops stored at 4°C

Storage time (days)	Treatment	Genera present, % of total				
		<i>Vibrio</i>	<i>Aero-</i> <i>monas</i>	<i>Coryne-</i> <i>forms</i>	<i>Pseudo-</i> <i>monas</i>	<i>Acineto-</i> <i>bacter</i>
0	V — Vacuum packaged	95	—	5	—	—
	S — Vacuum packaged + sorbate	95	—	5	—	—
	A — Aerobic	100	—	—	—	—
6	V — Vacuum packaged	100	—	—	—	—
	S — Vacuum packaged + sorbate	100	—	—	—	—
	A — Aerobic	100	—	—	—	—
13	V — Vacuum packaged	85	15	—	—	—
	S — Vacuum packaged + sorbate	25	25	—	35	5
	A — Aerobic	100	—	—	—	—

Bacterial flora. The initial bacterial flora of fresh scallops was similar to that found previously (Bremner and Statham, 1983) with a predominance of *Vibrio* spp. (Table 3). *Vibrio* spp. persisted throughout storage to become the spoilage flora of both aerobic and vacuum packaged scallops. The initial rapid decline in pH to below 6.0 may have excluded *Altermonas* spp. from the flora. The flora of scallops with added K-sorbate remained ~100% *Vibrio* spp. after storage for 6 days, but by 13 days this proportion had decreased to 25%. *Aeromonas* spp. and pseudomonads then represented 25% and 35% of the total flora, respectively.

Chung and Lee (1981) found *Pseudomonas* spp. were the dominant spoilage organisms in homogenates of English sole, whether K-sorbate at levels of 0.1 and 1.0% was present or not. In the present work, however, the initial flora was susceptible to the action of K-sorbate and a spoilage flora different from the initial flora resulted. In contrast, treatment A scallops were almost too objectionable, even to the authors, to be assessed after 29 days at 4°C. There are obvious differences in the nature of the odors of the scallops in treatments V and A even though the organisms in both were predominantly *Vibrio*, which appeared to be alike by all the tests used. The difference may be due to the fact that in treatment V the organisms were active anaerobically whereas in treatment A they were active aerobically. Fur-

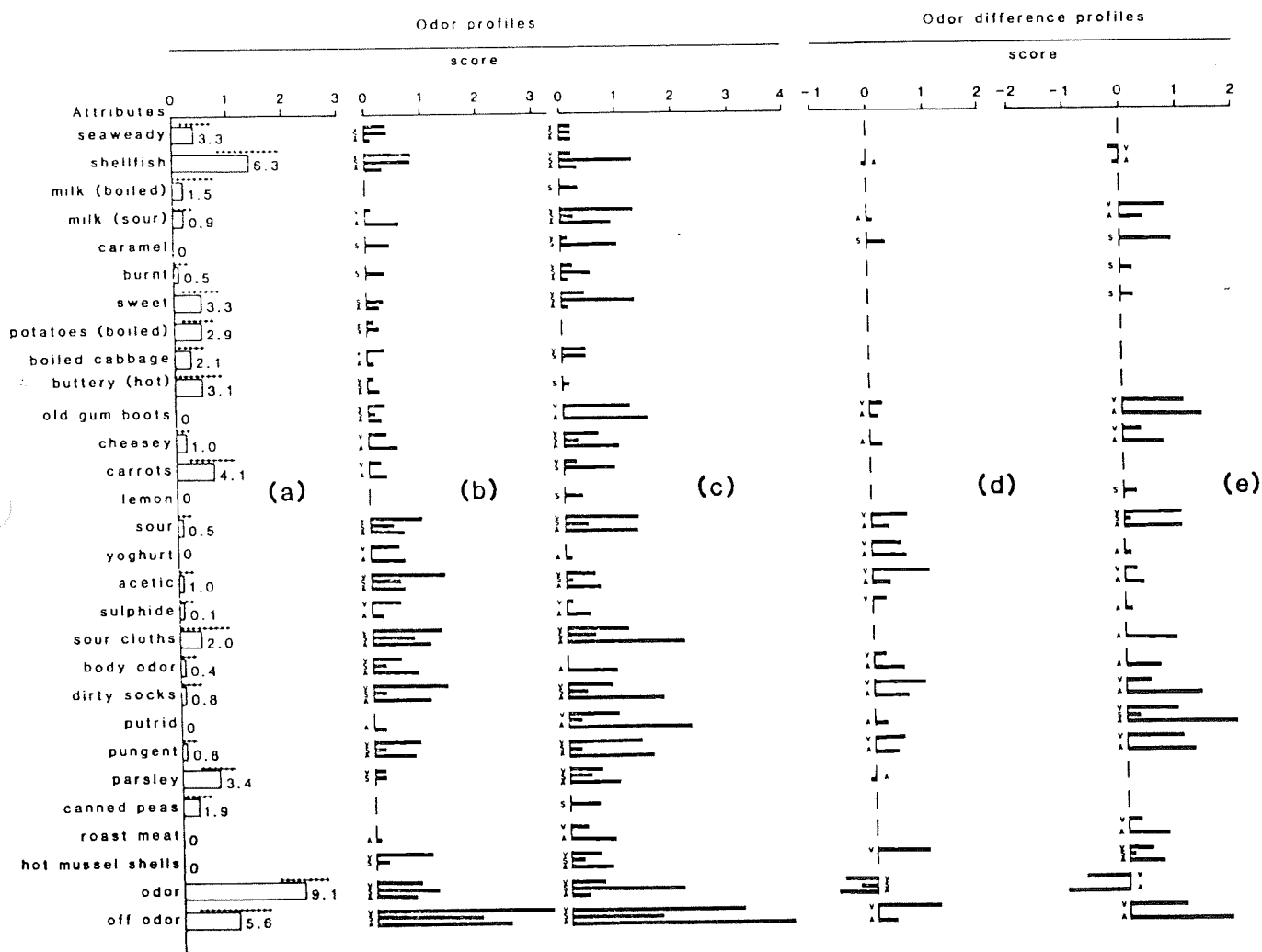


Fig. 3—Odor profiles of (a) fresh/frozen scallops, (b) scallops stored at 4°C for 6 days, (c) scallops stored at 4°C for 13 days and odor difference profiles (d) and (e) for the same stored scallops respectively. Odor difference profiles represent the amount by which attributes changed more than two S.D. from the means in 3a:  $\square$  mean and  $\cdots$  one S.D. range above and below mean of eight profiles for fresh/frozen scallops; bars represent in order treatment V, S and A (see Table 1). The numbers shown in Fig. 3a represent the mean number of panelists who scored the attribute as being present.

thermore, the packaging materials, barrier bags and polyethylene respectively, would have different permeabilities to the various odorants.

#### Panel profiles

The scores for both odor and flavor attributes generated by the profile panel were generally low. The higher categories, very strong and extremely strong were seldom scored. These categories are absent from the profile scales used by NLABS (1980). However, it was not mandatory for the panelists to score all attributes and hence the resulting zero scores (absent) contributed to the low means. Low scores also reflect the fact that many of the attributes listed are not necessarily desirable, but, when present at low levels, they give subtlety and nuance to the product. As a corollary it appears that higher scores for any one attribute result in lower acceptability. In both the odor and flavor profiles several attributes were not consistently scored and were deleted from the results. There were no differences of consequence between the profiles of the four lots of scallops stored frozen for 3, 6, 9 and 13 days and those of the four treatments assessed at the start of the experiment. The eight profiles were pooled and the overall mean, standard deviation (S.D.) and mean number of panelists registering each attribute are plotted for odor and flavor in Fig. 3a and 4a, respectively. The profiles for scallops in treatments V, S and A stored for 6 and 13 days at 4°C are shown in Fig. 3b and 3c for odor, and Fig. 4b and 4c for flavor.

Changes in profile for the scallops stored for 6 and 13 days at 4°C have been displayed as 'difference profiles'; Fig. 3d and 3e for odor differences, and Fig. 4d and 4e for flavor differences. These difference profiles represent values for those attributes which were greater or smaller by at least two standard deviations from the means for the fresh/frozen scallops. Where the attributes lay within the range of the mean  $\pm$  two S.D. no bar is plotted, while values greater than this range are plotted on the positive side of the ordinate with values less than the range on the negative side. On some occasions zero scores for some attributes resulted in a relatively large S.D. in comparison to a low mean score. When two S.D.s are subtracted from these low scores the result is a bottom range of zero. Thus some original attributes which were lost in the stored scallops do not appear in the difference profile. It is not certain whether the S.D.s for the fresh/frozen scallops are applicable to the stored scallops but despite this and the above consideration the difference profiles are a practical way of looking at the storage changes.

The major components of the odor profile (Fig. 3a) were described as shellfish, carrots, parsley, sweet, potatoes and buttery while the flavor profile was dominated by sweet, carrots and, to a lesser extent, other vegetable-like attributes.

