Improvements in product development and presentation of Australian prawns through investigation of their technological properties and development of new processing and packaging systems

H.N.Chinivasagam, H.A.Bremner and S.J Thrower

Project 91/100

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QUEENSLAND
DEPARTMENT OF
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FISHERIES
RESEARCH &
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Project concept and origin

IFIQ was encouraged by FIRDC in early 1990 to submit a proposal on the development and technology of prawn products. This was occasioned by the downturn in export prawn prices and a recognised need to look at better servicing of the domestic market. The initial proposal was that the technological work would be driven by market requirements and the first part of the proposal involved market research to investigate the types of prawn products required by the market. This market research component was deleted from the original proposal by FIRDC and subsequently a project that was technological in nature was approved in mid 1991.

The project was delayed in start for various reasons beyond IFIQ control, and a seafood technologist/microbiologist was appointed in May 1992. Effectively this has meant an active experimental project of 2.5 years duration, ceasing in December 1994.

Relevant dates

Project conceived	Latter part of 1990
Project submitted to FIRDC	December 1990
Negotiations with FIRDC: Project retitled and resubmitted	1991
Project altered and finally approved	mid 1991
Nominal starting date	1 July 1991
Request to delay start	December 1991
Project deemed to have started by M Walker & M Zagar	1 January 1992
Short report	January 1992
Scientist (N Chinivasagam) appointed	May 1992
Short report to FRDC	June 1992
Progress report	December 1992
Further extensive report	January 1993
New FRDC format application and short report	November 1993
(including notification of change of investigator from Thrower to Bremner)	
First construction of milestones	December 1993
Reporting on milestone	December 1993
(Plain summary and detailed paper)	
Illustrated progress report on milestone	June 1994
Project terminated	December 1994
Final report	October 1994

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Final report of Project 91/100 submitted to the Fisheries Research and Development Corporation in October, 1995.

Copies of this report may be obtained from:
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(II) NONTECHNICAL SUMMARY

Background

Australia is one of the few countries in the world where much of the domestic catch of prawns is landed chilled after being cooked onboard the vessel. In most other fisheries the catch is chilled thoroughly and then brought into port. This gives the buyer or processor a much greater range of options in determining which products to prepare according to the market demand. This flexibility of approach is currently not evident, although it is common overseas. Before these options can be fully explored, it is necessary to have reliable information on the storage life of prawns chilled and transported by appropriate methods. It is also essential to take into account variation between species and, most importantly, the nature of the bacterial flora which occurs naturally on the prawn and which is encouraged to predominate under the conditions of storage.

The work in this project was based on the assumption that the processing and development of prawn products will take place in land-based premises, not at sea. In order to provide sufficient options for product development, processors must receive chilled raw prawns in good condition.

Two major factors affect the storage life of prawns, bacterial action and development of blackspot. Control of these problems leads to higher standard products with an optimum storage life..

The project work thus first concentrated on the quality of typical product in the marketplace, then on handling practices and storage of chilled prawns and their microbiology before investigating the effects of packaging and storage.

Technical information

Prior to the commencement of the scientist on the project, information on prawns and processing was collected and some 600 articles were entered into the AUSEAS database. Very little information on basic spoilage patterns in Australian prawns was encountered. This technical information is thus available to industry through AUSEAS.

Market research

Market studies indicated that the industry was failing to meet its customer's requirements and that the first and most productive area in which to add value is to tackle major losses in prawn quality. There was thus an obvious need for QA and work on storage life.

QA programs

The market research led to the development of a QA program which was delivered in South Australia. Program proposals for Queensland were also developed and submitted. These proposals will underpin current initiatives in the QA field.

Quality of raw material

A survey of the prawns available in the near Brisbane area indicated that a large proportion had bacterial counts that were high indicating low remaining shelflife and that poor onboard and storage practices were resulting in inferior product entering the retail and wholesale outlets. Much of the product was only available in the cooked state and lacked variety, convenience and quality. Commercially available material would not make suitable raw material for better presented or value added products. Current handling needs to be upgraded.

Development of rapid method of assessment

A demerit point scoring system has been developed which is capable of giving a rapid assessment of the quality of prawns. This system can be used by industry to grade or select prawns.

Chilled storage of prawns in ice or ice slurry

Storage of prawns in ice slurry delays the formation of melanosis for several days more than does storage in ice. The degree of inhibition depends on the species with the delay being greatest in the deheaded form. This is likely to be due to the slurry restricting access of oxygen, since melanosis is an oxidative reaction. In addition the development of off odours and off flavours is much less in prawns stored in slurry than in ice.

The prawns stored in ice developed typical spoilage odours of rotting tropical fruit and sweaty sour dough since the main organism which dominated the flora was *Pseudomonas fragi*. In contrast these odours did not develop in the prawns stored in slurry but latterly sulphide odours developed. The dominant organism here was *Shewanella putrefaciens*. Thus different spoilage bacteria were selected by the different storage media despite being at the same temperature (0°C).

Therefore, for some applications, storage of raw prawns in ice slurry offers considerable advantage in that only slight changes occur in the first few days and metabisulphite is not required to prevent blackspot.

There were noticeable differences in the natural bacteria present on prawns caught in the different areas, a fact which may have greater importance if packaging of very fresh material in modified atmosphere packs is contemplated.

The total volatile nitrogen levels in all the prawns were high initially. These levels were near or exceeded limits normally regarded in other species as indicating spoilage and rejection of shipment. This is important since it demonstrates that inappropriate application of blanket standards to these species would result in rejection of even fresh caught material.

The demerit point scoring systems, previously developed for fish products (FIRTA 83/46), has been modified to be applied to prawns. This system has been found to differentiate well between treatments.

The changes in nucleotide levels occurred at different rates between the species but were largely unaffected by the storage form (head on or off) or storage medium (ice or slurry). Given that the changes are due to the inherent enzymes in the flesh, this result is to be expected.

Implications

The results of these trials have provided previously unknown information on the inherent bacterial flora in prawns from different areas and on the rate of transition to the dominant spoilage flora. They have shown that different spoilage bacteria are selected by different storage forms and that melanosis is inhibited for several days by immersion of the prawns in slurry.

This practice provides opportunity for the fishing sector to land good quality fresh prawns to the processor who can then explore appropriate product options.

Variability in flavour due to bacterial flora

The inherent flavour volatiles of some species were examined using the sophisticated techniques of gas chromatography coupled to mass spectrometry The range of specific volatile compounds produced by particular spoilage bacteria was also examined and strains of important spoilers were identified providing information on the type of spoilage interactions occurring with prawns information which will aid in the selection of suitable packaging techniques to inhibit the main spoilage flora.

Alginate glazes to decrease blackspot and inhibit bacteria

The effectiveness of alginate glazes to decrease blackspot formation and inhibit bacteria in chilled product were investigated. These glazes do not prevent blackspot nor do they inhibit bacteria.

Metabisulphite is more effective than glazes at inhibiting blackspot in frozen product. The glaze results in better separation of frozen prawns with less drip after thawing.

Packaging to inhibit melanosis

Vacuum skin packaging in the appropriate film is sufficiently effective to inhibit melanosis in frozen storage. Alternatively, packaging in a barrier bag with an oxygen absorber can also inhibit melanosis. Vacuum skin packaging and chill storage in a master outer containing carbon dioxide inhibits melanosis. The result is that methods are available to store prawns in both chilled and frozen form to inhibit melanosis without the use of metabisulphite or other chemicals.

Packaging to inhibit spoilage bacteria

Deheaded banana, tiger and king prawns were vacuum skin packed in films of various permeabilities to oxygen and stored at the practical temperature of 4°C. Storage lives were taken

as the period required for total bacterial counts to reach one million (10^6) organisms per gram. This is a conservative figure; prawns are still acceptable at this level. Under the appropriate circumstances, storage lives of 8-12 days for banana prawns, 7 days for tiger prawns and 13 days for king prawns can be obtained. These compare favourably with figures of about 5 days storage life predicted from the results of ice storage trials done on loose prawns. Storage of packs at temperatures near or just below 0°C would allow further increases in storage life. It appears that decreasing the oxygen tension in the packs has an initial inhibitory effect on Pseudomonad spoilage organisms. Vacuum skin packed prawn products with convenience and marketability can be prepared with sufficient shelflife to meet the requirements of distribution and the retail trade. Selection of appropriate films and trays with suitable permeabilities can provide conditions for excellent presentation without risk of botulism. Banana prawns appear to be the most versatile prawn for further processing. They are less prone to develop black spot and have lower counts of spoilage organisms naturally associated with them.

Total utilisation

The technical outlines for a small scale operation to process prawn heads and shells into a useful meal product have been developed. The meal has a favourable analytical profile with higher protein content and pigment level than imported products and prawns grow well on it. A process and recipe for utilising small, broken or reject prawns into prawn crackers has also been developed. The time temperature conditions for development of smoked prawn products have been investigated. The incorporation of non seafood ingredients into packages along with prawns has been tried. Adjustment of the acidity (eg with lemon) may lead to better inhibition of spoilage bacteria.

Reports, articles

A video tape on the work has been prepared for the "Deckhand" series produced from GBRMPA. Three papers have been published; one in 'Australian Fisheries', another in "Journal of Aquatic Food Product Technology" and a third in "Professional Fisherman". Other articles are in preparation.

(III) BACKGROUND (FROM ORIGINAL APPLICATION)

The recent slump in export markets for Australian prawns and the decline in the processing sector have highlighted the vulnerability of an industry that is heavily dependent on supplying a bulk commodity export market. There are two obvious alternative strategies. Firstly to target the domestic consumer and develop attractive products that will lift local consumption, while avoiding the pitfall of producing traditional items such as breaded products that compete directly with cheaper Asian imports, and secondly to explore alternative export markets for value-added products. These two approaches are not mutually exclusive, but have a high degree of compatibility. The factors which prevent an Australian household from eating more prawns are probably similar to those which inhibit consumption by American and European households. The main concerns are price and convenience. The opportunities for expanding exports of value-added shrimp products into Europe have been recently reviewed in a paper by Lambert (*INFOFISH International 4/90, p 11-14*).

Price

Most consumers regard prawns as an expensive gourmet food, and at present, most consumption is in restaurants rather than in the home. The popularity of imported Asian product is undoubtedly related to the lower price combined with a product of acceptable quality. It is likely that the restructuring of the industry that comes about as a results of the present downturn will lower the level of capital invested, providing some relief from the interest costs. This should in turn, allow for some easing in prices of raw material from local sources which will allow for some replacement of imports and provide scope for imaginative product development.

Convenience

If the marketing of prawns is to be significantly extended beyond the catering trade, a number of problems encountered by consumers must be addressed. The first of these is the highly perishable nature of the raw material. The "head" of the prawn contains the hepatopancreas, a gland which manufactures a battery of powerful proteolytic enzymes that attack the flesh, causing such conditions as loose head and mushy flesh. The most commonly used method of controlling such deterioration is to lower the temperature. Whilst this provides a partial solution, it is not very suitable for chilled products because enzyme reactions do not cease until the temperatures fall to the frozen storage range (below -20°C). Other options need to be explored for chilled product.

Another factor which makes prawns more perishable is the combination of a high initial bacterial load with a high pH which can lead to rapid bacterial spoilage. The development of new systems for modified atmosphere packaging allows far more effective control of bacteria during chilled storage.

A more practical problem is the difficulty of shell removal. Whilst the complete peeling of the prawns would put them into direct competition with cheap Asian peeled shrimp, it should be possible to develop a biochemical method of loosening the connective tissue which attaches

the shell to the flesh, thus easing the problems of peeling for the consumer. This would also assist in the utilisation of small prawns which have a limited market in this country at present.

Before new products and processes such as these can be developed, more information about our local species is needed. This project will provide key information for the industry on the properties of Australian prawns. In the past, such information has not been seen as necessary, since bulk prawns have sold well on the export market. Previous studies on the post-harvest handling of prawns have concentrated on three main areas, the prevention of melanosis ("black spot"), the development of taint, and general handling methods. While these topics were relevant to an industry that was based around supplying an overseas bulk commodity market with minimally processed product, the result is that there is no sound, systematic compilation of technical data on the post harvest properties of Australian prawns. Without it there is no baseline for modification or development of handling or processing procedures or for the development of new or improved products or methods of packaging for domestic and export markets.

(IV) NEED

Post harvest handling is a critical factor in obtaining initial raw material of prime quality, which creates the pathway to increased utilisation by preparation of more ready-to-eat marketable products of quality and convenience. This will enhance the market potential of prawns when compared to the limited emphasis on quality and scope for wider markets as it is at present as the domestic market.

(V) OBJECTIVES

To facilitate the development of domestic and export markets for value-added products made from Australian prawn species by:

(1) Developing improved methods of packaging, preservation and presentation suitable for "niche" markets and alternative distribution channels based on various properties of individual species.

(2) Providing a sound scientific and technological foundation on which to base the development of prawn products.

(VI) METHODS

(a) Plan of operation

(i) Scientific and technological investigation

Before new products are processes can be devised, it is necessary to put in place systems for establishing baseline properties and measuring changes in these over the shelflife of the product. The project will be approached in three phases. In the first phase, we will concentrate on demonstrating analytical methods. In the second phase this knowledge will be used to devise new methods of processing, packaging and presentation. The third phase will concentrate on disseminating information and assisting industry to implement the new technology.

Phase 1. In this phase methods will be developed for chemical and sensory analysis and initial studies will begin. This will involve analysis of volatile and non volatile flavour and compounds using Gas Liquid Chromatography and Mass Spectrometry, and nucleotide catabolism using High Performance Liquid Chromatography. Analytical studies of prawns will be correlated with taste panel trials which will be performed in association with IFIQ's sensory evaluation specialist using methods developed in Hobart CSIRO Laboratories to determine the significant flavour compounds. The catabolism of these compounds will be monitored in the immediate post mortem period to determine the effect of breakdown on taste acceptability.

This work will complement studies on *P. esculentus* being undertaken by IFIQ into live transport of this species (FIRDC grant 91/71). The two projects will be able to share similar raw material, thus offering depth to both projects and saving costs. At the end of this phase, analytical techniques will be in place, profile taste panels will be trained, and the biochemical mechanisms underlying flavour and spoilage will be defined. An additional spinoff from this project will be the collection of a considerable volume of data on the nutritional composition of the species. A more fundamental study of the nature and significance of the mechanisms of enzymic and bacterial spoilage will also be undertaken.

Phase 2. Once the spoilage mechanisms are understood, options for inhibiting enzymic and bacterial spoilage will be assessed with a view to developing alternatives to chemical such as metabisulphite. The main thrust of this work will be to explore different packaging films and gas mixtures in combination with chilled storage using a sophisticated facility for experimental studies into controlled atmosphere storage and modified atmosphere packaging which is being developed at IFIQ as part of a major project into tropical fruit products. The study will be widened to incorporate other commercial species and build up a library of technological information. In addition, the scope of the study will extend to include work on texture and ultrastructure using techniques and equipment developed for studies on collagen presently being done under a FIRDC sponsored project (88/90). This information will be useful in devising improved automated systems for heading and peeling prawns and suitable thermal pasteurisation regimes for preparing convenient, value-added products. Without such scientific input, the scope for the scope for product development will be limited by the lack of basic scientific knowledge of the raw material, its behaviour under different processing conditions, and interaction with other food ingredients in value-added products.

Phase 3. In this phase the results will be incorporated into the IFIQ extension program. The most effective systems of dissemination will be selected including published material, video, workshops and training courses.

(VII) PROGRESS ON METHODS

Phase 1

The methods for volatiles and non volatiles by GC/MS were developed and applied to prawns and to prawn broths inoculated with a series of isolated bacteria believed to be spoilers.

Nucleotide profiles of some species were done.

Sensory evaluation by profiling methods was regarded as too cumbersome after some preliminary testing and a modified demerit point score sheet was developed and used successfully.

Storage trials on 4 types of prawns stored in ice or RSW were done. Initial bacteria were isolated and spoilage flora characterised.

These spoilage bacteria were used in other tests.

Preliminary investigations into the role of bacteria in the development of blackspot were done.

Spoilage pattern of banana prawns stored at ambient temperatures was done.

A survey of the microbial status of prawns on the market place showed need for improvement.

Phase 2

Numerous trials using VSP and MAP were done.

Changes in spoilage flora were noted.

Oxygen absorbers were used to test if they could inhibit blackspot.

Work on texture and ultrastructure was not seen as being necessary at this stage.

Work on automated systems was not done nor was work on heading and peeling systems. The options of deheading were explored in packaging experiments.

Bacterial isolates were characterised according to new system (at U of Q by student).

Phase 3

Three articles published, others in preparation

Display packs made for FRDC and trade expositions.

Videos produced, one training course specifically from this project.

Basic information being incorporated in 'Quality Project' by strategic staff transfer.

SECTION 1 INTRODUCTORY REMARKS AND OUTLINE OF RESULTS

(VIII) DETAILED RESULTS

One nation fightback headland statement

There is need for a new approach to the handling, processing and presentation of prawn products for the domestic and export markets.

In domestic retail outlets most are sold as loose, chilled cooked prawns since this is the form in which they are landed. The practise of cooking prawns onboard boat is thought to have originated from the belief that they would keep better and that they provided consumers with a product in a form that was ready to use; simply peel and eat.

However, cooking is not necessary for preservation, prawns keep well when chilled properly and provision is made to prevent blackspot development. Purchasing habits have changed, most foods are bought in supermarkets and consumers require safe reliable products that are convenient, not messy and which result in little waste. Supermarkets and other retail outlets are geared more to handle packaged products of reliable standard with a guaranteed shelf life.

Prawns in the whole raw or cooked state are not a convenient, user-friendly product for the majority of consumers or for retail staff. Prawn waste is a nuisance in home refuse.

To meet market requirements a range of attractively presented, packaged, value added products must be developed. To do this the processors must receive good standard raw chilled prawns to provide them with the whole range of options for further processing and packaging.

Process control is notoriously difficult at sea and most processes, including cooking, are best done onshore. Better hygiene, lower bacterial counts and better product result from onshore processing where facilities are dedicated to that purpose. Boats are designed for catching, not processing and hygiene is often inadequate. Poor and variable quality product results.

Good quality chilled prawns can be delivered by the catching sector to the processor if the catch is well chilled. Refrigerated seawater or brine solutions are often used for this purpose but these can result in the uptake of unacceptable amounts of salt which limits the use of the prawns in the whole range of possible consumer products.

When warm water prawns are properly chilled in ice or ice-slurry the natural bacteria present on them generally fail to thrive and grow. Thus, there is a period of adjustment during which cold-tolerant spoilage bacteria gradually develop and bacterial counts are quite low. This period may last 4 - 6 days after catch and good products with low initial bacterial counts and acceptable shelf lives will result. Packaging the prawns in this period, under conditions which inhibit bacterial growth, will further ensure sufficient shelf life for the product.

One process of providing products that are attractive and well presented is that of vacuum skin packaging (VSP), where a clear film is drawn over the product on a tray such that it becomes a tight skin that fits snugly over the product.

Chilled deheaded prawns in these packs have good shelf life and the clear film allows the product to be shown to advantage against the contrasting colour of the tray. They can also be stored frozen and subsequently thawed for sale. Correct choice of the film can ensure blackspot doesn't occur.

Thus good, attractive, safe, sufficiently stable, well presented products can result from chilling prawns well after catch, processing on land by deheading or peeling and packing in vacuum skin packs.

If correctly prepared and stored, these products could have sufficient shelf life for both the export and domestic markets.

Outline of results

* Literature collected entered in database

* Survey of product type and quality
  - need for better raw material for value-adding
  - need for QA
  - market quality and variety poor
  - microbial safety suspect

* Raw Material - Chilled Studies
  - five species studied
  - shelflife studies done
  - demerit point scoring system proven
  - storage of prawns at same temperature in different media
  - spoilage profiles and volatiles produced by bacteria studied
  - spoilage and bacterial interactions studied

* Raw Material- Frozen Studies
  - conditions causing melanosis in prawns
  - use of glazes
  - use of alternatives to chemicals to prevent melanosis-absorbers, VSP

* Processing
  - varieties for packing
  - raw prawn
  - smoking
  - prawn crackers
  - mixed ingredients

* Packaging
  - use of different techniques
  - VSP use of films with different permeabilities to inhibit spoilers
  - use of oxygen absorbers to change atmosphere in the pack
  - VSP in combination with storage in CO_2
  - three major prawn species studied
  - effects of different storage temperatures noted
  - use of glazes studied
  - odour and taste profiles developed

* Utilisation of waste
  - meal process developed

* Preparation of articles, videos.
  - 'Deckhand' video produced
  - articles written and in preparation

SECTION 2 MARKET TRENDS AND QUALITY ASSURANCE

2.1 Market research

2.2 Quality assurance

2.3 Market trends

2.1 Market research

The marketing component of the original proposal was deleted by FIRDC , but some information was regarded as essential to help direct the project. Market studies were done inhouse by J McVeigh (IFIQ), prior to his joining the NSC, in conjunction with A Kriz, then market researcher with Agtrade (QDPI). They conducted interviews with suppliers, wholesalers and restaurateurs. The results of their enquiries indicated that the industry was failing to meet its customer's requirements and that the first and most productive area in which to add value was to tackle major losses in prawn quality. Common complaints included:

- poor size grading
- poor quality grading
- unreliable shelf life
- inconsistency of supply
- great variability in weight per pack
- inconvenient pack size
- foreign material
- considerable variation in cooked product

Outcome - Obvious need for QA and work on storage life.

These results were consistent with the presentations given at the International Seafood Conference, held by IFIQ in September 1992, by marketing manager P Cooper (TASSAL), restaurateur D Flockhart (Rumpoles), consultant M Marshall, educator J O'Brien and other members of the audience.

2.2 Quality assurance

The market research gave the impetus for the development of a QA program which was coordinated by S Thrower and delivered to the Spencer Gulf and Westcoast Prawn Fishermans Association by D Mahoney and D Milne of IFIQ. Project proposals for the Queensland industry were also developed; and submitted but these were put on hold pending the proposed national QA strategy.

2.3 Market trends

The following paper on marketing trends was drawn up during the project.

It was submitted and published in an abridged form by Professional Fisherman and a copy of this publication is attached

Trends in packaged seafood

Attractive, convenient seafood packs have gained market acceptance throughout the world. Nalini Chinivasagam and Allan Bremner report on these trends, on the Australian situation and on relevant recent research at the International Food Institute of Queensland.

The global scene

The global value of fish trade increased in 1992 reflecting considerably higher prices for a decreased volume of fish entering international trade (Josupiet, 1994). The FAO noted that fish production from most marine resources and from many inland water bodies, has reached or exceeded the level of maximum sustainable yield (Anon, 1993). These facts emphasise the need to adopt long term strategies for optimum utilisation of the available catch and of cultured seafood resources to obtain the highest returns for the industry. Coupled with this is the increasing need to meet changing consumer needs and market requirements and to reduce post-harvest losses.

The prepacked fresh seafood sector of the market in Europe is recording a high rate of growth and showing good development prospects (Monfort, 1992). The type of seafoods on offer ranged from those with a strong traditional approach (Spain, Italy, Germany), mainly smoked or preserved products to products with a high degree of innovation (UK, France) for which improved packaging and processing techniques were employed. These products are in the convenience ready-to-use range, and are predominantly sold chilled. Many use modified atmosphere packing and sous vide techniques to suit specific markets.

Such types of products have arisen due to an increasing consumer demand for fresh, minimally processed foods, as well as from an understanding of the health benefits of seafood. Better presentations in packs where the product is visible have led to increased consumer acceptance. The development of such products relies on a comprehensive understanding of the product-atmosphere-package interaction.

Modified atmosphere (MAP) or controlled atmosphere (CAP) packaging are being adopted in Europe, to provide the advantages of longer shelflife and better presentation of chilled seafood. Different gases or mixtures of gases are used to control or retard bacterial spoilage, thereby increasing the shelflife. Sous vide technology has also been used for most ready-to-use type of seafood products stored chilled. The success of such technology depends on its correct application to specific products under specific conditions as well as on the type of raw material used.

The Australian situation

The gross value of Australian fisheries in 1992-93 was estimated to be around $1374 m with seafood imports in 1992-93 being valued at nearby $490 m (Anon, 1993). The majority of the products are sold in the fresh or frozen form through fish shops or supermarkets. There is lack of variety, with consumers often having little knowledge about the particular type of fish, or on methods of preparation. Prepared seafoods are often limited to frozen crumbed fish portions or fillets. Some smoked and pickled products are produced locally but these only make up a small proportion of the market since mostly this type of product is imported. Australians are not known

as big eaters of seafood, which leaves much work for the industry to do to promote its products to the consumer for it to gain more market share and ensure its long term profitability.

The per capita consumption of seafood was 12 kg per annum in 1992. The 20% increase in seafood consumption since 1977 was attributed to consumption out of the home, while the number of seafood meals consumed at home fell from 1.55 meals per week in 1977 to 1 meal per week in 1990-92 (Anon, 1993), Table 1.

Table 1 Per person consumption of fish and seafood in 1977 and 1991.

	Fish		Seafood	
	1977	**1991**	**1977**	**1991**
	kg	**kg**	**kg**	**kg**
In home				
Fresh and frozen	2.90	4.26	0.80	0.68
Frozen packed	0.90	0.37	0.09	0.06
Canned	1.81	1.39	0.12	0.05
Total[a]	5.95	6.94	1.03	1.11
Out of home	1.84	2.38	1.24	1.64
Total	7.80	9.30	2.27	2.75

[a] Includes other types

Cited in ***Smith and Tran (1994)*** *- Source:* Fisheries Research and Development Corporation-1992

There is a need to increase seafood consumption both in and out of the home. The seafood consumption study indicated that consumers were concerned about the nature, quality and safety aspects of seafood products available in the domestic market.

The survey also showed that the use of packed frozen (or canned) seafood/fish decreased from 1977-1991. Thus there is a need to work towards developing products with better presentation, shelflife and safety (with use by dates), in addition to providing details of preparation and other nutritional information. The raw material used for the preparation of such products could also be from non traditional or under utilised species of fish. The Hassall report (1988) lists a variety of under utilised species which would benefit from value adding especially on a seasonal basis.

The report on "*A survey of value-added fish and seafood processors*" carried out for the Fishing Industry Research and Development Corporation indicates the following,

* relative to comparable Western countries, the Australian value-added fish and seafood processing industry has not achieved the same level of market penetration.

* in overseas countries fish processors have identified problems which consumers have with fish and seafood and have increased efforts to make fish more acceptable to the consumer by processing, pre-preparing and introducing other benefits, including packaging that overcomes these problems.

* given all international growth trends in value-added fish and seafood products, there appears to be a major opportunity in Australia to stimulate the processing industry to achieve greater penetration of the consumer market.

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The storage temperature was a critical factor in obtaining good shelf lives. Packaging prawns in (a) barrier film without oxygen (b) in a pack with oxygen absorbers or (c) in a carbon dioxide atmosphere prevented melanosis and resulted in a spoilage pattern different to prawns packed in a permeable film. A degree of melanosis occurred in untreated prawns stored in packs with permeable film. Although the bacterial flora and the spoilage patterns of prawns packed in these three ways was different to those packed in oxygen permeable films their overall shelflives were similar. A choice of the appropriate package needs to be made according to the circumstance (i.e. species, expected marketable shelflife). Oxygen absorbers were successful in preventing blackening in frozen king prawn packs over a period of 4 months.

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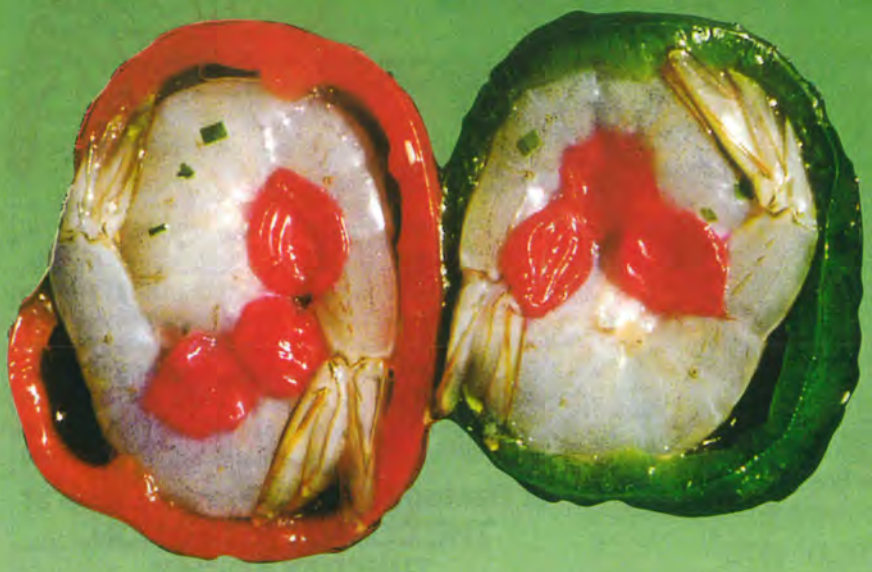
Figure 2.3.1 Prawns (raw) packed in vacuum skin packs in permeable film with capsicum and pickled onions - no additives. Stored chilled

Figure 2.3.2 Prawns (raw) packed in vacuum skin packs in a barrier film - no additives. Stored frozen

Figure 2.3.3 Prawns (cooked) packed in vacuum skin packs in a permeable film with brocolli -no additives. Stored chilled

Trends in packaged seafood



Prawns (raw) packed in vacuum skin packs in permeable film with capsicum and pickled onions - no additives. Stored chilled.

Attractive, convenient seafood packs have gained market acceptance throughout the world. Nalini Chinivasagam and Allan Bremner report on these trends, on the Australian situation and on relevant recent research at the International Food Institute of Queensland.

The global scene

The global value of fish trade increased in 1992 reflecting considerably higher prices for a decreased volume of fish entering international trade (Josupiet 1994). The FAO noted that fish production from most marine resources and from many inland water bodies, has reached or exceeded the level of maximum sustainable yield (Anon 1993). These facts emphasise the need to adopt long term strategies for optimum utilisation of the available catch and of cultured seafood resources to obtain the highest returns for the industry. Coupled with this is the increasing need to meet changing consumer needs and market requirements and to reduce post-harvest losses.

Table 1 Per person consumption of fish and seafood in 1977 and 1991

	Fish		Seafood	
	1977 kg	1991 kg	1977 kg	1991 kg
In home				
Fresh and frozen	2.90	4.26	0.80	0.68
Frozen packed	0.90	0.37	0.09	0.06
Canned	1.81	1.39	0.12	0.05
Total[a]	5.95	6.94	1.03	1.11
Out of home	1.84	2.38	1.24	1.64
Total	7.80	9.30	2.27	2.75

[a] Includes other types

Cited in Smith and Tran (1994) – Source: Fisheries Research and Development Corporation – 1992

The prepacked fresh seafood sector of the market in Europe is recording a high rate of growth and showing good development prospects (Monfort 1992). The type of seafoods on offer ranged from those with a strong traditional approach (Spain, Italy, Germany), mainly smoked or preserved products to products with a high degree of innovation (UK, France) for which improved packaging and processing techniques were employed. These products are in the convenience ready-to-use range, and are predominantly sold chilled. Many use modified atmosphere packing and *sous vide* techniques to suit specific markets.

Such types of products have arisen due to an increasing consumer demand for fresh, minimally processed foods, as well as from an understanding of the health benefits of seafood. Better presentations in packs where the product is visible have led to increased consumer acceptance. The development of such products relies on a comprehensive understanding of the product-atmosphere-package interaction.

Modified atmosphere (MAP) or controlled atmosphere (CAP) packaging are being adopted in Europe, to provide the advantages of longer shelflife and better presentation of chilled seafood. Different gases or mixtures of gases are used to control or retard bacterial spoilage, thereby increasing the shelflife. *Sous vide* technology has also been used for most ready-to-use type of seafood products stored chilled. The success of such technology depends on its correct application to specific products under specific conditions as well as on the type of raw material used.

The Australian situation

The gross value of Australian fisheries in 1992-93 was estimated to be around $1,374 m with seafood imports in 1992-93 being valued at near by $490 m (Anon 1993). The majority of the products are sold in the fresh or frozen form through fish shops or supermarkets. There is a lack of variety, with consumers often having little knowledge about the particular type of fish, or on methods of preparation. Prepared seafoods are often limited to frozen crumbed fish portions or fillets. Some smoked and pickled products are produced locally but these only make up a small proportion of the market, since mostly this type of product is imported. Australians are not known as big eaters of seafood, which leaves much work for the industry to do to promote its products to the consumer for it to gain more market share and ensure its long term profitability.

The per capita consumption of seafood was 12 kg per annum in 1992. The 20% increase in seafood consumption since 1977 was attributed to consumption out of the home, while the number of seafood meals consumed at home fell from 1.55 meals per week in 1977 to one meal per week in 1990-92 (Anon 1993), Table 1.

There is a need to increase seafood consumption both in and out of the home. The seafood consumption study indicated that consumers were concerned about the nature, quality and safety aspects of seafood products available in the domestic market.

The survey also showed that the use of packed frozen (or canned) seafood/fish decreased from 1977-1991. Thus there is a need to work towards developing products with better presentation, shelflife and safety (with use by dates), in addition to providing details of preparation and other nutritional information. The raw material used for the preparation of such products could also be from non traditional or under utilised species of fish. The Hassall report (1988) lists a

Above left: Prawns (raw) packed in vacuum skin packs in a barrier film - no additives. Stored frozen Above right: Prawns (cooked) packed in vacuum skin packs in a permeable film with broccoli -no additives. Stored chilled.

variety of under utilised species which would benefit from value adding especially on a seasonal basis.

The report on " A survey of value-added fish and seafood processors" carried out for the Fishing Industry Research and Development Corporation indicates the following:

* relative to comparable western countries, the Australian value-added fish and seafood processing industry has not achieved the same level of market penetration.
* in overseas countries fish processors have identified problems which consumers have with fish and seafood and have increased efforts to make fish more acceptable to the consumer by processing, pre-preparing and introducing other benefits, including packaging that overcomes these problems.
* given all international growth trends in value-added fish and seafood products, there appears to be a major opportunity in Australia to stimulate the processing industry to achieve greater penetration of the consumer market.

The report also concludes that the Australian value-added processing industry is fairly optimistic about future sales and almost two in three processors (63%) believed that sales would be greater in five years time. Thus there is a push for the type of value-added products suitable for the conditions of the Australian industry.

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SECTION 3 MARKET SURVEY

3.1 A survey of the microbiological status of prawns from retail and wholesale outlets in the Brisbane region

3.1 A survey of the microbiological status of prawns from retail and wholesale outlets in the Brisbane region

Abstract

A survey of samples of prawns from retail and wholesale outlets in the Brisbane region showed that a large proportion had bacterial counts that were high. This indicates that the prawns would have a short remaining shelflife. Much of the product was only available in the cooked state and there was a lack of variety and convenience in the presentation which was often of poor standard. Inferior product was entering the wholesale and retail trade indicating the prevalence of poor handling and storage practices. Lack of care for the product was noted from the point of capture all along the distribution chain to the retail outlets.

Much of the material available commercially available would not make suitable raw material for better presented or value added products. Current handling needs to be upgraded.

Introduction

Before commencing studies into packaging and product development it was necessary to survey the standard of product on sale at wholesalers and retailers. It was considered this would be a reasonable reflection of typical product currently available to processors for value-adding operations. In this it was also assumed that any further processing operations would be done onshore not at sea.

Accordingly a study area was selected from which product could conveniently be sampled. Some samples were also obtained directly from boats. In addition to taking samples of prawns, observations were made on the current practices of cooking, handling and transportation of prawns both on-board and onshore.

Materials and methods

A random selection of outlets in the northern suburbs of Brisbane was established as the study area. This region was selected as it contained a wholesale market, retail fish shops and supermarkets which regularly deal with the marketing, distribution and processing of fish and fishery products. The source, storage form, type and presentation of the samples are given in Table 1.

Samples were obtained at random intervals approximately on a monthly basis. The number of samples obtained on a particular day depended on what was available that day. The samples purchased from the particular source were sealed in bags, stored on ice and brought to the laboratory within an hour. Samples from the wholesale market were obtained under aseptic conditions in sterile bags and were similarly transported to the laboratory. It was not practical to measure the temperature of the product on display since this breached regulatory procedures in that it may contaminate the product. In one instance when staff attempted to take temperatures they were warned off by the proprietor. The temperature of some samples was taken after purchase when off the premises.

Table 1 Listing of the source, storage form and presentation of the seven types of prawns sampled.

Type of Prawn	Number of Samples	Source of Prawns											
		Boat		Wholesaler/Processor						Retail Outlet			
		Storage Form											
		Ice	Slurry	Ice		Chilled	Slurry	Frozen		Ice		Chill	
		Presentation											
		Raw	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw
Tiger	8		1	2	1	1	1		1			1	
King	18	1	2	1		1					1	9	2
Coral	4					1						3	
Greasy	9		1	1		4				1		2	
Endeavour	4					1		1				2	
Banana	7		2							1		1	3
Unknown	3*											3	
Total	53	7		16						30			

* Peeled prawn meat.

Microbiological analysis

Sample preparation

250 g of whole prawns were macerated in a "Colworth" stomacher to form a uniform composite sample. The bag was shaken to mix the sample and 10 g was weighed into sterile stomacher bags.

Tests carried out

Total bacterial counts were carried out by preparing serial dilutions using 0.1% peptone water for plating on nutrient agar. The plates were incubated at two temperatures, 30°C and 5°C. Testing for the pathogens *Salmonella, Vibrio parahaemolyticus, Staphylococcus aureus* and *E. coli* was carried out according to Australian standards AS 1766.2.5-1991, AS 1766.2.9-1991, AS1766.2.4-1986 and AS 1766-1987 respectively.

Statistics

The results were analysed by analysis of variance.

Results

Temperatures

The temperature of samples bought from six different retail outlets was measured outside the shop. One sample was 10°C, the others were between 5 and 6°C. Ten degrees is far too warm a temperature for prawns. If allowance is made for the delay in measuring temperature then the rest of the samples were probably at a temperature near 4°C. This is good but 0°C is much better.

Temperatures at the wholesalers were generally lower. Out of ten samples, six were below 2°C, two were at 3°C, one at 5°C, and one at 7°C.

Samples

Cooked prawns made up the majority (70%) of the samples reflecting the common manner in which the industry presents prawns to the consumer (Table 1). Most (64%) of the samples came from chill-storage cabinets while 17% were stored in ice, 13% were in slurry and 6% were frozen.

Most prawns were reasonably large, with 60% between 10-50 g in weight (Table 4) (note that this includes both cooked and raw prawns). Thirty percent of the samples were obtained from wholesalers, 57% from retailers with the remainder being obtained directly from the boat as the catch was landed.

Microbiology

Cooked prawns in the retail sector

The majority of the cooked prawns (62%), viz. 44% of the total sample, were stored in chilled display cabinets in the retail sector.

Those prawns stored in ice had a total bacterial count near 10^5/g and the count for those on display are shown in Table 2.

Table 2 Percentage distribution of total bacterial counts found on cooked prawns stored in retail chill displays.