The changes that occurred during storage are best seen from the difference profiles. After 6 days at 4°C there were significant changes in the profiles of the scallops in treatments V and A in which objectionable odors and flavors had formed (Fig. 3d, 4d). The cheesy, sour milk, yoghurt

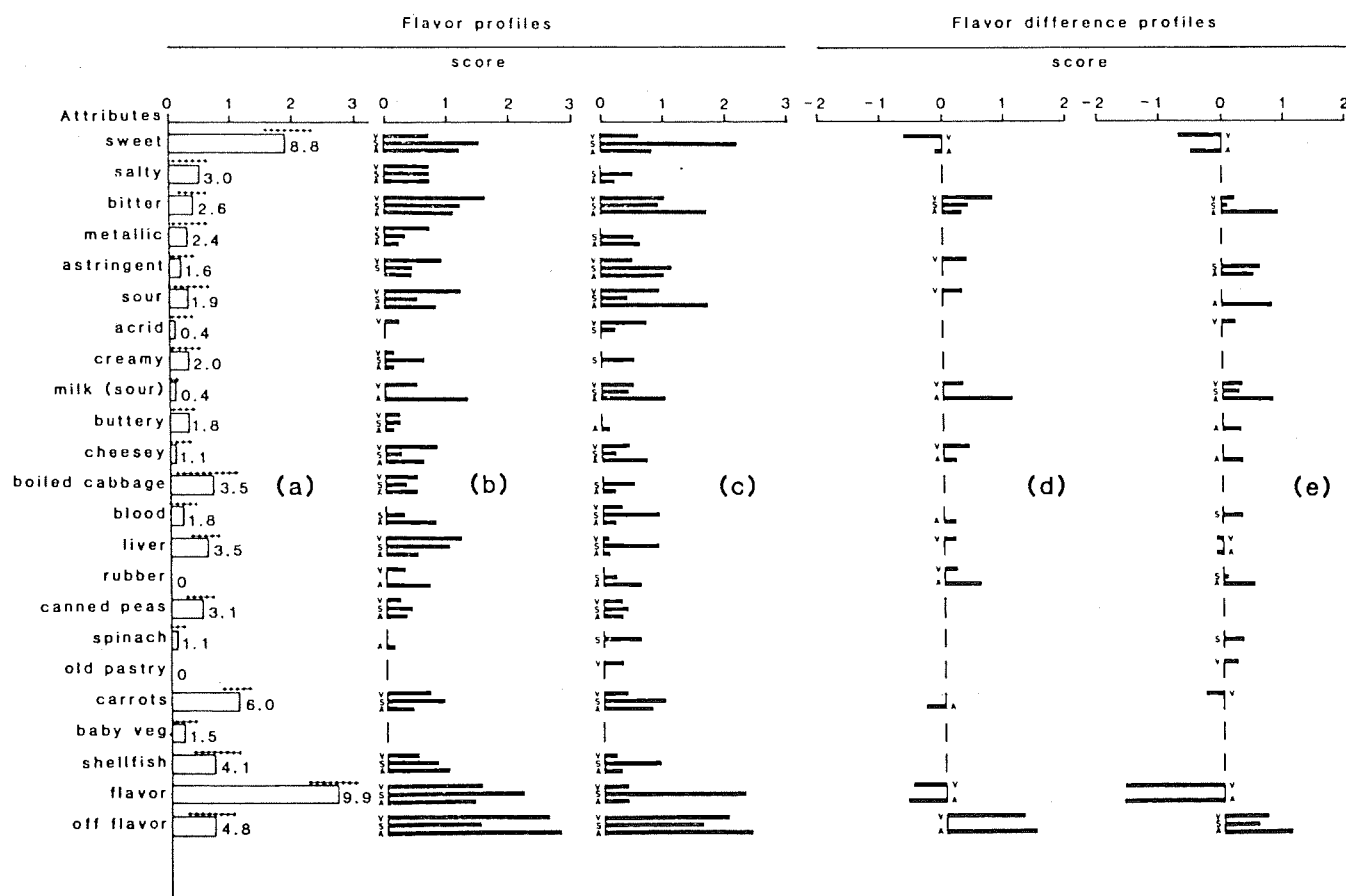


Fig. 4—Flavor profiles of (a) fresh/frozen scallops, (b) scallops stored at 4°C for 6 days, (c) scallops stored at 4°C for 13 days and flavor difference profiles (d) and (e) for the same stored scallops respectively. Flavor difference profiles represent the amount by which attributes changed more than two S.D. from the means in 4a:  $\square$  mean and  $\bullet\bullet\bullet\bullet$  one S.D. range above and below mean of eight profiles for fresh/frozen scallops; bars represent in order treatments V, S and A (see Table 1). The numbers shown in Fig. 4a represent the mean number of panelists who scored the attribute as being present.

## VACUUM PACKAGED SORBATE TREATED SCALLOPS...

and acid flavors and odors presumably were formed from fermentation of sugars as indicated by the concomitant decrease in sweetness. There were few changes in the profiles of scallops in treatment S (Fig. 3d, 4d) but after 13 days at 4°C some changes unique to this treatment such as burnt, sweet, caramel, lemon odors and spinach flavor were noted (Fig. 3e, 4e). Further deteriorative changes were evident in the profiles for scallops in treatments V and A after 13 days storage (Fig. 3e, 4e).

After 22, 29 and 48 days storage the frozen scallops (treatment F) had profiles similar to those in Fig. 3a, 4a. The profiles of treatment S scallops showed loss of some of the subtle attributes such as buttery (hot) odor and cabbage and blood flavors. By 48 days storage odor scores for

caramel and burnt had declined, lemon was still evident and the scores for boiled cabbage, cheesy and sour cloths were significantly larger. In the flavor profile there were significantly lower scores for sweet carrots and canned peas and very high scores for bitter (2.4 scale units), acrid, cheesy, rubber and a new attribute soapy (1.2 scale units). There were many similarities in the profiles of scallops in treatment S stored 48 days at 4°C to the profiles of scallops in treatments V and A stored for only 6 days, which is in agreement with the acceptability results on the Smiley scale (Fig. 5).

**Intensity attributes.** Analysis of variance was done on the odor, flavor, off odor, off flavor and Smiley scores. There were no significant differences between treatments after 2 days at 4°C. After 6 days storage treatments V and A scallops had significantly changed in all attributes (Table 4). No significant difference between treatment S and the frozen treatment was evident after 13 days storage, but by 22–29 days storage differences were apparent. Nevertheless, Smiley scores were not significantly lower (Table 5, Fig. 5) until 48 days chill storage.

**Comparison of raw odor with cooked odor and flavor.** Some of the odors detected in the raw scallops (Table 5) were still evident in the cooked scallops of treatment V (e.g. the cheesy notes), while the old pastry and sour dough smells typical of stored raw scallops in treatment A were not evident in either the odor or flavor profile.

### CONCLUSION

**ODOR AND FLAVOR SENSORY PROFILES** are a useful tool for describing the changes that occur during spoilage of chill stored scallops. Attributes found useful in indicating spoilage of untreated scallops (packed aerobically or in vacuum) were for odor: sour, sour milk, sour cloths, old gumboots, dirty socks, putrid and pungent, and for flavour: bitter, astringent and sour milk.

Table 4—Number of days at which significant sensory changes were detected in scallops stored at 4°C

Treatment	Attribute				
	Odor	Off odor	Flavor	Off flavor	Acceptability
V	6**	6***	6***	6***	6***
S	29*	29***	48***	29*	22*
A	6*	6***	6***	6***	6***

\*, \*\*, \*\*\* represent significant differences at  $p < 0.05$ , 0.01 and 0.001 levels, respectively, as estimated by analyses of variance.

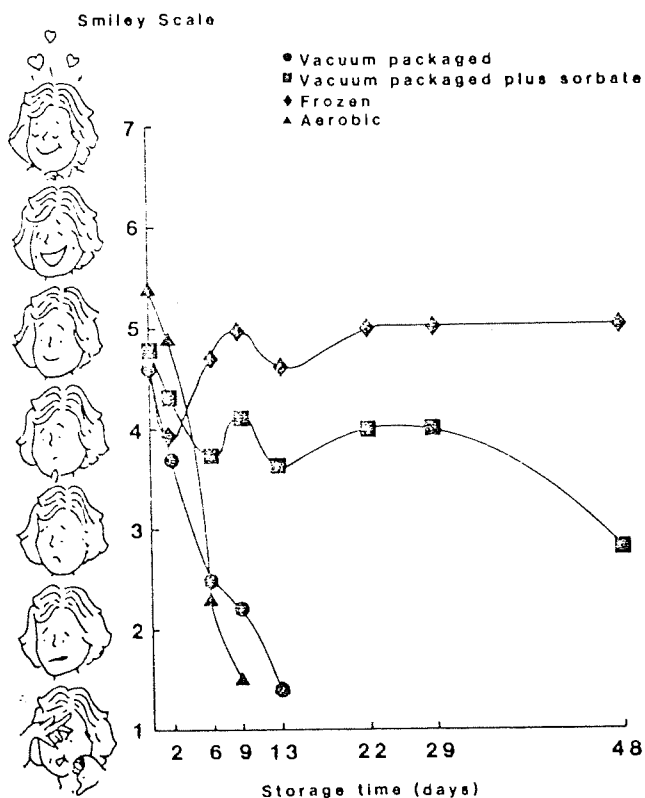


Fig. 5—General acceptability of stored scallops. Smiley scale courtesy of General Foods Corporation. (Freehand curves drawn through data).

Table 5—Raw scallop odors<sup>a</sup>

Treatment	Storage time (days) at 4°C						
	2	6	9	13	22	29	48
V	sharp, pungent, H <sub>2</sub> S, acetic, rotting seaweed	rubber, sulphide, gumboots	pungent, old socks, stale babies diapers	sour, onions, H <sub>2</sub> S, parmesan cheese, pungent	sweet, blue cheese, rotting tropical fruit, gumboots, seaweedy, very powerful	dog faeces, potent blue cheese, watery diarrhoea	—
S	trace H <sub>2</sub> S, slightly fruity	bland, plastic, floral	apple juice, fruity, stale apple	slightly pungent, not clean	pungent, sulphide	pungent, acid	pungent, acetic, fresh shellfish, fresh crayfish (nothing objectionable)
A	seaweed, doughy, acetic, not much odor	cheesy, old pastry	sour dough, pastry	stale scallop pie, sour pastry, bloody dark meat	sour dough, pastry, fruit, (very high off odor, but not like V).	sour dough, sweet, sickly vomit	—

<sup>a</sup> Odor listed did not appear in the thawed frozen scallops which were used as a comparison.

*Vibrio* spp. were confirmed as the sole spoilage organisms on vacuum packaged and aerobically packaged scallops. With scallops treated with 0.1% K-sorbate and vacuum packaged the population of spoilage organisms was heterogeneous. Suppression of spoilage and significant extension of shelf life was achieved by the addition of 0.1% w/w potassium sorbate to vacuum packaged scallops. The K-sorbate had an initial bactericidal followed by a bacteriostatic effect on the spoilage.

Conditions existing within vacuum packs of fish products have been shown to allow the growth of *Clostridium botulinum* type E if storage temperatures are allowed to rise above 3.3°C (Lindsay, 1982). The product normally spoils beyond acceptability before toxin production occurs (Bannar, 1979), but inhibition of the spoilage flora by the use of antimicrobial agents could result in edible products becoming toxic after extended periods in storage. Sorbic acid and K-sorbate have been shown to inhibit spore outgrowth in a number of products, including processed bacon (0.13%) (Huhtanen et al., 1981), uncured pork sausage (0.1%) (Tompkin et al., 1974) and in a spore germination medium (0.52%) (Smoot and Pierson, 1981). An investigation into the possibility of *C. botulinum* growth in K-sorbate treated scallops would be necessary to indicate the safety of this product over a 4-wk storage period.

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## PROPERTIES OF FRANKFURTER-TYPE SAUSAGES. . . From page 1038

when postrigor pork with 4.0 vs 2.0% salt was used, moisture contents of sausages decreased. Although not conclusive, the interaction between rigor-state and levels of salt in preblends suggests that the higher level of salt (4.0%) is more effective for hydrating proteins and retaining moisture in cooked sausages when prerigor pork rather than postrigor pork is used.

Correlation coefficients may be used to indicate the extent to which sensory properties are associated with physical/chemical properties (Table 4). Correlations between Instron stiffness and juiciness or firmness were more consistent in magnitude, within main effects and were slightly larger over all main effects, than correlations for processing shrinkage or moisture. Because of these relationships, Instron stiffness measurements may be the most reliable value for predicting juiciness or firmness of these frankfurter-type sausages. Although not conclusive, Instron stiffness values may also provide some evidence that sodium tripolyphosphate enhances the structural integrity of these types of sausage batters.

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# Editorial



## AUSTRALIAN ENGINEERING BULLETIN REACHES 15,000

**F**or the past 11 years, AEB publishers, Technical Indexes have been presenting engineering product stories, news and a lot more features too. We are now entering a new phase, what you could call a milestone in our history where we have increased our circulation from 4,000 to 15,000. This gives us a valuable opportunity of reaching more people and monitoring the feedback from a broader section of engineering personnel. I would like to personally thank the readers who have supported us over the years and helped us bring about our new circulation.

Technical Indexes have been in Australia for around 14 years, providing engineering information systems and data bases from the U.S.A., Europe and other parts of the world. The Australian Engineering Bulletin has shared in the growth of the company with the expansion of feature pages and a greater participation in industry. Our new thrust into the larger community reflects the upgrading of the Trade Press and the awareness that industry has towards the exchange of information. Readers are invited to make suggestions about a regular department, article or special feature — this sort of response will help us to serve the industry better.

## Research

# A spot test to identify molybdenum stainless steels

by S.J. Sykes†  
Engineer, CSIRO Tasmanian Food Research Unit

**F**or some time there has been available a simple and positive method for the shop floor classification of stainless and high alloyed steels.

Molybdenum is used as an alloying element in stainless and high alloyed steels which are required to have a high resistance to particular corrosive environments. These steels are recommended for applications where protection is required from highly corrosive non-oxidising environments, such as sea water and brine solutions.

Comsteel grades 444, 316, 316L and 317 have molybdenum concentrations of 2.0, 2.25, 2.25 and 3.25% respectively.

DAMAC DL-12 reagent\* is a commercially prepared solution used for the detection of molybdenum in steels. The amount of molybdenum present in a sample is roughly indicated by the colour change in a spot of the reagent applied to the sample.

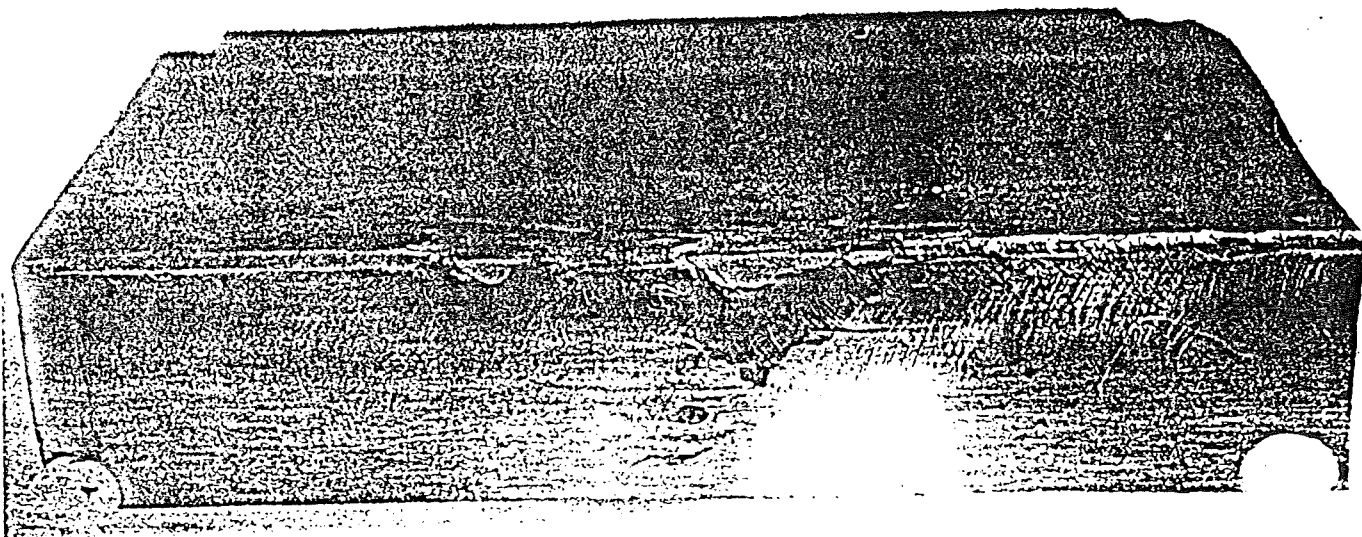
The use of DL-12 reagent to identify batches of stainless steel is advantageous in situations where analytical

facilities are not available. It is not difficult to envisage the disastrous consequences which may arise due to the use of an inappropriate grade of stainless steel.

The steel surface to be tested is prepared by cleaning and rubbing with dry emery paper. After thoroughly shaking the bottle, about two drops of the reagent, which is a yellow solution containing 28% hydrochloric acid, are applied to the surface. The drops of solution on the steel should be stirred slightly. If molybdenum is

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present in the steel, the yellow solution will become dark brown. The speed of the colour change is determined by the amount of molybdenum in the sample and by the temperature at which the reaction is taking place. If no molybdenum is present, the solution will simply dry on the surface, turning pale green with respect to the original solution.

Because the rate of reaction is dependent on temperature, the test should be performed simultaneously on two control samples of stainless steel. One of these controls should be of the composition required (eg. type 316 stainless steel), the other a grade which contains less than 0.15% molybdenum; the amount of molybdenum in each of the controls should be known to an accuracy of  $\pm 0.05\%$ . It is then possible to compare the response of the test sample to the two controls. Normally the response of the sample will match that of one of the two controls, thus clearly indicating the amount of molybdenum in it. If the response of the test sample differs substantially from either of the two controls, the material should be analysed by conventional methods.

Type 316 stainless steel's superior resistance to corrosive attack by sea water, led to its choice as the material to contact chilled sea water in a mobile chilled fish storage unit designed by officers of the CSIRO Division of Food Research. The function of this unit was to serve as the main component in experiments designed to study the storage of fish in mechanically refrigerated and ice-chilled sea water (Thrower and Stafford, 1981).