	Total Bacterial Counts per g of Prawn				
	$<10^4$	**10^4 - 10^5**	**10^5 - 10^6**	**10^6 - 10^7**	**10^7 - 10^8**
Samples incubated at 30°C	-	9.5	52.5	28.5	9.5
Samples incubated at 5°C	5	19	28.5	38	9.5

Cooked prawns in the wholesale sector

In the wholesale sector the majority of samples (2/3) were held in chilled display. The total bacterial counts for samples in ice ranged from 10^4 to 10^6 /g while for those in chillers 25% had counts below 10^5/g, 25% had counts between 10^5 and 10^6/g but 50% had counts between 10^7 and 10^8/g. Frozen cooked prawns had counts lower than 10^3/g.

These counts on cooked prawns give cause for concern. Trials in this laboratory have indicated that a rapid reduction of bacterial load occurs when prawns are cooked. If the prawns are not recontaminated there should be no reason why counts of 10^2/g or lower can not be obtained. The high counts obtained on cooked prawns are most likely due to a combination of post process contamination and elevated storage temperatures.

Raw prawns

Thirteen percent of samples were obtained directly as raw prawns from boats in ice or slurry Most of these samples were obtained as part of other experiments and they had thus been properly handled.

Eighty six percent of these had counts below 10^5 the remaining 10% had counts near 10^6/g.

In the retail sector raw prawns were mostly stored in chillers (71%) with counts mostly in the range of 10^5 - 10^6/g (Table 3).

A similar range of counts was obtained with samples taken at wholesalers.

Table 3 Percentage distribution of total bacterial counts found on raw prawns in retail outlets.

	Total Bacterial Counts per g of Prawn			
	$<10^4$	10^4 - 10^5	10^5 - 10^6	10^6 - 10^7
Samples incubated at 30°C	14	22	64	-
Samples incubated at 5°C	14	29	57.1	-

It has already been established that bacterial counts on freshly caught raw prawns in the region of 10^6 /g are quite usual. With chill storage these counts often decrease to levels nearer 10^4 /g since the Gram positive component of the flora fails to survive chilling and are thus not spoilage organisms.

Coliforms

Twenty two samples had detectable coliform counts. Of these five samples were below 10, nine were between 11 and 100, seven were between 101 and 1000 and one sample had a coliform count above 1000 /g. In three samples the coliforms were of faecal origin.

Coliforms should not be present in prawns caught from unpolluted waters and, if present, should be completely destroyed by cooking. With the exception of one sample all the coliforms found were present on cooked prawns. The presence of faecal coliforms is caused by poor hygiene. They must be due to post cooking contamination either in the cooling water used, the storage medium or on implements used to handle the prawns or by direct contact from humans. Fortunately only the one sample from a retail chill cabinet had 1000 coliforms/g.

Staphylococci

Twelve samples tested positive for staphylococci and most were the same samples that showed the presence of coliforms The levels were generally lower than 100 /g with only three samples having over 200/g. Two samples were of raw prawns indicating that contamination can occur in the raw state while the rest were on cooked prawns in both retail and wholesale display.

These are low levels, but they indicate contamination from humans after cooking. The problem with *Staphylococci* is that they produce toxin which is not inactivated on cooking.

Vibrio

Vibrio were present but none were the infective *Vibrio parahaemolyticus*.

Overall results

The overall microbiological results are presented in Table 4. Some general conclusions can be drawn from these results.

Table 4 **Total bacterial counts for various prawn samples obtained at two incubation temperatures listed as logarithms of the mean with group standard deviation in brackets.**

Comparison	Number	30°C Incubation		5°C Incubation	
Type of Prawn					
Tiger	16	5.40^{bc}	(1.65)	5.14	(1.85)
King	36	5.35^{bc}	(1.15)	5.40	(1.16)
Coral	8	6.06^{ab}	(0.76)	6.07	(0.90)
Greasy	18	5.30^{bc}	(0.64)	5.18	(0.71)
Endeavour	8	4.65^{c}	(1.92)	4.11	(2.46)
Banana	14	4.88^{c}	(0.81)	4.77	(1.21)
Unknown	6	6.60^{a}	(0.54)	5.78	(1.12)
Source of prawn					
Boat	14	4.30^{a}	(0.81)	3.89^{a}	(0.86)
Retail	60	5.57^{b}	(0.98)	5.46^{b}	(1.08)
Wholesaler	32	5.43^{b}	(1.50)	5.33^{b}	(1.75)
Storage form					
Ice	18	5.07^{a}	(0.80)	4.99^{a}	(0.94)
Chill	68	5.81^{b}	(1.04)	5.72^{b}	(1.20)
Slurry	14	4.61^{a}	(0.85)	4.22^{a}	(1.01)
Frozen	6	2.89^{c}	(0.50)	2.54^{c}	(0.85)
Mode of presentation					
Cooked	74	5.67^{a}	(1.24)	5.56^{a}	(1.42)
Raw	32	4.63^{b}	(0.77)	4.41^{b}	(0.92)
Weight range					
0-10	32	5.69^{a}	(0.83)	5.59	(0.90)
11-20	62	5.36^{a}	(1.37)	5.18	(1.61)
21-50	4	4.94^{ab}	(0.39)	4.79	(0.70)
>51	8	4.25^{b}	(0.64)	4.19	(0.87)

Values significantly different ($P \leq 0.05$) to one another within a row, within a comparison are marked with different superscripts.

It must be borne in mind that in this general analysis the results for both cooked and raw prawns are combined. The unknown types and the coral prawns had the highest bacterial counts; the tigers, kings and greasys form a group while the bananas and endeavours had the lowest counts.

The unknown samples were of cooked peeled meats and were mostly imported. Coral prawns do not fetch a high price, are not very popular are generally incidental to a main catch and are not necessarily well looked after.

Samples obtained directly from the boat had low counts. These bacteria were typical of the natural flora present on the prawn after catching and chilling and the average count of 10^4/g could be considered to be quite low.

Of the storage forms the frozen prawns had low counts and those in slurry (mostly obtained directly from the boat) were also quite low.

The storage form with the highest counts was in the chill store cabinets and these were mostly in the cooked (83%) ready to eat form in which most product is displayed and from which most is sold to the consuming public.

This agrees with the fact that the cooked prawns all had much higher counts than the raw prawns.

Observations on hygiene

Many of the storage facilities particularly the tanks of refrigerated seawater contained water which was contaminated and was obviously not changed for several days (Figure 3.1). This allows the selection and development of a 'soup' of cold tolerant spoilage organisms which can provide a massive contaminating microbial dose to the fresh new product which may have had few, if any, of these organisms as part of its natural flora when it was captured. In addition to this both cooked and raw product were often stored in the same tank. Thus the prawns, which after cooking should have only very low bacterial counts, are recontaminated with high levels of cold tolerant spoilage bacteria. The water in the prawn cookers was often not changed for several days (Figure 3.2). This results in bacterial growth and contamination with bacterial by products in the time when the cooker is not in use between trips. These byproducts then contaminate fresh incoming prawns. The baskets and buckets used for onboard sorting, storage and transport of cooked and raw product were often only poorly cleaned (Figure 3.3) These are poor practices which allow cross contamination and recontamination of product.

In retail display many opportunities for cross contamination were observed with the same utensils such as tongs or scoops being used for the whole range of cooked and raw products as well as to serve or replenish ice. Cooked product was often found in contact with raw product (Figure 3.4) and raw prawns adjacent to other seafood such as oysters which would be eaten raw (Figure 3.5).

Discussion and conclusion

This survey provides a good indication of the bacterial status of prawns in outlets in SE Queensland and is probably typical of the status in other areas. The observations and the microbial counts indicate there is a need for improved procedures at every step in the chain from the boat through to the retail outlets. Better practices are needed onboard boat to prevent cross contamination of cooked and raw product and better control procedures on cooking and cooling are required with more frequent changes of water and attention given to the better cleaning of buckets and other utensils.

The facilities on most boats provide good opportunities for cross contamination of cooked product and this situation persists through the distribution chain. Thus the potential available shelflife of the product is wasted by the time it reaches the retailer. At the retailer more consideration should be given to controlling the temperature and to prevention of cross contamination. The product at the retail stage is thus close to the end of its usable life instead of being only at the beginning. Given that the majority of prawns currently sold retail are cooked better handling and storage procedures starting at the point of catch are essential.
The hazard points where procedures need to be tightened are displayed in Figure 3.6.

One thing is clear, that much of the current material available is not of sufficiently good standard for making into packaged value-added prawn products for export or domestic sale.

This reinforces the need for Quality Assurance programs based on the concepts of Hazard Analysis Critical Control Point resulting in manuals of Best Practice. Such an approach is vital if the industry is to be profitable and survive or diversify. Too much valuable product is currently written down such that it does not reap the full potential return of which it is capable.

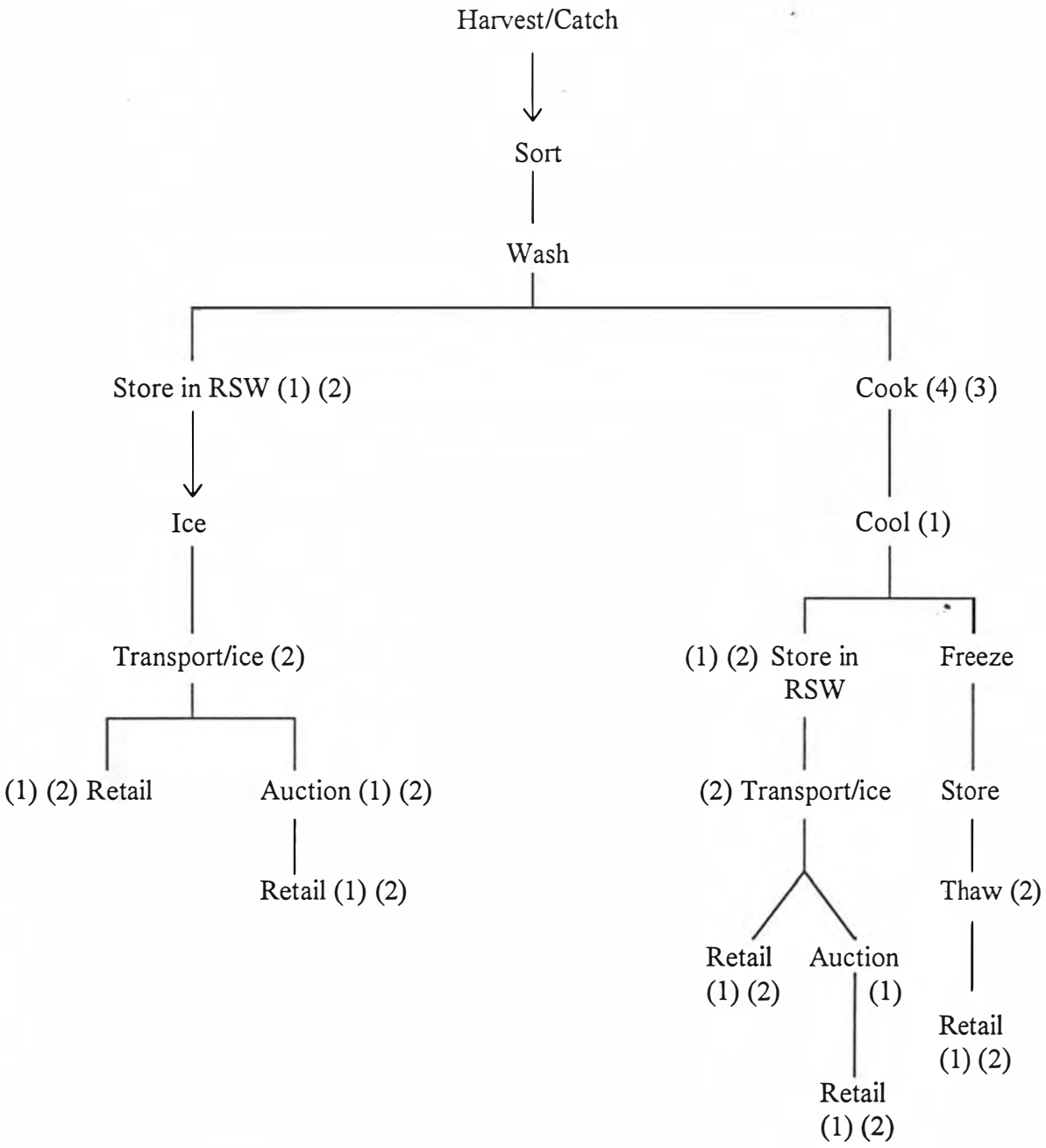
Figure 3.1 Contaminated refrigerated seawater used for storage of prawns

Figure 3.2 Dirty cookwater used to cook prawns in.

Figure 3.3 Dirty buckets into which fresh caught prawns are sorted.

Figure 3.4 Cooked and raw prawns stored adjacent to each other. Note the cooked prawn in the raw tray with another balanced on the edge between them.

Figure 3.5 Close storage of cooked and raw seafoods allowing opportunity for cross contamination



(1) hazard/cross contamination
(2) hazard/microbial growth
(3) hazard/survival of bacteria
(4) pathogen elimination possible

Figure 3.6 Hazard analysis points in prawn processing.

SECTION 4 CHILL STORAGE OF PRAWNS

4.1 Paper published in the Journal of Aquatic Food Product Technology

4.2 Paper published in Australian Fisheries

4.1 Paper published in the Journal of Aquatic Food Product Technology

Spoilage pattern of five species of Australian prawns: deterioration is influenced by environment of capture and mode of storage

This paper contains all the major technical methodology used in the investigations.

4.2 Paper published in Australian Fisheries

Scope for Value Added Prawns

This paper conveys the results and concepts from the above technical paper in plainer language.

SPOILAGE PATTERN OF FIVE SPECIES OF AUSTRALIAN PRAWNS: DETERIORATION IS INFLUENCED BY ENVIRONMENT OF CAPTURE AND MODE OF STORAGE

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Stephen J. Thrower
Stephen M. Nottingham

ABSTRACT. Five species of commercial prawns *Penaeus plebejus, P. merguiensis, P. semisulcatus/P. esculentus* and *M. bennettae*, were obtained from South-East and North Queensland, chilled soon after capture and then stored either whole or deheaded on ice and ice slurry, until spoilage. Total bacterial counts, total volatile nitrogen, K-values and total demerit scores were assessed at regular intervals. Their shelf lives ranged from 10-17 days on ice and >20 days on ice slurry. Initial bacterial flora on prawns from shallower waters (4-15 m) were dominated by Gram-positives and had lag periods around 7 days, whereas prawns from deeper waters (100 m) were dominant in *Pseudomonas* spp. with no lag periods in bacterial growth. The dominant spoiler in ice was mainly *Pseudomonas fragi* whereas the main spoiler in ice slurry was *Shewanella putrefaciens.* Bacterial interactions seem to play a major role in the patterns of spoilage in relation to capture environment and pattern of storage.

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INTRODUCTION

There are over fifty recorded species of penaeid prawns in Australian waters of which ten are considered commercially important (Grey *et al.*, 1983). These are caught in a range of habitats and climatic conditions ranging from shallow sandy coastal and estuarine waters to deeper offshore waters from the tropical areas of the Gulf of Carpentaria in the north to the cooler waters of Spencer Gulf in the south. The catch is valued at about A$260 million (Anon., 1993) and much of this is frozen unprocessed for export. There has been little development of processed chilled product for the domestic market. Prawns are commonly sold loose in the cooked, chilled state in fish retail outlets and supermarkets. Pre-packaged chilled products should have a market advantage provided they are well presented and have sufficient shelf life in the distribution and sales system so it is essential to understand the pattern of microbial spoilage in order to process such products.

The storage life of chilled seafood is dependent on the nature and number of the initial microbial flora as well as the conditions of storage. The initial flora is a function of the environment of capture and most work regarding this influence has been done on fish species not on crustacea.

Liston (1992), citing several studies on tropical fish from both cooler and warmer regions, concluded that the temperature of the water in which fish live is an important determinant of shelf life. Fish from warmer waters (Adriatic sea, Indian and Pacific Oceans, South Africa and Australia) carry more mesophilic Gram-positive bacteria such as *Micrococcus*, coryneforms and *Bacillus* (Shewan, 1977), rather than Gram-negatives which are common in colder waters. Wood (1952) described several species of *Corynebacterium*, micrococci (common in estuarine mud but rare in sea water), *Bacillus* and *Pseudomonas* from marine

environments, off eastern Australia. Commercial species of Australian prawns are trawled from both off-shore and in-shore fishing grounds and studies have indicated that the initial bacterial flora present varies with locality (Gillespie, 1980). The spoilage bacteria *Shewanella putrefaciens* appears to occur naturally in waters and soil as well as spoiling fish, crustacea and poultry, (Shewan, 1976). Thus, in addition to other factors the bacterial species initially present as a consequence of the environment of capture would influence the rate of spoilage which, in turn, determines shelf life.

The shelf lives of tropical shrimp under chill storage conditions have been reported as 16 days on ice (Cann, 1974), 13 days on ice (Jayaweera and Subasinghe, 1988) and 13 days at 0°C (Shamshad *et al.*, 1990) whereas non-tropical shrimp (*Pandalus* and *Nephrops)* species are totally spoiled after 8-10 days (Walker *et al.*, 1970). Bacterial spoilage occurs mainly on the surface of crustaceans at chill temperatures (Miget, 1991).

The heads of prawns contain 75% of the prawn's bacterial population (Green, 1947) as well as high levels of enzymes that are responsible for the development of melanosis (blackening) of prawns. Deheading reduces the risk of melanosis. Cobb (1977) reported that the shelf lives of prawns depended on whether they were stored head-on (5 days) or deheaded (> 14 days). In contrast, work from Thailand, indicates that "beheading of shrimp did not improve the storage life to the extent found in European and North American shrimp" (Cann, 1974).

The present study evaluates the pattern of storage life of five commercially important species of prawns in relation to their capture environment and chilled storage environment.

MATERIALS AND METHODS

Transport, method of storage and sampling

All species were trawled from commercial fishing locations as listed in Table 1. A random sample of prawns (about 12) was aseptically removed from the sorting tray prior to sorting of catch (except for tiger prawns) for microbiological analysis, while the rest were sorted from by-catch, rinsed to remove surface debris in sea water and held according to usual practice in refrigerated sea water tanks till landed ashore (1-4 hours). After this they were taken to the laboratory in an ice slurry (1-2 hours). The tiger prawns were kept alive for 24 hours; they were then placed in ice, a sample taken as stated above and then flown from Cairns to Brisbane, so that initial tests could be done a few hours after death as with the other species.

The king and banana prawns were stored both whole and deheaded both on ice and ice slurry (3:1, ice:water), whereas tiger and greasy prawns were stored whole both on ice and ice slurry. The boxes containing all prawn treatments were stored in a 1-3°C cold room for easy maintenance of temperature. The temperature of prawns in ice and ice slurry was monitored daily and maintained at 0-1°C. The slurry was replenished as required. Iced prawns were stored in fish boxes, perforated to allow drainage.

Every 2-3 days, a random sample of prawns (10-16) was taken from each treatment for microbiological, chemical and sensory analysis, and this continued until the prawns were spoiled.

Sensory Analysis

A demerit scoring system was developed (Table 2) in which the visible and taste characteristics of king, greasy, tiger and banana prawns were assessed by an experienced panel of eight. Nine major characteristics were assessed (Table 2): eight characteristics pertained to

changes in raw prawns and one (taste) to cooked prawns. Individual prawns were deheaded and placed (unwashed) in a sealed bag which was immersed in rapidly boiling water for 3 minutes, then cooled in ice water.

Microbiological Analysis

Total bacterial counts (at incubation temperatures of 5° and 30°C) were estimated by plating serial dilutions of prawn samples, prepared by homogenising (in a Colworth Stomacher) 10 g of prawn (by macerating 3-5 prawns aseptically) in 90 mL of sterile peptone (0.1% w/v) diluent. Surface plating techniques were used to obtain a total bacterial count. This analysis was done in duplicate. Total H_2S producers were tested according to Gram *et al.* (1987). All colonies from a sector of the plate were restreaked onto nutrient Agar (BBL). Once pure colonies were obtained, they were transferred to peptone yeast extract agar slopes and stored for biochemical identification. All colonies were tentatively identified by the following reactions: (a) Gram reaction in 3% KOH (Gregersen, 1978), (b) hanging drop for testing motility and morphology, (c) oxidase reaction using oxidase test strips (dp diagnostics, Australia) , (d) catalase using 3% H_2O_2. Gram-positive isolates were classified according to Sneath *et al.* (1986). The Gram-negatives were tested for glucose metabolism as in Hugh and Leifson (1953). Those tentatively identified as Pseudomonads or *Shewanella* spp. were tested for (a) reduction of trimethylamineoxide (TMAO) in TMAO medium (Gram *et al.*, 1987), (b) extracellular hydrolases (on gelatine, starch and DNA test agars), (c) diffusible fluorescent pigments and (d) ornithine and lysine decarboxylases (tested as in Hendrie and Shewan, 1979). The initial flora was classified into *Pseudomonas* I, II, III/IV according to the scheme of Shewan *et al.* (1960). The flora from spoilage was further classified to species level according to Hendrie and Shewan (1979), Jorgensen and Huss (1989). Under these schemes *P. fragi*

would be classified in group II. *S. putrefaciens* (previously *P. putrefaciens*) would occur in groups III/IV.

Chemical Analysis

Total volatile nitrogen (TVN), pH and K-value were analysed according to Wong *et al.* (1991).

Estimation of storage life

Storage life was based on the time taken for bacterial counts to reach 10^8 CFU/g and TVN increase above a level of 30 mg N/100 g flesh.

RESULTS

Sensory Analysis

Total demerit scores

Total demerit scores obtained for all prawns steadily increased with storage; total scores for prawns stored in ice were always higher than for those stored in slurry (Figures 1 and 2). Higher scores represent a gradual loss of quality associated with deterioration. Melanosis, odour, and taste were the three characteristics most associated with deterioration of prawns.

Melanosis

Ice: Stored prawns had a high occurrence of melanosis, especially in whole prawns.

Slurry: Stored prawns had developed only *slight* melanosis, (scores less than 1, Table 2) for almost all the storage period. Deheaded banana and king prawns had the lowest scores.

Off odours

Ice: In king prawns (whole and deheaded), perceivable off odours (scores 1-2, *slight* to *moderate* odour) developed on the 10th day, whereas, in tiger and banana prawns, perceivable

off odours developed after the 14th and 21st days. In greasy prawns off odours were first perceived on the 3rd day and were not spoilage odours.

Slurry: Off odours developed later in all species than when they were stored on ice (after 10 days in king, 12-20 days in banana and 21 days in tiger prawns). Off odours in king and banana prawns were sulphurous, but they developed more in the slurry itself, rather than in the prawns.

Taste

Ice: In whole king, banana, tiger and greasy prawns, taste scores were rated as *very good to good* up to 9, 7, 6 and 6 days, respectively.

Slurry: Scores in ice slurry were rated *good* for 4, 2 and 1 days for banana, greasy and tiger prawns, after which they remained *fair* till spoilage. Lower scores were given in the early stages of storage and panelists commented on the loss of typical prawn flavour. Thus, these low scores in the early stages of storage arose from loss of flavour rather than spoilage. In contrast, scores for king prawns remained *good* for >10 days.

Microbiological Analyses

Total bacterial counts

The counts obtained at 5°C incubation (not presented) were similar to counts obtained at 30°C incubation temperatures indicating the psychrotrophic nature of the spoilage flora. Towards the end of storage, the flora on all prawns stored on ice slurry were dominant in H_2S producers (not presented), whereas the flora on those stored on ice, were not.

At the time of capture, initial bacterial counts for king and banana prawns (Figure 3) ranged between 10^3-10^4 CFU/g (30°C incubation), whereas in tiger and greasy prawns (Figure 4) they were higher, 10^5-10^6 CFU/g (30°C incubation). On the 2nd day the counts in

tigers (10^4 CFU/g on ice and 10^2-10^3 CFU/g on slurry) were lower (Figure 4). A similar decrease was observed in the greasy prawns as well, indicating the mesophilic nature of the initial flora at the time of capture and their failure to thrive in chilled conditions.

Ice: Bacterial counts reached 10^8 CFU/g (30°C incubation) after 10 days for whole king prawns (11 days deheaded) and 15 days for banana prawns stored either whole or deheaded in ice (Figure 3). Counts of 10^8 CFU/g are often associated with the limit of acceptability. A lag in bacterial counts from the 0-8th day was observed in banana prawn, whereas periods of decrease in bacterial counts were observed with tiger and greasy prawns (Figure 4). Such patterns were not observed in king prawns. Total bacterial counts reached 10^8 CFU/g in tiger and bay prawns on the 14th and 17th day.

Slurry: For prawns stored in ice slurry total bacterial counts took 21 days to reach 10^8 CFU/g (30°C incubation) for king (whole and deheaded), tiger (whole) and banana (deheaded) prawns. Whole greasy and deheaded banana prawns reached 10^8 CFU/g by 17 days.

There were no clear differences in total bacterial counts between storage in ice or slurry for up to 2, 5 and 7 days with king, greasy and tiger prawns. In banana prawns a clear difference was observed only after 14 days of storage in ice and slurry.

Total counts of 10^8 CFU/g were associated with off odours, and total counts of 10^9 CFU/g were associated with strong spoilage odours.

Identity of initial flora

The initial flora on king prawns was dominated by pseudomonads (65%) along with 24% irregular non-sporing Gram-positive rods, whereas banana prawns had fewer pseudomonads (only group II, 19%) and 38.5% irregular non-sporing Gram-positive rods

(Table 3). Some of the flora in banana (11.5%) and tiger (80%) prawns showed no growth on subculturing and so could not be identified (Table 3 and 4). The tiger prawns were obtained from the far north of Queensland, a more tropical region, unlike the others which came from the Southern Queensland region. The greasy prawns were dominant in Gram-positives (91%), comprising mostly irregular non-sporing Gram-positive rods (76%) as well as micrococci (15%) and a few pseudomonads (1.5%) (Table 4).

Ice: After 2 days of storage in ice, king prawns showed considerable increase in pseudomonads (75%), whereas total pseudomonads in banana prawns remained at (11.5%), with a decrease in the proportion of group II . There was an increase in the irregular non-sporing Gram-positive rods, whereby the total percentage of Gram-positives was 51%. The distribution of the flora in tiger and greasy prawns was not analysed on the 2nd day of storage.

Slurry: After 2 days in slurry, total pseudomonads (groups I-IV) in king and banana prawns were 79% and 19% of the population respectively. Similarly in king and banana prawns group III/IV pseudomonads increased to around 10% of the population. The Gram-positives in banana prawns also increased to 62%. Thus, the handling at the early stages of storage caused some changes in the flora but did not completely alter its composition or its incidence.

Spoilage flora

As spoilage proceeded, the counts of H_2S producers showed a gradual increase with time in prawns stored in ice slurry. *Shewanella putrefaciens* dominated at the end of spoilage (in ice slurry), Table 5, and strong sulphurous odours developed. In contrast, the spoilage flora in all ice-stored prawns were dominated by *P. fragi* where fruity odours intensified towards the end of spoilage.

Biochemical Analyses

Total volatile nitrogen

The initial TVN levels ranged between 22-34 mg N/100g flesh (as high as rejection levels), varied between species (Figure 5). At the initial stages, TVN levels remained almost the same, or even decreased slightly, until later in the storage period.

Ice: When bacterial counts reached 10^8 CFU/g, TVN values were also around 30 mg N/100 g flesh (Figure 5), after which there was a rapid increase in TVN after 10 days for king prawns and after 14 and 15 days for tiger and banana prawns respectively.

Slurry: For prawns stored in ice slurry, the TVN values appeared to rise marginally towards the end of storage (>20 days) in all species of prawns.

K-values

Increases in K-value occurred over the whole storage period (Figure 6) whether prawns were stored in ice or in slurry, although there was an apparent increase followed by a decrease in the king prawns. The initial K-value in the tiger prawns was remarkably high. No differences were observed for deheaded king and banana prawns stored either in ice or in slurry.

With the exception of the tiger prawns, the slopes for change in K-value were similar, in the range 1.6-1.4% per day over 20 days. The slope for the tiger prawns (which had a much higher starting value) was nearer 0.7% per day.

Shelf lives of prawns

A storage life of 10-11 days on ice was obtained for king prawns, whereas greasy, banana and tiger prawns had shelf lives of 17, 15 and 14 days respectively. In contrast, all species of prawns stored on ice slurry had shelf lives in excess of 20 days.

Correlation coefficients

Significant ($P<0.05$) relationships between demerit scores and total bacterial counts (30°C incubation) were found, r^2 = 0.902, 0.919, 0.633 and 0.800 for king, banana, tiger and greasy prawns respectively. A similar relationship was obtained for demerit scores and total bacterial counts at 5°C incubation, r^2 = 0.819, 0.923, 0.773 and 0.537 for king, banana, tiger and greasy respectively.

DISCUSSION

The five species of prawns obtained from commercial grounds and handled under optimum chilled storage conditions exhibited a variation in shelf life. The difference in storage lives found with these four types of prawns (one type being made up of two similar species) was attributed to the nature of their initial bacterial flora as well as to their subsequent spoilage flora which developed on them. The initial flora was environment dependent, where as the spoilage flora, mainly *Pseudomonas* spp. (on ice) and *S. putrefaciens* (on slurry) were dependent on the storage environment. Storage lives of 10-17 days were obtained on ice in contrast to longer storage lives (>20 days) with storage of prawns in ice slurry.

There was little difference in bacterial counts between whole and deheaded prawns. The decrease in bacterial numbers at the initial stages and the failure of some of the species to grow (especially for tiger prawns) at chill temperatures, indicate that the high counts were a factor of the environment of capture rather than deterioration. These bacterial species would have little to do with spoilage. High initial bacterial counts (10^5-10^6 CFU/g) on the tiger and greasy prawns have previously been reported for Australian prawns (Gillespie, 1980). The onboard rinsing , handling procedures and storage in refrigerated seawater did not drastically alter the dominant bacterial profiles.

The influence of initial flora on pattern of spoilage

The distribution of the initial flora seems to be influenced by the environment of capture. Prawns obtained from shallower waters (banana 4 m and greasy 10 m) were dominant in soil-related Gram-positive bacteria (50% and 75% respectively) while king prawns obtained from off-shore deeper waters (110 m) were dominant in cold-loving *Pseudomonas* (65%) on capture. King prawns occur in off-shore oceanic waters up to 220 m deep, while greasy and banana prawns occur on muddy bottoms up to 14 and 45 m deep respectively (Grey *et al.*, 1983). Freshly caught (at 9 m) mixed species of Indian shrimp have a flora dominant (60%) in Gram-positives (Krishnamurthy and Karunasagar, 1986), a pattern observed in the present study. Thus the difference in bacterial spoilage potential is related to the time taken for the main spoiler to gain dominance over the initial flora present on prawns on capture.

There appeared to be a lag phase lasting for 5-6 days in the growth of bacteria on the banana prawns. This lag period coupled with the real decreases in bacterial numbers seen with the tiger and greasy prawns is responsible for the longer shelf lives of these tropical prawns. High counts of *Pseudomonas* lead to shorter shelf lives in prawns (present study) and nile perch, (Gram *et al.*, 1989). Low counts and slower growth of tropical psychrotrophic bacteria on nile perch results in a long storage life which is shortened considerably if they are inoculated with *Pseudomonas* (Gram *et al.*, 1989). The longer shelf lives for banana, tiger and greasy prawns are related to the initial dominance of a non-spoiling flora which is directly related to the type and nature of the capture environment. The implication (unproven, as yet) is that the same species of prawn from different locations may have a different storage life according to the natural microflora present on it.

Difference between ice and ice slurry

At the initial stages of storage there was little difference in quality between prawns stored in ice and ice slurry though ice slurry gave better protection against melanosis. The high taste scores given to tiger, banana and greasy prawns stored in slurry was due to a decrease in flavour intensity attributed to the gradual leaching out of flavour components. This occurred while bacterial counts were still low and no incipient spoilage was evident. Loss of amino acids and water-soluble phosphates (Rajendrath and Bose, 1965) can seriously affect taste. It is not known why the king prawns did not suffer the same loss of flavour.

The implications are that prawns with higher initial loads of Gram-positive non-spoilers could be stored in any storage medium but flavour retention (an important aspect in further processing) should be taken into account with respect to the duration of storage. For prawns with high pseudomonad counts on capture, storage in slurry would be preferable, particularly for king prawns where flavour loss was not a problem.

The storage environment and spoilage

The environment of storage also seems to determine the type and nature of the main spoilers under chilled storage conditions, where oxygen availability as well as levels of available free amino acids could be major factors. The spoiler dominant in ice stored prawns was *P. fragi*). This species is associated with fruity off odours and was responsible for spoilage of tropical prawns (Cann, 1974; Krishnamurthy and Karunasagar, 1986). The spoiler dominant in ice slurry, *S. putrefaciens*, was responsible for production of the strong sulphurous off odours and is a well documented spoiler of fish from mostly colder regions (Shewan, 1977). The organism *P. fragi* is a spoiler of fish and prawns from tropical areas. Gram (1993) observed that strains of *Pseudomonas* inhibit *S. putrefaciens* and suggested that the selection of microflora during storage is not determined by growth rate alone but is also influenced by microbial

interaction. The selection of two different spoilers in the present study could be due to the bacterial interactions related to the environment of storage.

Sensory changes and the assessment of storage life

The demerit scoring system developed by Bremner *et al.* (1986) was modified for prawns and showed high correlation with total bacterial count especially for king and banana prawns (r^2 = 0.902, 0.919 respectively). This system was thus suitable for assessing the total changes associated with visual and taste variations in evaluating quality loss rather than using either of them individually. The system is also useful in evaluating flavour/taste changes occurring independent of bacterial spoilage, which could affect storage life estimations.

Biochemical changes and the assessment of storage life

TVN values at the initial stages were high (22-34 mg/100 g) and showed little change until spoilage, similar to observations in tropical prawns (Cann, 1974) and tropical brackish water prawns (Reilly *et al.*, 1984). TVN levels proved useful in corroborating the occurence of spoilage along with increase in total bacterial counts (10^8 CFU/g) at the latter stages of spoilage. This increase was more evident with prawns stored on ice. Cheuk *et al.* (1979) obtained a good correlation between total bacterial counts and TVN where levels remained constant for 11-15 days (similar to the present study) on brown and pink shrimp (Gulf of Mexico). In the present study TVN values were only useful indicators at the latter stages of storage. Some initial values for TVN were higher than previously reported initial values (Lannelongue *et al.* 1982) and limits of acceptability of 30mg/100g flesh for fish (Connell 1990). This demonstrates that standard rejection values cannot be applied to all species of prawns.

The initial K-value for tiger prawns was high (50%), closer to the range (35-40%) reported for spoiled brackish water prawns (Reilly *et al.*, 1984). A higher rate of accumulation

of hypoxanthine in chilled Australian king prawns than in banana prawns, was reported by Gillespie (1980), a trend similar to the present study. In other work carried out at this laboratory (Paterson *et al.*,1995*)* found a K-value of 57% in a sample of live *P. esculentus* caught by trawling. The present high K-value in tiger prawns, could be related to the stress during capture, handling and icing. Thus K-value is not neccessarily a good indicator of freshness for prawn. Deheading as well as the storage of prawns in ice or slurry made no difference to the rate of change in K-value.

Presentation style and storage life

The presentation style (whole/deheaded prawns) is an important factor in the processing (packaging) and marketing of different products. There was little difference in the storage life of whole and deheaded prawns. The main purpose of deheading, which proved effective for deheaded king and banana prawns, was to reduce the risk of melanosis (due to elimination of the hepatopancreas and certain tissues, which reduce the polyphenyl oxidase content and associated precursors). Work on Australian prawns indicates that compounds responsible for development of iodoform and garlic off flavours (occurring in some species of prawns) are also concentrated in the head (Whitfield, 1990).

CONCLUSION

This work has shown that considerable shelf lives can be achieved with some types of prawns according to the circumstances of capture environment and storage medium.

The storage lives of the four types of prawns studied here was greatly influenced by the initial bacterial flora from the capture environment. If this flora was proportionately high in Gram-positive organisms, they failed to thrive and played little or no part in spoilage. It then

took several days for a Gram-negative spoilage flora to become established and then to cause spoilage.

Storage in ice resulted in a flora mainly comprising *P. fragi* which produced spoilage compounds with odours and flavours typical of rotting tropical fruits that gradually increased in intensity. Storage in ice slurry resulted in the selection of mainly *S. putrefaciens* which produced strong sulphurous odours and flavours at the latter stages of storage.

This knowledge of the interaction of the capture and storage environments provides basic information on which industry can make informed choices in adoption of handling and storage practices. The work also confirms the role of *P. fragi* as a spoilage organism of great significance in chilled stored warm water prawns.

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Table 1: Prawn species and catch details

Type of Prawn	Location	Trawl Depth (m)	Average[4] weight (g)	Trawl Duration (min)	Month/ water temp.
[1]Eastern King *Penaeus plebejus* Hess, 1865.	23° S 153° E Mooloolaba	110	60	30	Oct. approx. 25°C
Banana *P. merguiensis* de Man, 1888.	27° 18.5'S 153° 15.4'E Moreton Bay	4	30	30	May approx. 20°C
[2]Brown & Grooved Tiger *P. esculentus* Haswell, 1879b *P. semisulcatus* de Haan, 1850.	14° 54' S 145° 16' E Cairns	9	30	30	June approx. 25°C
[3]Greasy *Metapenaeus bennettae* Racek & Dall, 1965	27° 15' S 153° 27' E Moreton Bay	15	4	30	Feb. approx. 27°C

1 Simplified to king prawn throughout the text; obtained off shore

2 The brown tiger and the grooved tiger prawn are found together, caught and marketed together and are very closely related. Simplified to tiger prawn in text.

3 Known locally also as bay prawn

4 Based on a random sample of 20 prawns

Table 2: Visual observation

Name: ..... Date: .....