The equipment had been in service for two months after delivery when welds on the internal lining of one of the tanks were found to be severely corroded. The welding procedure used in the fabrication of this tank was immediately suspected. When approached on this subject the manufacturer stated that all 90° joints were fusion welded from the outside using the TIG process. Strips guillotined from the parent plate were used as filler wire where necessary. There was no postweld heat treatment of the seams.

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*"With the use of DL-12  
costly repairs  
can be avoided . . ."*

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The CSIRO Division of Manufacturing Technology examined and analysed samples taken from welds of two tanks. One sample displayed no corrosion of either the weld or parent plate; its composition was consistent with it being made wholly of type 316 stainless steel.

The other sample (Sample 2, Fig. 1), which was taken from a similar location in another of the tanks, showed "sporadic perforation of the weld bead by a corrosion process. The accelerated corrosion was quite clearly associated with the use of an inappropriate material in the construction of the sea water tank." (G.J. Ogilvie, pers.comm.) Analysis of the sample showed its composition to be:

Cr	18.2%
Ni	9.5%
Mo	Nil
Remainder	72.3%

This analysis is consistent with the material being AISI type 302 stainless steel. These results showed that although the corrosion was initially observed in the region of the welds, the failure was caused by the use of type 302 stainless steel for the construction of the whole tank. This conclusion was subsequently confirmed by the discovery of holes in the tank which were caused by the same corrosion process but were removed at least 100 mm from the region of the welds. It now became necessary to identify those other parts of the equipment which were not made of the specified material; DAMAC DL-12 reagent was employed for this purpose.

It is incumbent upon managers and foremen to ensure that adequate supervision of processes on the factory floor is maintained. In processes which involve the cutting and welding of stainless steel it is critical that the grade of material being used is correctly identified. If the manufacturer of the sea water tanks had used DL-12 reagent as a matter of course to check the material used in construction, costly repairs could have been avoided.

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†S.J. Sykes is employed as an Experimental Office under a grant from the Fishing Industry Research Trust Account.

\*1. DAMAC DL-12 reagent is manufactured by Damac, 74 rue Jules-Guesde, B.P. 60 92302 Levallois Perret Cedex and marketed in Australia by Selby's Scientific Ltd.

\*2. The work discussed in this article was done with DAMAC DL-12 and there may be other materials commercially available that may be used for the same purpose. It is not intended that this article be read as a recommendation of DAMAC DL-12 over other such products.

## Taster Response to Salt in Minced Fish

H. ALLAN BREMNER

### ABSTRACT

Cooked fish minces, alone and with four added levels of salt, were tasted at the same sessions as five solutions of similar salt concentration. Saltiness was measured by the tasters using the method of magnitude estimation. The magnitude estimation exponent for salt in cooked fish mince was 1.05 whereas that for the solution was significantly higher at 1.51.

### INTRODUCTION

REFRIGERATED SEA WATER (RSW) provides an effective medium for the chilling and holding of fish from the point of catch through to processing. In RSW the fish absorb salt due to osmotic imbalance and salt uptake is one of the factors limiting duration of storage in this medium. There is an extensive literature on many aspects of salt ingestion (e.g. Kare et al., 1980), its potential hazards (IFT, 1980), and on the taste of salt in solution, particularly with reference to the elucidation of the mechanisms of taste. The technique of magnitude estimation (Stevens, 1957) based on the power function  $S = KI^n$  where  $S$  is perceived stimulus intensity,  $I$  is physical intensity,  $K$  a constant, and  $n$  the exponent or magnitude estimator, has been applied to salt solutions. In those experiments where a sip procedure has been followed, exponents ranging from 0.92–1.6 (Table 13, Meiselman, 1972) have resulted. If the exponent for cooked fish was in the upper range, say 1.5, and if fish stored in RSW for about 3–4 days increased in salt content from the naturally occurring level of 0.2% (approx.) to 0.6% then by substitution in the equation we have  $S_{\text{natural}} = K(0.2)^{1.5}$  and  $S_{\text{stored}} = K(0.6)^{1.5}$ . Then  $S_{\text{stored}}/S_{\text{natural}} = 0.6^{1.5}/0.2^{1.5} = 5.2$  which implies that the stored fish would taste five times more salty. This experiment was therefore done to ascertain the response to salt in cooked fish and, as a reference, salt solutions were tasted at the same time. The author has found no other references making this comparison between simple solutions and complex foods except for the work of Moskowitz et al. (1974) where the sweetness of sucrose was estimated in a solution, a beverage, a pudding and a cake to give magnitude estimates of 1.06, 1.22, 1.06 and 0.77 respectively. To the author's knowledge this comparison between salt in solution and in cooked fish has not been made before.

### MATERIALS & METHODS

FIVE SOLUTIONS of sodium chloride A.R. were prepared in glass distilled water at concentrations of 0.2% (0.0342M), 0.4% (0.0684M), 0.6% (0.102M), 0.8% (0.1369M), and 1.0% (0.1711M). No traces of chloride were detected in the distilled water. Minces were chosen as a means of reducing the variability which otherwise would occur between and within fillets. The minces were prepared from fresh jackass morwong (*Nemadactylus macropterus*) by trimming, skinning then dicing the fillets, mixing them and passing the flesh through a grinder plate with 4 mm holes. The mince was then re-mixed. Minces with approximate salt concentrations of 1.0%, 0.8%, 0.6% and 0.4%

were prepared by slowly adding 10% w/v salt solution dropwise while thoroughly mixing the mince with a fork. The well mixed minces were divided into two 300g portions for taste sessions which were held at 11:30 a.m. and 2:30 p.m. The minces were cooked to a temperature near 75°C in covered stainless steel bowls set in a water bath at 87°C. The cooked minces were mixed and juices allowed to settle and the solids were sampled in duplicate for analyses for chloride and moisture content (AOAC, 1980) and these results were used in the calculations. The cooked mince solids were then served hot, in pre-warmed metal baking trays divided into six compartments. The salt solutions were served at room temperature (15°C), for experimental convenience, in waxed paper cups. There were eight tasters, all of whom had previous experience at tasting salt solutions and salted minces but not both at the one session. Each taster received five salt solutions and five cooked minces (four with added salt, one with no addition); four tasted the solutions before the minces, the other four tasted the minces first. This sequence was reversed in the second session. The order of concentration was randomized within each set (mince or solution) and was changed at each session. The solutions were marked with the alphabetical symbols K, L, M, N or O and the minces as P, R, S, T or U. Tasters were told to adhere strictly to the given order. Distilled water was provided and tasters were instructed to rinse their mouths between sets (solutions or minces) but not between samples in the same set. The tasters set their own pace and the duration of time each stimulus was held in the mouth was not controlled. The duration between tastes either within or between sets was also not controlled.

Tasters were instructed to judge the intensity of saltiness of the samples using the method of magnitude estimation (Stevens, 1957). No modulus was used and to the first sample they received tasters assigned any number for intensity they considered appropriate. To subsequent samples they assigned numbers proportional to perceived magnitude. The only restriction stipulated was that zero scores were not allowed.

The experiment was repeated 1 wk later. The results were normalized by dividing each score by the value given to the first sample tasted and these normalized ratios were examined by analysis of variance. Taster and session effects were removed and the logarithm of the normalized magnitude estimate was regressed on logarithm of salt concentration.

### RESULTS & DISCUSSION

THE RESULTS, EXPRESSED as ratios, plotted in Fig. 1 on log-log coordinates, were assumed to be fitted best by straight lines. This assumption was subjected to a goodness of fit test which showed that the points did not deviate significantly from straight lines. The slope for the response to the salt solutions is 1.51 (s.e. 0.1) which is in the upper range of the values listed by Meiselman (1972) agreeing closely with 1.59 found by Ekman (1961), 1.58 by Ekman and Akesson (1965), and 1.43 by Meiselman (1968).

The results for the response to salt in minces are plotted in Fig. 1 as salt concentration expressed as a percentage of the moisture content of the cooked mince, giving a line with a slope of 1.05 (s.e. 0.1). Alternatively, if salt concentration is expressed in terms of percentage in the cooked mince (g/100g) then the result is a line with the same slope (1.05, s.e. 0.1), but with a higher intercept i.e. parallel to the line in the figure. It appears that the response to salt in cooked minced fish is different to that in solution ( $P < 0.001$ ). The reasons for this difference would be difficult to ascertain and are certainly beyond the scope of the present investigation, and the considerations given here are obviously speculative.