	Sample				General Comments
	A	B	C	D	
APPEARANCE 0 High sheen/neutral/characteristic translucence 1 Slightly dark/less translucent 2 Dull dark/opaque					
HEAD 0 Firmly attached/no discolouration/organs distinct 1 Slightly loose or drooped/slightly red or green 2 Loose/membrane broken/moderate discoloration 3 Very loose or absent, or washed-out colour					
SHELL CONDITION 0 Normal/smooth 1 Coarse/rough 2 Gritty/slimy					
MELANOSIS 0 No melanosis 1 Slight blackening of head or shell margins 2 Head black/moderate blackening of segments 3 Extensive blackening of head and shell					
CHARACTERISTIC ODOUR 0 Absent or fresh sea smell 1 Moderate prawn smell 2 Slight prawn smell 3 Loss of characteristic odour					
"OFF" ODOUR 0 None 1 Slight 2 Moderate 3 Strong					
PEELED MEAT 0 Firm/elastic 1 Slimy/rough					
FLESH APPEARANCE (in cross-section) 0 Translucent 1 Slight opaque 2 Opaque					
TASTE 0 Very good 1 Good 2 Fair 3 Poor (*reject*)					

Table 3: Initial bacterial flora[1] on king and banana prawn[2] on capture (0 d) and after 2 days (2 d) of storage on ice and ice slurry

Bacterial Species	Types of prawn					
	King			Banana		
	0 d %	2 d/ice %	2 d/slurry %	0 d %	2 d/ice %	2 d/slurry %
Pseudomonas GpI	10.3	25.0	13.8	-	-	-
Pseudomonas GpII	51.7	46.9	55.2	19.2	8.5	8.1
Pseudomonas GpIII/IV	3.4	3.1	10.3	-	3.0	10.8
Irregular Non Sporing Gram-positive rods	24.1	-	13.8	38.5	43.0	56.8
Micrococci	3.4	-	-	7.7	8.6	5.4
Other Gram-positives	-	-	-	-	-	-
Other Gram-negatives	-	-	6.9	23.0	37.2	18.9
No Growth on subculturing	6.8	25.0	-	11.5	-	-
Total Colonies (Nos.) isolated	(29)	(32)	(29)	(26)	(35)	(37)
Total count/g	app.10^4	app.10^6	app.10^5	app.10^4	app.10^4	app.10^4

1 from 30°C incubation

2 whole and deheaded

Table 4: Initial bacterial flora[1] on tiger and greasy prawn[2] on capture

Bacterial Species	Types of prawn	
	Tiger %	Greasy %
Pseudomonas GpI	-	-
Pseudomonas GpII	-	-
Pseudomonas GpIII/IV	-	1.5
Irregular Non Sporing Gram-positive rods	-	75.8
Micrococci	3.3	15.1
Other Gram positives	-	-
Other Gram negatives	16.6	7.5
No Growth on subculturing	79.9	-
Total Colonies (Nos.)	(30)	(66)
Total counts/g	app. 10^5	app. 10^5

1 from 30°C incubation

2 whole and deheaded

Table 5: Spoilage flora[1] on king, banana, tiger and greasy prawn[2] stored in ice and ice slurry

Prawn species		Bacterial species		
		P. fragi	*S. putrefaciens*	others
		%	%	%
[3]Banana	- ice	87.0	8.0	5.0
	- slurry	-	100.0	-
[4]King	- ice	88.4	11.2	-
	- slurry	31.2	58.3	10.4
[5]Tiger	- ice	61.3	9.7	29.1
	- slurry	11.1	88.9	-
[6]Greasy	- ice	53.3	6.6	39.9
	- slurry	6.7	80.0	13.3

[1] 30°C incubation
[2] whole and deheaded
[3] after 23 days/(64 colonies)
[4] after 21 days/(105 colonies)
[5] after 22 days/(58 colonies)
[6] after 19 days/(30 colonies)

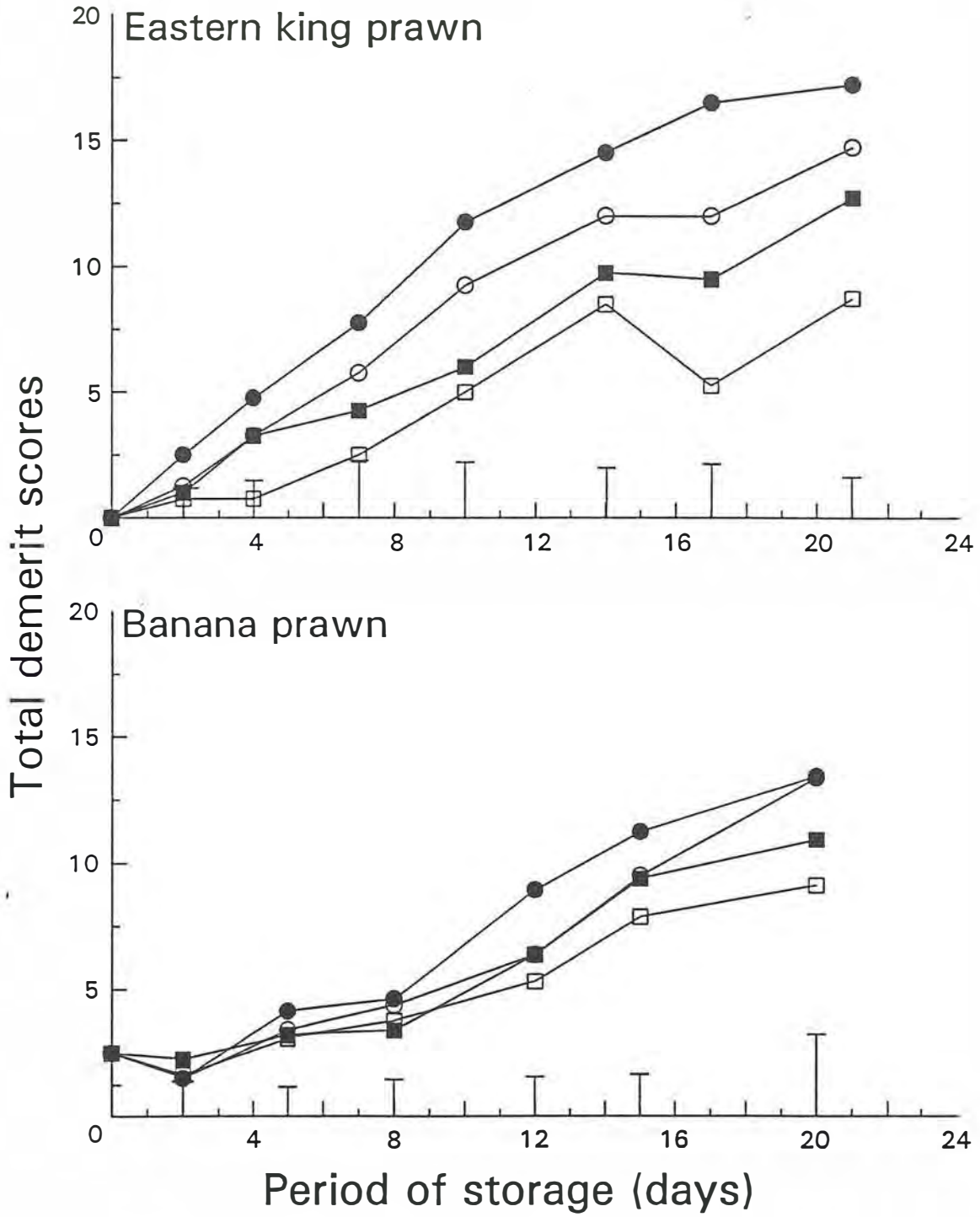


Figure 1: Change in total demerit score for prawns stored whole in ice (●), whole in ice slurry (■), deheaded in ice (○), and deheaded in ice slurry (□).

vertical lines represent the LSD at the 5% level

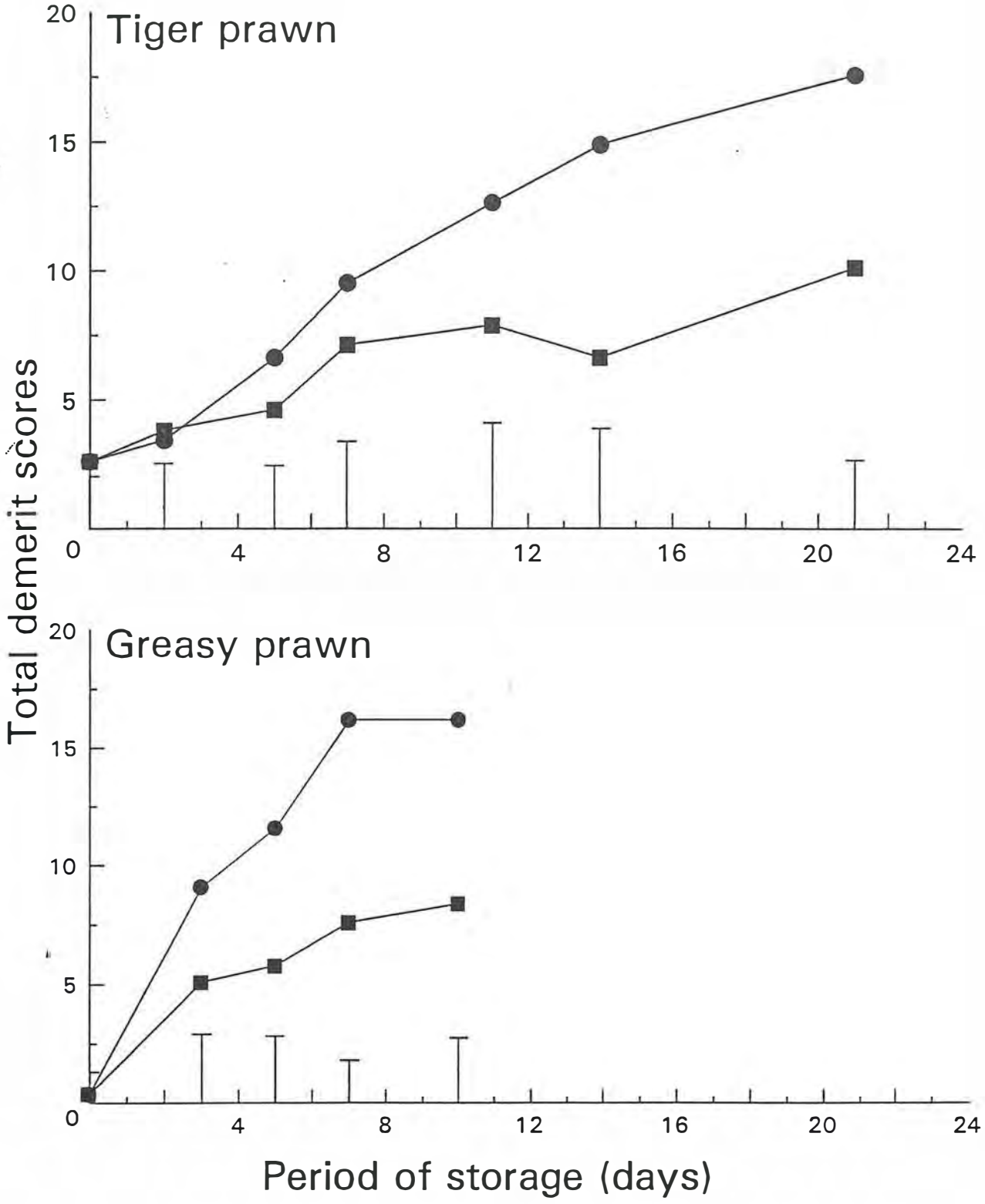


Figure 2: Change in total demerit score for prawns stored in whole in ice (●), whole in slurry (■).

vertical lines represent the LSD at the 5% level

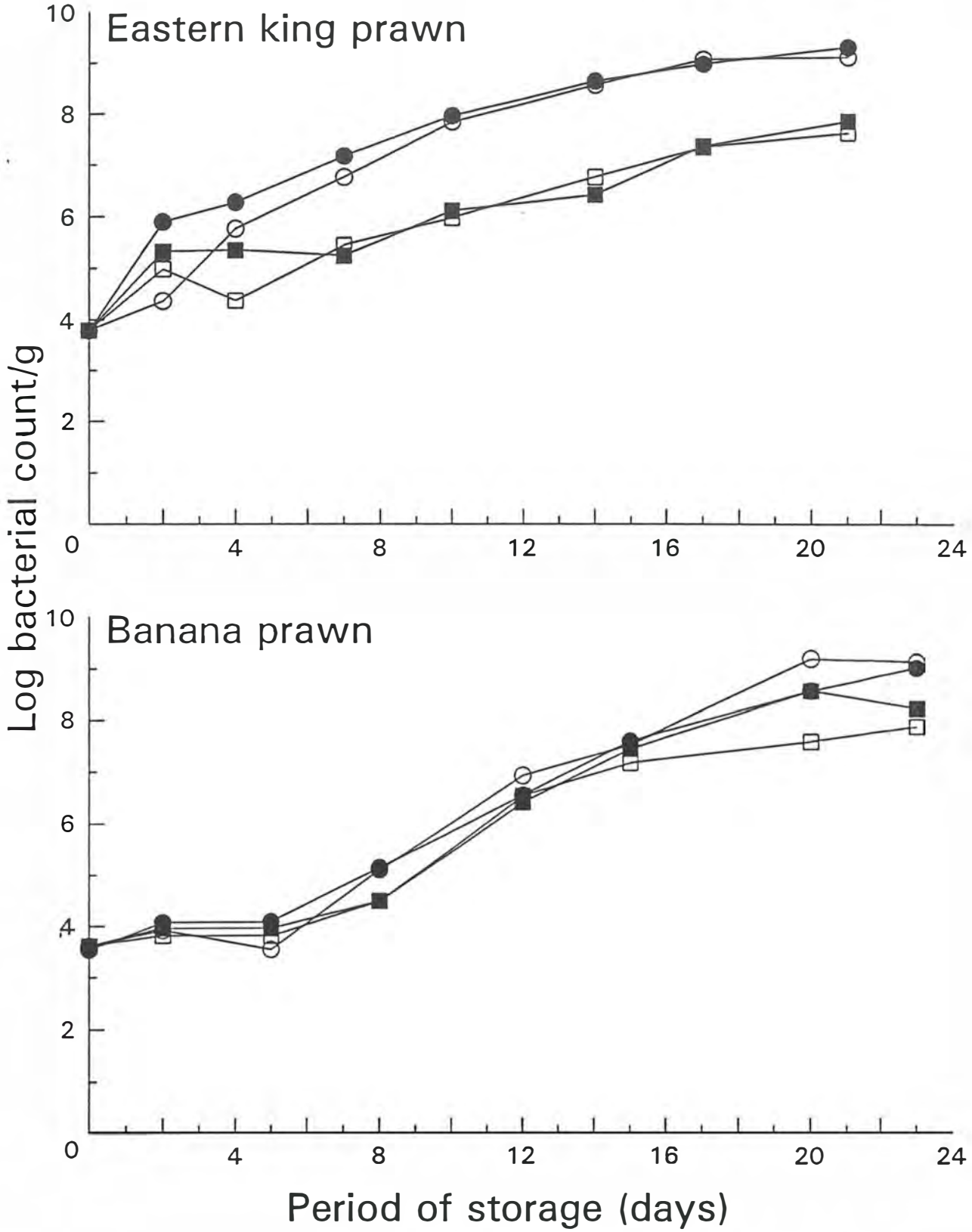


Figure 3: Change in total bacterial count (30° incubation) for prawns stored whole in ice (●), whole in slurry (■), deheaded in ice (○), and deheaded in slurry (□).

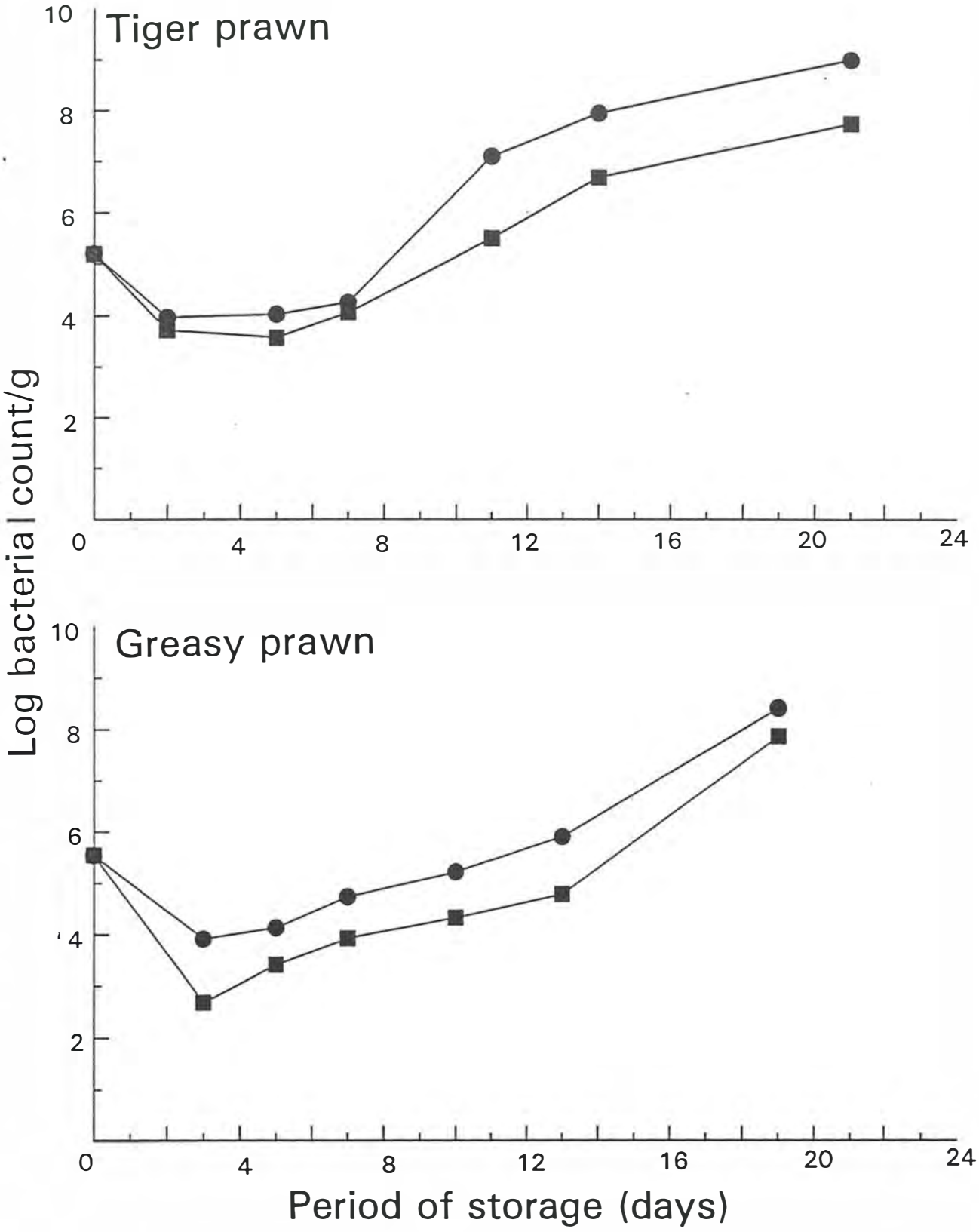


Figure 4: Change in total bacterial count (30°C incubation) for prawns stored whole in ice (●), and whole in slurry (■).

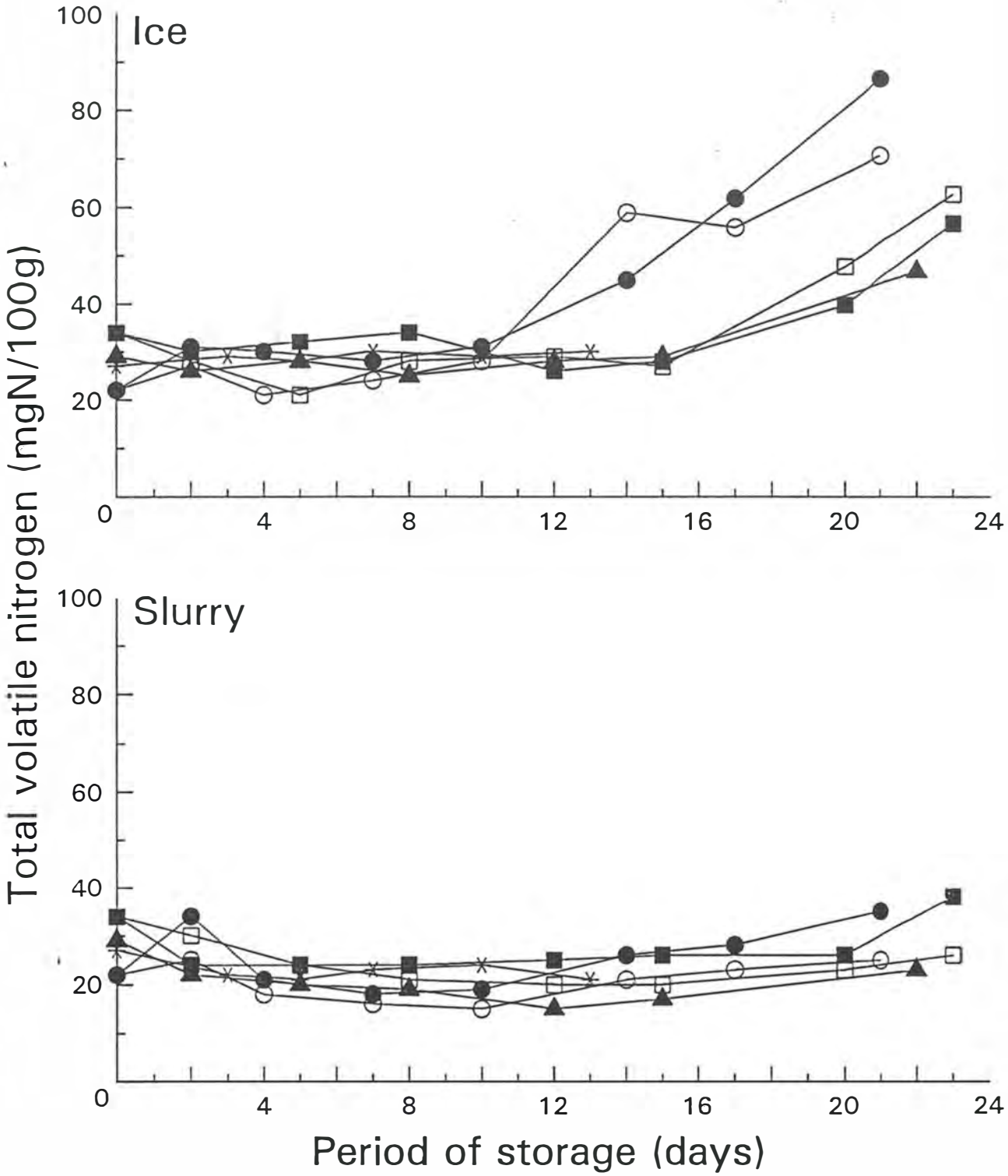


Figure 5: Change in total volatile nitrogen in king-whole (●), king-deheaded (○), banana-whole (■), banana-deheaded (□), tiger-whole (▲), and greasy-whole (∗).

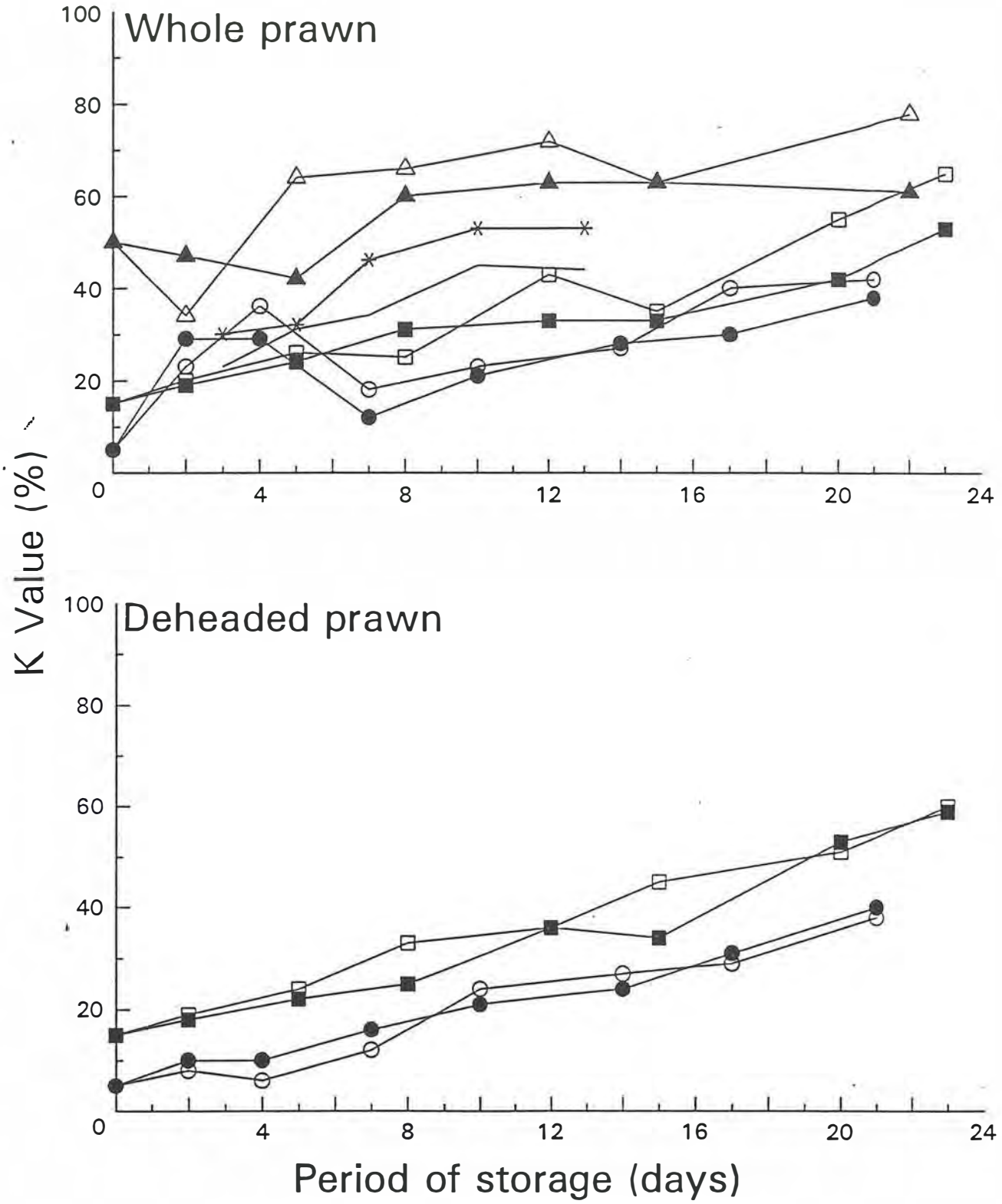


Figure 6: Change in K-value in king in ice (●), king in slurry (○), banana in ice (■), banana in slurry (□), tiger in ice (▲), tiger in slurry (△), greasy in ice (∗), and greasy in slurry, (–).

Scope for value adding prawns

There is much potential for placing chilled and value added prawn products on the supermarket shelves according to the results of work done recently at the International Food Institute of Queensland. Nalini Chinivasagam, Allan Bremner and Steve Thrower report.

Researchers from the International Food Institute of Queensland (IFIQ) have examined the technical problems involved in storing raw untreated prawns destined for further processing. They found that different storage conditions result in different types of spoilage bacteria and also that prawns from different environments harbour different bacterial types when caught. These results can be used to advantage to plan the logistics of production and distribution according to species available.

There has been little development of chilled prawn products for the Australian retail market. Prawns are generally sold by weight in the cooked, chilled form and are displayed loose in supermarkets, delicatessens, fishmongers and fish and chip shops. Many displays are substandard and not attractively presented.

This form of product is quite messy, not convenient, often smelly and is certainly not well liked by supermarket staff. In short, to many people, this is an inconvenient, unattractive way to purchase a product.

Furthermore, the whole prawn is offered for sale leaving the customer with the task of heading, peeling, de-veining and preparing it for the table. These discarded heads and shells must then be disposed of and as highly perishable materials, are notoriously offensive in domestic rubbish.

There is a need to attract consumers who do not specifically eat seafood due to the lack of knowledge of preparation, uncertainty about its quality and because of the bother of cleaning and disposal of the waste.

There is, therefore, considerable interest in developing pre-prepared, convenient products for the retail trade. These must be of sound microbial quality, with sufficient shelf life in the distribution system. They also must have sufficient remaining shelf life to allow storage in the home for a convenient length of time without the development of unacceptably high levels of bacteria.

Understanding bacterial flora

Before these aims can be achieved it is necessary to understand the nature, number and growth rate of the bacterial flora on the prawns. These factors determine the shelf life and safety of chilled prawns and prawn products. Despite a long history of domestic sales of prawns in this country there is very little information on the nature and numbers of the bacteria associated with them from the point of catch through to the point of sale.

Recent surveys of material available in south east Queensland in fish shops, supermarkets and wholesalers indicate that microbial standards are poor and that product offered for sale has little or no remaining shelf life and should be cooked or consumed that day.

To prepare products with reasonable shelf lives it is necessary to aim for a low initial bacterial count and to use means of inhibiting or slowing the growth of spoilage bacteria. Prawns handled well at capture could have a low initial count. Higher initial counts would not necessarily indicate a short shelf life if bacteria present are of non spoiling types.

The bacteria on the prawns are a reflection of the environment from which they are captured. The proportion of non-spoiling bacteria at the time of capture has an influence on the chilled shelf life of the prawn. Different types of bacteria could be found on the same species of prawn captured from different environments or areas with respect to depth or temperature.

The work reported here is a preliminary study on which to base manufacturing protocols that will result in products with reliable, stable properties.

Chilled storage studies

The first steps were to examine the natural flora present on the prawns and to note the spoilage patterns in chilled storage. Four types of prawns: king, tiger, banana and greasy were obtained under commercial conditions as fresh as possible in an untreated state (not dipped in sodium metabisulphite). These prawns were obtained from Moreton Bay, Mooloolaba and Cairns.

They were stored either in ice (0°C, with removal of drip water), in ice slurry (2:1, ice:water and temperature 0–1°C) or refrigerated sea water (RSW). The ice and slurry were replenished daily, with removal of all excess liquid in the slurry and drip liquid from melting ice.

The king and banana prawns were stored both whole and headless while the greasy and tiger prawns were stored whole. The prawns stored in RSW became too salty (natural prawn flavour masked) after a few days and thus were found not suitable for further processing. The work was continued with only ice and ice slurry.

What's best: ice, ice slurry or RSW?

Ice is preferred to ice slurry, especially if melanosis is not a problem in the particular prawn; in ice the loss of flavour components is minimal. Leaching of the enzyme, polyphenyl oxidase (responsible for melanosis) could occur, but ready access of oxygen contributes to melanosis or blackening of prawns.

During storage in ice slurry, oxygen has less access to the prawn. The enzymes, as well as melanin pigments which may have formed in the prawns, leach out into the slurry (turning the slurry black). Flavour giving compounds also are leached out leaving the prawn rather bland. This also depends on the type of prawn.

Storage of prawns in RSW leads to salt uptake in the prawn which may limit opportunities for further preparation into the best quality products.

Assessing prawn spoilage

Bacterial counts were continued until the prawns were considered spoilt. Prawns were assessed by a trained panel using a systematic demerit point scoring scheme (for visual and flavour changes). Chemical tests were also done at regular intervals on the stored material.

Shelf life of prawns

There was little difference between the bacterial growth pattern of prawns stored whole or headless either in ice or ice slurry. Some advantage was obtained by storage in ice slurry and beheading these prawns resulted in much less potential for melanosis to occur. Headless prawns are more convenient to handle and package for better presentation to the consumer.

No delays in bacterial growth occurred with king prawns and they had the shortest storage life. This was attributed to the fact that they had an initial flora high in pseudomonads which are known spoilage organisms on chilled seafood.

The flora on the other prawns comprised other types (non-spoilers) which were acclimatised to their natural warm environment. These types failed to thrive in the chilled conditions and it took eight to ten days for a cold adapted flora to emerge and finally dominate. This fact alone has considerable positive implications in that if warm water prawns are properly chilled (at catch), and have a flora of non-spoiling bacteria, it could take a few days for the spoilers to dominate and degrade the prawns.

The typical situation is shown in Figure 1, where, although the king prawns had lower initial levels of bacteria, these were spoilers which grew well in the chilled conditions resulting in shorter shelf life. In contrast, the tiger prawns had higher initial counts but these bacteria were not adapted to the chill environment and they failed to thrive. Counts were thus low for up to seven days before a spoilage flora became dominant.

The presence of such lag periods in bacterial growth gives considerable scope for the preparation of chilled product.

Banana prawns can be kept in ice slurry for up to eight to ten days (lag period) without melanosis or a subsequent rise in total bacterial count. Processors thus have a range of options open to them.

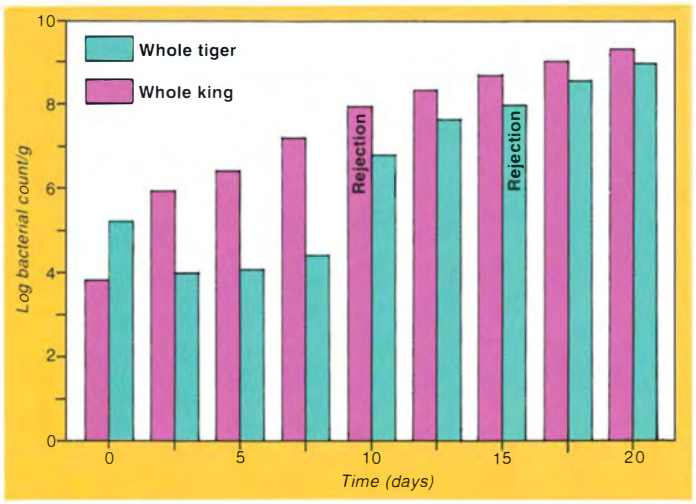
Tiger prawns stored in ice are very prone to melanosis but by storing them in slurry the processor has several days in which to turn them into product.

Greasy prawns can also be kept for a considerable time in slurry before processing.

Spoilage patterns in ice or ice slurry

Storage on ice resulted in the gradual selection of a particular type of bacteria mainly the species *Pseudomonas fragi*. These bacteria produced odours and flavours typical of rotting tropical fruit that gradually increased in intensity throughout the storage period.

Figure 1. Change in total bacterial counts in king and tiger (whole) prawns stored in ice.



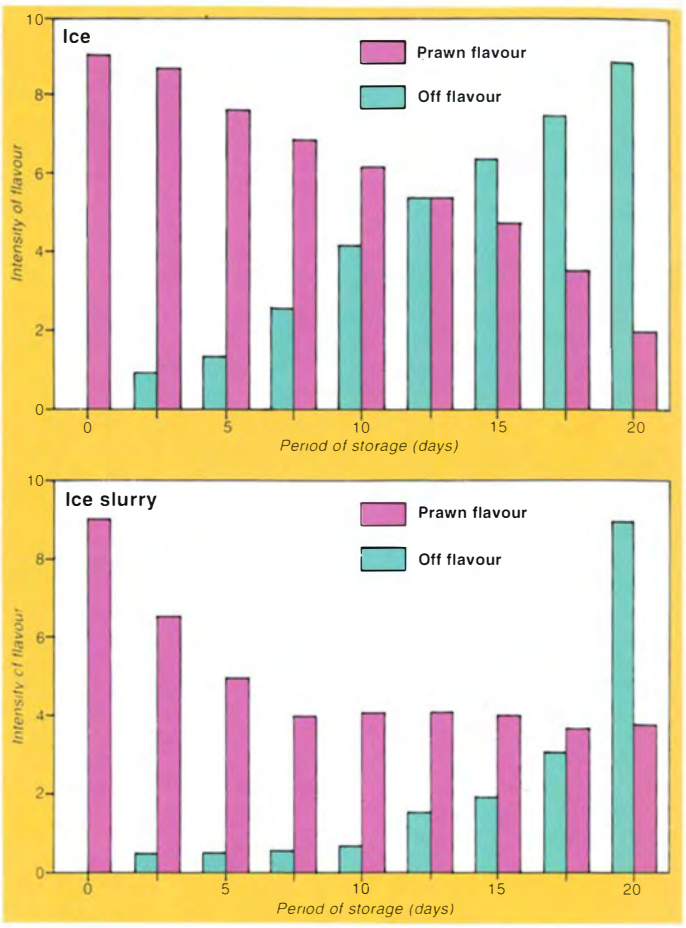


Figure 2. Change in flavour intensity in prawns stored in ice and ice slurry.

Table 1

Storage lives* of chilled whole prawns

Type of prawn	*Storage form (days)*	
	Ice	*Ice slurry*
King	10–11	20
Banana	15	20
Greasy	17	20
Tiger	14	20

*Storage life based on bacterial rejection levels

In contrast, storage in ice slurry more effectively slowed bacterial growth and resulted in the selection of the bacteria, *Shewanella putrefaciens*. This organism is a well known spoiler which produced unpleasant sulphide like odours and flavours. These were not evident until the latter stages of storage. For much of the storage period there were no objectionable changes, but, when the changes came they were dramatic. The typical situation is shown in Figure 2. The odours developed more in the slurry than in the prawns themselves.

In ice the intensity of prawn flavour slowly decreases during storage while the intensity of off flavour increases. In ice slurry there is a rapid decrease in intensity in flavour with only slight increase in off flavour, until the latter stages of storage.

The decrease in flavour intensity in prawns stored in slurry occurred in all species except in king prawns. The flavour loss is probably due to the leaching out of components such as free amino acids and organic phosphates. This means that prawns stored in slurry may be rated low in 'quality' even though they are in prime bacteriological condition. If prawns are packaged soon after catch no loss of flavour occurs.

An estimate of the storage lives is given in Table 1.

Chemical and physical influences on storage

Headless prawns did not blacken till the latter stages since a major source of the enzyme that causes the problem is in the

Table 2

Time taken for black-spot* to develop to a rating of 'slight' for four types of prawns stored in ice or in slurry

Type of prawn	*Storage form*			
	Whole		*Headless*	
	Ice	*Ice slurry*	*Ice*	*Ice slurry*
King	2	6	3	21+
Banana	5	20+	12	20+
Greasy	1	10+	ND	ND
Tiger	3	21+	ND	ND

ND Not done

* Based on mean demerit scores from a panel of 8 members

head. Black-spot did not occur on the prawns stored in slurry till the latter stages of storage (Table 2) since this process requires oxygen and the ice slurry prevents access of oxygen to the prawn. Such biochemical changes could depend on moult stage of the prawns as well. Optimum temperature control and minimum physical abuse are also essential.

Flexibility in processing

The specific knowledge of the storage life allows the fisher the opportunity of bringing the catch to the processor in the prime, raw, chilled state leaving open the options for processing into a variety of forms.

In turn the use of appropriate chilling regimes can give the processor the opportunity to prepare fresh chilled prawn products for the market.

This work has also helped establish the time frame in which an operator must work in order to place chilled fresh product on the market or to further process it.

Further studies on preparation of value added prawn products are in progress based on these current results.

Opportunity for value adding

Storage and processing without additives gives the opportunity for value-adding. Selected packaging techniques as well as rigid temperature control aid in development of such a product.

Results from this study indicate that banana prawns provide the best option for preparation of raw chilled products. Prawns that have a shorter shelf life or have problems of melanosis could be smoked. Smaller prawns such as greasy could be processed whole into dried products. The heads could also be used for preparation of other products for industrial use or as a prawn meal, leading to total utilisation of the prawns.

When chilled correctly, Australian prawns have a significant shelf life giving processors many options and an appropriate time frame in which to produce value added products.

Prawns from different locations can have different bacterial types on them which can result in different shelf lives.

There is potential for value-adding to headless prawns by preparation of ready-to-eat or ready-to-cook products incorporating attractive presentation with longer shelf-life. Thus the loss of weight due to beheading is compensated for in the market value of the product.

Nalini Chinivasagam is a Microbiologist, Allan Bremner is a Senior Principal Scientist and Steve Thrower is a Principal Scientist with the Department of Primary Industries Queensland (IFIQ), 19 Hercules Street, Hamilton, Queensland 4007.

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SECTION 5 SPOILAGE PROCESSES

5.1 Volatile components and tropical spoilage

5.2 Bacterial melanosis

5.3 Spoilage at ambient temperatures

Introduction to section 5

In order to design packaged products that have a significant shelf life it is essential to know and understand the nature of the major spoilage bacteria present. Only then can conditions be selected to prevent or minimise their deleterious effects.

Most of the work on the microbiology of spoilage of seafood has been done on Northern hemisphere cold water species and the work on prawns has all been done on the one major commercial prawn species in the north, *Pandalus borealis*. In contrast, Australia has several major commercial species caught over a wide range of habitats in tropical and subtropical areas.

Tropical and subtropical bacteria have different characteristics and cause different spoilage odours and flavours than those which from cold water.

To elucidate this a number of bacterial isolates were placed on sterile prawn broth and the volatile compounds they produced were identified and comparatively quantified using gas chromatography/mass spectrometry.

This work has never been attempted before and has established firmly that *Pseudomonas fragi* is a major spoilage species on warm water prawns. This bacteria is relatively unknown in cooler waters.

5.1 Volatile components associated with bacterial spoilage of tropical prawns

Abstract

Prawns stored in ice slurry are effectively in an environment of low oxygen tension and as a result have a spoilage flora dominant in *S. putrefaciens*. In contrast oxygen is not restricted when prawns are stored in ice and *P. fragi* is the normal spoilage organism.

The free amino acid profiles of two major species of prawns were high in arginine (12-16%) and lower in cysteine (0.1%) and methionine (0.1-0.2%). These free amino acids could be source substrates for bacterial degradation in banana and king prawns.

Analysis, using gas chromatography/mass spectrometry (GC/MS) of filter sterilised raw prawn broth inoculated with a total of 15 cultures of *P. fragi* and *S. putrefaciens* showed the presence of 17 major volatiles after 2 weeks of incubation at 5°C. They were mainly amines, sulphides, ketones and esters. The absence of TMA and H_2S was evident.

Similarly the GC/MS analysis of chilled stored prawns indicated the presence of amines at the early stages of storage (less than 8 days) irrespective of the nature of the storage media. Esters were present only in prawns stored in normal oxygen conditions at the latter stages of storage (more than 8 days).

Principal component analysis of the volatiles produced by pure cultures inoculated into sterile prawn broth indicated three major groups (a) *P. fragi* from a particular geographic location (b) *S. putrefaciens* from another geographic location and (c) a mixture of *P. fragi* and *S. putrefaciens* from a different of geographic locations

Production of sulphides (and amines) occurred irrespective of the type of bacteria, whereas esters were only produced by *P. fragi*.

This information suggests that bacterial spoilers are selected on the basis of availability of oxygen and nutrients. The biochemical pattern of volatiles produced is influenced by the different bacterial strains which rely on the environment of capture.

A knowledge of the relationship of the interactions between *S. putrefaciens* and *P. fragi* is critical in understanding the spoilage of chilled tropical seafood.

Introduction

The available oxygen and nutrients, such as free amino acids, determine the spoilage pattern associated with different bacterial species. Work on temperate fish indicates that pseudomonads belonging to groups I, II, III/IV (for English sole) or groups II, III/IV (haddock) were major spoilers (Herbert and Shewan, 1975; Shaw and Shewan, 1968) respectively. There is limited literature on such work on temperate crustaceans.

Shewanella putrefaciens is an important spoiler of seafood, while other pseudomonads have been reported to spoil seafood as well (Shewan, 1977). *Pseudomonas* spp. have been reported (Gram *et al.*, 1993) to spoil tropical Nile perch resulting in the development of fruity odours

Prawns also spoil due to breakdown of proteins residues by enzymes from the hepatopancreas etc. which lead to the release of free amino acids. These can act as substrates for bacteria which utilise them to form breakdown products and volatile compounds. Such volatile compounds play a major role in the acceptability of different package/chilled seafood products during storage before overt spoilage occurs

Compounds of major sensory significance from pseudomonads on beef were ethyl methyl esters of C_2 - C_8 fatty acids, sulphur containing compounds thiols, sulphides and thio esters (Edwards, Dainty and Hibbard, 1987). A pyrazine derivative was found responsible for production of musty potato odours by *P. perolens* on sterile fish muscle (Miller *et al.*, 1973).

The main aim of this study was to establish the bacterial profile of two major spoilers and their spoilage potential in sterile prawn broth. These volatile compounds were compared with those obtained from prawns stored under two different chilled storage conditions in order to establish which were of bacterial origin. This knowledge is necessary to help establish suitable package characteristics.

Materials and methods

Isolation and identification of bacterial isolates

Bacterial were isolated from spoiling prawns and identified as in Section 4.1. Further microbiological tests were performed using the API 20NE (France) test kit and the spoilers were tentatively identified. The identification was confirmed using the BIOLOG system (microlog^{TM} 3 release 3.01A) for Gram negative bacteria.

Preparation and inoculation of sterile muscle broth

Freshly caught prawns (2-3 h) were transported aseptically to the laboratory in ice. On arrival the prawns were rinsed in sterile distilled water and aseptically cut at the anterior and posterior region through the flesh (care was taken not come in contact with any gut contents). The shell was removed aseptically. The muscle was chopped and blended with chilled sterile distilled water (1:3 flesh:water) in a high speed blender for 2 min. The chilled contents were centrifuged at 20,000 rpm for half an hour. The supernatant was collected chilled again and filtered first using a millipore filtration system though an 0·45µ filter and then through an 0·22µ filter. The resultant broth was checked for sterility prior to dispensing in 2 mL lots into sterile bottles (5 mL with septum on lids), after which individual bottles were also checked for sterility prior to inoculation. Aseptic conditions were maintained at all stages of sterile muscle production. A loopful (ca 10^5 - 10^6/mL) of an over night pure culture (previously identified) was introduced into the bottle which was then incubated at 5°C for 14 days. The sterile broth was used within three days of preparation and was held at a temperature of 0-1°C.

Preparation of prawns for analysis of volatile compounds

Four species of prawns used for volatile analysis were obtained at initial and spoilage stages of storage as in Chinivasagam et *al.* (1995) They were held frozen (-40^{0}C) till analysed.

Isolation and identification of volatile components from prawns and sterile prawn broth

Volatile components were analysed as in Wood, Aston and Douglas (1994).

Analysis of free amino acids of prawn flesh

Free amino acids in freshly caught banana and eastern king prawn flesh were analysed on an extract at the CSIRO Division of Food Research, North Ryde, NSW.

Estimation of H_2S producing bacteria

The H_2S producing bacteria were estimated on iron-sulphide agar as outlined in Section 4.1.

Results

The development of the H_2S producing bacteria varied whether prawns were stored on ice or ice slurry as indicated in Figure (5.1.1). There was a gradual increase in the percentage of H_2S producers in king (2 days), tiger (5 days) and banana (6 days) prawns stored in ice slurry, but after this the increase was rapid.

With prawns stored on ice, the percentage of the H_2S producers were around 10% or less during the entire course of the trial. Thus the environment of storage was responsible for selecting different growth patterns of the H_2S producing bacteria.

The free amino acid profile banana and king prawns indicated low levels of cysteine and methionine (0.07 - 0.23 mg/g), with higher levels of arginine (11.93 - 15.84 mg/g) (Table 1). Glycine levels ranged from 5.47 - 9.61 mg/g.

Volatiles produced in banana, greasy and king prawns within less then 8 days of storage in ice slurry were mainly amines (Table 2a), with sulphides developing after 8 days of storage.

Amines were also produced in the above prawns in less then 8 days when stored in ice, whereas after 8 days, sulphides as well as esters were the dominant volatiles. The presence of sulphides was also detected in less than 8 days when prawns were stored on ice, but not as much as at the later stages of storage (Table 2b).

Table 1 Free Amino acid Profile of Prawn Flesh.

Amino Acids	Prawn Type	
	King mg/g	Banana mg/g
Asparagine	0.52	0.25
Aspartic acid	0.02	0.01
Arginine	15.84	11.93
Alanine	0.86	0.75
Cysteine	0.07	0.07
Glycine	5.47	9.61
Glutamic acid	0.28	0.11
Glutamine	2.26	2.16
Histidine	2.22	0.13
Isoleucine	0.16	0.11
Leucine	0.36	0.59
Lysine	0.35	0.17
Methionine	0.23	0.11
Proline	5.35	4.21
Phenylalanine	0.12	0.10
Serine	1.10	0.35
Tyrosine	0.16	0.14
Threonine	0.48	0.13
Valine	0.31	0.20

Table 2a Volatiles compounds detected in the headspace from banana, greasy and king prawns stored in ice slurry.

	trimethylamine	2 methyl-2-propyl amino	0-2-methyl propyl hydroxylamine	0-3 methyl butyl hydroxylamine	2-methyl-2 propane amino	n-methyl methane amine	methyl disulphide	carbon disulphide	dimethyl disulphide	methyl-methyl thio methyl sulphide	methyl propyldi sulphide	dimethyl trisulphide	2 methyl thio propane unknowns	2 methyl propanoic acid ethylester	ethyl butyrate	2 methyl-2-butanoic acid ethylester	ethanoic-S-ethyl ester
	AMINES						SULPHIDES							ESTERS			
Banana (<8)	✓	-	✓	✓	-	-	✓ *	-	-	✓ *	-	-	-	-	✓	-	-
Bay (<8)	✓	✓	✓	✓	-	-	✓	-	-	✓	-	-	-	✓	-	-	-
King (<8)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Banana (>8)	✓	-	✓	-	-	-	✓	✓	✓	✓	✓	✓	-	-	-	-	-
Bay (>8)	✓	-	✓	✓	-	-	✓	✓	✓	✓	✓	-	-	✓	-	-	-
King (>8)	-	-	-	-	-	✓	-	-	-	-	✓	-	-	-	-	-	-

ND - Not done

* - Present on day 0

✓ - Detected in whole prawn

Table 2b Volatiles compounds detected in the headspace from banana, greasy and king prawns stored in ice.

	trimethylamine	2 methyl-2-propyl amino	0-2-methyl propyl hydroxylamine	0-3 methyl butyl hydroxylamine	2-methyl-2 propane amino	n-methyl methane amine	methyl disulphide	carbon disulphide	dimethyl disulphide	methyl-methyl thio methyl sulphide	methyl propyldi sulphide	dimethyl trisulphide	2 methyl thio propane unknowns	2 methyl propanoic acid ethylester	ethyl butyrate	2 methyl-2-butanoic acid ethylester	ethanoic-S-ethyl ester
	AMINES						**SULPHIDES**							**ESTERS**			
Banana (<8)	✓	✓	✓	✓	-	-	✓ *	✓ *	-	✓ *	-	-	-	-	✓	-	-
Bay (<8)	✓	✓	✓	✓	-	-	✓	✓	-	✓	-	-	-	-	-	-	-
King (<8)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Banana (>8)	✓	-	-	-	-	-	✓	✓	✓	✓	✓	✓	-	✓	✓	-	-
Bay (>8)	✓	-	-	✓	-	-	✓	-	✓	✓	✓	-	-	✓	✓	✓	-
King (>8)	-	-	-	-	-	-	-	-	✓	-	✓	-	✓	✓	-	✓	✓

ND - Not done

* - Present on day 0

✓ - Detected in whole prawn

Table 3 **Description of the odour of the volatile compounds produced by spoilage bacteria (*P. fragi* and *S. putrefaciens*) in sterile banana prawn broth.**

Culture Number	Identity	Type	Source	Odour
348	*P. fragi*	King	Mooloolaba	fruity/sulphide
395	*P. fragi*	King	Mooloolaba	fruity/sulphide
813	*P. fragi*	Banana	Moreton Bay	slightly fruity
830	*P. fragi*	Tiger	Cairns	strong fruity
363	*P. fragi*	King	Mooloolaba	fruity/sulphide
397	*P. fragi*	King	Mooloolaba	neutral
437	*S. putrefaciens*	King	Mooloolaba	neutral
777	*S. putrefaciens*	Banana	Moreton Bay	sulphidy
824	*P. fragi*	Tiger	Cairns	slightly fruity
827	*P. fragi*	Banana	Moreton Bay	neutral
749	*S. putrefaciens*	Banana	Moreton Bay	sulphidy
755	*S. putrefaciens*	Banana	Moreton Bay	sulphidy
771	*S. putrefaciens*	Banana	Moreton Bay	sulphidy
858	*S. putrefaciens*	Tiger	Cairns	neutral
864	*S. putrefaciens*	Tiger	Cairns	neutral

The nature of the odours produced by the various isolates is listed in Table 3. All 15 cultures used in the trial were identified using conventional methods, API kits and finally identified using the BIOLOG identification system. Eight cultures represented *P. fragi* and 7 cultures represented *Shewanella putrefaciens*.

The distinct differences between the species was the ability of *S. putrefaciens* to reduce TMAO and produce H_2S in the media of Gram *et al.*, 1993, where as all pseudomonas cultures did not have this ability. The *Shewanella putrefaciens* were light pink to darker pink, while the *Pseudomonas* was white in colour. The biochemical profile of the two bacterial species is summarised in Table 4 along with the major tests performed.

Table 4 Biochemical profile of the two groups of cultures.

	P. fragi	*S. putrefaciens*
Gram reaction	-	-
Oxidase reaction	+	+
Catalase activity	+	+
O/F (oxidn./fermtn. test)	Acid/oxi	Base/-
Motility	+	+
TMAO reduction	-	+
H_2S production	-	+
Gelatine hydrolysis	+	-
DNAse activity	-	+
Casein hydrolysis	+	-
Ornithine decarboxylase	-	+
Nitrate reduction	+	-
Arginine dihydrolase	-	+/-
Maltose assimilation	+	slight

Volatiles from sterile broth

There were some obvious differences in the range of volatiles produced by the different cultures (Table 4) which lists peak area/mL of head space volatiles produced by different bacterial cultures. This gives a relative ratio of production of different volatiles in relation to the control (sterile broth). A wide variety of complex sulphides were produced by most isolates. Higher volumes of ketones and esters were produced by *P. fragi.* Culture number 830 (*P. fragi*) was unique in producing high amounts of pyrrolidine and other esters. Most of the *P. fragi* cultures additionally produced 2-propanethiol.

Principal Component Analysis

These peak areas were subjected to Principal Component Analysis using the UNSCRAMBLER package on the log transformed data. The initial analysis using 16 cultures and 21 peaks (compounds) provided little information regarding peak groupings and object separation. It confirmed that culture 830 was different to the rest and it could be removed from the model. By doing this a number of peaks became redundant or contained little extra information and these too were removed from the model.

Further analysis and refinement produced a model in which only 6 peaks were required to describe the cultures (Figure 5.1.2). The first component PC1 was described by a combination of 1-propanethiol, bis (methylethyl) disulphide and 2-propanethiol in one direction and dimethyl disulphide in the other. The second component (PC2) was less defined but included methanethiol and thiobismethane. Thus it is sulphide compounds that differentiate between these cultures.

Table 5 Head Space Volatiles (peak area/mL) produced by *P. fragi* and *S. putrefaciens* in Sterile Prawn Broth.

Culture Number	methanethial	methanethiol	unknown	Thiobismethane	1-propanethiol	2-methyl-1-propanethiol	2-(methylthio)-propane	dimethyldisulphide	1-2-Bis(ethylthio)-ethane	methyl propyldisulphide	Dimethyl trisulphide	bis(-methylethyl) disulphide	Fluoromethyl methyldisulphide	Ethyl acetate	Acetic acid, methyl ester	pyrrolidine	propanoic acid ethyl ester	2-methyl-propionic acid ethyl ester	3-methyl butanoic acid, methyl ester	3-methyl butanoic acid, ethyl ester	2-propanethiol
348	55	173	-	-	2204	-	-	-	342	1378	774	2074	-	-	-	-	-	-	-	-	4242
395	-	103	-	-	1256	-	-	-	250	617	716	1055	-	-	-	-	-	-	-	-	1849
813	-	72	-	70	-	-	-	126	253	354	642	245	-	-	-	-	-	-	-	-	247
830	261	461	1381	1658	6242	669	1331	558	272	2191	2872	1248	383	5316	223	173459	1063	1366	522	4777	9167
363	-	-	-	-	635	-	-	121	271	880	-	516	-	-	-	-	-	-	-	-	1351
397	-	173	240	239	131	-	-	777	276	403	4885	-	-	-	-	-	-	-	276	-	-
437	-	3177	-	145	-	-	-	4896	240	719	2105	177	163	-	-	-	-	-	-	-	101
777	1268	6874	-	-	-	-	-	5396	375	490	345	-	-	-	-	-	-	-	-	-	-
824	-	180	-	84	-	-	-	1101	230	401	2017	-	194	-	-	-	-	201	-	-	-
827	-	444	1392	2068	-	-	-	2056	276	531	1458	-	154	-	-	-	-	-	-	-	-
749	5203	6066	-	114	-	-	-	6132	152	441	5238	-	-	-	-	-	-	-	-	-	-
755	318	455	-	-	-	-	-	4305	404	412	2857	-	-	-	-	-	-	-	70	-	-
771	314	5695	133	-	-	-	-	5092	365	350	3197	-	-	-	-	-	-	-	-	-	-
858	-	682	-	159	-	-	-	2004	319	343	2043	-	-	-	-	-	-	-	-	-	-
864	308	4404	-	-	-	-	-	1375	212	277	1232	-	-	-	-	-	-	-	-	-	-
Control	277	230	176	124	-	-	-	433	378	264	74	-	-	-	-	-	-	-	-	-	-

Based on the above compounds, the bacterial cultures were distributed into 3 major groupings (Figure 5.1.3).

Cultures 363, 348, 395 (all *P. fragi*) formed one grouping and all originated from one geographic area (Mooloolaba).

Cultures 749, 777, 771, 755, 864 formed another grouping (all *S. putrefaciens*) and with the exception of 864 were from a different geographic location (Moreton Bay).

In contrast cultures 824, 858, 397, 827, 437, 813 were either *S. putrefaciens* or *P. fragi* and all were from either Mooloolaba, Moreton Bay and Cairns and thus formed third grouping of variable nature.

Culture 830 (*P. fragi*) which was distinctly different and was not a part of the PCA due to its distinct variation was from Cairns.

Discussion

The cultures isolated and identified in the present study were from 3 distinct environments, i.e. off Mooloolaba, Moreton Bay and Cairns from king, banana and tiger prawns respectively.

The prawns were stored in either ice or ice slurry till spoiled. Thus the bacterial isolates were from specific environmental conditions such as in ice (normal oxygen availability), and ice slurry (low oxygen availability).

S. putrefaciens was isolated as the dominant organismstures isolated from prawns stored in ice slurry, whereas *P. fragi* was the dominant organism isolated from those stored in ice.

The major volatiles from stored prawns were isolated and identified using GC/MS and comprised of 3 major groups, mainly, amines, sulphides and esters.

In all three types of prawns amines were more prevalent during the *initial* stages of storage in prawns stored both on ice and ice slurry. They were mainly trimethylamine 0-2, methyl propyl hydroxylamine, 0-3 methyl butyl hydroxylamine.

Sulphides developed in the *latter* stages of storage on all prawns irrespective of storage media, while methyl disulphide, methyl-methyl thio methyl sulphide was present on day 0 and thus could not have occurred as a spoilage volatile of bacterial origin (all prawns were handled well at capture).

Esters and ketones were dominant in prawns stored on ice especially in the latter stages of storage. They were mainly 2 methyl propanoic acid ethyl ester, ethyl butyrate, 2 methyl-2-butanoic acid ethyl ester, ethanoic-8-ethyl ester.

All the above volatiles could be of chemical origin or products of bacterial or chemical degradation. Products of bacterial degradation would be more significant at the latter stages of spoilage in relation to product acceptability.

In the second stage of the study a sterile muscle broth was prepared where all the 15 cultures were inoculated and stored chilled so as to ascertain the types of volatiles produced as a result of bacterial degradation of the prawn muscle. A total of 21 volatile compounds were isolated, eleven of which were mainly compounds of sulphide origin, the others were mostly esters and ketones. Pyrrolidine was produced in large amounts by one *P. fragi* culture which also produced other unique esters and ketones. This culture produced strong fruity odours.

Other *P. fragi* cultures developed mild fruity and sulphide odours. The *S. putrefaciens* cultures developed sulphide odours which were stronger in intensity than those produced by *P. fragi.*

The free amino acid profile of banana prawn sterile muscle broth was high in arginine (11.93 mg/g), glycine (9.61 mg/g) and proline (4.21 mg/g). Methionine (0.11 mg/g) and cysteine (0.07) were in low levels and, unless there are other non-protein sources of sulphur that can be used by the bacteria as a substrate, it must be assumed that these are the source of the volatile sulphur compounds

There were 21 volatile compounds isolated and identified by GC/MS that were due to bacterial degradation. Of these sulphides were produced by all isolates, whereas ketones and aldehydes were produced by *P. fragi*. These two groups of compounds comprised the major spoilage volatiles that developing as the odours of spoiled prawns. Amines were not detected as a part of the spoilage volatile compounds developed by the isolates, though amines developed as a part of the spoilage volatile compounds in prawn flesh.

The notable lack of TMA production by *S. Putrefaciens* was unexpected and it is assumed the organism preferred to metabolise other components of the sterile prawn broth.

Conclusion

This work has confirmed the conclusions previously described in Section 4 that *P fragi* is a major spoilage organism in tropical prawns and that the nature of the volatile components produced by spoilage bacteria depends on the environments of storage and of capture.

An understanding of the relationship between. *P. fragi* and *S. putrefaciens* is necessary in order to design packs with the correct conditions to select the preferred spoilage pattern.

There are a variety of sulphide compounds, more than hydrogen sulphide alone, produced by the various strains of bacteria which contribute to the odour of spoiling prawns.

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Acknowledgment

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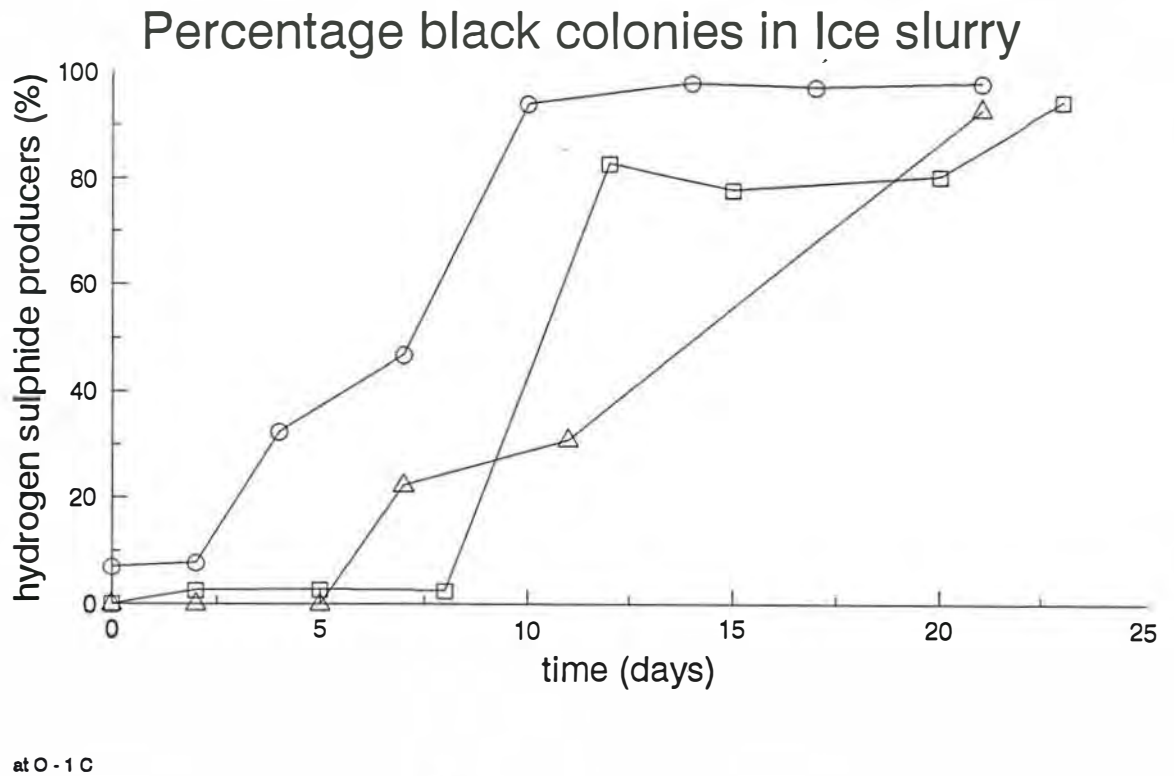
Figure 5.1.1



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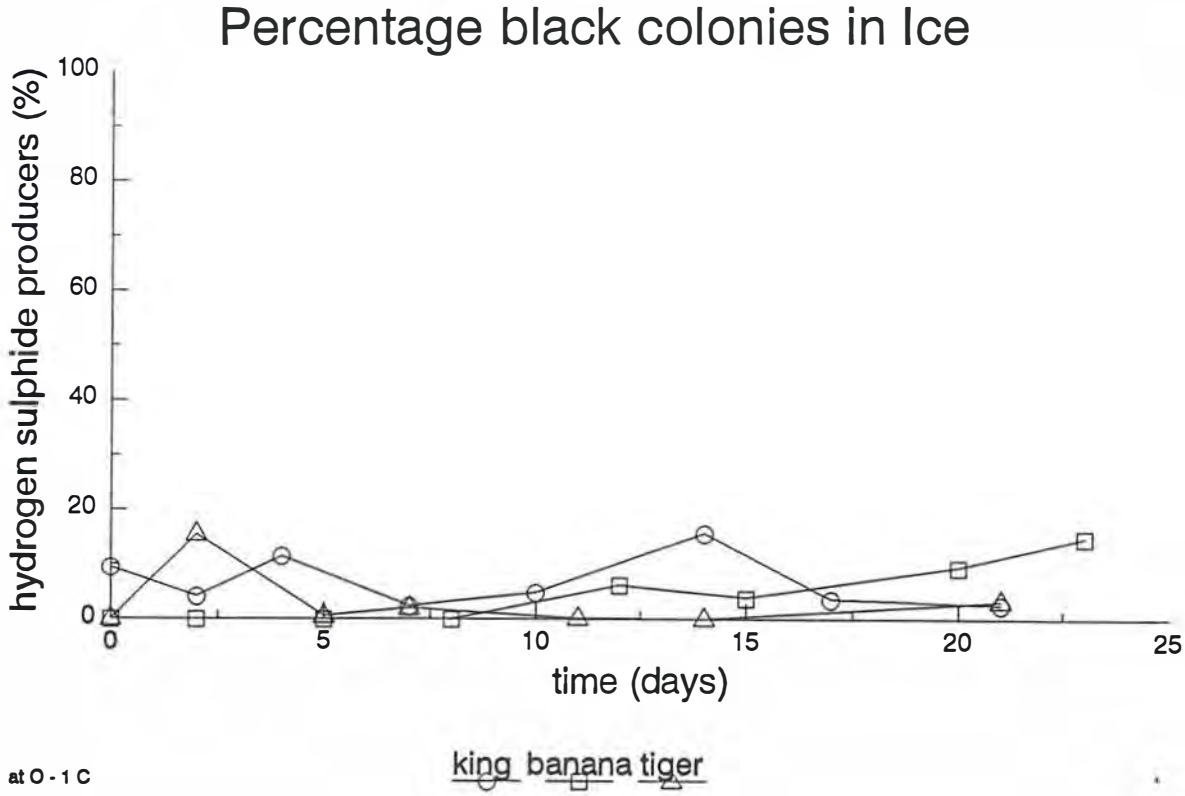


at O - 1 C

Figure 5.1.2

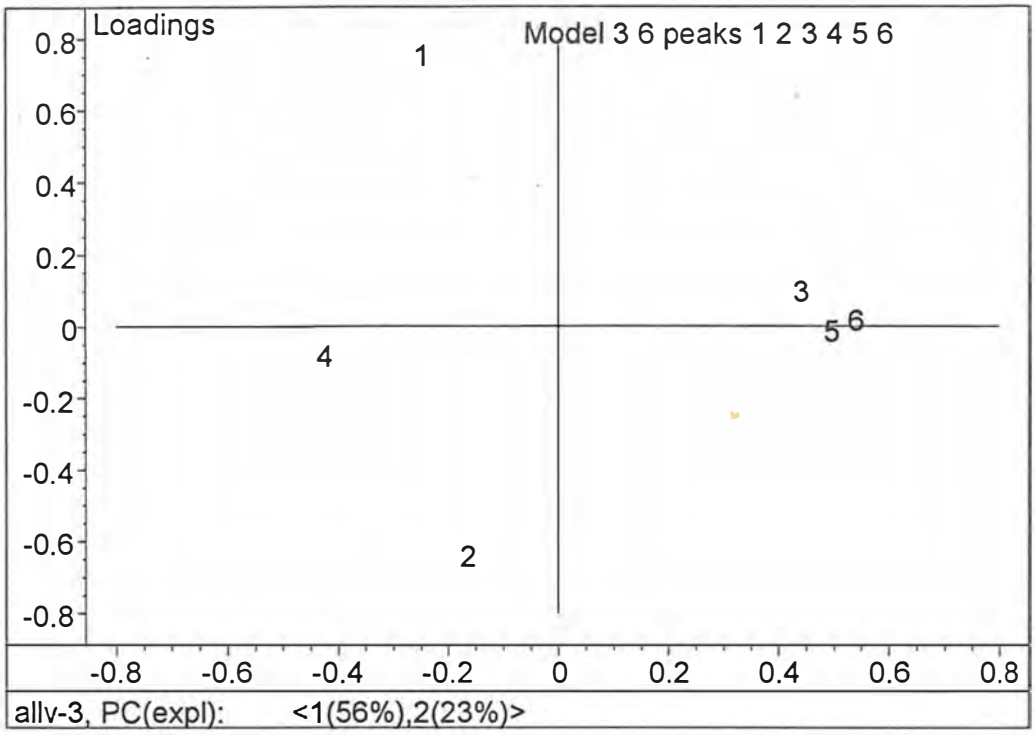
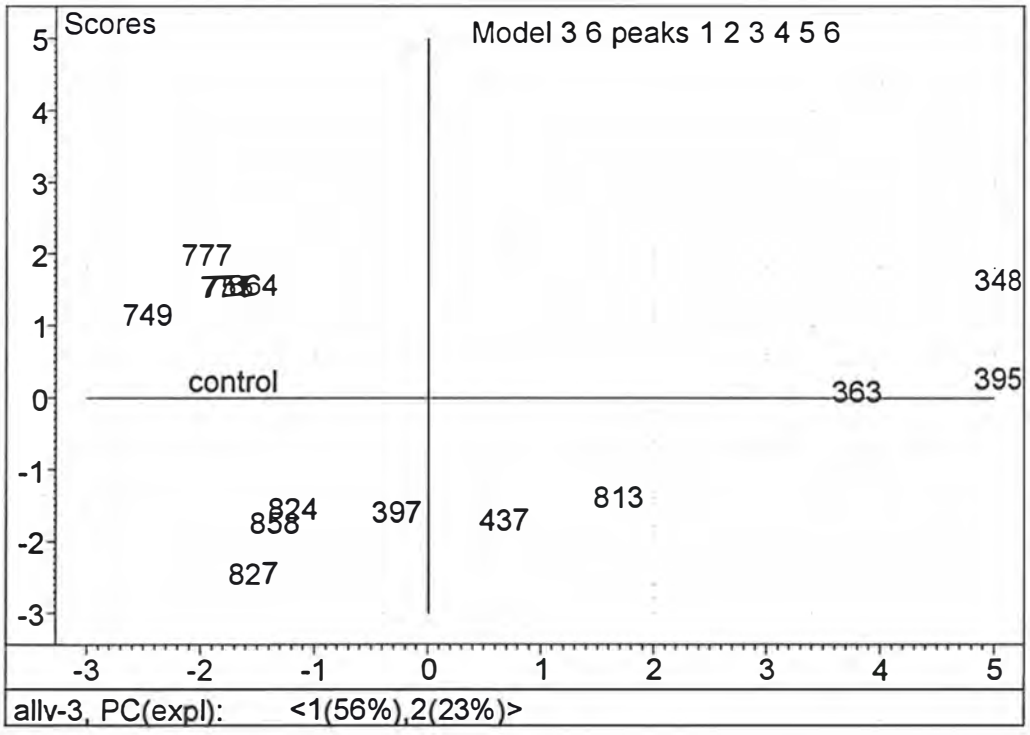


Figure 5.1.3



5.2 Bacterial melanosis

Introduction

Melanosis is a problem in prawns to which the common answer is treatment by sulphur dioxide in the form of sodium metabisulphite. This is effective, but, it is common knowledge that, during storage, melanosis may still occur even though there are apparently sufficient levels of SO_2 to prevent it. This suggested the possibility that some bacteria may be capable of producing melanosis.

Materials and methods

A range of bacterial isolates from spoiling prawns were available from the previous storage trials.

Forty eight different isolates were inoculated into broths of a minimal media containing the amino acid tyrosine, and into the same broth with the addition of peptone water. Tyrosine is the substrate for enzymic production of melanin. Broths were incubated at temperatures of both 30°C and 5°C for a period of days over which they were examined for development of colour. The colour was judged arbitrarily on the following scale:

0 no colour, 1 slight pink, 2 pink red, 3 red brown, 4 brown, 5 black (see Figure 5.2.1).

Results

Several of the cultures were capable of producing melanin (Figure 5.2.2). The overall scores for melanin production are shown plotted as an average for each bacterial type, either *Pseudomonas fragi* or *Shewanella putrefaciens* at the two incubation temperatures 30°C and 5°C in the two media, minimal (Figure 5.2.3) and, with peptone (Figure 5.2.4).

The *P. fragi* cultures had clearly more ability to produce melanin as indicated in Figure 5.2.3.

When incubated at 5°C melanin production was slow. Many of the isolates of *S. putrefaciens* could not grow well in the minimal media. When peptone was added- making the media more nutritional closer to that 'naturally' occurring on a prawn- *S. putrefaciens* grew well and produced melanin at 30°C. The *P. fragi* grew equally well whether peptone was included in the media or not. At 5°C only that *P. fragi* cultures produced significant melanosis within 8-11 days.

The melanosis scores produced by the individual cultures incubated at 5°C in peptone tyrosine broth are shown in Table 1.

Only 6 of the 16 isolates which proved to be *S. putrefaciens* managed to turn the medium pink after 19 days indicating the first step to producing melanosis. After 11 days at 30°C all these cultures had produced a brown broth indicating they had the capacity to produce melanin.

Thus, although they have the capability to produce melanin it is unlikely they would contribute to melanin production at 5°C or lower.

On the other hand 25 of the 31 *P. fragi* isolates had indicated they could produce melanin after 17 days incubation. The majority of these isolates had produced red-brown to brown broths at 30°C after 8 days incubation. It is likely that the prawns would provide better natural conditions and a more complete nutritional supply for growth than these artificial media ,so that if prawns or packs of prawns were not kept properly chilled some melanin due to strains of *P. fragi* may be formed

Conclusion

Several stains of *P. fragi* are capable of producing melanin, but more work would be required to find the practical significance of this. It may explain why melanosis can occur during storage of sulphite treated prawns but it also may have implications for the stability of prawns which have not been treated with sulphite.

Table 1 Melanosis scores (0-5) obtained by incubating pure cultures at 5°C in peptone tyrosine broth.

Type	Culture Number	Melanosis score: Period of incubation (days)						
		6	7	8	11	14	17	19
S. putrefaciens	741	-	-	-	-	-	-	1
	755	-	-	-	-	1	1	1
	771	-	-	-	-	-	1	1
	858	-	-	-	-	-	-	1
	873	-	-	-	-	-	-	1
	874	-	-	-	-	-	-	1
P. fragi	333	-	-	-	-	1	1	3
	348	-	-	-	-	-	1	1
	353	-	-	-	-	1	1	3
	363	-	-	-	-	-	1	2
	367	-	-	-	1	1	2	4
	377	-	1	1	1	2	3	3
	382	-	-	-	-	1	2	3
	389	-	-	1	1	1	2	3
	395	-	1	1	1	1	2	4
	397	-	1	2	2	2	3	3
	399	-	-	-	-	-	1	1
	406	1	1	2	2	2	3	3
	428	1	1	1	2	2	3	3
	430	-	-	-	1	1	3	3
	781	-	-	-	-	-	1	1
	787	-	-	-	-	-	1	1
	793	-	-	-	1	1	2	3
	794	-	-	-	-	-	1	1
	803	-	1	1	1	1	2	3
	811	-	-	-	-	1	1	1
	813	-	-	-	1	1	2	3
	824	-	-	-	-	-	1	1
	827	-	1	1	1	1	2	3
	830	-	-	-	-	-	-	1
	833	-	-	-	-	1	2	3

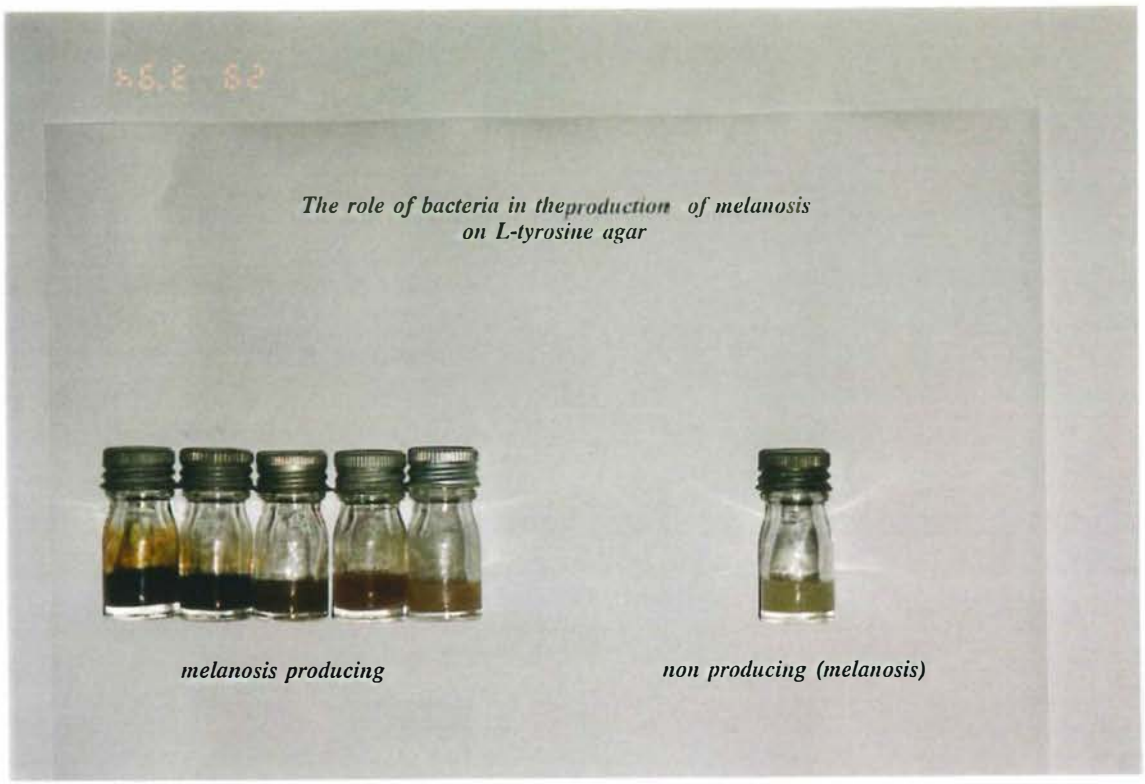


Figure 5.2.1 Examples of the grades used to mark melanosis production in tyrosine broth.