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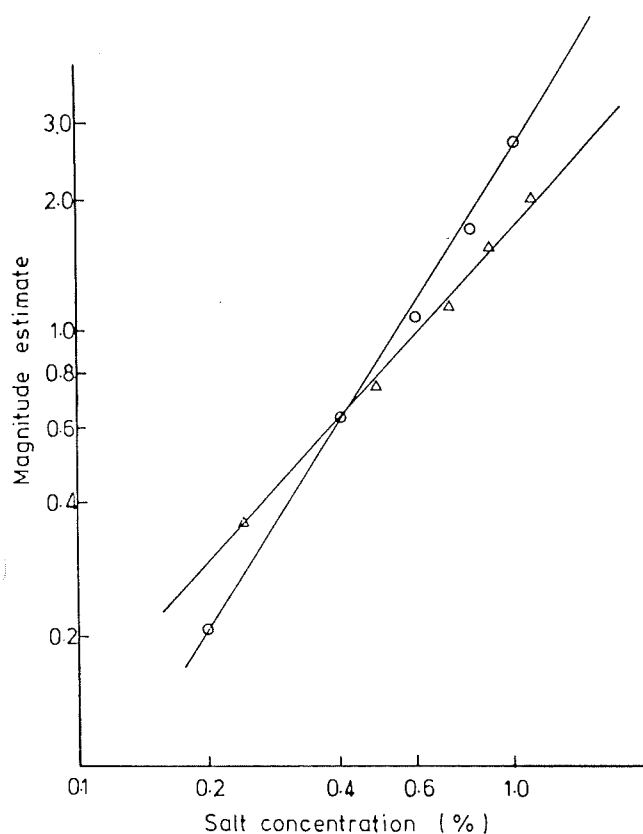


Fig. 1—Log-log plot of normalized magnitude estimates vs salt concentration in solution (○) and in the cooked fish mince (△) expressed as percentage of the moisture content (g/100g moisture content).

Moskowitz (1973) found little influence of temperature on magnitude estimates for salt solutions so it is unlikely that the temperature difference between the solutions and the minces was responsible for the difference in slopes.

Obviously the mince presented a different stimulus delivery system from the solutions and this resulted in a different effective stimulus. Some of the influencing factors could be, suppressants and synergists in the minces, effects of adaptation, variations in salivary responses, and possibly lower effective flow rates in the case of the minces with consequently a greater duration of stimulus. This may be analogous to the situation where solutions have been flowed over whole or part of the tongue resulting in lower magnitude exponents from 0.41–0.91 (Meiselman, 1972). It is no easy matter to compare stimuli in complex foods, prepared and eaten in the normal manner, with simple solutions and these results indicate that estimates obtained using solutions should not be applied to real foods but that a stimulus should be tested in the food itself. The present work indicates that small increases in the salt level of fish stored in RSW will not result in large increases in perceived saltiness of the cooked flesh. Further work to estimate the response in other forms of cooked fish will be undertaken.

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# On-board handling of gemfish

## Part I: Importance of chilling and gutting

THE fishery for gemfish (*Rexea solandri*) in south-eastern Australian waters has suffered recently from poor demand and low prices. Two of the main causes of these problems have been poor quality, and competition from imported species, especially orange roughy (*Hoplostethus atlanticus*) from New Zealand (Ref. 1). Quality loss was caused mainly by poor handling, processing and packaging (Ref. 2).

Gemfish present special problems for the industry because large hauls are taken over a short season, straining the capacities of fishermen and processors. It is not practical with the small crew on a typical trawler to gut gemfish on board. Effective chilling either with ice or refrigerated sea water (RSW) is often not accomplished. When fish enter accelerated rigor at high temperatures (above 15°C) gaping of the fillets can result (Ref. 3a).

This article describes an experiment designed to identify quality loss due to delays in chilling and gutting. The work was funded by a grant from the Fishing Industry Research Trust Account.

The fish used were caught in one haul by *Kapala* in July 1981 in 260 fathoms east of Long Reef, Sydney (FRV *Kapala*, Cruise Report No 72, NSW State Fisheries). The temperature of the fish when caught was 17°C. Fish varied from 60 to 76 cm long and 1.5 to 3.1 kg in weight. Some of the fish had well developed roes and their digestive tracts were relatively empty.

It took an hour at an ambient temperature of 20°C to sort the fish from the bulk of the catch and randomly allocate them to

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five treatments. These five treatments were divided into two broad categories, those stowed in refrigerated sea water (RSW) at -1°C for 24 hours (three treatments) and those held at ambient temperature (15-20°C) for 24 hours (two treatments) before being frozen to -18°C.

Of the chilled fish, one group was gutted and bled in RSW as soon as possible after catching (treatment 1) then lowered into the RSW tank in a net bag, while two groups were similarly stowed ungutted in RSW. Of the two chilled ungutted groups, one was frozen ungutted (treatment 2) and one was gutted and bled in RSW for 60 minutes before freezing (treatment 3).

Of the two groups held on deck at ambient temperatures for 24 hours, one was gutted and bled in running seawater for 60 minutes before freezing (treatment 4) and one was frozen (-18°C) ungutted (treatment 5). Fish in treatment 4 still bled freely after 24 hours on deck. The treatments are summarised in Table 1.

The fish were shipped frozen to the CSIRO Tasmanian Food Research Unit in Hobart for quality evaluation. This was done after frozen storage (-18°C) for 6 and 18 weeks from the time of catching.

It was considered that a 24-hour delay at ambient temperature would simulate some of the

poorer handling practices known to occur in the gemfish fishery. It has been shown that storage of fish for 24 hours at 17°C will produce the same amount of bacterial spoilage as 44 hours at 10°C, nearly four days at 4°C and 7.3 days at 0°C, the temperature of melting ice (Ref. 4). Because of the relatively small quantity of fish needed for this experiment, the ratio of RSW:fish was high (20:1). This would have resulted in a rapid rate of cooling which would be representative of the first haul on a commercial trawler.

The fish were thawed by holding for 65 hours in still air at 4°C, after which skin samples were removed for microbiological examination and the whole raw fish were assessed visually. The fish were then filleted for further visual assessment, with some fillets being used for chemical analysis and the others for evaluation by three different taste panel techniques.

### Visual assessment

Immediately after thawing, the whole fish from each of the treatments were examined in turn by four experienced staff for appearance, skin condition, stiffness, eye clarity, gill condition, condition of belly, and vent or belly cavity. The raw fillets were then examined for blood stains, color, staining, bruising, smell, elasticity, wetness and degree of gaping. Ranking of individual attributes did not consistently place the treatments in the same order. Treatments were thus ranked by giving equal weight to all characteristics and summing the rankings for both whole fish and fillets (Table 2).

Mechanical damage in the net

may account for some defects which were randomly distributed amongst the treatments. It was noted, for example, that some fillets from all treatments were stained with a bright yellow pigment in the area adjacent to the kidney.

### Laboratory tests

#### Trimethylamine

The level of trimethylamine (TMA), a compound produced by bacteria, was measured immediately after the gemfish were thawed and filleted (Fig. 1).

All three of the chilled treatments resulted in fish flesh with levels of TMA below 1.5 mgN%, the level recommended for good quality cod (Refs. 3b, 5).

Fish in the two treatments which were left for 24 hours at ambient temperature before freezing and storage showed much higher levels of TMA, with fish from treatment 5 showing values in excess of 10 mgN%, the level at which fish is considered too spoiled for most uses (Ref. 3b).

The fish from treatment 4 had nearly as high a level of TMA (8 mgN%), a level used as a cut-off point in product specifications by major companies (Ref. 3c).

Microbiological tests showed similar results to the TMA analysis; for example the skin of fish from treatment 2 had 400 bacteria/sq cm and that of treatment 5 fish had over one million bacteria/sq cm.

#### Salt

The chilled fish took up salt from the RSW. Fish from treatment 1, which had cut flesh surfaces exposed to salt water, took up most salt (Fig. 2). These results were confirmed in the saltiness ratings by the analytical taste panel.

#### Taste panel assessment

Three types of taste panel were used to assess the quality of unskinned fillets (Table 3). These are referred to as the consumer panel, analytical panel and profile panel.

#### Consumer panel

The consumer panel scored for

Table 1: Summary of treatments.

1	RSW		Left on deck	
	2	3	4	5
Gut	RSW 24 h.	RSW 24 h.	Ambient 24 h.	Ambient 24 h.
RSW 24 h.	—	Gut	Gut	—
Freeze	Freeze	Freeze	Freeze	Freeze

Table 2: Treatment ranking ([I] best to [V] worst) by the different assessment techniques used.

Assessment technique	Ranking of treatments				
	I Best	II	III	IV	V Worst
Visual					
Whole fish	1	2&4		3	5
Fillets	3	1	2	4	5
TMA	2	1	3	4	5
Taste panels					
Consumer (fried)	1	3	2&4		5
Analytical (steamed)		2	1&3	4	5
Profile (grilled)	2	1	3&4		5

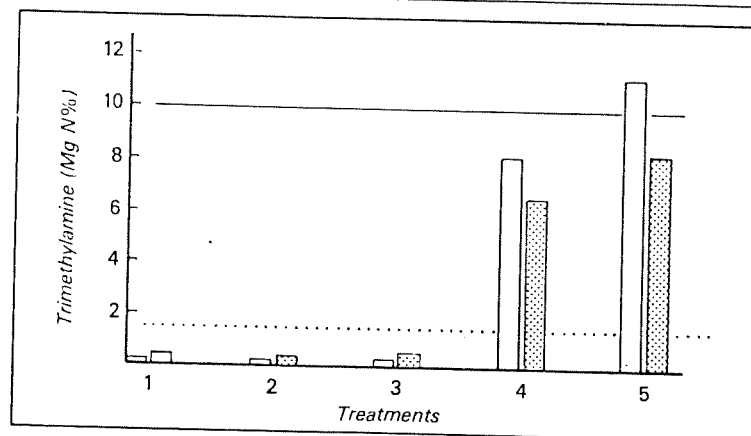


Figure 1: Levels of trimethylamine in fillets. Blank columns — 6 weeks' storage at  $-18^{\circ}\text{C}$ . Shaded columns — 18 weeks' storage at  $-18^{\circ}\text{C}$ . Horizontal lines indicate cut off level for good quality fish (.....) and level at which fish is too spoiled for most uses (—).