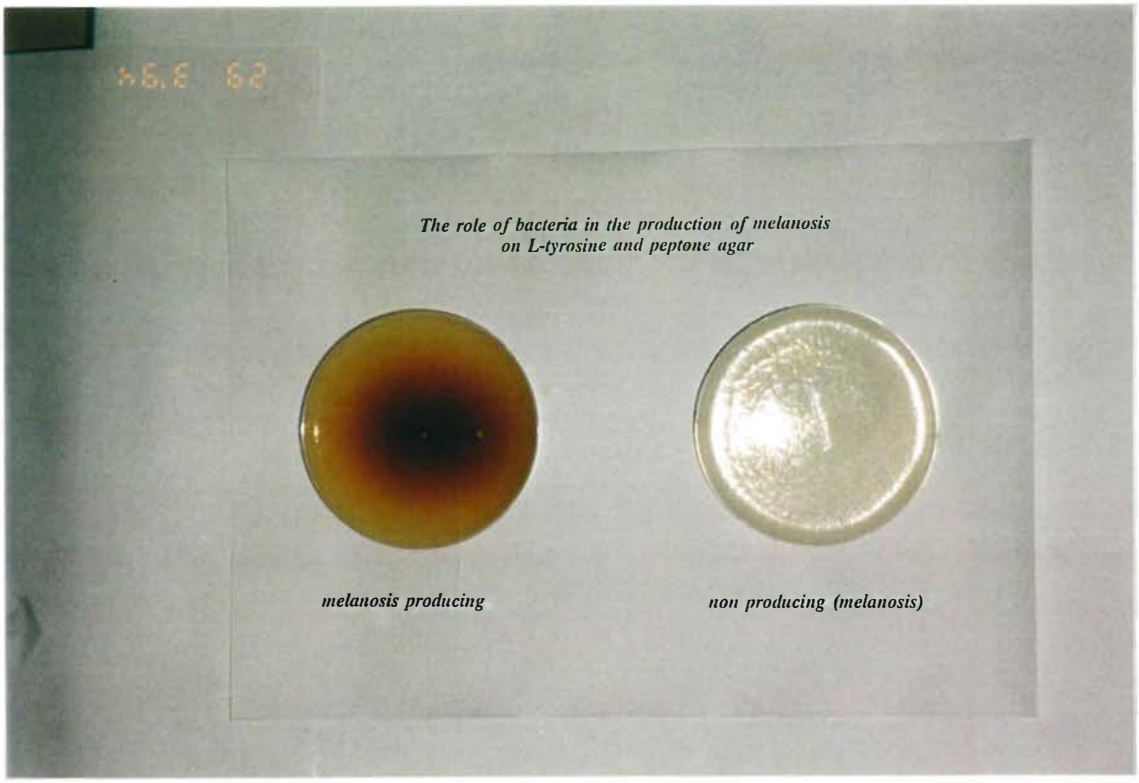
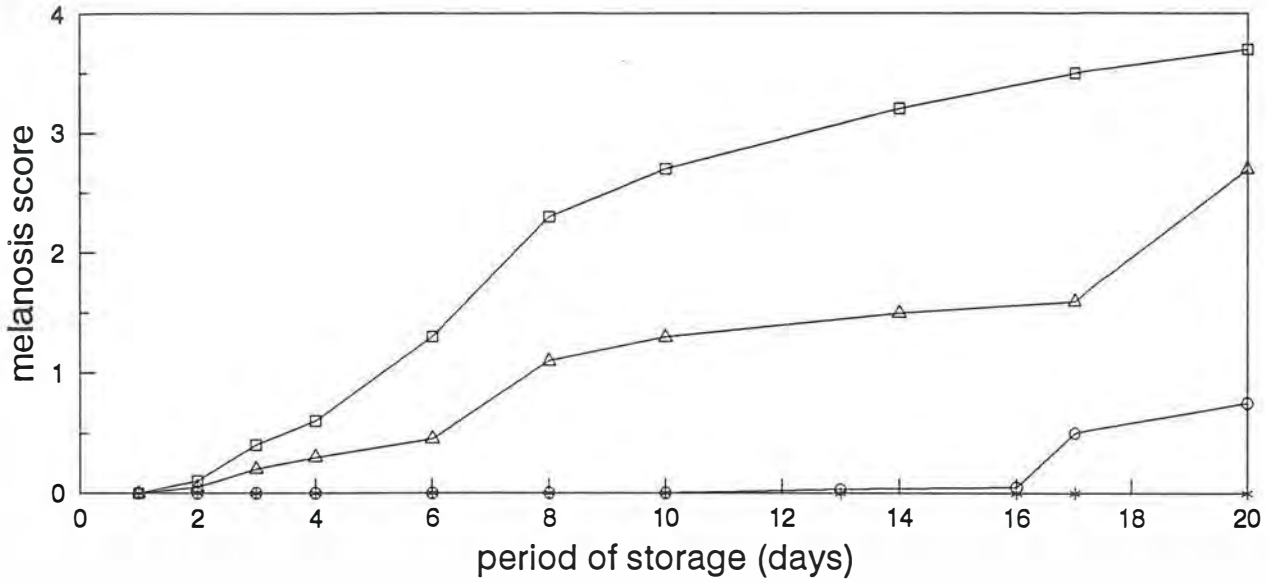


Figure 5.2.2 Melanosis production by *P.fragi* on tyrosine peptone agar.

Figure 5.2.3

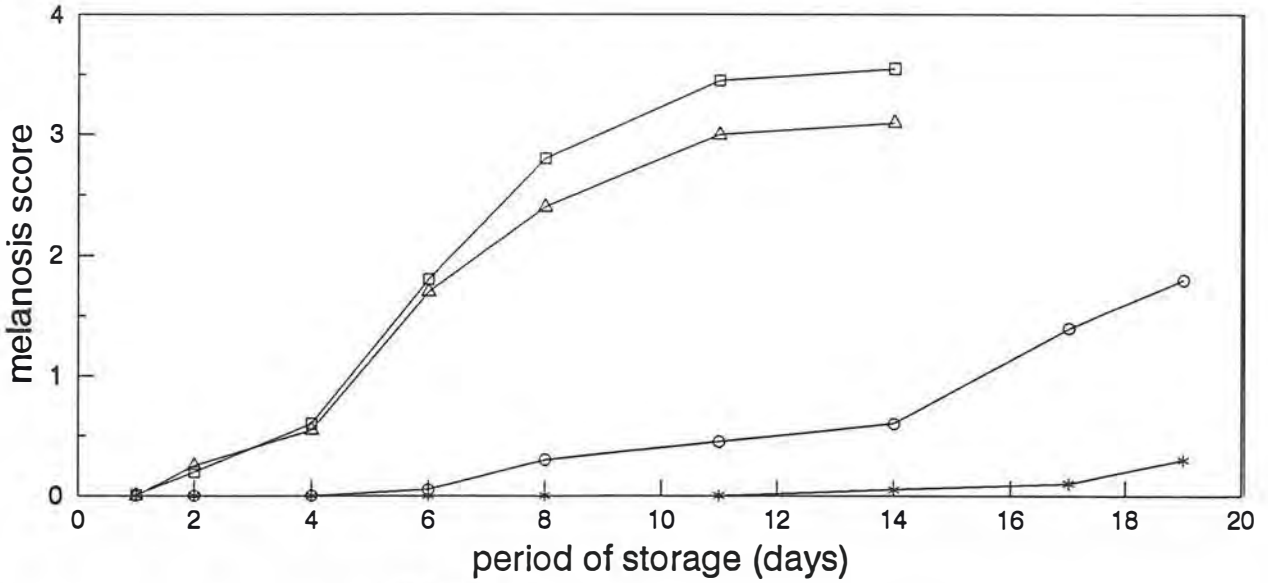
Average melanosis score for bacterial cultures grown in minimal tyrosine broth incubated at either 5°C or 30°C



P. fragi @ 30°C S. putrefaciens @ 30°C P. fragi @ 5°C S. putrefaciens @ 5°C

Figure 5.2.4

Average melanosis score for bacterial cultures grown in tyrosine broth with peptone added incubated at either 5°C or 30°C



P. fragi @ 30°C S. putrefaciens @ 30°C P. fragi @ 5°C S. putrefaciens @ 5°C

5.3 Spoilage at ambient temperatures

Aim

To assess the effects of storage of banana prawns for extended periods at ambient temperatures.

Introduction

On occasions prawns are not chilled after catch and they appear not to deteriorate significantly if the period at high temperatures is relatively short.

These experiments were done to assess the effects of leaving prawns unchilled at ambient temperatures.

Materials and methods

Banana prawns were obtained on two occasions 12/5/93 and 21/5/93 in Moreton Bay inside Cribb line west of Brisbane Channel (Bramble Bay) at around 1600 hours.

The temperature on 12/5/93 was around 30°C and on 21/5/93 it was 22°C. The prawns were washed with the deck hose, not chilled, but kept moist.

At the laboratory they were stored in a plastic container covered to keep the prawns moist .

The prawns were assessed throughout the night at two-hourly intervals and were sampled for total bacterial counts, K-value, pH and total volatile nitrogen (TVN). Samples were also frozen for visual assessment by an eight member panel.

Results

A set of photographs showing the slight changes in appearance that occurred in the first few hours with the prawns stored at 22°C (21/5/93) are shown as Figure 5.3.1 a & b.

The total bacterial counts did not change over the first 8 hours in the prawns stored at 30°C (Figure 5.3.2) and for about 6 hours in those stored at 22°C (Figure 5.3.3).

At 30°C the total bacterial counts took about 16 hours to reach 10^6 cfu/g. In contrast, the bacteria on whole prawns in ice (Figure 3 Section 4.1) took about 9.5 days (228 hours) to reach this level. Thus the rate of growth of bacteria was 14 times faster at 30^0C than at 0°C. Note that the flora may be of different compositions at the two temperatures.

At an ambient temperature of 22°C the bacterial count reached 10^6 cfu/g in about 11 hours, viz about 22 times faster than occurred previously on banana prawns stored at 0°C.

These different apparent relative growth rates between storage at 30°C and 0°C are likely to be due to a different flora being selected at the higher temperature (all other factors being considered equal, i.e. assuming the prawns are of similar composition, not true as seen by pH and TVN results). That is the prawns from 21/5/93 may have developed a more psychrotrophic flora at 22°C, whereas a temperature of 30°C is more in the mesophile range so that different flora would be selected by the temperature.

The pH did not alter much in those stored prawns at 30°C (12/5/93) for at least 12 hours whereas the pH in those from 21/5/93 started about 0.4 units higher and gradually increased over the first 8 hours (Table 1). Despite there being only nine days between the catches from the same area, these are relatively large differences. This indicates that the characteristics of different catches cannot be taken for granted.

The TVN values (Table 1) were high initially in comparison to reported values. In fact a value of 30 mg N/100 g is regarded as spoilage for many seafood products. High values like this have been reported in other trials (Section 4.2) and so are regarded as common for some Australian prawns. The TVN values for the prawns stored at 30°C (12/5/93) showed a steady rapid increase even though bacterial growth was negligible for the first 8 hours. This TVN production must be due to inherent enzymic or chemical reaction.

The K values showed a steady increase indicating that enzymic activity proceeded quite rapidly. The initial values were relatively high and were probably due to stress in the trawl.

The results for visual assessment are given in Table 2 as means for eight assessors. It was not possible to assess all sample times.

Table 1 Changes in pH, TVN and K value in banana prawns stored at temperatures of 30°C (12/5/93) and 22°C (21/5/93).

	Period of storage (hours)								
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
pH									
12/5/93	-	6.5	6.5	6.5	6.5	6.6	6.7	6.8	6.7
21/5/93	6.8	6.9	7.1	7.0	7.2	7.2	7.3	7.3	-
TVN (mg N/100 g)									
12/5/93	-	34	33	37	39	44	52	49	60
21/5/93	30	30	32	35	25	31	42	45	-
K-value (%)									
12/5/93	-	15	18	18	19	24	29	24	35
21/5/93	12	12	13	24	49	42	41	56	-

Table 2 **Demerit point scores for banana prawns stored at ambient temperatures on 12/5/93 (30°C) and 21/5/93 (22°C).**

Storage period hours	12/5/93 (30°C)	21/5/93 (22°C)
0	3	-
2		3
4	5	3.5
6	-	4.5
8	5.7	6.5
10	10.5	-
14	12	-
16	-	8

There was a progressive increase in demerit scores although, in both instances the scores were not high until after six hours. The relevant comparisons are in Figure 1 Section 4.1 where whole prawns stored in ice were given a score of 5 after 8 days (192 hours). In relative terms this means that the visual changes were occurring 48 times faster at temperature of 30°C than in ice at 0°C to reach the score of 5.

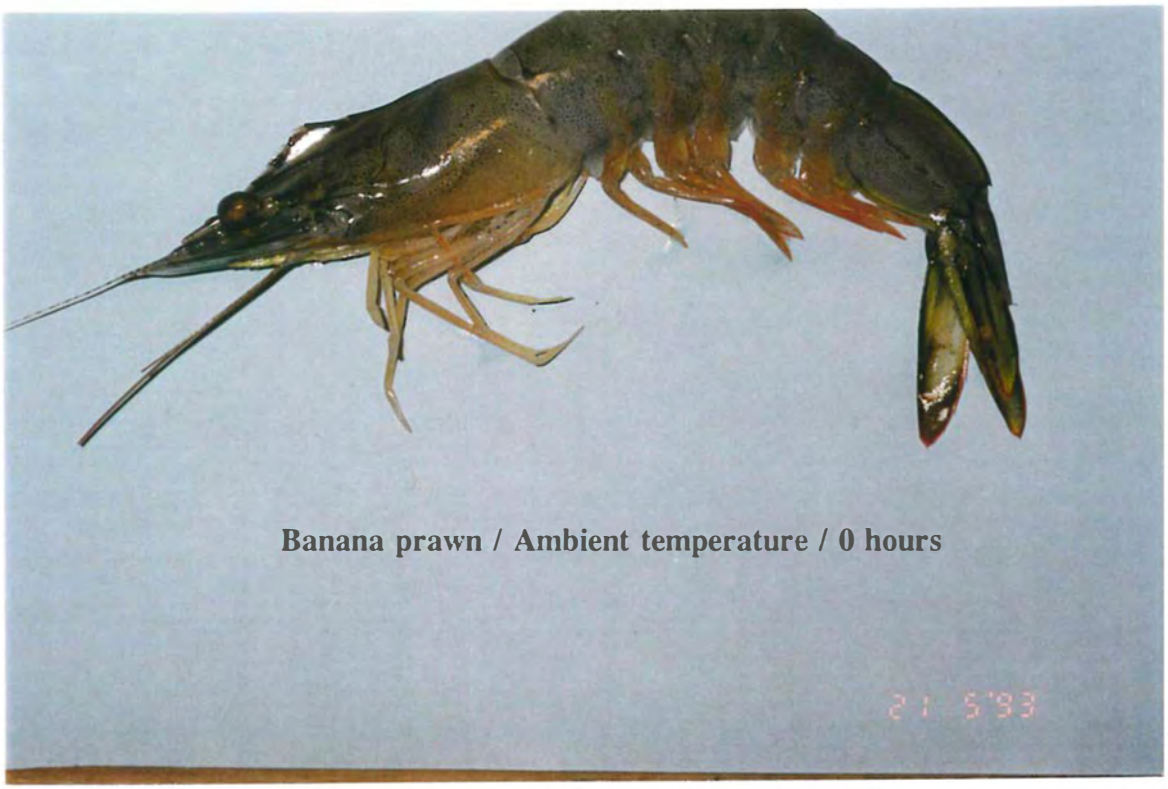
On the other hand the prawns took 10 hours at 30°C and 13 days (312 hours) to reach a demerit score of 10, a relative 31 times faster than the previous results for banana prawns stored in ice.

Changes were slower at 22°C (21/5/93) with 16 hours taken to reach a score of 8 (see also Figure 5.3.1a & b. With whole banana prawns in ice this score was reached after about 11 days (264 hours) the relative rate being about 16.

Conclusion

Even though banana prawns stored at ambient temperature do not apparently exhibit much change for the first few hours and are still acceptable in appearance, underlying enzymic and chemical changes are proceeding at a considerable rate. The bacteria present are undergoing a period of adjustment, after which they can rapidly grow and spoil the prawns.

After 8-10 hours at high temperatures the banana prawns are still good for consumption but they are of little use for further processing or storage. It is essential that prawns are chilled unless they are to be consumed directly.



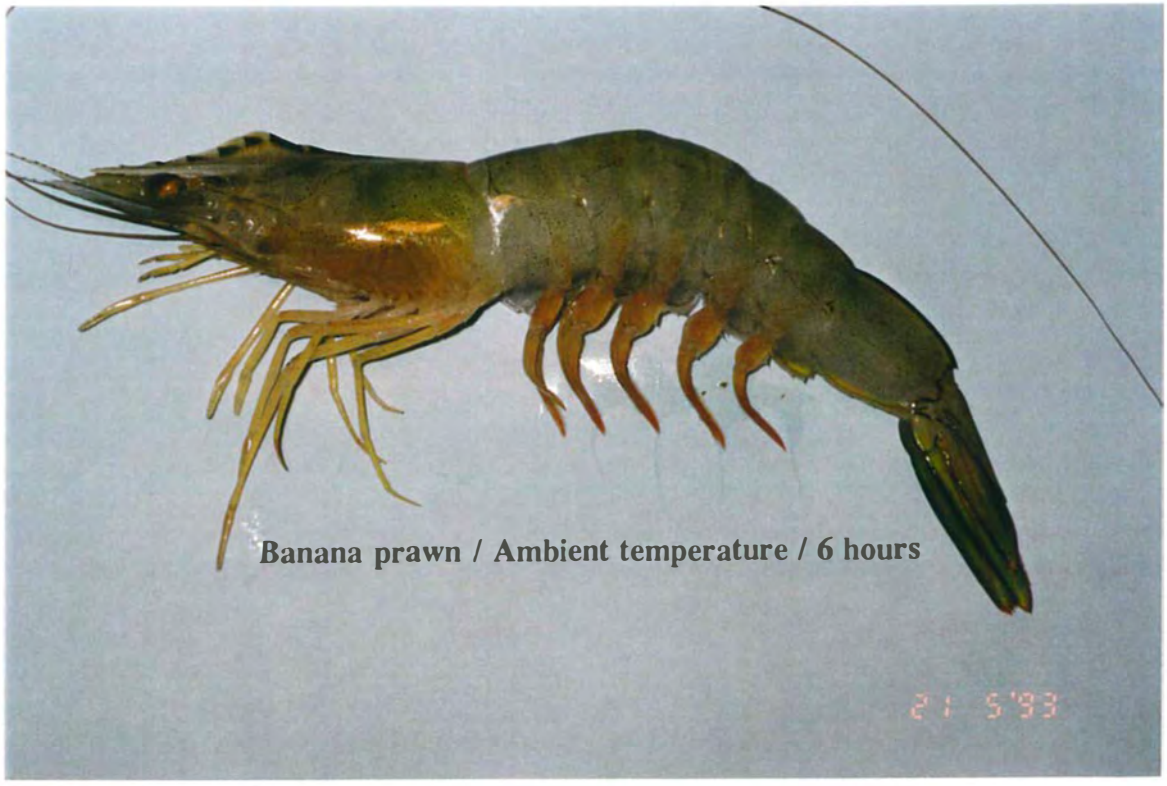


Figure 5.3.1 (a) Banana prawns stored at 22^{0}C for 0 and 6 hours.

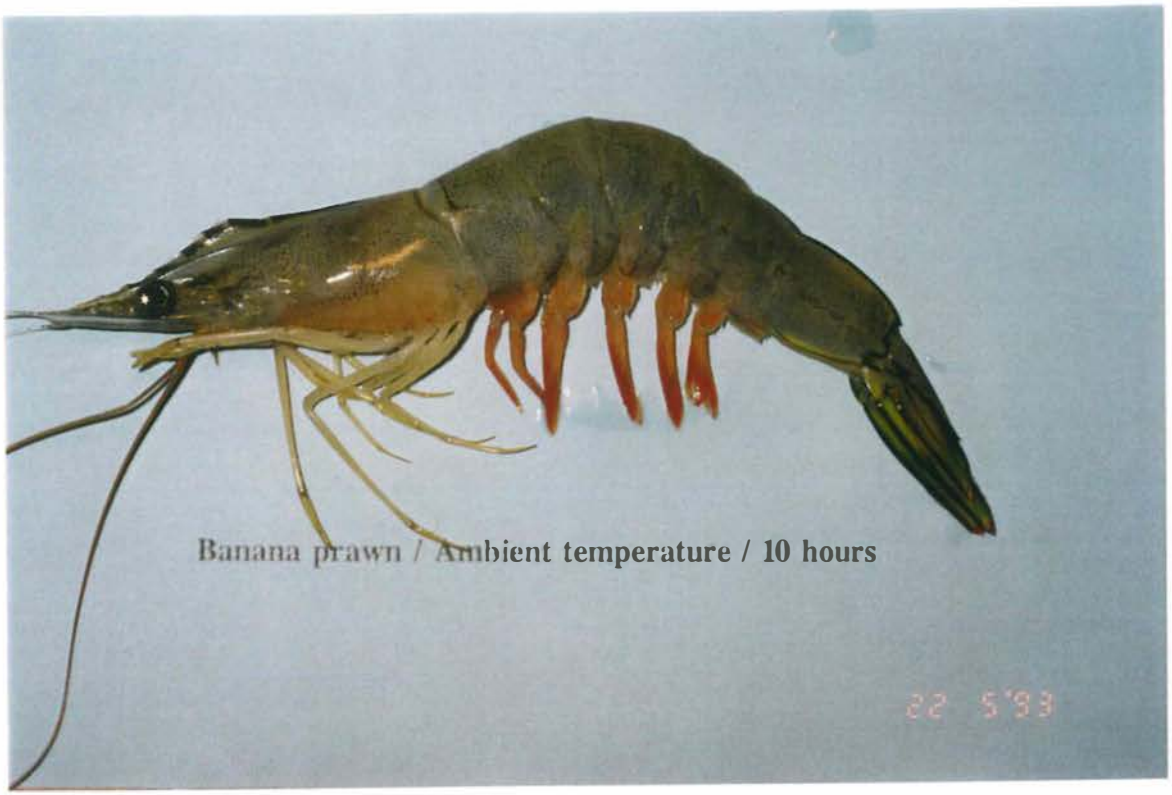


Figure 5.3.1 (b) Banana prawns stored at 22^{0}C for 10 and 16 hours.

Figure 5.3.2

Total bacterial counts (cfu/g) on banana prawns stored at an ambient temperature of 30°C

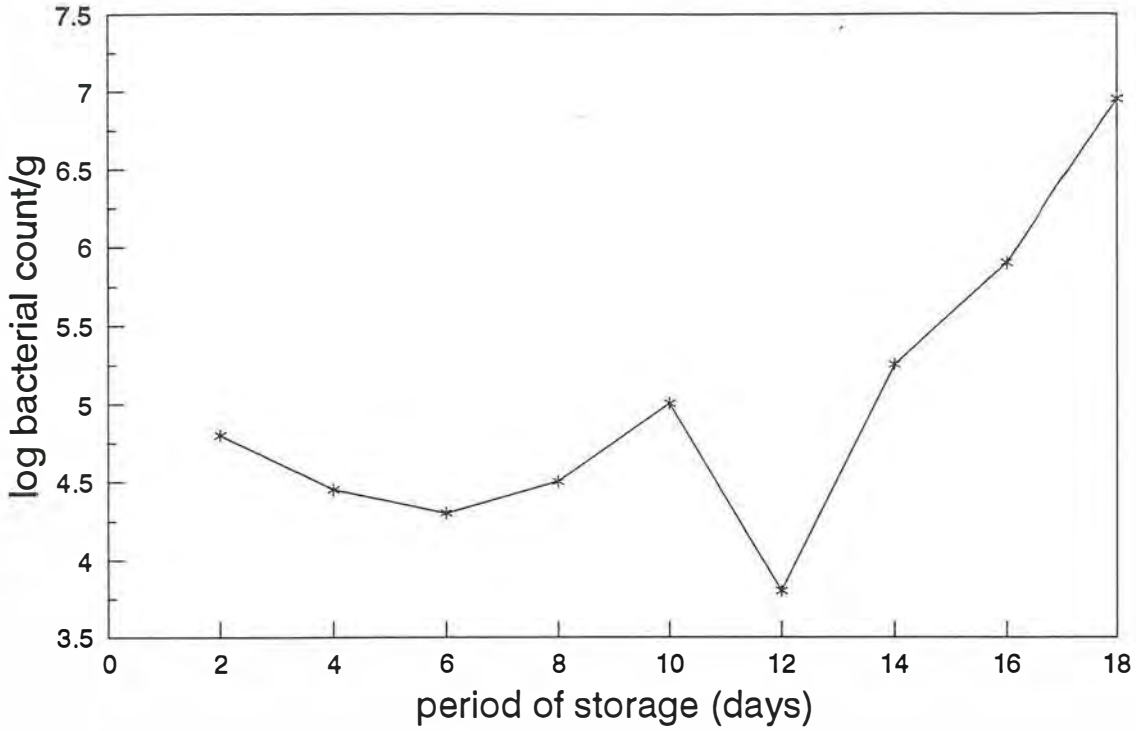
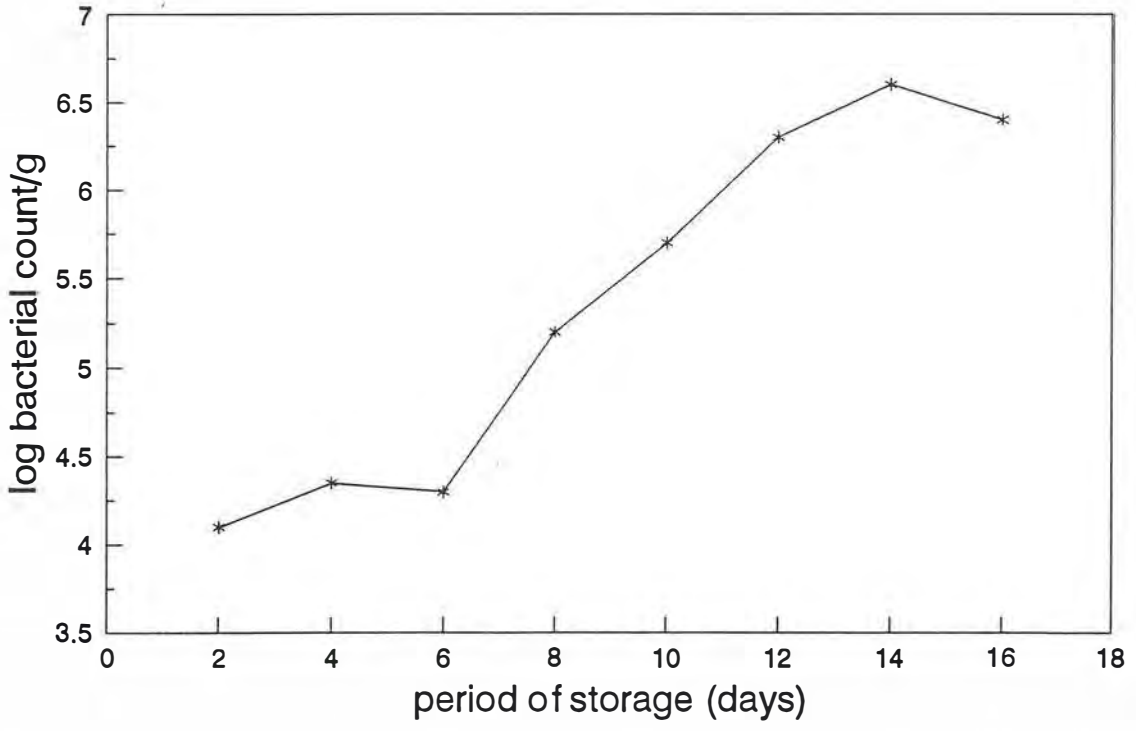


Figure 5.3.3

Total bacterial counts (cfu/g) on banana prawns stored at an ambient temperature of 22°C



SECTION 6 CHILL STORAGE OF PACKAGED PRAWNS

6.1 Vacuum skin packaging of deheaded king prawns

6.2 Vacuum skin packaging of banana and tiger prawns using barrier film

6.3 Packaging of banana prawns

6.1 Vacuum skin packaging of deheaded king prawns

Aim

The aim of this experiment was to assess the use of vacuum skin packaging on the appearance, microbiology and potential shelf life of deheaded king prawns (*Penaeus plebejus*).

A secondary aim was to explore whether a glaze of alginate would improve the appearance and shelflife of the packaged prawns

Introduction

King prawns are a readily available resource from which value added packs can be made. The vacuum skin packaging (VSP) process, involves heat shrinking a thin transparent film over a product arranged on a rigid tray resulting in very attractive convenient packs. The transparent film shows off the product to advantage, giving it a surface gloss and bright appearance. The system produces a very effective pack for prawns particularly if they are first deheaded.

Prawns are best packed in VSP in the deheaded form for several reasons:- (1) the product is more ready to use with less waste (2) the head is more prone to melanosis and (3) the sharp feelers and spikes of the rostrum which are likely to pierce the film and cause product failure are not present.

The film used for the pack must be clear and strong and can be permeable or impermeable to gases but should not transmit water vapour. There are advantages and disadvantages in using permeable films for packaging. Vapour permeable films allow odours to diffuse so that strong smells do not concentrate in the pack and become very obvious to the user when the pack is opened. They maintain an aerobic bacterial flora which produces customary, rather than unusual, spoilage odours and they eliminate the remote possibility of *Clostridium botulinum* growth in the pack. The disadvantage is that the aerobic atmosphere may not be as effective as an anaerobic atmosphere in retarding the growth of spoilage bacteria.

Alginate glazes can be used as a means of keeping prawns separate during freezing. A dip in alginate was included as a treatment in this trial to test whether it could act as a partial barrier to oxygen to prevent melanosis and whether it would improve the shelflife of the packaged prawns.

Materials and methods

The king prawns were obtained directly from the boat and were caught off Mooloolaba. After catch they were stored in refrigerated sea water without treatment in metabisulphite or other compounds normally used to prevent melanosis. On receipt the prawns were stored in chilled seawater in insulated containers and transported to the laboratory (Hamilton, Brisbane) where they were weighed, deheaded and reweighed, washed in fresh water and allocated to each of four separate treatments.

Samples were taken to obtain initial microbiological and chemical information.

One quarter of the prawns were then dipped in an 0.5% alginate solution (Manugel DMB, Kelco Industry Pty Ltd) for 30 sec, drained for 30 sec before being packed. All the prawns were packed, 6 at a time on PVC trays 380 micron thick and then covered in a 100 gauge film ("Permeable Intact") and sealed under vacuum film-to-tray (Figure 6.1.1) using a Mini RM 331 packaging machine. The film had an oxygen permeability of 2100 mL/ m^2/24 h/bar at 23^0C and a water vapour permeability of 6 g/m^2/24h at 90% relative humidity. The packs were stored in chillers set at temperatures of 0°, 5°C and 10°C with a total of 24 packs per treatment at each temperature. Three packs from each treatment were examined at each of the sampling times, set at about three day intervals unless the prawns were clearly spoilt.

Sampling and observations

Three packs were opened for each treatment at each sampling time. Immediately after opening they were evaluated by the experimentors.

Samples for microbiological analysis were aseptically taken from each pack and 10 g prawns were homogenised with 90 mL sterile peptone (0.1%) water in a Colworth Stomacher.

Demerit points

Prawns from each treatment were identified with random 3 digit numbers and were displayed on a bed of ice for evaluation by an experienced group of eight observers using the demerit point score sheet. Samples of prawns were cooked by immersing them in a polythene bag in boiling water for 5 mins then cooling them in ice slurry. Segments of the prawns coded with the appropriate number were presented to each observer for evaluation of taste at the same time as they scored the visual observations.

pH

Two prawns from each treatment were homogenised in an equal volume of distilled water and the pH taken using a Metrohn Universal pH meter .

Total volatile nitrogen (TVN)

Three prawns from each treatment were macerated and TVN estimated on them by the method outlined in Section 4.1.

Microbiological analysis

Total bacterial counts (at incubation temperatures of 5°C and 30°C) were estimated by plating serial dilutions of prawn samples. Surface plating was used to obtain a total bacterial count. Sulphide producing bacteria were identified on iron agar by the method of Gram outlined in Section 4.1.

K-value

The total nucleotides and K-values were estimated by the method outlined in Section 4.1.

Results

Observations

The whole prawns and ranged in weight from 35 to 44g, median 41g, and the deheaded prawn weights ranged from 26 to 40g with a median of 33g (Figure 6.1.2). This represents a mean recovery of 80%.

The experimentors observations are listed in Table 1. Melanosis had not occurred within 3 days in the packs stored at a temperature of 0°C and 5°C but was evident in those at 10°C. After 6 days, slight melanosis occurred on prawns in all the packs and this would effectively limit the storage life. No off odours were detected in packs stored at 0°C and 5°C up to 10 days of storage, whereas at 10°C strong sulphide smells were present.

Table 1 Experimentors observations on VSP prawns.

Date	Storage Removal (Days)	glazed	unglazed		
		Storage Temperature			
		0°C	0°C	5°C	10°C
1/10/93	0	fresh	fresh	fresh	fresh
4/10/93	3	fresh	fresh	fresh	medium melanosis
7/10/93	6	slight melanosis, no off odour	slight melanosis, no off odour	slight melanosis, no off odour	melanosis
11/10/93	10	no off odour, sea smell, melanosis	no off odour, sea smell, melanosis	no off odour, slight sulphide in one pack	strong sulphide smell
14/10/93	13	moderate melanosis, medicinal odour, 4 mL drip	moderate melanosis, slight sea weedy/ prawns smell, no drip	sulphide odour, moderate melanosis, 7-10 mL drip	strong sulphide, melanosis, 12 mL drip
18/10/93	17	sulphide odour, moderate melanosis, 4 mL drip	slight sulphide odour, moderate melanosis, 1-3 mL drip	no sulphide odour, melanosis, 6-14 mL drip	nd

Date	Storage Removal (Days)	glazed	unglazed		
		Storage Temperature			
		0°C	0°C	5°C	10°C
21/10/93	20	fruity odour, NH_3, slight sulphide 1 mL drip	sulphide odour, 4 mL drip	nd	nd
25/10/93	24	melanosis in 35% of body, old sock odour 2.5 mL drip	melanosis on 35% of body, old socks odour	nd	nd

The glaze made little difference to the appearance of the prawns or to the amount of free drip present in the packs.

Demerit scores

The presence of the glaze made no difference to the demerit point scores given by the observers to the prawns (Figure 6.1.3). The change was much more rapid for prawns stored at 10°C. There was no difference between the scores for prawns stored at 5°C and 0°C. This was unexpected, since the rate of deterioration at 5°C is usually twice that at 0°, while at 10°C the rate is four times.

It took 12 days for prawns stored at 0°C to be given a score of 12 points. From this, scores of 6 days and 3 days would be predicted for prawns stored at 5°C and 10°C whereas it took 11 and 4 days respectively to reach this score. Although this experiment was limited in scope these results may indicate an effect of the package in inhibiting change.

Microbiology

The total bacterial counts obtained from incubation at 30° C are shown in Figure 6.1.4. The initial counts were low indicating what can be achieved by good practice.

It took twenty days for counts on prawns stored at 0°C to reach 10^6/g. The glazed prawns supported higher bacterial growth than the unglazed presumably because the glaze provided a good moist nutrient rich surface. Bacterial growth on prawns stored at 10°C was initially much faster than at 5°C or 0°C (expected) but slowed soon after counts approached 10^6/g (about 7 days). It appears that some chemical inhibitory process was involved.

This inhibitory pattern was less evident for the psychrotrophic bacterial counts obtained by incubation at 5° C (Figure 6.1.5). Indeed the bacterial counts at this incubation temperature were slightly higher for all treatments indicating the adaptable nature of the inherent flora.

The results obtained for the sulphide producing bacteria are shown in Figure 6.1.6 as a proportion of the total flora and as the number present. In the packs stored at 5°C the H_2S bacterial count reached 10^6/g (nearly 100% of total flora) between 13 and 17 days storage coincident with the time the experimentors recorded the presence of sulphide odours in the packs. The H_2S count for the unglazed prawns stored at 0°C was only about 10^4/g (80% of total flora) after 20 days of storage although sulphide odours were evident to the experimentors by 17 days of storage. Not all sulphide odours are due to H_2S and bacteria producing them may not be selected on this growth medium.

Total volatile nitrogen

The glaze did not affect the production of total volatile nitrogen (TVN) on the prawns and as expected storage at higher temperatures increased the rate of production (Figure 6.1.7).

pH

There was an increase in pH in all treatments particularly during the first 10 days of storage. The rate being related to temperature (Figure 6.1.8).

K-value

The total nucleotide pool of the initial samples was 10 μ mole/g, a common value for prawns, by the end of the trial the levels were near 8 μ mole/g indicating the usual decrease with chilled storage. The initial K-values were high, 66%, and did not change over the total storage period. High initial values are related to stress during capture.

Summary of results

Table 2 Time (days) taken to reach certain specified levels and rate of change relative to the rate at 0°C (in brackets).

Temperature of storage (°C)	Specified levels and rate of change relative to the rate at 0°C (in brackets)						
	Bacterial count (30°C)	Two log cycle increase (30°C)	Bacterial count (5°C)	H_2S producing bacteria 10^6/g	Ten demerit points	Experimentor detection of sulphide odours	TVN 50 mg/ 100 g
0	20 (1)	17 (1)	18 (1)	23 (1)	12.5 (1)	17 (1)	23 (1)
5	13 (1.5)	9 (1.8)	9 (2)	15 (1.5)	11.5 (1.1)	10 (1.7)	11 (2.1)
10	7 (2.9)	3 (5.7)	6 (3)	nd	4 (3.1)	6 (2.8)	4 (5.8)

The experimental results have been summarised comparatively in Table 2 along with calculation of the rates of change relative to that in prawns stored at 0°C. The results on relative rates for TVN and time taken for a 2 log cycle of bacterial growth compare favourably.

In general the relative rates of change observed in prawn packs stored at 5°C is a little lower than the rate of 2 anticipated. The rate of change in demerit points is markedly lower than anticipated in theory and lower than would be expected from the bacterial counts. The increase in bacterial numbers does not seem to have been accompanied by the production of off-odours and the physical appearance did not markedly change. Nor were there drastic textural or flavour changes. The first signs of change such as dullness in appearance and off odours and off flavours appeared at bacterial levels of 10^6/g. Even the bacterial growth appears somewhat slower than in previous experiments. King prawns which were stored unpacked in ice or in ice slurry took 2 and 10 days respectively to reach bacterial counts of 10^6/g from a commencing level of 10^4/g.

Conclusions

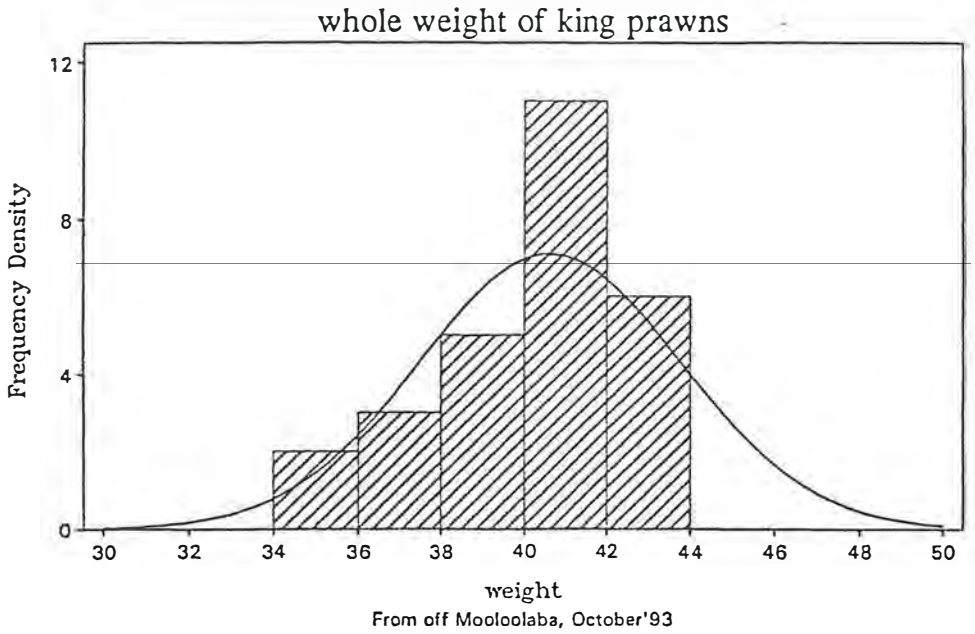
Deheaded king prawns packed in vacuum skin packs have a storage life at 0°C limited by melanosis to about 6 days. If melanosis was controlled then a storage life of 13 days at 0°C and 6 to 10 days at 5°C should be achievable. This relies on the starting material being of good standard with a low bacterial count.

King prawns are prone to melanosis, more so than banana prawns. This is a physical, visual problem that in no way renders the prawn inedible or bacteriologically unsound. It is made worse by poor handling and physical damage. The prawns in this study were handled well. The permeable film did not afford protection from melanosis and chemical additives or other protective packaging must be used in order to achieve the full potential shelf life estimated at about 13 days.

The glaze did not inhibit bacterial growth or act as a barrier to oxygen to prevent melanosis nor did it have a beneficial effect on the appearance or properties of the stored prawns.

Figure 6.1.1 A vacuum skin pack of deheaded king prawns

Figure 6.1.2 Distribution of weights



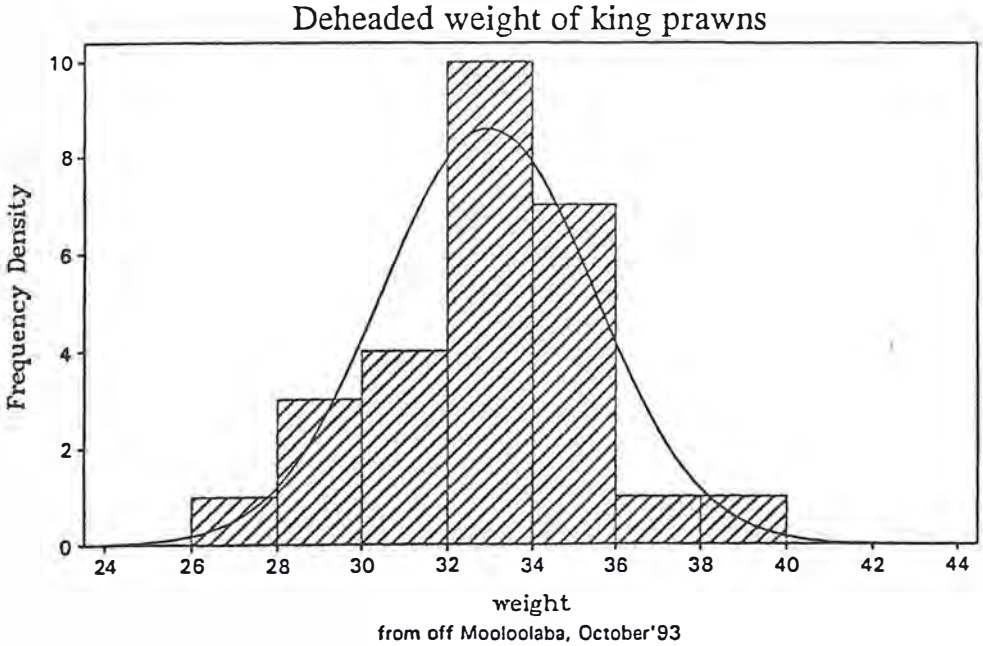


Figure 6.1.3

Change in demerit scores of vacuum skin packed king prawns in permeable film and stored at 0°C (with/without glaze), 5°C and 10°C.

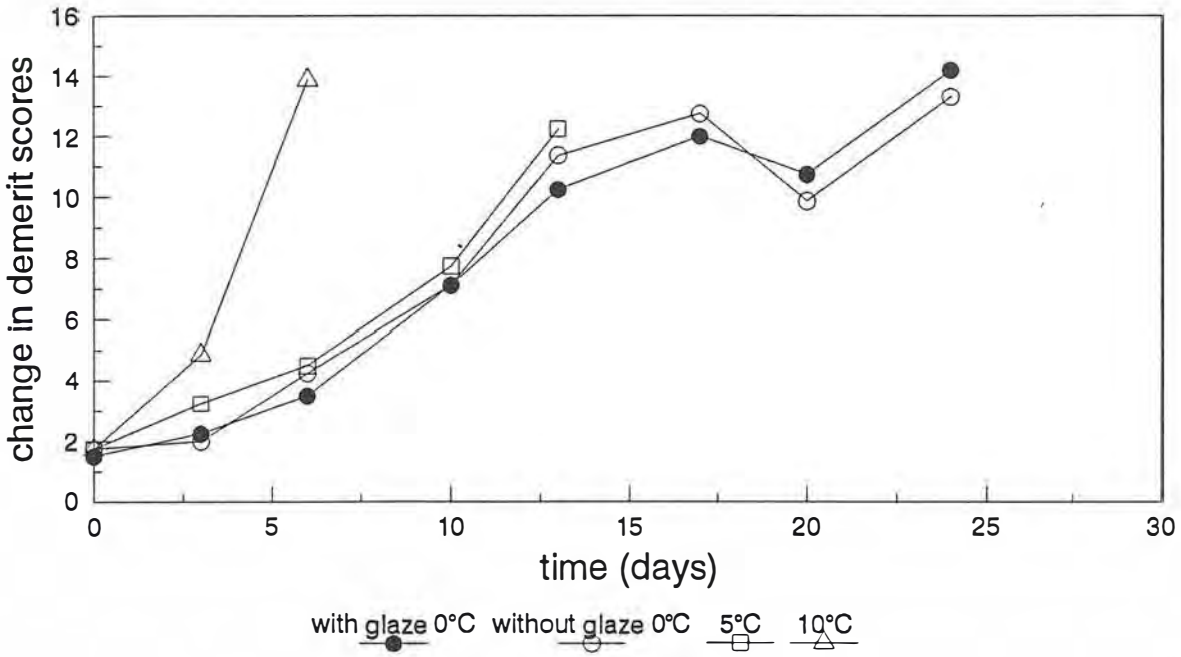


Figure 6.1.4

Change in total bacterial counts(30°C) in vacuum skin packed king prawns in permeable film, stored at 0°C (with/without glaze), 5°C & 10°C

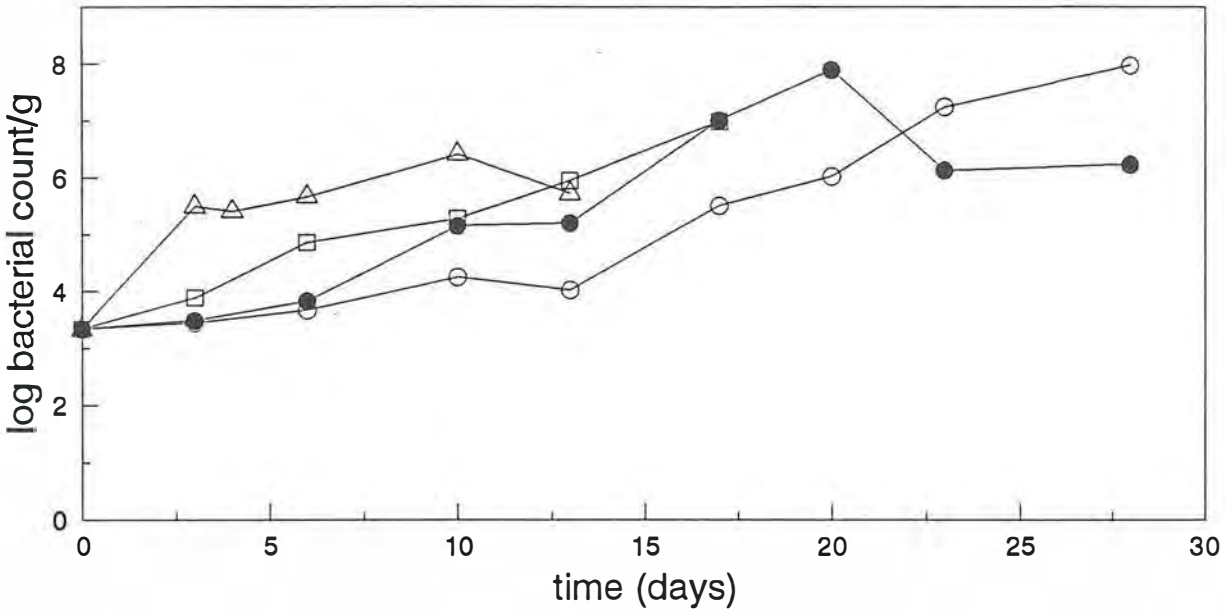


Figure 6.1.5

Change in total bacterial counts (5°C) in vacuum skin packed king prawns in permeable film, stored at 0°C (with/without glaze), 5°C & 10°C

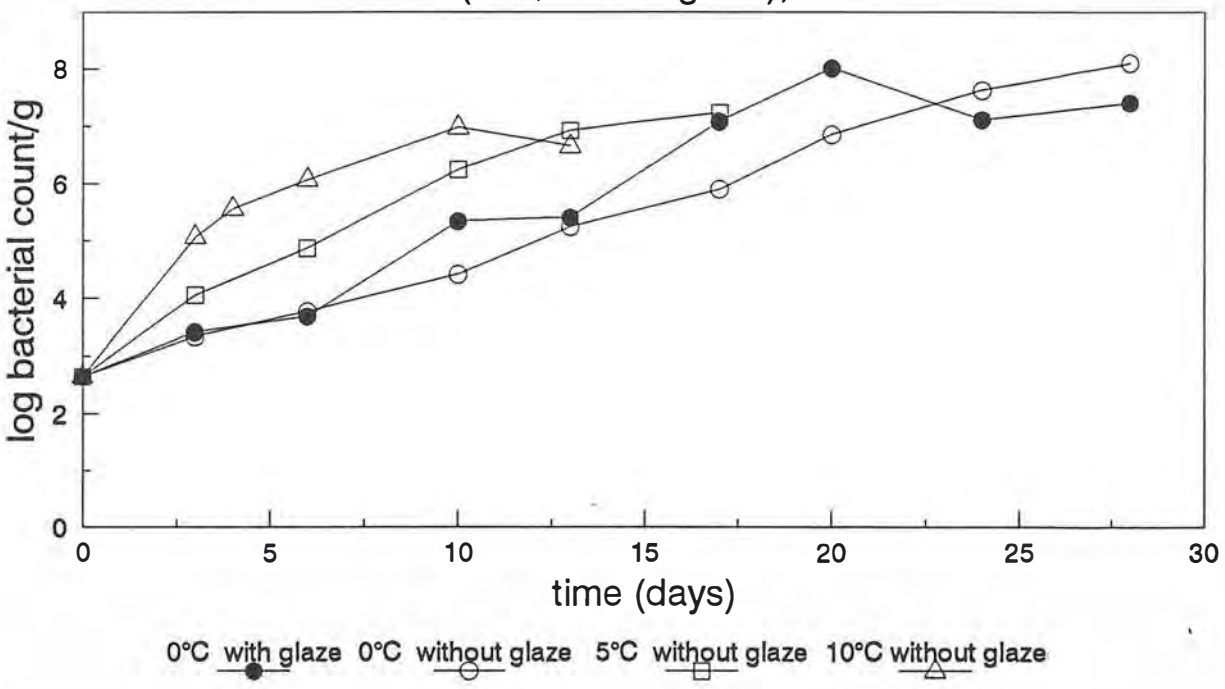


Figure 6.1.6

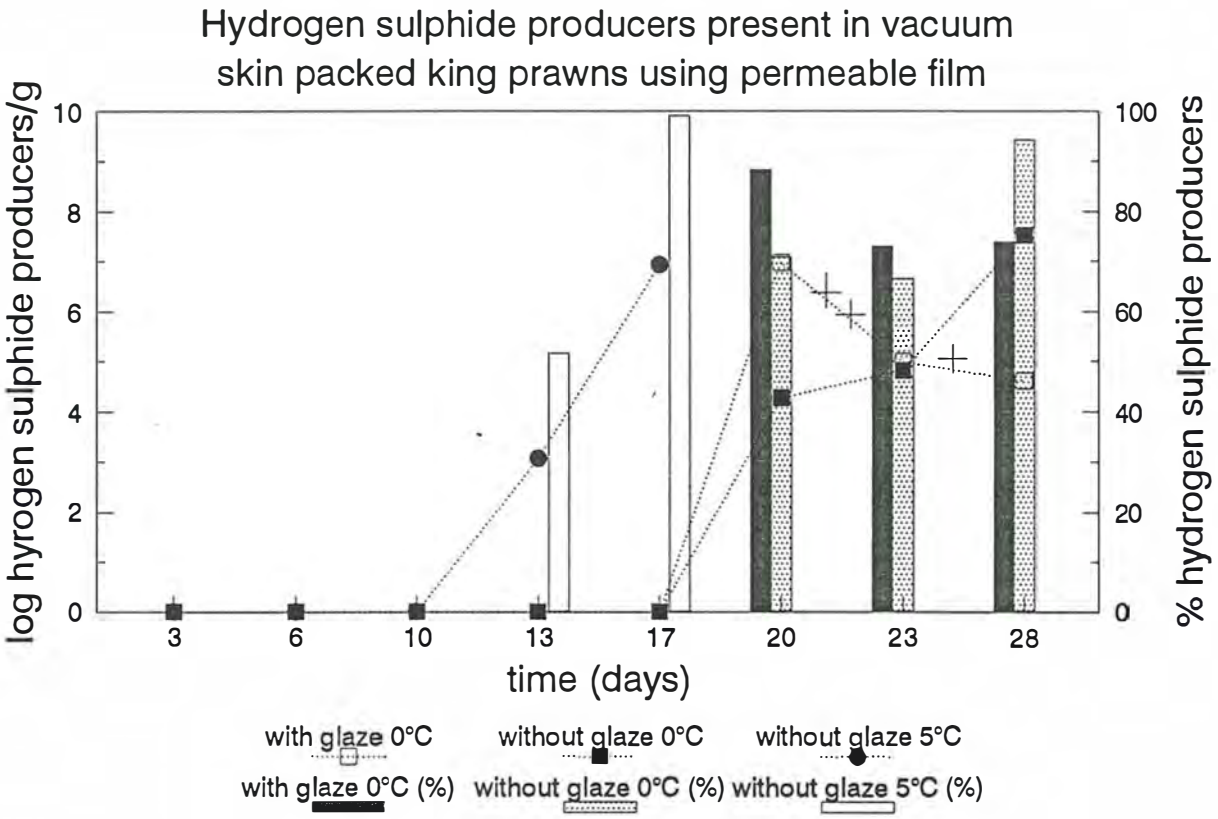

Hydrogen sulphide producers present in vacuum skin packed king prawns using permeable film
log hyrogen sulphide producers/g
% hydrogen sulphide producers
0
2
4
6
8
10
0
20
40
60
80
100
3
6
10
13
17
20
23
28
time (days)
with glaze 0°C
without glaze 0°C
without glaze 5°C
with glaze 0°C (%)
without glaze 0°C (%)
without glaze 5°C (%)

Figure 6.1.7

Change in total volatile nitrogen in king prawns vacuum skin packed in permeable film and stored at 0°C(with/without glaze), 5°C and 10°C

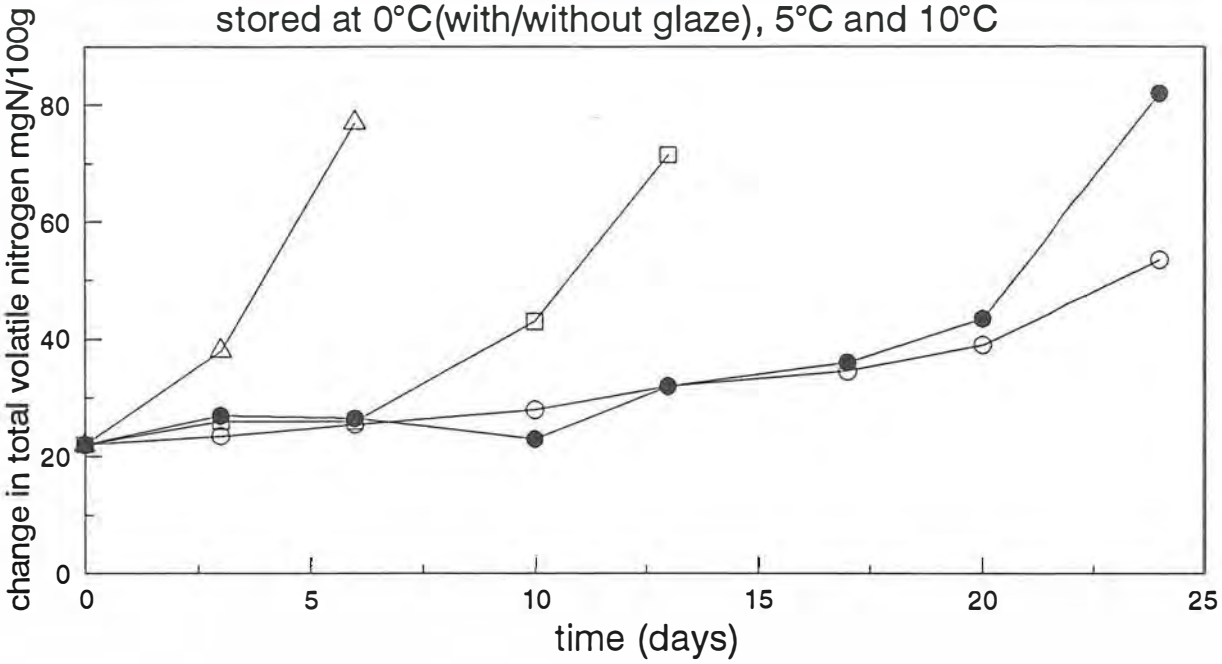
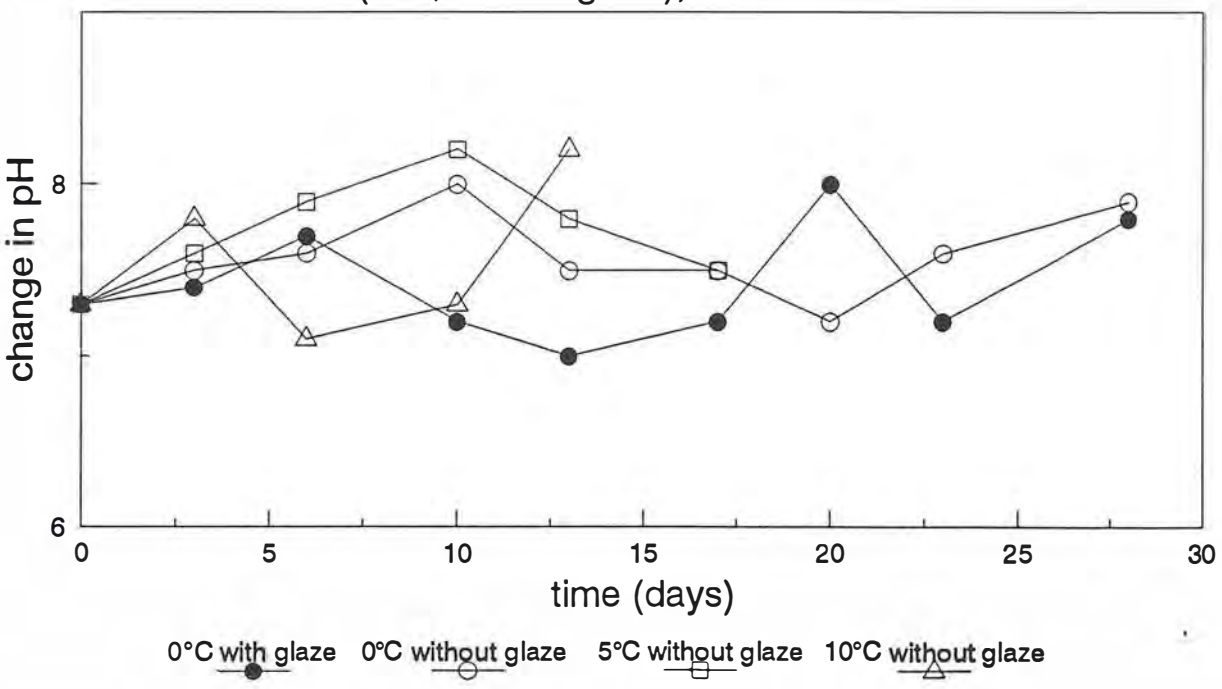


Figure 6.1.8

Change in pH in vacuum skin packed king prawns packed in permeable film, stored at 0°C (with/without glaze), 5°C and 10°C.



6.2 Vacuum skin packaging of banana and tiger prawns using barrier film

Aim

To investigate whether vacuum skin packaging of prawns in barrier film leads to greater shelf life and different spoilage characteristics.

Introduction

The process of vacuum skin packaging (VSP) provides an attractive convenient means of presenting prawns to the consumer. A previous trial has shown that if prawns of good standard with low microbial counts are packed then products can be obtained that have good microbial stability.

The shelf life however was limited by melanosis since the film employed was permeable to oxygen and no agents such as metabisulphite were used to retard its development. If melanosis can be retarded by the packaging without the use of chemicals then this would allow the processor the opportunity to place a premium product on the market.

The next logical step was therefore to package prawns in barrier films where access to oxygen was restricted.

Materials and methods

Materials

The tiger prawns (*Penaeus esculentus*) were caught in Moreton Bay in the early morning of 21/4/94 and were obtained from a local supplier. The banana prawns (*Penaeus merguiensis*) were caught in the afternoon (1200-1400 hours) of the same day on Moreton Bay off Scarborough near the mouth of the Caboolture river. After sorting they were stored in refrigerated sea water until they arrived at the laboratory about 2 hours after catch.

The banana prawns were in excellent condition but it was noted that the tiger prawns had a high enzyme activity in the head region.

The prawns were weighed and deheaded, reweighed, then washed in tap water, drained and packed on trays, six to a tray or a bag.

Packs of tiger prawns were prepared both in oxygen permeable polyethylene bags and on trays sealed with a barrier film. There were insufficient banana prawns for both treatments and only the barrier film was used. Twenty five packs of each treatment were prepared. The barrier film was 75 gauge thickness with an oxygen permeability of 40 mL/m^2/24 h/bar at 23°C and water vapour permeability of 6 g/m^2/24 h at 90% relative humidity.

Low density polyethylene bags (poly bags) were used as a film highly permeable to oxygen. The trays were 380 micro PVC and the machine a mini RM 331 from Trigon Packaging systems. The packs were stored in a chiller at a temperature of 4°C (range 3-5°C).

Sampling and observations

A sample of the prawns was taken before deheading for microbial counts and other analyses.

The packs, 3 per treatment, were sampled at about 3 days intervals for up to eight sampling times. The experimentors took observations on each pack as it was opened.

Samples for microbiological analysis were aseptically taken from each pack and 10 g prawns were homogenised with 90 mL sterile peptone (0.1%) water in a Colworth Stomacher.

Demerit points

Prawns from each treatment were identified with random 3 digit numbers and were displayed on a bed of ice for evaluation by an experienced group of eight observers using the demerit point score sheet. Samples of prawns were cooked by immersing them in a polythene bag in boiling water for 5 mins then cooling them in ice slurry. Segments of the prawns coded with the appropriate number were presented to each observer for evaluation at the same time as they scored the visual observations.

pH

Two prawns from each treatment were homogenised in an equal volume of distilled water and the pH taken using a Metrohn Universal pH meter.

Total volatile nitrogen (TVN)

Three prawns from each treatment were macerated and TVN estimated on them by the methods previously outlined in Section 4.1.

Microbiological analysis

Total bacterial counts (at incubation temperatures of 5° and 30°C) were estimated by plating serial dilutions of prawn samples, prepared by homogenising (in a Colworth Stomacher) 10 g of prawn (by macerating 3-5 prawns aseptically) in 90 mL of sterile peptone (0.1% w/v) diluent. Surface plating techniques were used to obtain a total bacterial count. Identification of genera and sulphide producers were done according to methods outlined in Section 4.1.

Results

Weights

There was quite a range of weight of the tiger prawns from 18 to 48 g with no clear median size. After deheading the range was from 9 to 27 g (Figure 6.2.1). The banana prawns ranged

from 14 to 38 g with a median of 27g (Figure 6.2.2) and after deheading from about 13 to 37g with a median size of 23g (Figure 2).

Experimentors observations

These are listed in table 1.

Table 1 Experimentors observations.

Date	Period of storage (days)	Observations		
		Tiger prawns in poly bags	Tiger prawns in barrier VSP	Banana prawns in barrier VSP
18/4/94	0	very good appearance	very good appearance	very good appearance
19/4/94	1	normal appearance, fresh odour	very good appearance, fresh odour	very good appearance, fresh
24/4/94	5	slight melanosis, all colours intact, slight prawn odour	very good appearance, fresh	very good appearance, fresh
27/4/94	8	slight melanosis, amine, basic odour	good appearance, no off odour, fresh	fresh odour, very good appearance, colours intact
31/4/94	12	slight melanosis, digested appearance, sour and amine odour	slight sulphide odour, no melanosis, good appearance	very good appearance, colour intact, no off odour
4/5/94	15	medium melanosis, digested appearance, sour, putrid odour	good appearance but slightly dull, weedy, sulphide, 'old socks' odour	good appearance, sweet slight sulphur
8/5/94	19	digested slight amine	sweet odour, earthy, weedy, appearance OK	appearance OK, sulphur, amine
11/5/94	22	-	-	good appearance slightly dull sulphide, amine, sweet
15/5/94	26	-	-	good appearance but slightly dull, sulphide amine, sweet

Date	Period of storage (days)	Observations		
		Tiger prawns in poly bags	Tiger prawns in barrier VSP	Banana prawns in barrier VSP
19/5/94	30	-	-	strong sulphide, amine, 'old' odour, sweet

Tiger prawns

The prawns in the poly bags had developed slight melanosis by 5 days in storage and by 8 days had amine, basic off odours as well as melanosis (Table 1, Figure 6.2.3). By 12 days they were sour and by 15 days were putrid and showed substantial melanosis (Figure 6.2.4).

In contrast those in the barrier film exhibited no off odours after 8 days storage; they had slight sulphide odours by 12 days but no melanosis (Table 1). Their appearance remained good for up to 19 days (Figure 6.2.5 & 6.2.6) with no real signs of melanosis.

Banana prawns

The appearance of the banana prawns was still good after 22 days storage (Table 1). No off odours were noted up to 12 days but a sweet, slight sulphur smell was noted by 15 days storage. No melanosis occurred up to 19 days of storage (Figure 6.2.7 & 6.2.8).

Demerit points

The mean number of demerit points are plotted in Figure 6.2.9. The panel results concur with those of the experimentors that the tiger prawns in the permeable packs deteriorated more rapidly than those in the barrier packs. The banana prawns showed a pattern typical of the 'long lingering type' that occurs also with fish.

pH

Despite the obvious differences in visual scores, the pH results for all packs were remarkably similar (Figure 6.2.10). Since there were considerable differences between treatments in the microbial counts it must be assumed that the increase in pH is more due to enzymic and chemical action than to bacterial activity.

Microbiology

The bacterial counts obtained at incubation temperatures of 30°C and 5°C were identical throughout for all treatments (Figure 6.2.11) indicating an adaptable psychrotrophic flora.

The starting counts were quite low just over 10^4/g for tiger prawns and 10^3/g for banana prawns. The counts reflect the experimentors observations and the demerit point scores.

There was quite a rapid increase in bacterial growth on the tiger prawns in the poly bags reaching 10^6/g in about 4 days. By eight days the counts were quite high, over 10^8/g. There was no lag phase since the psychrophilic flora could thrive in the conditions. In contrast, bacterial growth in the barrier VSP was slower with counts reaching 10^6/g after 7 days and 10^7/g after 8 days. Thus the barrier pack appeared to inhibit microbial growth; a lag phase of up to 5 days was noted and the total counts never became as high as those in the polyethylene bags.

The counts on the banana prawns did not reach 10^6/g until about 12 days of storage and there was evidence of a lag phase of about 8 days as the flora adjusted to its new environment.

Sulphide producing bacteria

The sulphide producing bacteria are plotted in Figure 6.2.12 as total counts and as a percentage of the total microbial flora.

These results demonstrate that with the tigers packed in the poly bags after 5 days storage virtually the total microbial flora (10^6/g) was comprised of bacteria capable of sulphide production. Similarly the flora of the tiger prawn in the barrier bags was also predominantly sulphide producing bacteria although after 5 days the counts were only 10^4/g. These results indicate that packaging in barrier film did not alter the nature of the microbial flora but retarded its growth.

With the banana prawns after 8 days storage the sulphide producing bacteria were about 50% of the total flora but then effects were not evident since the counts were very low, below 10^2/g. By 12 days of storage the counts were approaching 10^6/g and the total flora was comprised of sulphide producers.

These results again show this lag phase in development of a spoilage flora under these circumstances. The sulphide producing flora increased after 12 days to near 10^7/g after which some inhibitory process slowed the rate of growth.

However it appears that a flora of the same nature is selected so that spoilage proceeds along the same lines just at different rates.

Microbial flora

The flora on the tiger prawns at the time of catch and one day after packaging in either VSP or in poly bags was dominated by *Moraxella* species (Table 2) which are known to spoil fish. In contrast the flora on the banana prawns at harvest was mostly *Bacillus* species and other Gram positive organisms which failed to grow when subcultured. These organisms are not spoilers. Some *Moraxella* were present at harvest but none were detected one day later in the packs.

Table 2 Bacterial flora (30°C incubation) on tiger and banana prawns packed in poly bags and barrier film. Chill stored at 3-5°C.

Type of Flora	Tiger (VSP)			Banana (VSP)			Tiger (Poly)	
	Harvest	Initial (Day 1)	Spoilage (Day 15)	Harvest	Initial (Day 1)	Spoilage (Day 27)	Initial (Day 1)	Spoilage (Day 15)
Gram positive flora	19	8	6	5	-	6	4	-
Moraxella spp	48	53	-	19	-	2	37	23
Pseudomonas spp	-	-	-	-	-	8	12	-
Other gram negative flora	-	-	-	-	-	6	-	-
Lactobacilli	7	-	-	2	-	-	2	-
Shewanella putrefaciens	-	-	92	-	-	70	-	77
Acinetobacter	7	2	-	-	-	-	4	-
Vibrio spp	-	-	2	-	-	2	-	-
Dead	19	37	-	73*	100*	6	32	-
Total bacterial count	5×10^4	8×10^3	$\sim 10^8$	10^4	7×10^3	$\sim 10^8$	9×10^3	$\sim 10^9$

* Presumptively identified as Bacillus spp during initial identification.

The spoilage flora on tiger prawns in both VSP and poly bags was *Shewanella putrefaciens*, 92 and 77% respectively along with some remaining Moraxella in the poly bags. *Moraxella* are spoilers which could be expected to survive in these conditions. On the other hand *Shewanella putrefaciens* normally prefers a low oxygen concentration. This low concentration may have occurred in these bags where the prawns were not spread in a single layer as in VSP but were together in one mass.

The banana prawns also spoilt due to *Alteromonas putrefaciens* and this would account for the detection of the sweet slight sulphur odours after 15 days storage since the film used in the VSP packs would not allow these odours to dissipate.

Summary of results

A summary of the results is given in Table 3 to draw out the differences between the tiger prawns packed in the two different forms and the banana prawns in the barrier packs. The greater stability to change of the banana prawns can clearly be seen and despite the growth of bacteria they remained good in appearance for a considerable time.

It also appears that when a sulphide producing flora of about 10^6/g occurs that sulphide odours are detectable when the pack is opened and that soon after this off odours and off flavours will be detectable in the cooked prawns.

Table 3 Time (days) taken to reach certain specified indicator levels in packaged prawns stored at 4°C.

Prawns and Treatment	Specified Levels						
	Bacterial count		H_2S producing bacteria		Observations		
	10^6/g	10^7/g	10^6/g	10^7/g	10 demerit points	Off odours	pH 7→pH 8
Tiger in poly bags	4	6	5	7	9	8	12
Tigers in barrier packs (VSP)	7	8	7.5	10	15	12	14.5
Bananas in barrier packs (VSP)	12.5	15	12	15	never	15	18

Comparison with other trials

The comparison between the present trial and previous chill storage trials in which the prawns were not packaged but stored in ice or ice slurry is shown in Table 4.

It appears that the tiger prawns in the poly bags give comparable results to the tiger prawns stored in ice, since the spoilage at a temperature of 6°C is normally at least twice that at 0°C.

Viz 4.5 days at 4°C is equivalent to 9 days at 0°C, and these are the times taken for the bacterial counts to reach 10^6/g.

The barrier pack is acting more like storage in slurry in which oxygen is also restricted. For example the time taken for the counts on tiger prawns to reach 10^7 in the barrier packs at 4°C is 8 days which is equivalent to 16 days at 0°C as observed in the slurry.

With the banana prawns the time taken for the count to reach 10^7/g in the barrier packs (at 4°C) was 15 days which would be equivalent to 30 days at 0°C double that actually observed. To put it in the reverse way the time predicted from the slurry experiments for banana prawns in barrier packs at 4°C would be more like 7.5 days rather than the 15 days observed.

Therefore the circumstances in the barrier pack inhibited bacterial growth on the banana prawns but not on the tiger prawns. The circumstances, or environment, of storage in a pack are a combination of many factors. In this case the pack and the temperatures were the same but the available nutrients on each type of prawn would be different and the interactions between the original microflora and the flora which can grow in these conditions would also be different.

Table 4 Time (days) taken to reach certain specified indicator levels in banana and tiger prawns in two different storage experiments.

Specified level	Time (days)						
	Tiger prawns in packs 4°C		Banana prawns in packs 4°C	Tiger prawns stored at 0°C		Banana prawns stored at 0°C	
	Poly bags	Barrier	Barrier	Ice	Slurry	Ice	Slurry
Bacterial count							
10^6/g	4.5	7	12.5	9	12	10	11
10^7/g	6	8	15	10.5	16	14	15
10 demerit points	15	9	never	7	21	13	15

Conclusion and discussion

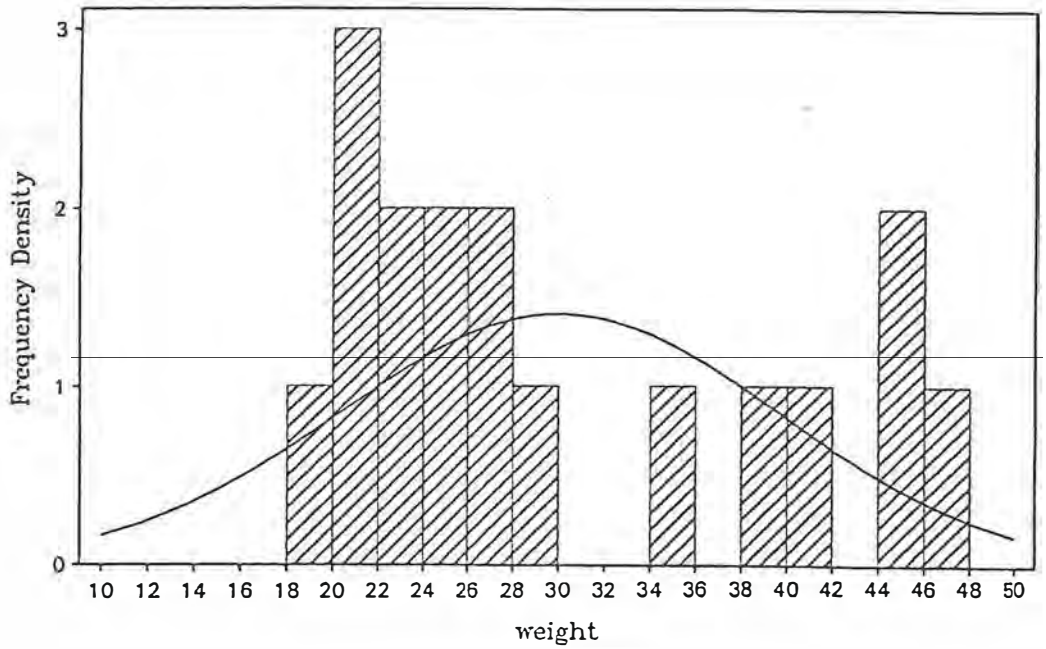
Packaging of tiger prawns in barrier film using vacuum skin packaging can increase their shelflife over that obtained in a permeable pack such as a poly bag. These results were similar to those obtained by storing tiger prawns either in ice or in slurry in a previous experiment.

Banana prawns have been previously found to be quite stable in storage and vacuum skin packaging can result in a product with a relatively long shelflife. However the spoilage organism *Shewanella putrefaciens* can still survive and grow in the packs to produce unpleasant spoilage odours of a sulphide like nature. Therefore the spoilage pattern is not radically different from storage in other oxygen limiting situations such as in ice slurry. Nor is it very different from other forms of chilled storage, it just takes longer to reach a level at which the prawns would be rejected.

The VSP process inhibits melanosis; none was observed in either the tiger or the banana prawn packs. Thus the use of chemicals can be avoided.

The nature of the microflora found at capture depends on the environment of the prawn. The tiger prawns had a high level of *Moraxella* which grow in chill storage and which are capable of spoilage activity. As a result there was only a short (if at all) lag phase in bacterial growth. On the other hand the largely Gram positive flora of the banana prawn did not survive chill conditions or subculturing and took little or no part in the spoilage process. Thus there was a lag phase while the flora adjusted and *Alteromonas, Pseudomonads* and other Gram negative bacteria became established.