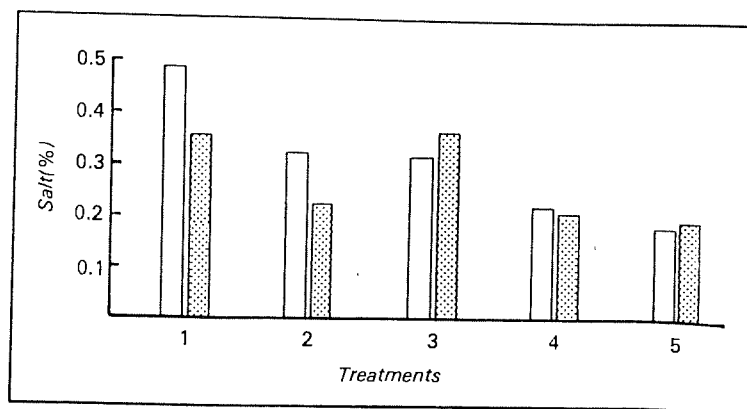


Figure 2: Levels of salt in fillets. Code as for Figure 1.

**Table 3: Description of taste panels.**

Type of panel	Post-thawing storage	Cooking method	Treatments assessed per session	Number of panelists	Number of sessions	Scoring scale
Consumer	Fillets vacuum packed and frozen for 2 days	Deep fried in bread crumbs	All 5	20	2	1-7*
Analytical	0°C overnight	Steamed 75°C	All 5	11	8	1-9
Profile	Fillets vacuum packed and frozen for 1 week	Grilled to 72°C shredded and reheated in a microwave oven to 70-80°C	All 5	10-15	1	1-7*

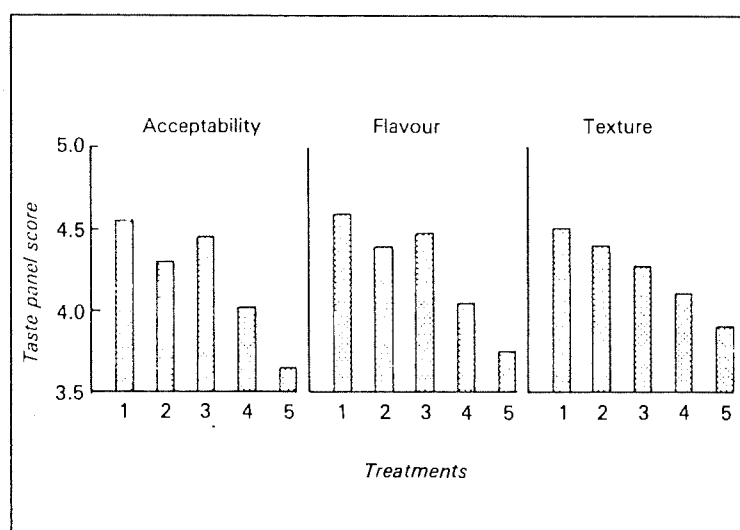
\*A score of 1 represents inedible, a score of 7 superb.

flavour, texture and overall acceptability on the General Foods 'Smiley' Scale of seven faces representing degrees of acceptability. Samples from the chilled treatments were scored more highly than the fish left at ambient temperature for 24 hours (Fig. 3).

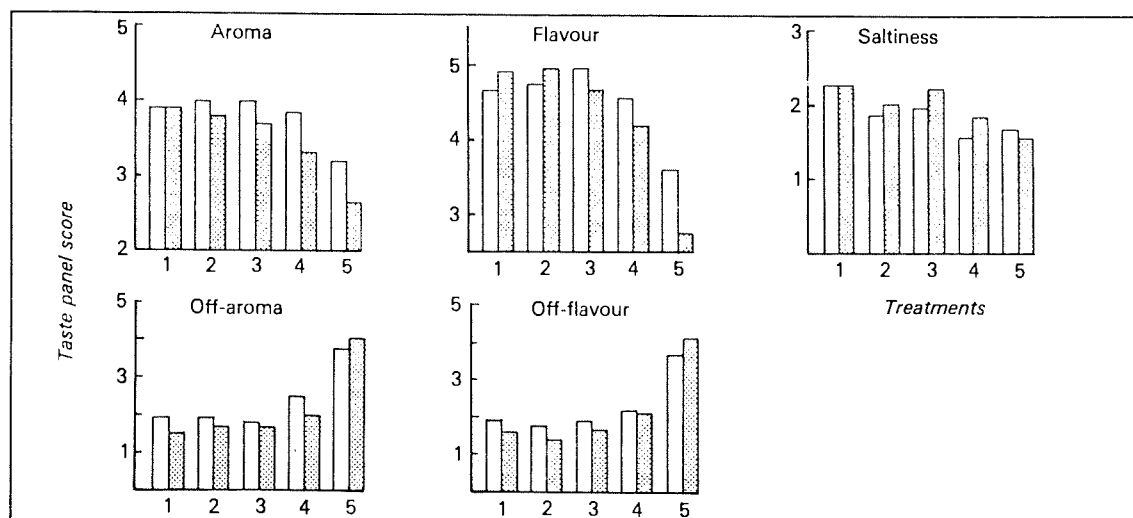
#### Analytical panel

The analytical panel scored the intensity of 11 characteristics (aroma, off-aroma, flavour, off-flavour, saltiness, dryness, firmness, springiness, toughness, succulence and fibrousness).

Aroma and flavour scores for fish from treatments 1 to 4 were similar (Fig. 4) after six weeks storage at -18°C but those from treatment 5 were much lower. Storage for 18 weeks resulted in



**Figure 3: Consumer acceptability taste panel results, mean values for removals 1 and 2. Note that the overall acceptability results reflect the flavour ratings.**



**Figure 4: Results of the analytical taste panel assessment of flavour and aroma. Codes as for Figure 1.**

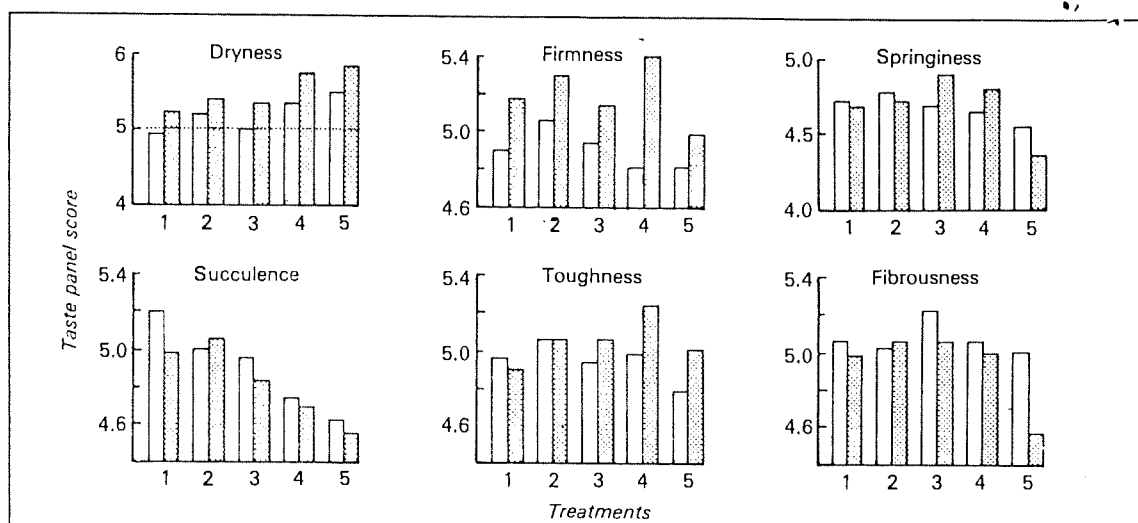


Figure 5: Results of the analytical taste panel assessment of textural properties. Codes as for Figure 1. The horizontal line on the dryness histogram indicates the value normally expected for fish, neither wet nor dry.

reduced scores for aroma and flavour for both treatments 4 and 5.

Off-flavour and off-aroma scores were markedly higher for treatment 5 fish at the samplings performed at six and 18 weeks. It is possible that spoilage compounds, formed during the holding period at ambient temperature, migrated from the guts during frozen storage. Furthermore, it is likely that the bacteria and enzymes in the guts of treatment 5 fish became active during thawing.

Treatments 4 and 5 resulted in lower scores for succulence and higher scores for dryness than the chilled treatments (Fig. 5), while the springiness of treatment 5 fish was low and decreased with frozen storage. All fish were firmer after frozen storage for 18 weeks.

#### Profile panel

The profiling technique describes the odour and flavour of the samples in terms of other familiar sensations, materials and foods. The acceptability of the prepared material was also judged.