Whole weight of tiger prawns



Deheaded weight of tiger prawns

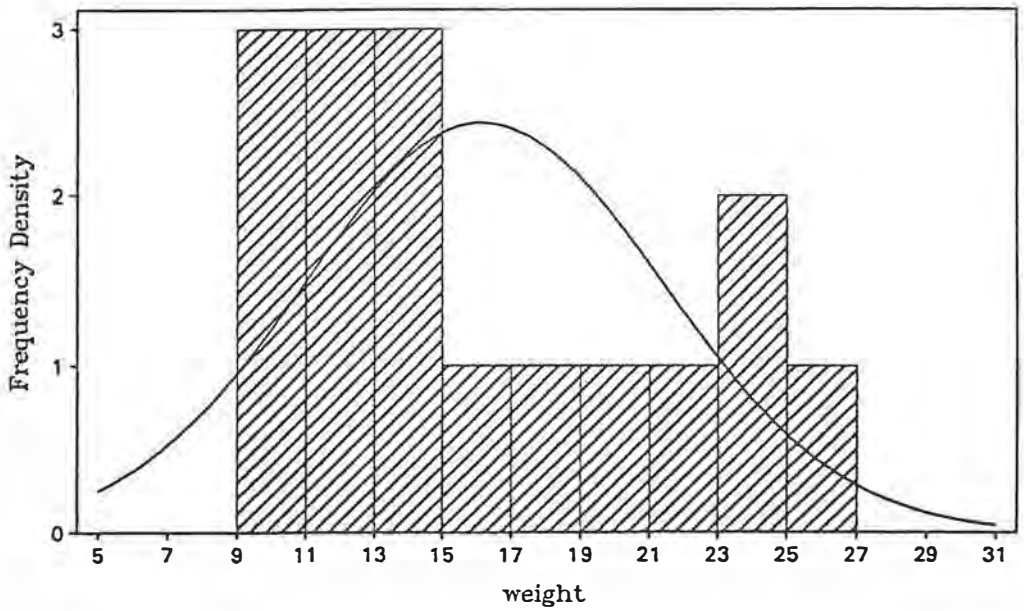
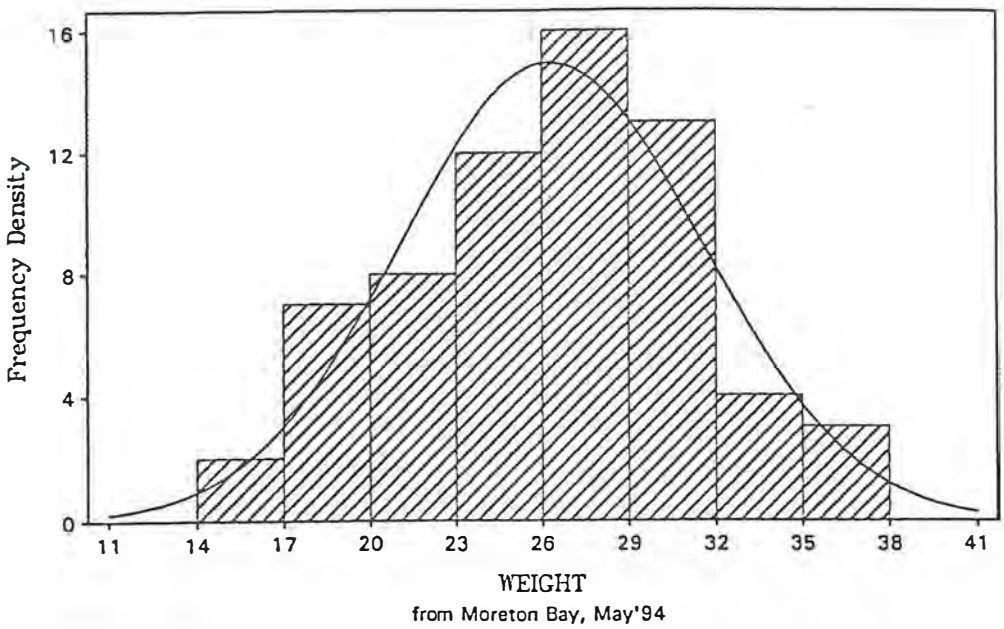


Figure 6.2.1 Weights of whole and deheaded tiger prawns

Whole weight of banana prawns



Deheaded weight of banana prawns

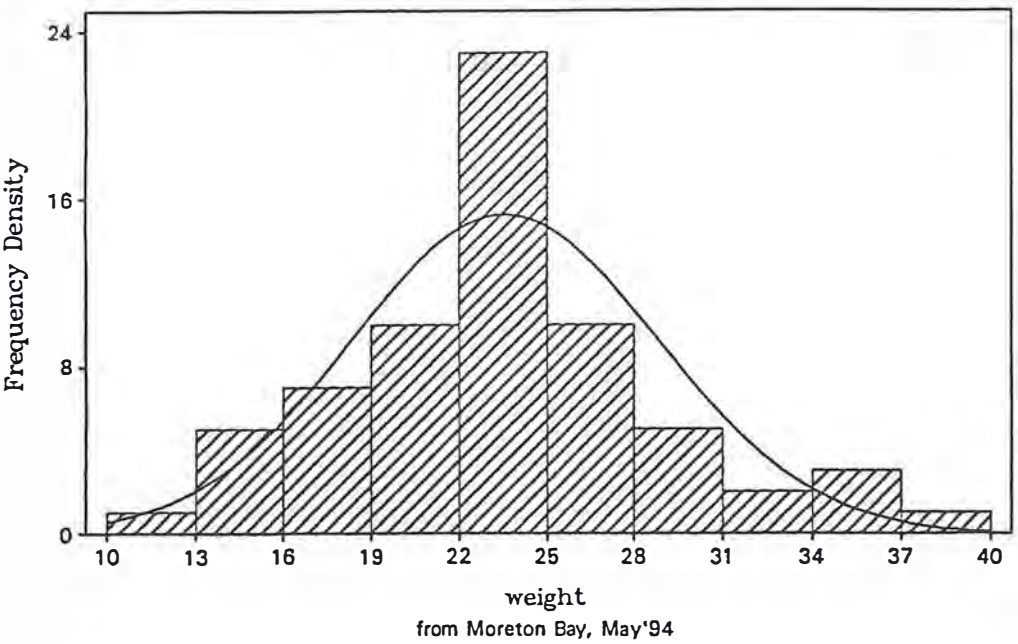


Figure 6.2.2 Weights of whole and deheaded banana prawns

Figure 6.2.3 Tiger prawns stored five days in poly bags.

Figure 6.2.4 Tiger prawns scored 15 days in poly bags. Extensive melanosis, darkening of the flesh and other deteriorative changes (Table 1) have occurred.

TIGER PRAWNS - *P. esculentus/semisulcatus* - 5 days
Chill Storage - Vacuum skin packaging in barrier film

Figure 6.2.5 Tiger prawns in barrier vacuum skin packs stored chilled for 5 days.

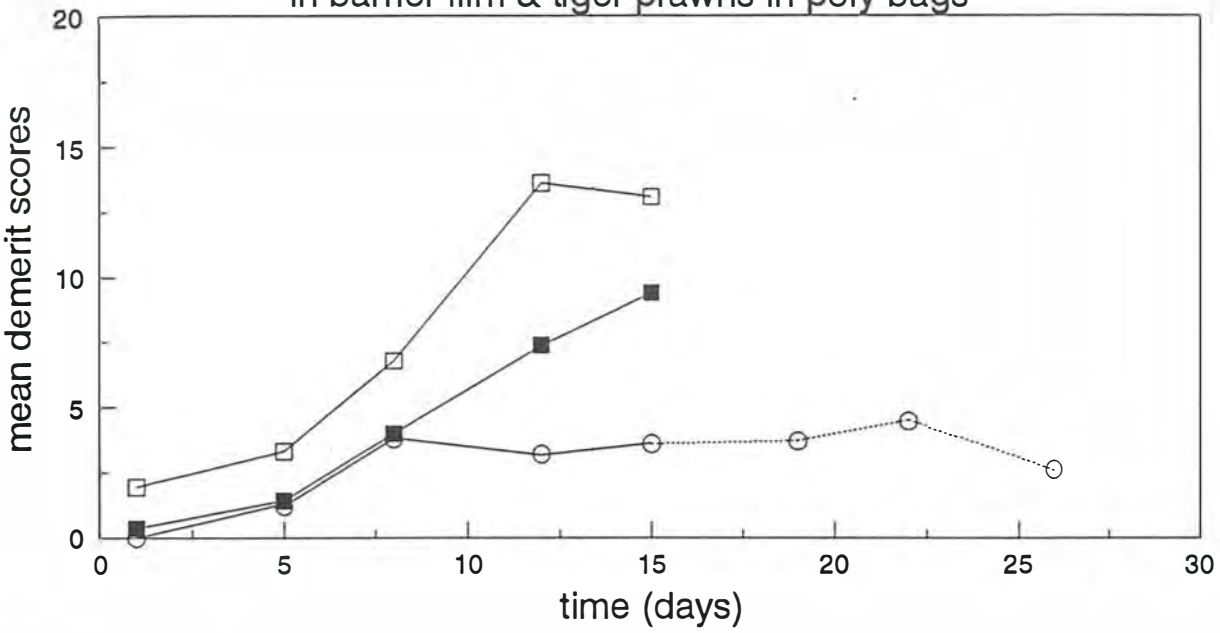
Figure 6.2.6 Tiger prawns stored chilled in barrier vacuum skin packs for 19 days. They show no signs of discolouration or melanosis but were spoilt otherwise (Table 1).

Figure 6.2.7 Banana prawns in barrier vacuum skin packs chill stored for 5 days.

Figure 6.2.8 Banana prawns chill stored 19 days in barrier vacuum skin packs. No melanosis or discolouration evident.

Figure 6.2.9

Change in demerit scores of vacuum skin packed banana & tiger prawns in barrier film & tiger prawns in poly bags



p <0.01 = 4.8447 for comparing storage times of a particular treatment

Figure 6.2.10

Change in pH in tiger and banana prawns vacuum skin packed in barrier film and tiger prawns packed in poly bags.

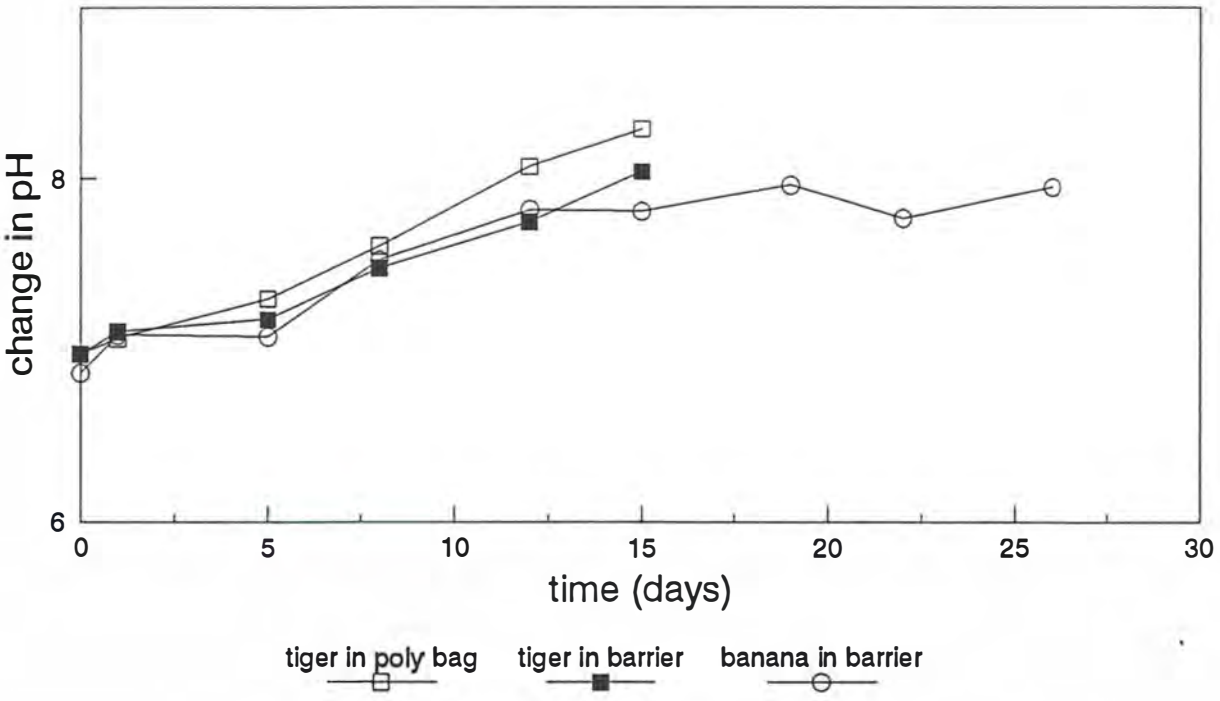


Figure 6.2.11

Change in total bacterial count in tiger and banana prawns vacuum skin packed in barrier film and tiger prawns packed in poly bags.

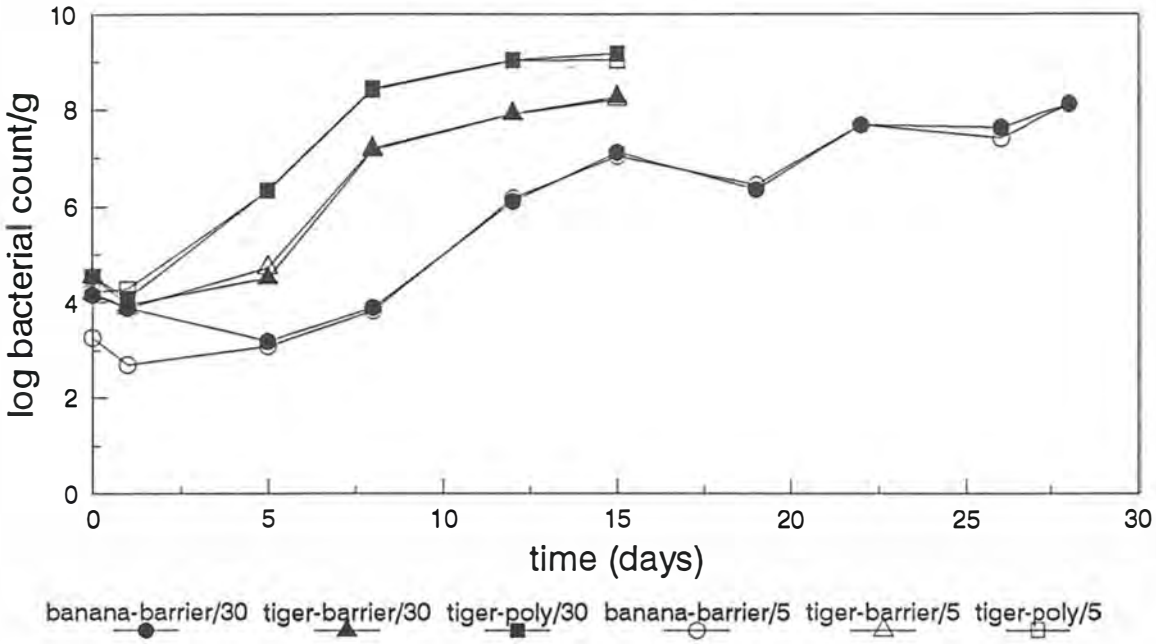
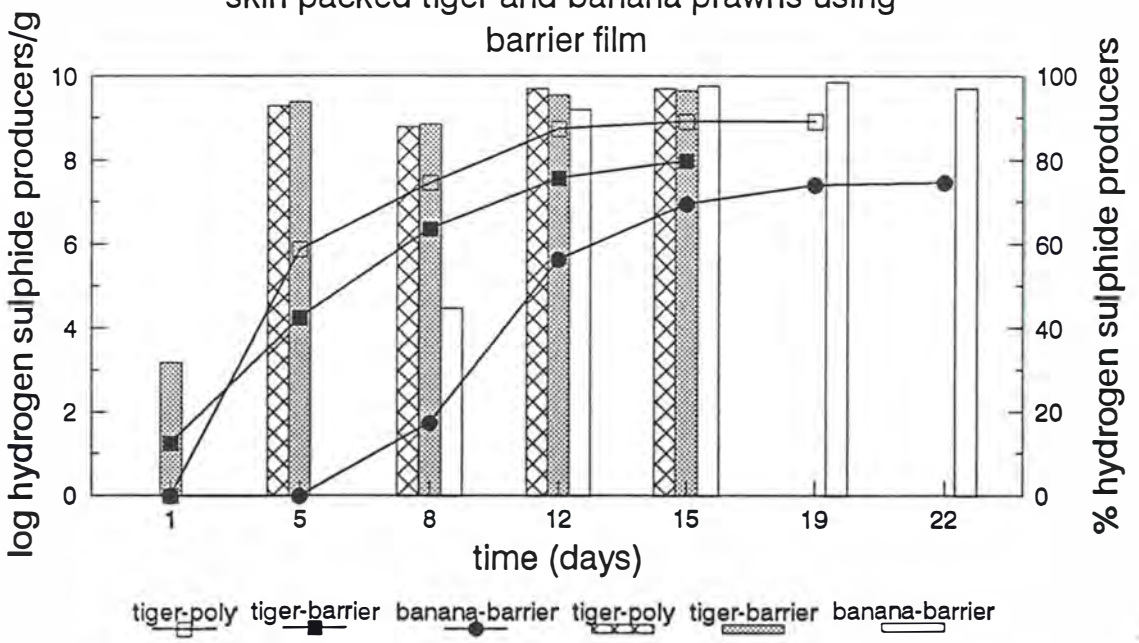


Figure 6.2.12

Hydrogen sulphide producers present in vacuum skin packed tiger and banana prawns using barrier film



non-hydrogen sulphide spoilers present on 1&2 packs on the 22nd and 26th day of storage.

6.3 Packaging of banana prawns

Aim

The aim of this trial was to establish whether packaging in an oxygen limiting atmosphere would alter the microbial flora during chilled storage and extend the shelf life of banana prawns in vacuum skin packs.

Introduction

Previous experiments have shown that the shelf life of prawns in vacuum skin packs can be limited by melanosis but that if the oxygen is limited then inhibition of melanosis may occur. One means of achieving this is to pack in an atmosphere rich in carbon dioxide which is known to inhibit both melanosis and the normal spoilage flora which develops on chill stored seafoods. This requires sophisticated packaging equipment fitted with a backflushing device and specialised trays and films. Another option is to place the normal vacuum skin packs sealed with permeable film into an outer master pack containing CO_2. The CO_2 permeates effectively through the packs. Yet another option to packaging in CO_2 is to use oxygen absorbing sachets to scavenge the residual oxygen in a package. This has the advantage of apparent simplicity, since only a normal sealer without vacuum is required.

This experiment was done to test the effectiveness of these approaches at:

- modifying the atmospheres in the packs
- inhibiting melanosis
- preventing off odours accumulating in the packs
- modifying the spoilage flora
- presenting an attractive pack.

Materials and methods

Banana prawns (*Penaeus merguiensis*) were caught in Moreton Bay in 5 shots in 4-8 metres of water from off Scarborough harbour to the mouth of the Caboolture river between 2200 and 2400 hours on 17/5/94. The prawns were sorted into an ice slurry and transported, after landing, to the laboratory in Hamilton, Brisbane.

They were then weighed, deheaded, reweighed, washed in fresh chilled water, drained and allotted to each of five packaging treatments.

The prawns were packed either in plastic sachets or by vacuum skin packaging (VSP). Within each VSP treatment group the prawns were packed on rigid 380 micron PVC trays six to a tray and were covered with a heat sealed film using a Trigon Mini RM331 packaging system.

The treatments were:

(1) Vacuum skin pack with a permeable film stored in a CO_2 filled outer master pack.
VSP/perm/CO_2 Prawns were packed using 100 μ SP film with an oxygen permeability of 2100 mL/m^2/24 h/bar at 23°C and water vapour permeability 6 g/m^2/24 h at 90% relative humidity. The trays were then placed in 18 x 24 cm barrier bags of oxygen permeability 10 mL/m^2/24 h/bar at 23°C and the bag was evacuated then filled with CO_2.

(2) Vacuum skin pack with a permeable film.
VSP/perm 1 As for (1) above except that trays were not placed in barrier bags or stored in CO_2.

(3) Vacuum skin pack with a permeable film
VSP/Perm 2 As for (2) above with a similar film.

(4) Packed in a bag used for primal cuts of lamb
Lamb Prawns were placed in a plastic bag used for packaging of lamb carcasses and portions. Size 180 x 450 mm. Oxygen permeability 2150 m/m^2/24 h/bar at 23°C.

(5) **Absorber** Prawns were placed in the barrier bags as used in 1 and 2 and an absorbing sachet [Ageless®-S (200) Mitsubishi Gas Chemical Company Japan supplied by W.R. Grace] was added to each bag which was not evacuated but heat sealed.

Twenty four packs of VSP trays per treatment and eight lots of lamb bags were prepared and all treatments were stored in a chiller set at a temperature of 3-5°C.

Sampling and observations

A sample of the prawns was taken before deheading for microbial counts and other analyses.

The packs, 3 per treatment, were sampled at about 3 day intervals for up to eight sampling times. The experimentors took observations on each pack as it was opened.

Analysis of gas composition

The oxygen and CO_2 composition in the headspace of the packs was estimated using a gas analyser.

Demerit points

Prawns from each treatment were identified with random 3 digit numbers and were displayed on a bed of ice for evaluation by an experienced group of eight observers using the demerit point score sheet. Samples of prawns were cooked by immersing them in a polythene bag in boiling water for 5 mins then cooling them in ice slurry. Segments of the prawns coded with

the appropriate number were presented to each observer for evaluation at the same time as they scored the visual observations.

pH

Two prawns from each treatment were homogenised in an equal volume of distilled water and the pH taken using a Metrohn Universal pH meter as in previous experiments.

Microbiological analysis

Samples for microbiological analysis were aseptically taken from each pack and 10 g prawns were homogenised with 90 mL sterile peptone (0.1%) water in a Colworth Stomacher.

Total bacterial counts (at incubation temperatures of 5°C and 30°C) were estimated by plating serial dilutions of prawn samples. Surface plating was used to obtain a total bacterial count. Sulphide producing bacteria were identified on iron agar. The flora was identified by methods previously described.

Results

Weights

The prawns ranged in weight from 11 to 49 g with a median weight near 28 g (Figure 6.3.1).

The deheaded prawns ranged in weight from 7 to 40 g, median 21 g (Figure 6.3.2).

Experimentors observations

The experimentors observations are listed in Table 1. Slight melanosis was observed in the VSP/perm 1, VSP/Perm 2 and lamb treatments by 9, 6 and 9 days respectively. Whereas prawns in the VSP/perm/CO_2 and absorber treatments showed no signs of melanosis, throughout the experiment.

The prawns in the lamb bag had a slight ammoniacal odour by 6 days of storage and those in the VSP/perm 1 and VSP/Perm 2 treatments had off odours by 9 and 13 days storage. Off odours were apparent also in the absorber treatments by 13 days and by 13-16 days in the VSP/perm/CO_2 treatment.

Storage conditions

The chiller temperature was constantly monitored electronically and it varied between 3 and 5°C. A typical daily plot is shown as Figure 6.3.3.

Gas composition

The gas composition in the headspace of the absorber and CO_2 packs is given in Table 2. The absorbing sachet kept the oxygen levels down to just below 1·5% with 1 to 2% CO_2 whereas those packs in the master outer mostly had less than 0·4% oxygen but over 98% CO_2.

Table 1 Experimentors observations.

Date	Period of Storage (days)	Treatment				
		VSP/Perm	Poly	Lamb	Absorber	VSP/Perm/CO_2
18/5/94	0	fresh	fresh	fresh	fresh	fresh
20/5/94	2	no off odour, no melanosis	no off odour, no melanosis	no off odour, no melanosis	no off odour, no melanosis	no off odour, no melanosis
24/5/94	6	no off odour, no melanosis	no off odour, slight melanosis	slight NH_3, no melanosis	no off odour, no melanosis	no off odour, no melanosis
27/5/94	9	"musty", dull appearance, slight melanosis	no off odour, slight melanosis,	slight amine, melanosis	no off odour, good appearance, no melanosis	no off odour, very good appearance no melanosis
31/5/94	13	slight amine, melanosis, dull	amine, onion, slight sulphide, melanosis	amine, oily, melanosis	good appearance, shiny, slight sulphide	very good appearance, shiny, 'onion' but not bad odour
3/6/94	16	amine, dull, extensive melanosis	extensive melanosis, slightly sweet, slight sulphide	extensive melanosis, amine	good appearance, amine, sulphide odour	good appearance
7/6/94	20	bad appearance, amine sweet sulphur	bad appearance, sulphide normal odour	no melanosis, no sulphur	good appearance, strong aromatic odour in outer part	sulphide, sweet in outer bag
10/6/95	23	-	-	-	no melanosis sulphur, stale, aromatic, amine	slight sulphide, old prawn, smell, no melanosis
17/6/95	30	-	-	-	-	good appearance, sulphur, old prawn

Table 2 Oxygen and carbon dioxide levels in packs (absorber and carbon dioxide).

Date	Oxygen Absorber		VSP/Perm/CO_2	
	O_2 %	CO_2 %	O_2 %	CO_2 %
20/5/94	1.57	2.9	2.34	96.9
	1.41	0.8	-	-
24/5/94	1.42	1.7	0.30	98.7
	1.39	1.1	-	-
27/5/94	1.38	1.0	0.41	98.2
	1.37	1.1	-	-
31/5/94	1.27	1.5	0.32	98.4
	1.30	0.8	-	-
3/6/94	1.28	1.64	0.32	98.56
	1.54	1.18	-	-
7/6/94	1.32	1.25	0.32	99.18
	1.37	2.10	-	-
10/6/95	1.32	2.10	0.33	98.85
	3.20	1.21	-	-
17/6/94	1.25	4.1	0.22	98.9
	-	-	-	-

Visual assessment

After 7 days chill storage the prawns in the poly bag were showing the first signs of melanosis (Figure 6.3.4) and, by 16 days storage, melanosis, discolouration of the flesh and decomposition were well advanced (Figure 6.3.5). Slight traces of melanosis could be seen after 7 days storage at one or two of the joints of the carapace on the prawns stored in the VSP permeable packs (Figure 6.3.6). By 20 days these dark areas were quite visible and the flesh had started to darken (Figure 6.3.7) but these changes were less noticeable than for the prawns in the poly bags (Figure 6.3.5) The prawns in the packs containing the oxygen absorber did not exhibit melanosis even after 30 days chill storage (Figure 6.3.8) and neither did those packed in the CO_2 outer (VSP/Perm/CO_2) (Figure 6.3.9).

Decreasing the available oxygen by either absorbing it from the pack or by vacuum packing and storing in an oxygen free CO_2 -rich atmosphere effectively inhibits melanosis.

Demerit points

The change in demerit scores is shown in Figure 6.3.10. There was little difference between the treatments up to 6 days of storage, but, after the lowest scores were given to the prawns in the CO_2 master pack. The VSP/Perm and the Lamb bag showed the most rapid change.

Microbiology

The total bacterial counts at incubation temperatures of 30°C (Figure 6.3.11) and 5°C (Figure 6.3.12) are very similar.

The initial counts were quite low, 10^4/g at 30°C and 10^2/g at 5°C. The count at 30°C showed a slight lag in the first couple of days as the flora adjusted in composition to be more psychrophilic in nature.

The slow rate of growth of bacteria in the VSP/perm/CO_2 packs was most striking, indicating strong inhibition of the spoilage bacteria.

The absorber treatment showed similar inhibition of the flora up to 9 days of storage.

After the initial adjustment, the spoilage flora grew well in all the aerobic treatments.

The only treatment in which H_2S producing bacteria were detected insignificant numbers was in the absorber treatment (Figure 6.3.13), where by 13 days of storage they were 50% of the flora at levels near 10^4/g.

The low levels counts of H_2S producing bacteria found in the permeable and lamb bags after 2 days storage were not evident after this and presumably the organisms responsible failed to grow and were replaced by others.

A proportion of the flora isolated after 5 days of storage could not be recultured after being kept chilled until it could be characterised. These are reported as dead isolates. It is likely they were Gram positive organisms. Since they failed to survive in the refrigerator it is unlikely they would be major contributors to spoilage in chilled stored prawns.

Composition of bacterial flora

The composition of the flora (Table 3) on the prawns was first examined after they had been in the packs four days. This flora in the VSP/Perm packs had a high proportion of *Moraxella* whereas the prawns in the Lamb bag had high levels of *Pseudomonas* and *Lactobacilli.* Both the flora in the VSP/Perm/CO_2 packs and the absorber packs had high proportions of organisms that failed to survive subculturing. Both these packs had *Lactobacilli*, 52% in the absorber packs.

The major spoilage organisms in the VSP/Perm 1 and VSP/Perm 2 packs were *Pseudomonad spp., Lactobacilli* and *Alteromonas putrefaciens.* The Lamb bag had mostly *Pseudomonas spp.* and *Vibrio spp.* as the spoilage flora.

Table 3 Bacterial flora on banana prawns (*P. merguiensis*) packed using permeable film and stored under normal and carbon dioxide atmospheres.

Type of flora	VSP/Perm 1		VSP/Perm 2		Lamb Bag		Oxygen Absorber		VSP/Perm/CO_2	
	Initial Day 4	Final Day 20	Initial Day 4	Final Day 20	Initial Day 4	Final 17 days	Initial Day 4	Final Day 23	Initial Day 4	Final Day 27
Gram positive flora	22	-	13	-	6	4	22	-	14	-
Moraxella spp.	44	-	64	-	6	6	-	-	14	-
Pseudomonas spp.	10	44	3	32	29	56	-	-	-	-
Other Gram negative flora	6	-	-	-	-	-	-	-	-	-
Lactobacilli	-	35	8	32	47	8	52	70	14	100
Shewanella nutrefaciens	-	10	5	14	-	-	-	24	-	-
Acinetobacter spp.	-	6	8	20	6	2	-	6	-	-
No growth when subcultured	18	4	-	2	6	2	26	-	57	-
Vibrio	-	-	-	-	-	22	-	-	-	-
Total bacterial count	10^3	3×10^7	10^3	5×10^7	10^3	10^8	8×10^3	6×10^7	7×10^3	3×10^5
Total number/colonies	50	45	39	50	49	50	50	50	49	50

The absorber packs were different again with *Lactobacilli* (70%)and *Shewanella putrefaciens* (24%) as the major spoiler. Only *Lactobacilli* survived in the low oxygen tension in the VSP/Perm/CO_2 treatment and the total counts remained low.

Thus, at very low oxygen levels the normal spoilage flora cannot survive, whereas, at slightly higher levels as found in the absorber pack, Shewanella can grow quite well after a period of adjustment to cause spoilage. The Lactobacilli were apparently not in high enough concentration to be totally inhibitory. A lower proportion of Shewanella was found in the more permeable packs and the high proportions of Pseudomonads were the spoilers. It is interesting to note that there were reasonably high levels (35 and 32%) of Lactobacilli in the VSP/Perm 1 &2 packs. The different flora in the different treatments explains the differences in the observors comments (Table 1).

The anaerobic flora is given as a percentage of the total count in Table 4. The lowest proportion of anaerobes was found in the Lamb bag, with the next lowest in the two VSP/Perm treatments. The proportion of anaerobes in the absorber treatment remained near 20-30% throughout while that in the VSP/Perm/CO_2 treatment started very high then levelled out near 20 to 30%. The high counts initially were probably due to some of the organisms which failed to subculture in aerobic media. Also the total aerobic count was at its lowest at the start so the relative proportions could be distorted. Some Lactobacilli survived and were enumerated in the anaerobic count.

pH

There were few differences in pH between prawns in all of the treatments (Figure 6.3.14). The reason for the apparent rise in those prawns stored in CO_2 over the first 5 days is not simple to explain. It is possibly a side effect of the higher anaerobic count leading to some basic amine formation. Alternatively, an enzyme system may have been stimulated by the CO_2 but this is speculative. It would be expected that the pH would fall due to dissolution of the CO_2 in the prawn tissue and consequent formation of carbonic acid.

It is probable that the decrease in pH in this treatment observed at the later stage of the trial was due to lactic acid production from Gram positive lactobacilli, although no characterisation of the flora was done to the level of genera to support this assumption.

Table 4 Percentage of Anaerobic Count (AN) in relation to total bacterial count/g (TBC) in banana prawns in the various treatments.

Day	VSP/Perm 1 % AN/TBC	VSP/Perm 2 % AN/TBC	Lamb Bag % AN/TBC	Oxygen Absorber % AN/TBC	VSP/Perm/CO_2 Carbon dioxide % AN/TBC
0	-	-	-	-	-
2	12	20	7	25	250
6	10	5	3	29	201
9	2	3	5	30	25
13	8	20	-	13	7
16	13	3	4	20	21
20	9	14	-	21	22
23	-	-	-	28	27
27	-	-	-	-	23

Summary and discussion

The results are summarised in Table 5.

Table 5 Time (days) taken to reach certain specified levels.

Treatment	Specified levels				
	Bacterial count at 30°C		Ten demerit points	Amine or sulphide odour	Melanosis detected
	10^6/g	10^7/g			
VSP perm	10	14	11	13	9
Poly	11	14	11	13	6
Lamb	8	12	12.5	6-9	9
Absorber	12	14	14	13	never
CO_2	never	never	16	16	never

The lamb bag is probably the most oxygen permeable and the bacterial count on the prawns reached 10^6/g by 8 days. This is equivalent to 16 days at a temperature of 0°C a result similar to that obtained when prawns were stored in slurry. However the flora in the bag was probably *Pseudomonas fragi* (although it was not identified to species level) whereas that in slurry was *Shewanella putrefaciens*.

The prawns packed in the VSP permeable films took 10-11 days to reach counts of 10^6 at 4°C. This is equivalent to 20 days at 0°C which exceeds that observed for banana prawns stored either in ice or in slurry.

The prawns packed in the CO_2 deteriorated despite the fact that the bacterial counts were low throughout. They exhibited 'oniony' odours by 13 days and developed sulphur/sulphide odours from then on. One interpretation of this is that inherent enzymic action in the prawn is responsible for the development of these off odours. If this is the case then irrespective of whether the spoilage flora is inhibited then there are definite limits to shelf life. It is also known that the type of lactobacilli present has a bearing on whether off odours and off flavours are generated or not. In packaged meats hetero-fermentative *Lactobacilli* produce these while homofermentative do not but the situation with prawns has not been investigated.

Conclusion

Vacuum skin packaging of banana prawns in permeable films provides a product with an attractive appearance in which melanosis is slightly delayed over storing them in ice. Exclusion of oxygen either partially using absorbers or by placing permeable packs in master outers can completely prevent the development of melanosis for at least 23 days at a temperature of 4°C.

Packaging in the CO_2 outer inhibits the spoilage flora and encourages the development of *Lactobacilli* which inhibit other bacteria, have less spoilage potential and which grow relatively slowly.

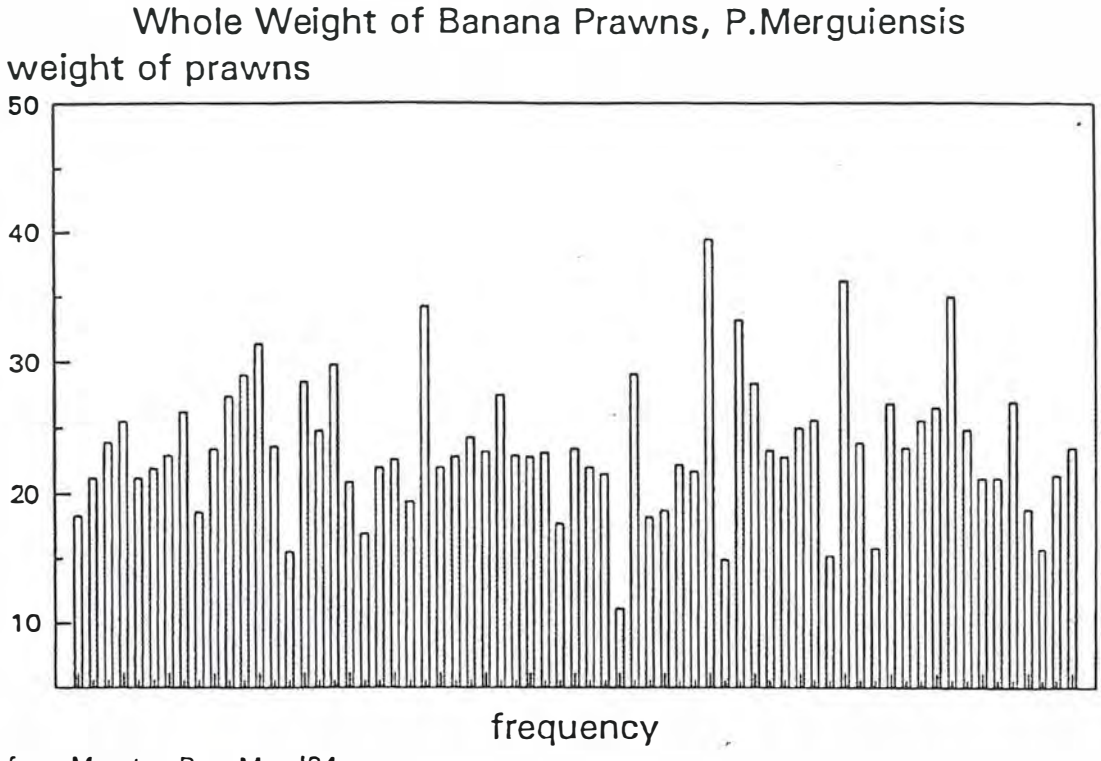


Figure 6.3.1 Distribution of weights of whole prawns.