The detailed profiles for aroma and flavour will be discussed in Part 2 (Ref. 6).

#### Results of assessments

The results of all the assessment techniques ranked from best to

worst are shown in Table 2.

It can be clearly seen that fish in treatment 5 which were left unchilled for 24 hours and not gutted before freezing, showed the poorest quality by every criterion, but it is difficult to identify the best of the three chilled treatments. Treatment 4, where fish left at ambient were gutted before freezing, rated second poorest after frying, grilling or steaming and by chemical analysis. Holding at higher temperatures for 24 hours did not result in an increased incidence of gaping.

#### Conclusion

Prompt on-board chilling is the most important factor in achieving top-quality gemfish. If chilling is delayed, inadequate or absent it is essential to gut the fish before freezing, but even then the fish will not be of top quality. If the fish had been actively feeding and were left ungutted before freezing they would have deteriorated even more rapidly.

#### Key points

Prompt chilling is the key to maintaining gemfish quality.

Gutting of fish on board may be unnecessary if chilling is prompt and effective.

Gutting of gemfish before freezing is essential if chilling of

the fish has been delayed or ineffective.

Many of the deteriorative changes are not obvious by visual inspection of whole fish or fillets.

Chilled and frozen storage magnifies damage caused by poor handling.

A quality-assurance scheme based on prompt effective chilling is essential for production of quality gemfish.

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- 3a. Connell, J. J. (1975). *Control of Fish Quality*. Fishing News (Books) Ltd., West Byfleet, Surrey England, p. 32.
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# On-board handling of gemfish

## Part II: Sensory profiles

HIGH-QUALITY gemfish fillets can be produced only if due care and attention is given to all steps of handling and processing from net to plate.

Inadequate chilling, or worse still no chilling at all, coupled with poor handling and storage, results in unpleasant odours and flavours caused mainly by bacteria on the skin and in the guts. Digestive juices in the gut can also contribute to spoilage.

The odours and flavours of seafoods are subtle, but trained taste panels can describe these natural characteristics in terms of other foods or well-known odours and flavours. Similarly, taste panels can also describe the changes that occur during spoilage. A list of words used to describe odours and flavours (called here 'descriptors') can be used to build up a profile of a particular fish and to provide graphic evidence of changes due to poor handling.

In Part I of this article changes in the quality of gemfish under five different handling treatments were discussed. These treatments are reproduced for convenience in Table 1. The fish were examined after six and 18 weeks frozen storage at  $-18^{\circ}\text{C}$ . After visual inspection the fillets from the five treatments were vacuum-packed and stored at  $-18^{\circ}\text{C}$  for one week.

### Chilled storage

The vacuum-packaged fillets from fish in each treatment were thawed overnight at  $4^{\circ}\text{C}$  and presented to the panel zero, four and seven days after thawing and storing at  $4^{\circ}\text{C}$ .

This procedure simulated a distribution chain in which the fish were landed from a boat,

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frozen, held in cold store, thawed, filleted, packaged, refrozen, thawed and 'distributed' through retail outlets. The profiles of these fish provide a description of how gemfish change during retail storage.

### Profiling technique

A panel of 10 to 15 people were grouped around a table. Each was provided with two score sheets listing words that have been used to describe seafoods.

Samples were prepared by grilling unskinned fillets until a centre temperature of  $72^{\circ}\text{C}$  was reached. To minimise variability, the cooked fillets were shredded with a fork and the material mixed. The mixed flesh was distributed between two bowls which were reheated to  $70-80^{\circ}\text{C}$  in a microwave oven immediately before being presented to the panel.

The panelists selected the appropriate words for odour and flavour which described the sensations they experienced and scored them according to the scale shown in Table 2. Members of the panel were familiar with the use of the

score sheets and, indeed, had helped to compile the list of appropriate descriptors at training sessions.

Panelists made their initial assessments of odour individually and then discussed their findings. They were free to amend their sheets as a result of the discussion. They then recorded their flavour scores in the same way.

A seven-point facial 'Smiley' scale (see Fig. 1) was on display to the panel and this attribute (overall acceptability) was marked independently with no discussion.

Of the 67 descriptors listed for odour, the panel scored 54 at least twice, of the 61 listed for flavour, 46 were scored at least twice. For economy of space only those descriptors which increased or decreased with acceptability, or which applied exclusively either to those fish which had been in refrigerated sea water, or to those which had been left on deck at ambient temperature before freezing, were selected as 'prime' descriptors for gemfish.

Figures 2 and 3 show total panel scores in graphic form; 'desirable' descriptors are coloured blue, 'undesirable' descriptors are coloured red and overall acceptability values are coloured black.

In order to make a readily understandable display, both the total acceptability scores and the total descriptor scores of the whole panel were brought to a common denominator and

Table 1: Summary of treatments.

1	RSW		Left on deck	
	2	3	4	5
Gut	RSW 24 h.	RSW 24 h.	Ambient 24 h.	Ambient 24 h.
RSW 24 h.	—	Gut	Gut	—
Freeze	Freeze	Freeze	Freeze	Freeze



Solid line: 6 weeks storage at  $-13^{\circ}\text{C}$   
 Broken line: 18 weeks storage at  $-13^{\circ}\text{C}$

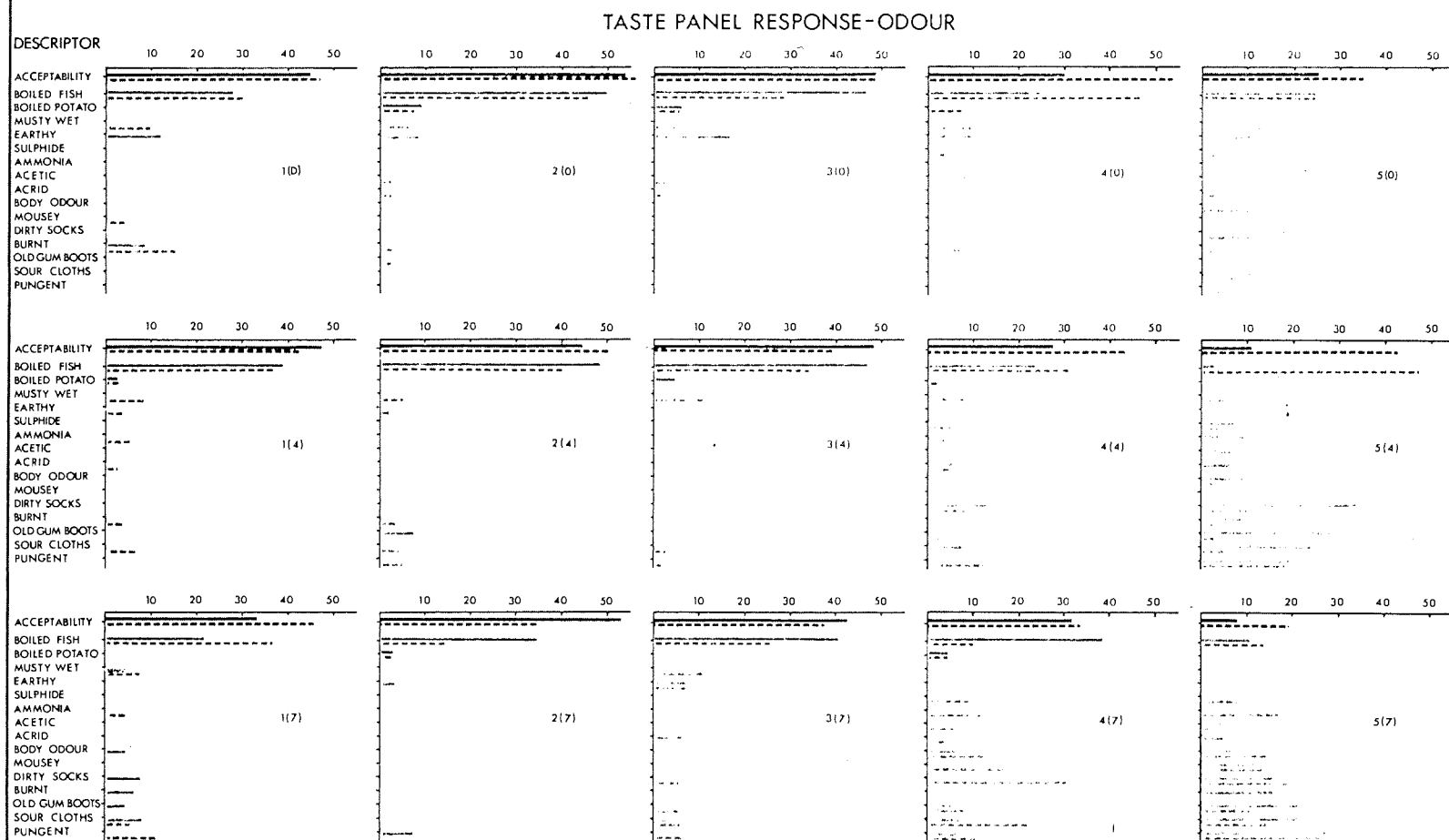


Figure 2: Odour profiles for treatments 1-5. Figures in parenthesis following treatment numbers indicate days at  $4^{\circ}\text{C}$ .

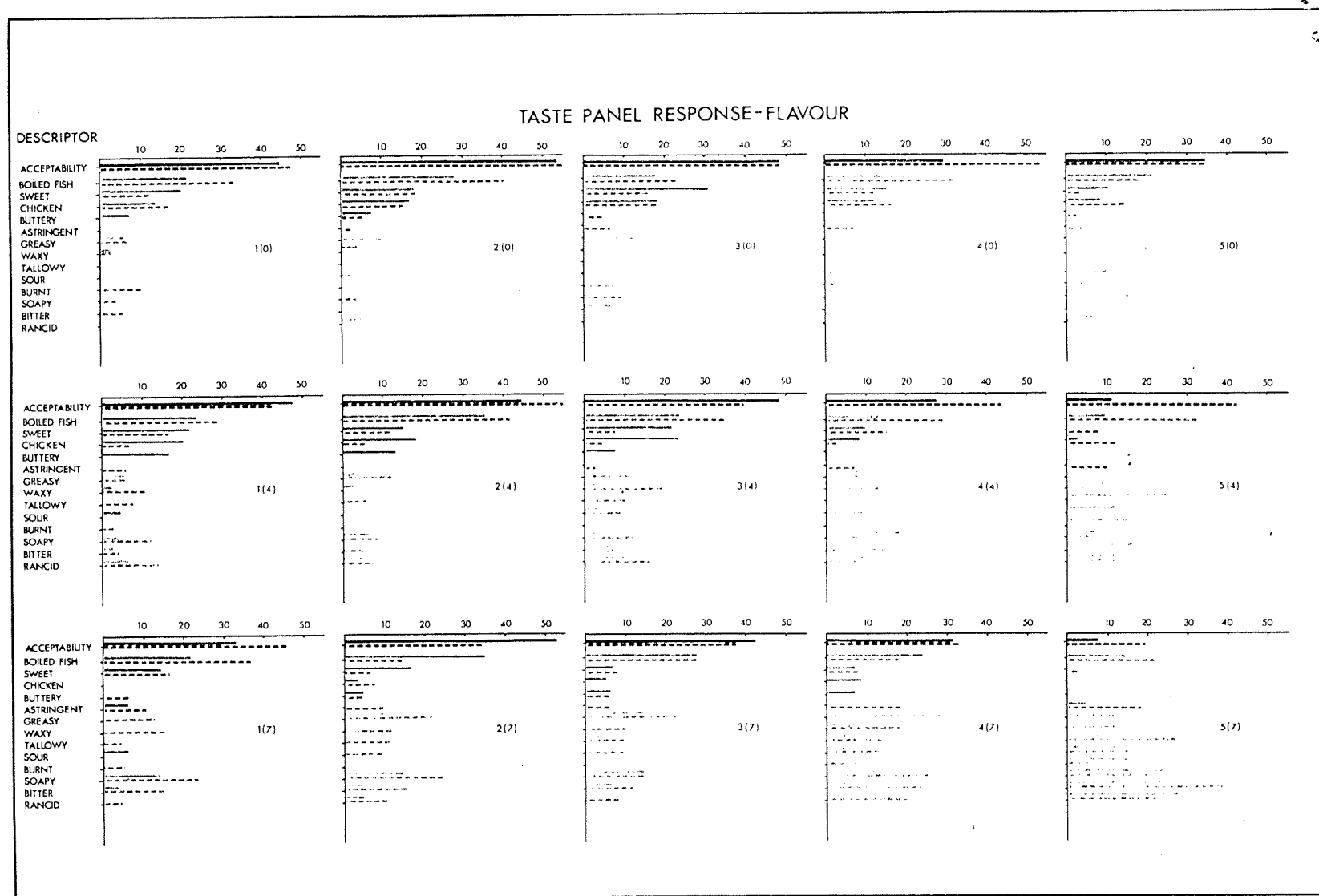


Figure 3: Flavour profiles for treatments 1-5. Numbering as for Figure 2.

Colour profiles prepared by Catherine Bryden.

Table 2: Scoring of descriptors.

Intensity of descriptor	Score	Total panel score as a % of maximum panel score
Absent	0	
Very slight	1	20
Slight	2	40
Moderate	3	60
Strong	4	80
Very strong	5	100

presented on a scale from zero to 100. These scores can be converted back to intensity by reference to Table 2 and Figure 1.

### Effects of treatments

Inspection of the odour profiles in Fig. 2 shows the great difference in the number of undesirable descriptors for treatments 1, 2 and 3 (only a few red bars) as all chill storage times compared with treatments 4 and 5.

The comparison is less pronounced for the flavour profiles in Fig. 3 although fillets from fish in treatment 5 which were left on deck ungutted before freezing evoked the largest undesirable response from the panel, directly after thawing, and after subsequent chill storage at 4°C. Fillets from treatment 5 also showed the greatest fall in acceptability after seven days at 4°C.

The taste panel used fewer desirable descriptors to describe odour than it did to describe flavour, and both odour and flavour profiles showed that a decrease in quality was accompanied by an increase in negative descriptors rather than a decrease in positive descriptors.

Absence of sour and rancid off-odours are mentioned in product specifications for frozen portions of white fish (Ref. 1). These odours were present in treatment 5 at all chill storage times and developed rapidly in treatment 4. Sour and rancid flavours were present after thawing in treatment 4 and 5, with detectable traces in treatment 2, but developed in all treatments on chill storage, the increase being most marked in treatments 4 and 5.

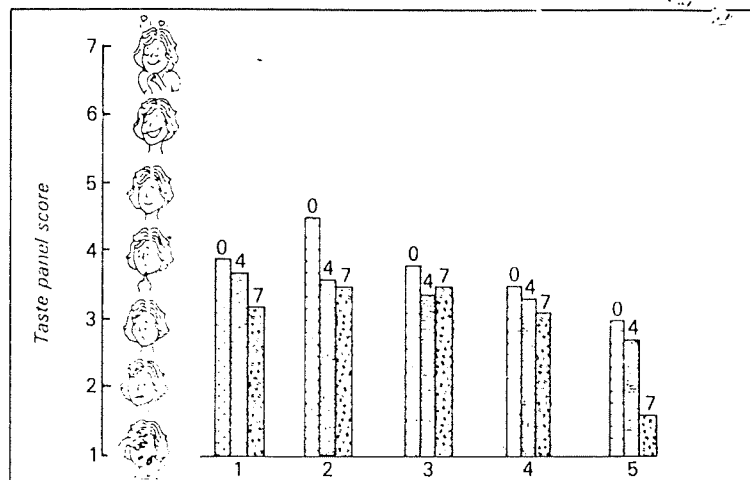


Figure 1: Profile taste panel ratings of overall acceptability of fillets after 0, 4 and 7 days storage at 4°C. 'Smiley' scale for acceptability (courtesy of General Foods Corporation). It should be noted that scores of 7 could only be expected from very fresh, high priced varieties of fish.

### Overall acceptability

The mean acceptability scores for all treatments are shown in Figure 1. They obviously decreased during packaging at 4°C and were significantly lower initially for treatment 5. None of the initial acceptability scores were high and were probably a result of the manner in which it was necessary to cook and present the samples. Even the 'best' samples had been subjected to two freezing and thawing cycles before being finally cooked and tested. The higher acceptability scores given by the profile panel (Figs. 2 & 3) for fish from treatments 4 and 5 in the second assessment is unexplained.

Profiles made on fresh gemfish obtained from a commercial outlet in Hobart were characterised by their lack of negative descriptors. However, when these fish were frozen and thawed they gave profiles similar to those obtained from fish in treatments 1 to 3 in this experiment. Thus freezing and thawing alone can result in the production of less desirable odours and flavours and a reduction in acceptability.

### Conclusion

Odour and flavour profiling have confirmed the conclusions made in Part 1 of this paper. Rapid chilling of gemfish on

board the vessel produces fillets with fewer off-odour and flavour notes and increases the potential retail shelf life.

Fish which are left ungutted on deck at ambient temperatures for 24 hours before freezing have considerably more flavour off-notes and a markedly reduced potential retail shelf life compared with those which have been left on deck, then gutted before freezing.

Fillets from fish left on deck at ambient temperatures and ungutted before freezing would not meet the product specification of major companies; fish gutted before freezing would be marginal.

To produce good gemfish fillets the following factors are important.

Chill the catch to 0°C or just below; either ice or RSW is suitable.

Keep chilled at all stages.

Fish must be gutted before freezing if delays in chilling have been unavoidable.

Use efficient freezers that reduce temperature rapidly.

Never allow thawed or packaged fillets to warm up.

### References

1. Connell, J. J. (1975). *Control of Fish Quality*. Fishing News (Books) Ltd., West Byfleet, Surrey, England, p. 138.

BRIEF   FIRC 27   FINAL REPORT

PROJECT

- .   80/7   Development of fish handling, processing and packaging systems and their influence on the quality of Australian Seafood products.

BACKGROUND

.   Project received	1980/81	\$72,400
	1981/82	\$79,200
	1982/83	\$73,300

COMMENTS

- .   Has been a major project and has generated a considerable volume of useful data of relevance to Australian fish handling and processing
- .   Project has been value for money

ATTITUDE

- .   Accept report