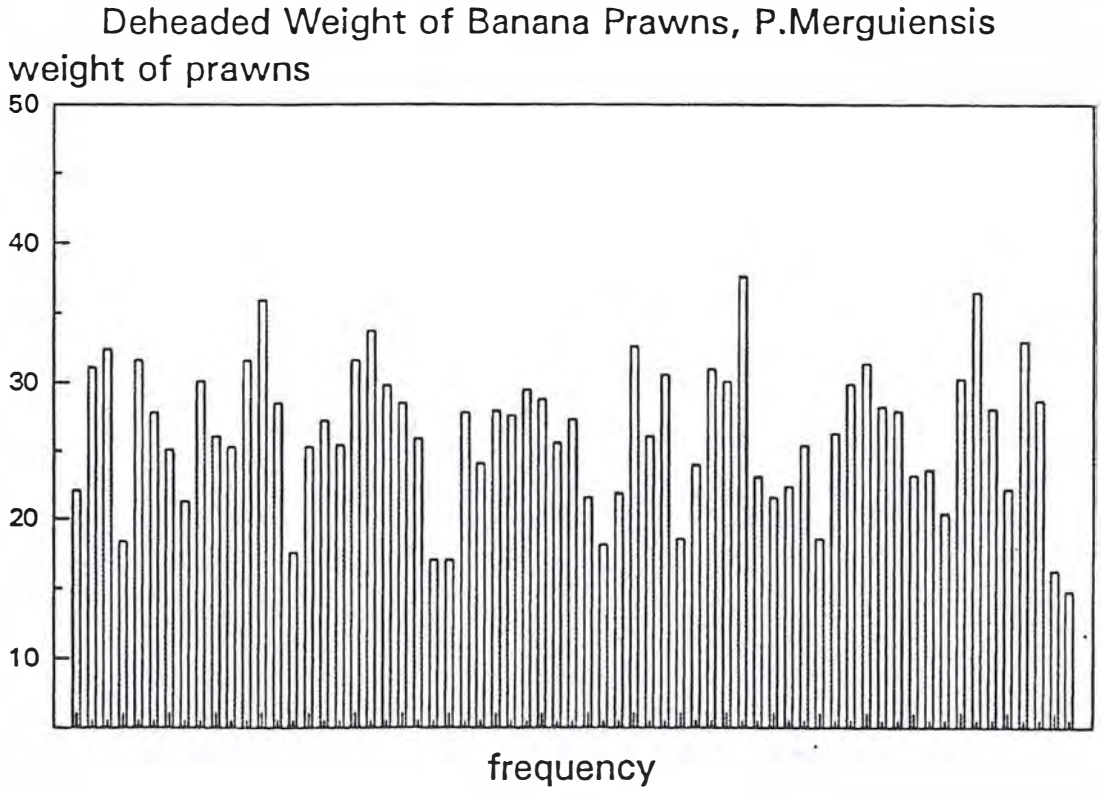


Figure 6.3.2 Distribution of weights of deheaded prawns

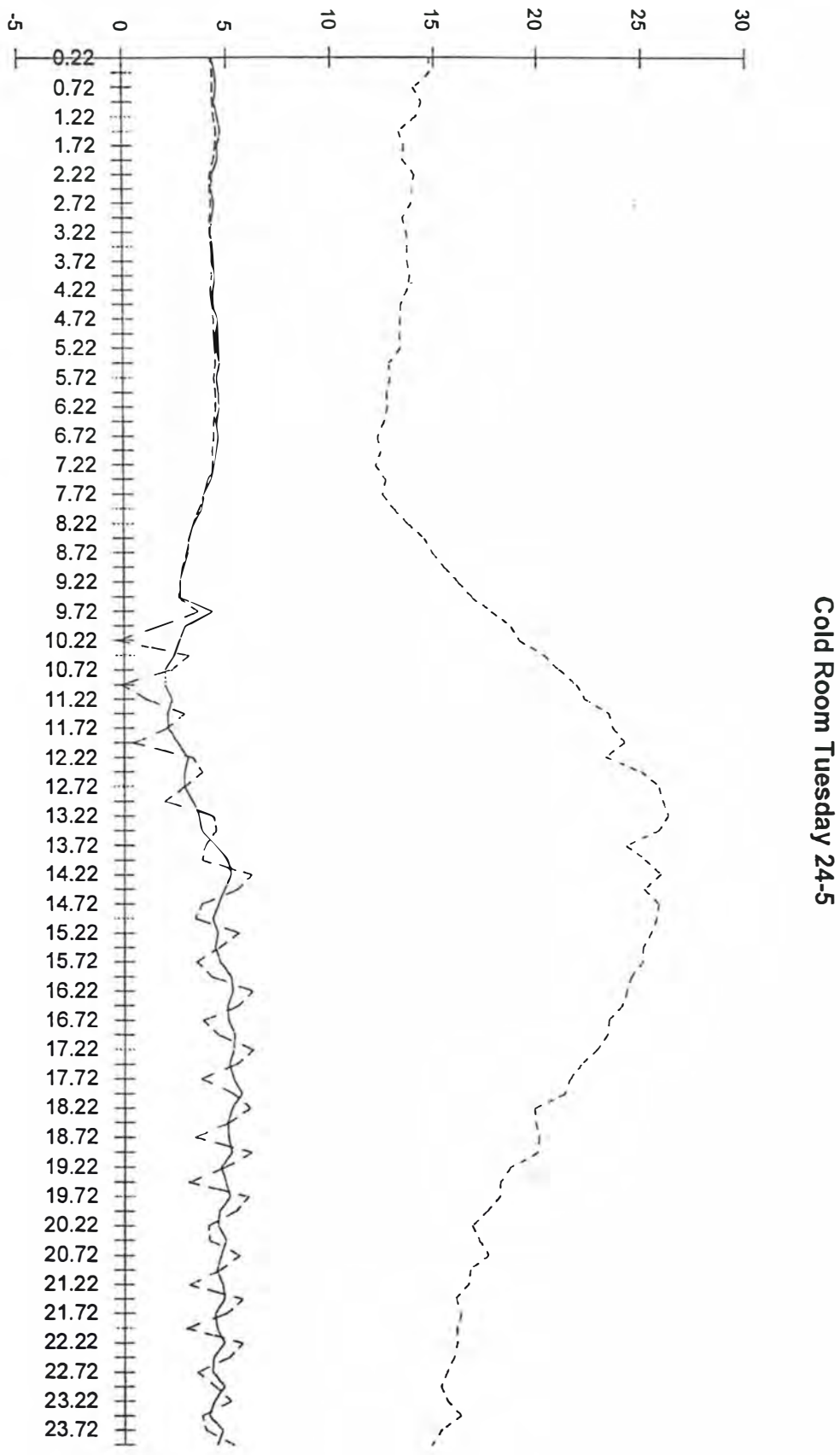


Figure 6.3.3 Typical trace of chill room temperature.

Figure 6.3.4 Banana prawns chill stored in poly bags for 7 days. Some melanosis on tail areas.

Figure 6.3.5 Banana prawns chill stored for 16 days. Note extensive melanosis, flesh discolouration and composition.

Figure 6.3.6 Banana prawns in vacuum skin packs covered in permeable film (VSP/Perm) chill stored for 7 days. Slight traces of melanosis on some prawns at the carapace joints.

Figure 6.3.7 Banana prawns VSP/Perm packs stored 20 days. Noticeable melanosis at carapace joints.

Figure 6.3.8 Banana prawns in barrier bags with oxygen absorbing sachet (Absorber) chill stored 30 days. No melanosis.

Figure 6.3.9 Banana prawns vacuum skin packed in permeable film chill stored for 30 days. No signs of melanosis.

Figure 6.3.10

Change in demerit scores of vacuum skin packed banana prawns in permeable film and stored normally or in carbon dioxide

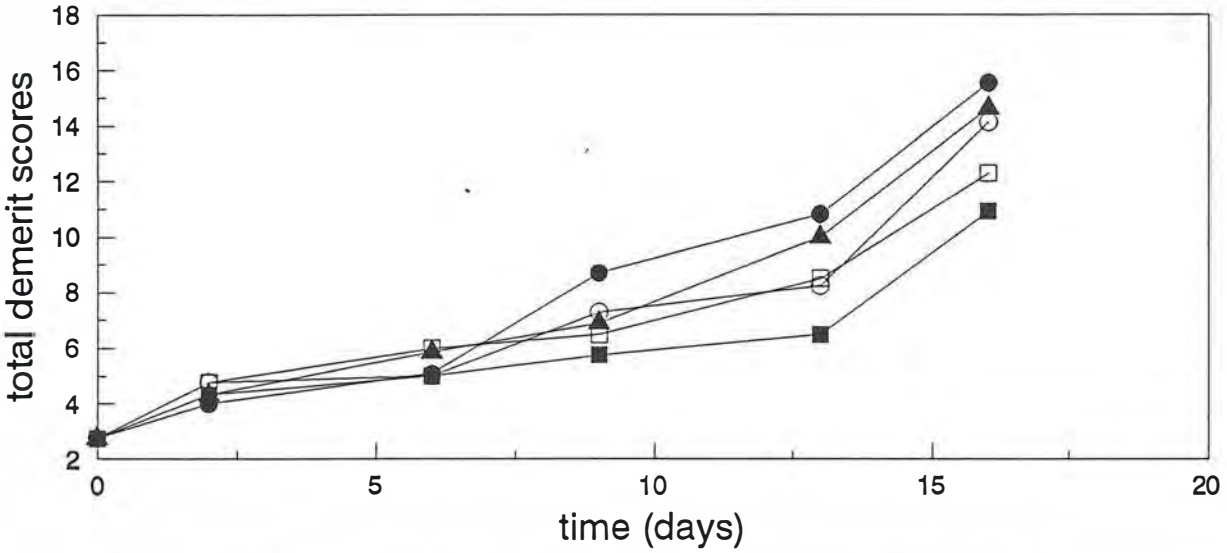


Figure 6.3.11

Total bacterial counts (30°C) in prawns packed using permeable film and stored under normal or carbon dioxide atmospheres.

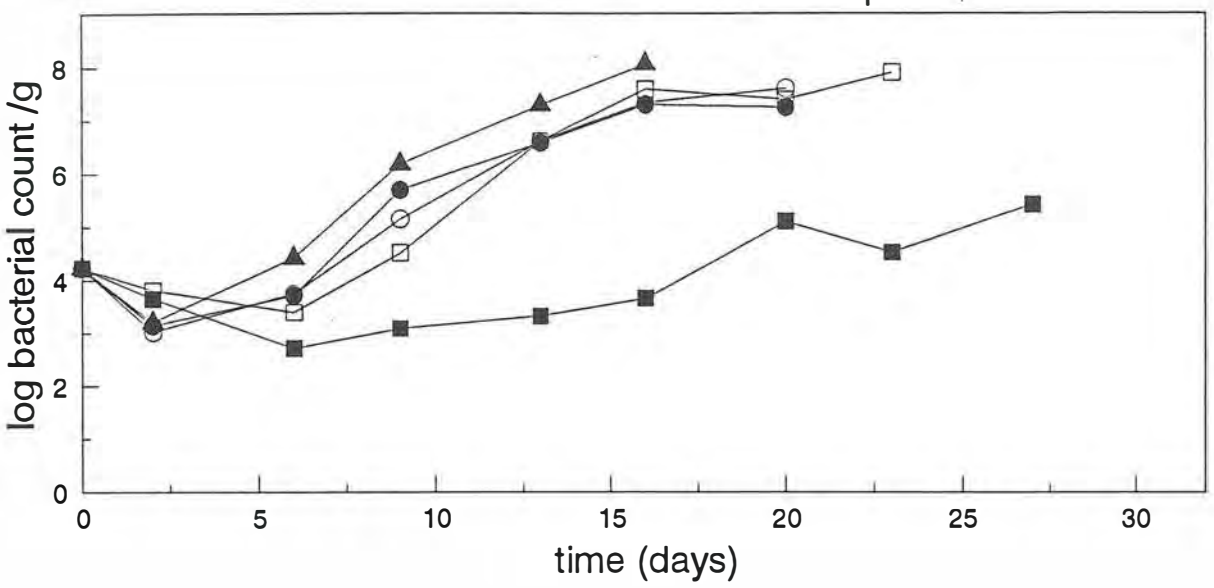
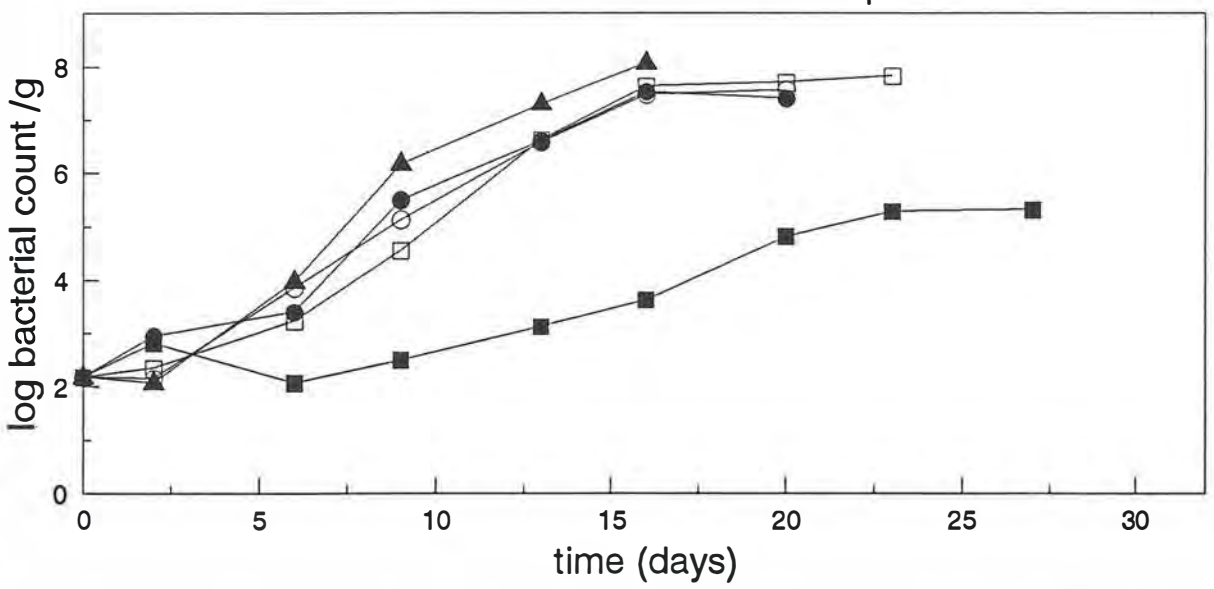


Figure 6.3.12

Total bacterial counts (5°C) in prawns packed using permeable film and stored under normal or carbon dioxide atmospheres.



permeable-1 "Trigon" | permeable-2 "Trigon" | lamb poly bag "Trigon" | Oxygen-absorber "Ageless" | carbon dioxide master pack

Figure 6.3.13

Hydrogen sulphide producers present on banana prawns packed in vacuum skin packs using permeable film or poly bags

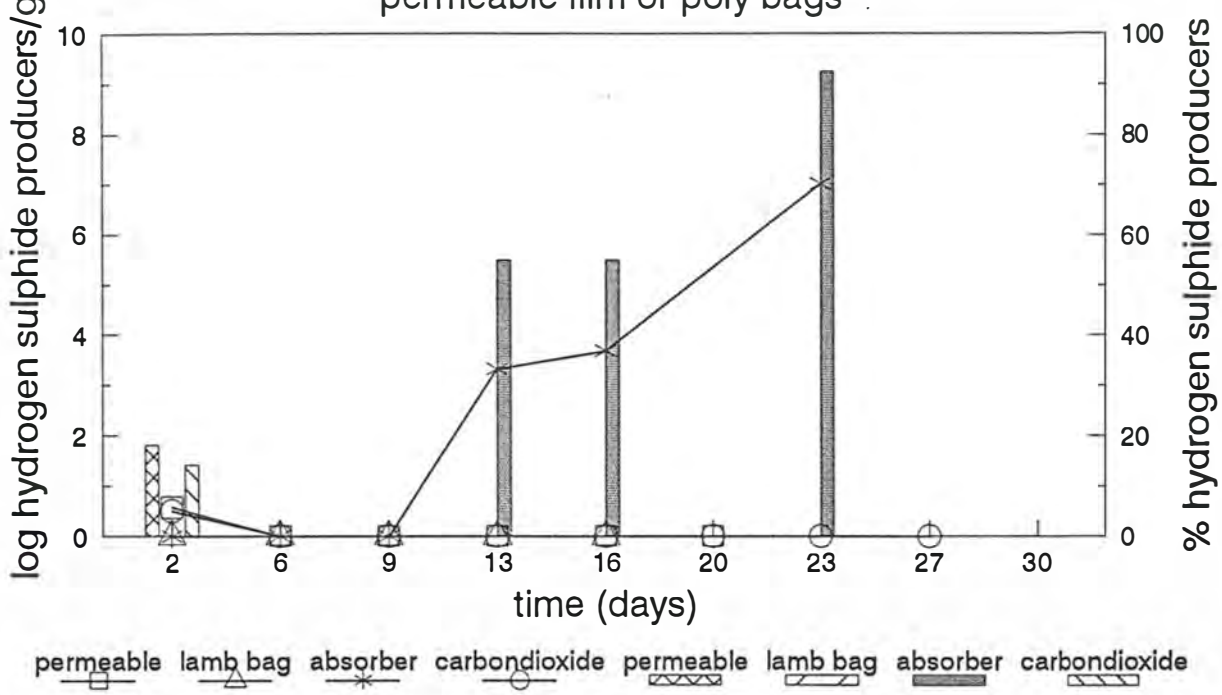
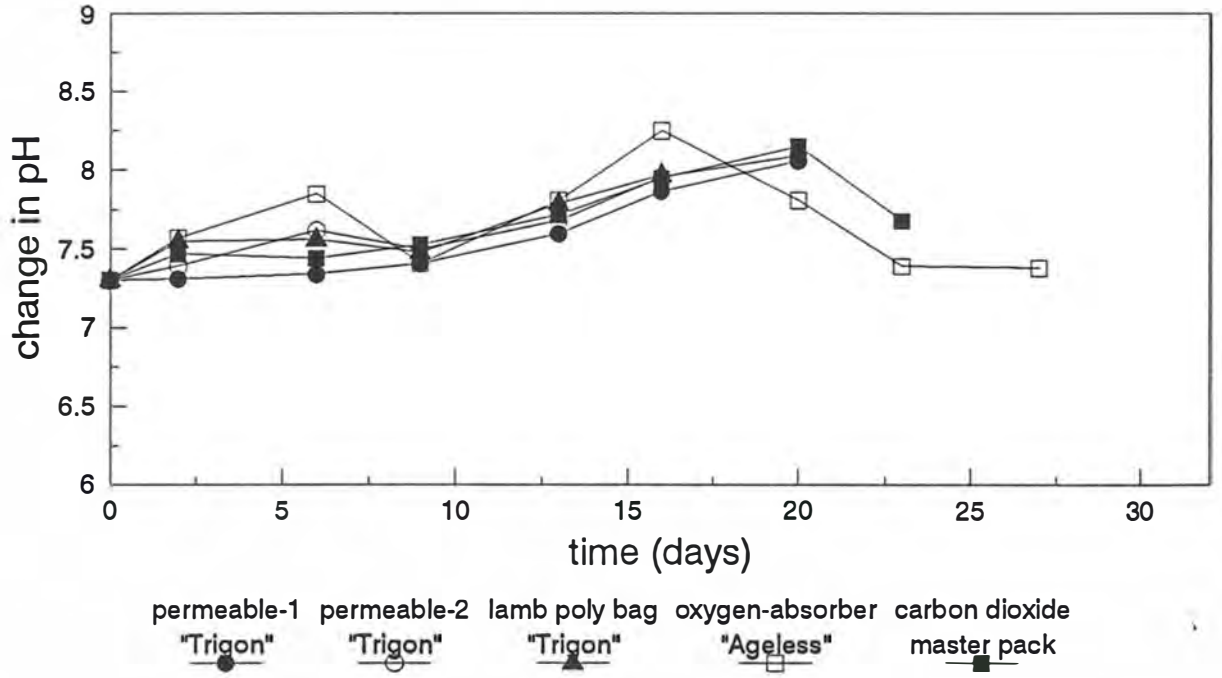


Figure 6.3.14

pH in banana prawns packed using permeable film and stored under normal or carbon dioxide atmospheres.



SECTION 7 FROZEN PACKAGING AND STORAGE TRIALS

7.1 Frozen storage of king prawns with oxygen absorbing sachets

7.2 Frozen storage of tiger prawns in poly bags

7.3 Storage of banana prawns frozen onboard boat

7.1 Frozen storage of king prawns with oxygen absorbing sachets

Aim

To test the effectiveness of oxygen absorbing sachets to inhibit melanosis during frozen storage.

Introduction

Common experience and the results from previous experiments have shown that king prawns are quite susceptible to melanosis. Oxygen is a key factor necessary for the formation of the black pigment melanin. If prawns can be stored in an oxygen limiting environment then melanosis should not occur.

The normal method of preventing melanosis is to use the reducing agent sodium metabisulphite but there is a growing trend to restrict its use. Also a premium price can be obtained for sulphite free prawn.

If an alternative to metabisulphite is effective on king prawns then it should be more effective on other species such as banana prawns which are less prone to melanosis.

Materials and methods

The king prawns used in this experiment were from the same catch as those described in a previous section. They were caught off Mooloolaba and transferred to the laboratory in an ice slurry. At the laboratory they were dipped 30 sec. in an 0.5% solution of alginate then drained for 30 sec.

Six whole prawns were packed into a barrier bag and an oxygen absorbing sachet Ageless®-S 200 (Mitsubishi Chemical Co obtained through W R Grace Pty Ltd) placed in each. The bags were held for 8 h at a temperature of 0°C to allow the sachet to absorb the oxygen. The packs were blast frozen then held in frozen storage (-18°C). A few packs were also stored at -30°C for six months after which they were stored chilled at 1°C for one month.

At each of six subsequent sampling times after 0, 35, 49, 62, 76 and 114 days frozen storage 4 packs were taken at random. They were thawed in a cool room (4°C) before inspection.

The prawns were photographed then assessed using the demerit point system.

Total bacterial counts were estimated after 19 and 81 days of frozen storage.

Results

The total bacterial counts were low (Table 1) indicating good handling.

Table 1 Total bacterial counts on frozen king prawns.

Date of sample	Period of frozen storage (days)	Incubation 30°C	Temperature 5°C
1/10/93	0	3×10^3C	7×10^2
20/10/93	19	5.1×10^3	2.9×10^3
20/1/94	81	2.1×10^3	2.0×10^3

There was no decrease in count due to the freezing or frozen storage.

Melanosis and visual assessment

No melanosis was observed up to 114 days frozen storage (Figure 7.1.1 and 7.1.2). Neither was there any significant change in the scores for visual assessment (Table 2).

Table 2 Scores for melanosis and total demerit points.

Period of storage (days)	Melanosis	Total demerit point
0	0.06	2.82
35	0.25	2.00
49	0.25	1.94
62	0.00	2.38
76	0.44	2.19
114	0.13	1.88

These are all very low scores.

There was no melanosis in prawns in the packs stored for six months and after one further month at 1°C they were still free (Figure 7.1.3). Thus storage with the absorber provides protection after thawing for a substantial time.

Conclusion

Melanosis and general deterioration in king prawns can be prevented for at least 114 days frozen storage (-18°C) by packing in a barrier bag with an oxygen absorber.

The oxygen absorber additional protection after the pack is thawed.

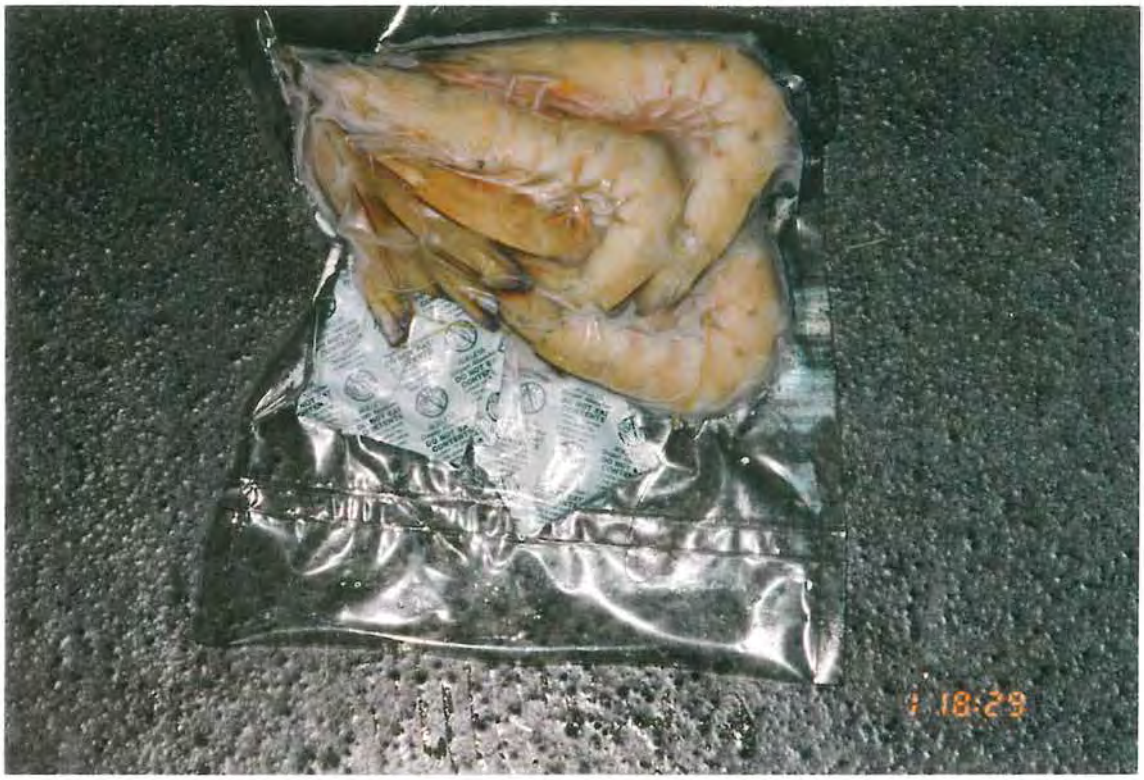


Figure 7.1.1 Frozen King prawns in a barrier bag with oxygen absorbing sachets.

Figure 7.1.2 King prawns after 114 days frozen storage in a barrier bag with an oxygen absorbing sachet. There is slight melanosis on some prawns where they have suffered damage in the catch.

Figure 7.1.3 King prawns stored frozen for six months (-30°C) then chill stored for one month (1°C). No signs of melanosis.

7.2 Frozen storage of tiger prawns in poly bags

Aim

To establish the rate of development of melanosis in tiger prawns stored frozen that had not been treated with sodium metabisulphite (meta).

Introduction

Frozen prawns can be used to produce value added packs such as vacuum skin packs. It has been established that if the film used has barrier properties or, that if the packs are placed in an outer master pack filled with carbon dioxide, then melanosis will not occur and bacterial growth will be retarded. The success of these approaches relies on having good standard prawns available.

This trial was aimed at testing the frozen storage life of tiger prawns that had not been treated with meta.

Materials and methods

Tiger prawns, a mixture of grooved (*Penaeus semisulcatus*) and brown (*Penaeus esculentus*) were caught off Mooloolaba on 4/2/94. These are the same prawns used in a previous chill storage trial. They were not dipped in meta and were brought to the laboratory in ice slurry.

Six prawns were packed in each of 18 low density polyethylene bags which were sealed then blast frozen for 2 h and stored at a temperature of -18°C. Three bags were taken from storage after 0, 17, 31, 45, 60 and 73 day.

Chemical analyses were done on samples after 4, 45 and 73 days frozen storage.

Results

There were only slight increases in total volatile nitrogen (TVN) (Table 1) and in K-value by 73 days storage.

Table 1 Results of chemical analyses.

Period of storage (days)	K-value %	TVN mg N/100 g
4	10	16
45	6	25
73	16	24

Melanosis

The results are shown in Table 2.

Blackening was more prominent in the tail region than in the head. Substantial melanosis had occurred by 45 days in storage.

Table 2 Percentage number of prawns exhibiting melanosis in three regions during frozen storage.

Period of storage (days)	Head region	Tail region	Edge of shell
0	0	0	0
17	4	8	0
31	6	27	0
45	39	66	63
60	73	80	72
73	94	100	100

Conclusion

Tiger prawns can probably be stored no longer than about 20 days frozen if they are to be used whole or with the shell on for value added products unless other steps are taken to prevent melanosis.

7.3 Storage of banana prawns frozen onboard boat

Aim

To assess different handling practices such as delays in chilling and the effects of glazing on banana prawns.

Introduction

The fishery for banana prawns (*Penaeus merguiensis*) in the Gulf of Carpentaria is one of Australia's most important. It is well established and although a number of practices have arisen in the industry for handling the prawns, discussions with catchers and processors indicated the need for further work.

As a result, a set of related experiments were designed to be done on a commercial boat of the Raptis fleet during the 1993 season.

Experimental aims

1. To test effectiveness of a water glaze at preventing melanosis in comparison to treatment with sodium metabisulphite (meta).

2. To test the effects of a delay of two hours before chilling or freezing the prawns.

3. To test the effectiveness of a water glaze applied to prawns after they had been chilled in ice for 2 hours.

Materials and methods

The banana prawns used were all from the one haul landed about 0900 hours on 19 June 1993 by a Raptis trawler in the Gulf. The prawns were being sorted into groups as the treatments were applied.

After treatment they were all packed (finger laid) into the 3 kg waxed board cartons lined with a sachet as used commercially. The glaze was applied to the appropriate treatments by placing 100 mL seawater into the carton and ensuring each prawn was well wetted. Each carton held between 20 and 30 prawns and 3 cartons per treatment were prepared (Table 7.3.1).

The cartons were held frozen aboard boat until they were despatched to the IFIQ laboratories Hamilton, Brisbane where they were stored in a freezer (-20^0C) awaiting examination. A typical carton containing glazed prawns is shown in Figure 1.

They were first examined 46 days after catch. These samples were then refrozen and examined 86 days after catch along with another set which were held frozen for 81 days in order to assess the effects of double freezing.

Melanosis

This was assessed by the method of Ruello (1975) in which the number of prawns with blackspot is calculated as a percentage of the total number in the whole sample.

Table 1 Description of treatments.

Sample No.	Description	Time sorted hours	Temp. ^{0}C	Treatment	Time placed in freezer hours
1	Meta	0930	23.8	Dipped 4 min. in Meta (23.5^{0}C)	1055
2	Unglazed	0910	23.5	Washed in seawater 23.7^{0}C	0930
3	Glazed	0910	23.5	Washed in seawater 100 mL seawater glaze added	0935
4	Ambient glazed	0930	23.5	Left exposed at ambient (24.6^{0}C) for 3 hours then sprinkled with water	1235
5	Seawater 2 h	0910	23.5	Held in seawater (23.5^{0}C) 2 h	1140
6	Ice	0935	23.5	Held in ice 2 h (4.5^{0}C)	11.50
7	Ice/glaze	0910	23.5	Held in ice, 2 h (4.5^{0}C) 100 mL seawater glaze added	11.50

Results

The results of the chemical analyses are listed in Table 2.

Table 2 Results of chemical analyses.

Treatment	pH	TVN (mgn/100 g)	K-value (%)
1. Meta	7.0	45	17
2. Unglazed	7.0	35	15
3. Glazed	7.0	39	13
4. Ambient/glazed	7.0	56	14
5. Seawater	7.0	28	10
6. Ice	7.0	33	29
7. Ice/glaze	6.8	36	24

None of the results of the chemical analyses appear to discriminate between the treatments to any great degree. The pH of the material held at ambient temperature or in seawater did not increase although the TVN for the ambient treatment was the highest.

Melanosis

The results are listed in Table 3.

Table 3 Percentage number of prawns in each treatment showing melanosis after frozen storage.

Treatment	Storage period (days) at -18^{0}C		
	46	**81**	**86 refrozen**
1 Meta	0	5	0
2 Unglazed	0	56	100
3 Glazed	0	89	74
4 Ambient/glazed	20	61	91
5 Seawater	9	78	78
6 Ice	13	63	92
7 Ice/glaze	0	65	67

The prawns which were frozen without delay showed no melanosis after 46 days storage. When they were refrozen and examined after a further 45 days, only those that had been treated with meta exhibited no blackspot.

The seawater glaze gave no protection at all from blackspot. Leaving prawns exposed at ambient encourages blackspot. Restricting access of oxygen by keeping them in seawater improves the situation in the short term. Chilling in ice and glazing also has short term beneficial effects.

Only those prawns treated with meta have a reasonable storage life with regard to blackspot. Double freezing generally makes the situation worse.

These results are illustrated in the accompanying figures. The full complement of treatments are not shown. It is unfortunate that background lighting and conditions of film development have not provided uniform colouration throughout. Those prawns treated in meta are shown in Figure 7.3.2 and, although the photo is dark no melanosis is evident even on the refrozen prawns. Those not dipped in meta exhibit melanosis in the tail area and, after refreezing on the body near the legs (Figure 7.3.3). Melanosis is quite evident on those prawns which were held at ambient temperature after 46 days, and this too increased with further storage and refreezing (Figure 7.3.4). Slight melanosis is evident on those that were stored 2 hours in ice (actual temperature 4.5°C) which increases after further storage and after refreezing (Figure 7.3.5).

Visual and taste assessment

The demerit point system provides an overall approach to assessment of quality attributes of prawns.

The lowest scores after 46 days storage were given to the prawns treated with meta. Glazing had some beneficial effects. All the scores increased with storage time and this was due not just to increased melanosis but to a deterioration in taste attributes. All refrozen prawns were scored worse than those which had only been frozen once.

This assessment confirms general knowledge that continual deterioration occurs during frozen storage of prawns and that thawing and refreezing makes them worse.

Table 4 The results of visual assessment of stored frozen prawns.

Treatment	Storage period (days) at -18^{0}C		
	46	**81**	**86 refrozen**
1 Meta	1.3	2.7	3.4
2 Unglazed	2.1	3.8	5.3
3 Glazed	1.7	2.5	3.4
4 Ambient/glazed	2.7	4.0	5.8
5 Seawater	2.0	3.4	5.1
6 Ice	2.4	2.8	5.3
7 Ice/glaze	2.2	2.6	5.6

Conclusion

Delays on deck even if prawns are chilled lead to a greater degree of blackspot formation.

Metabisulphite gives good protection against blackspot. If there is need to avoid its use other measures must be taken.

A glaze gives negligible protection against blackspot.

Reference

Ruello, J H 1975. Quality control in the prawn industry. First National Prawn Seminar Maroochydore 1973, Australian Government Publishing Services. pp 192-203.

Figure 7.3.1 Typical carton of glazed prawns

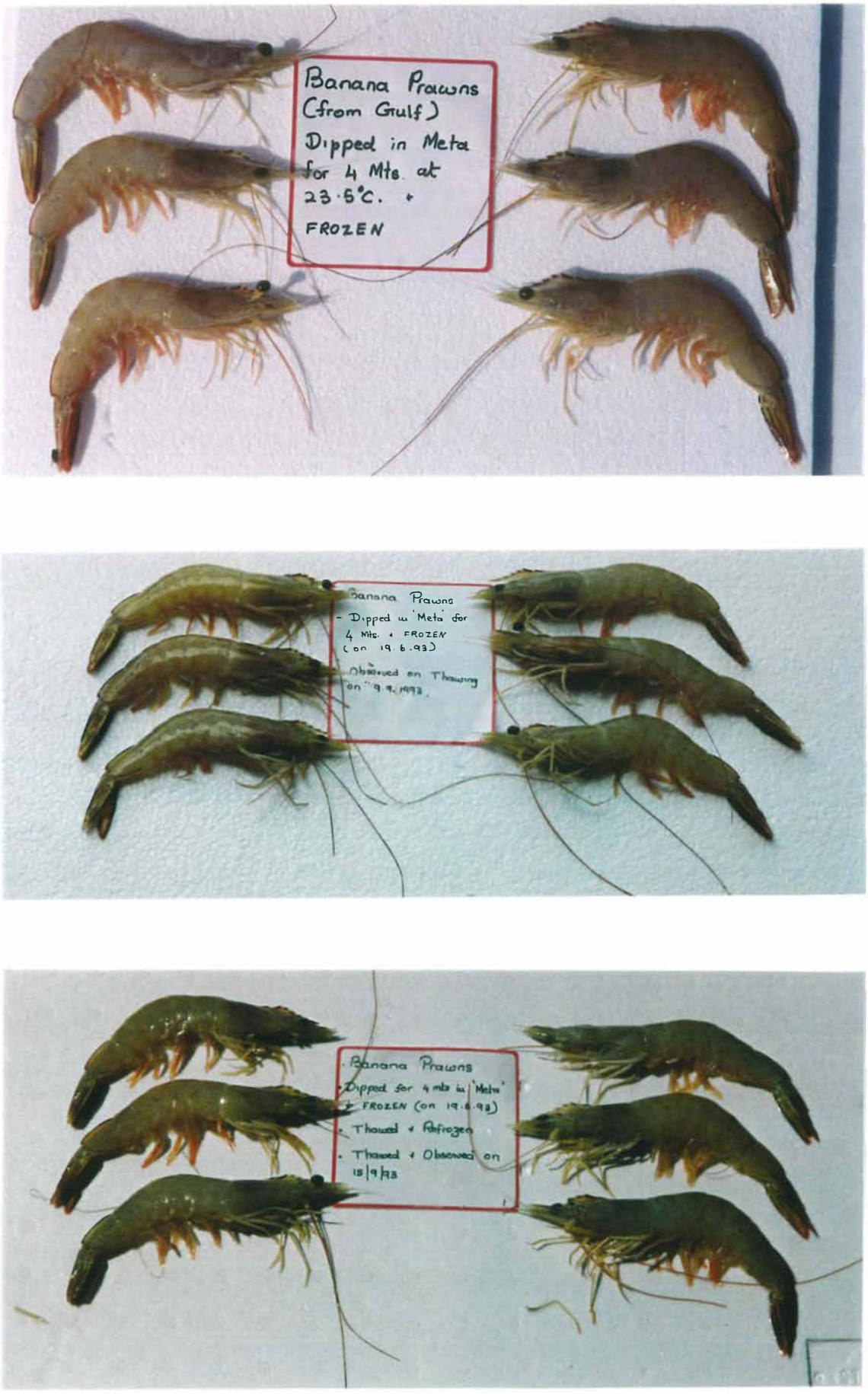


Figure 7.3.2 Banana prawns dipped in meta then frozen stored (a) for 46 days (b) for 81 days and (c) sample (b) refrozen for a further 41 days.

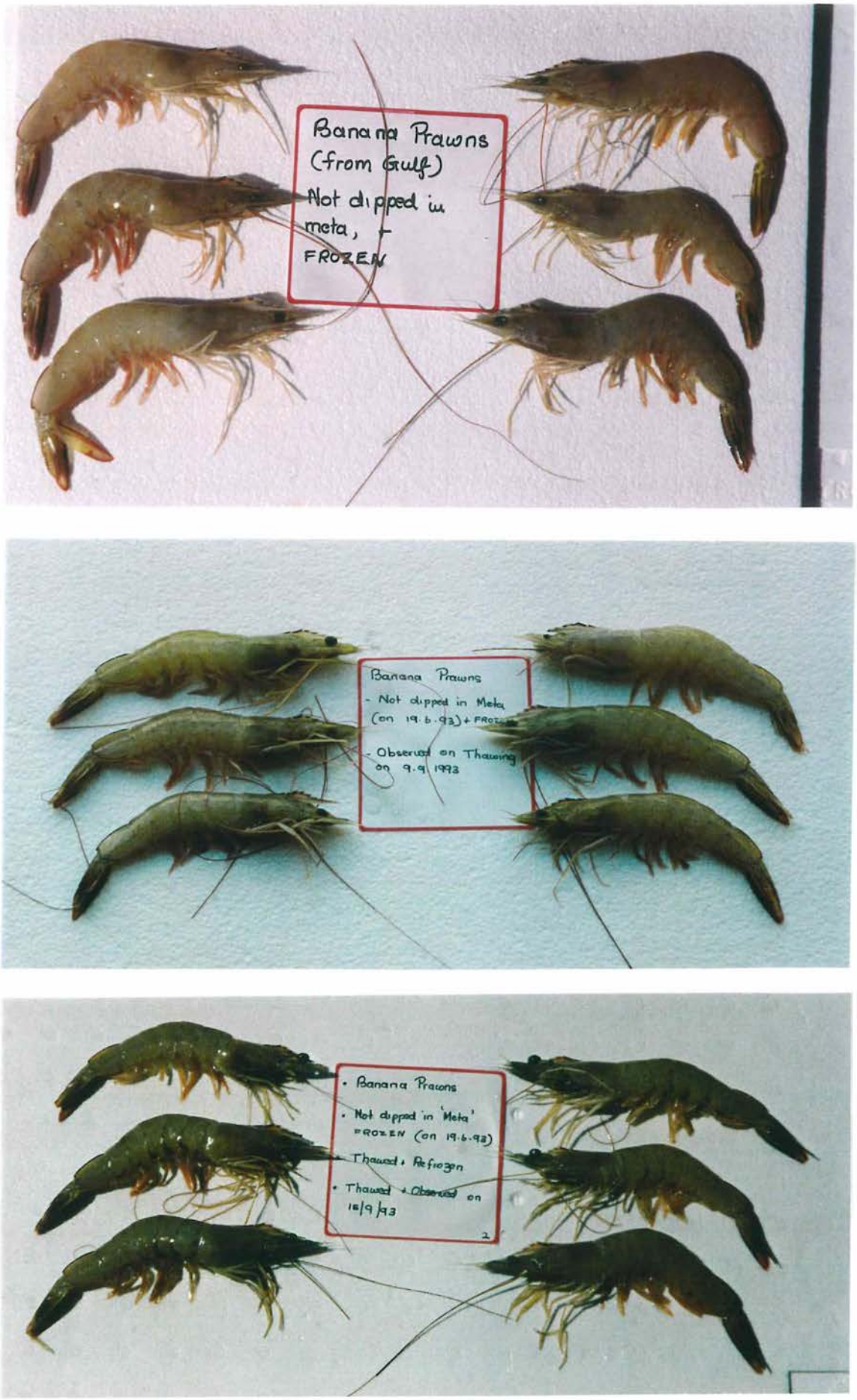


Figure 7.3.3 Banana prawns untreated then frozen stored (a) for 46 days (b) for 81 days and (c) sample (b) refrozen for a further 41 days.

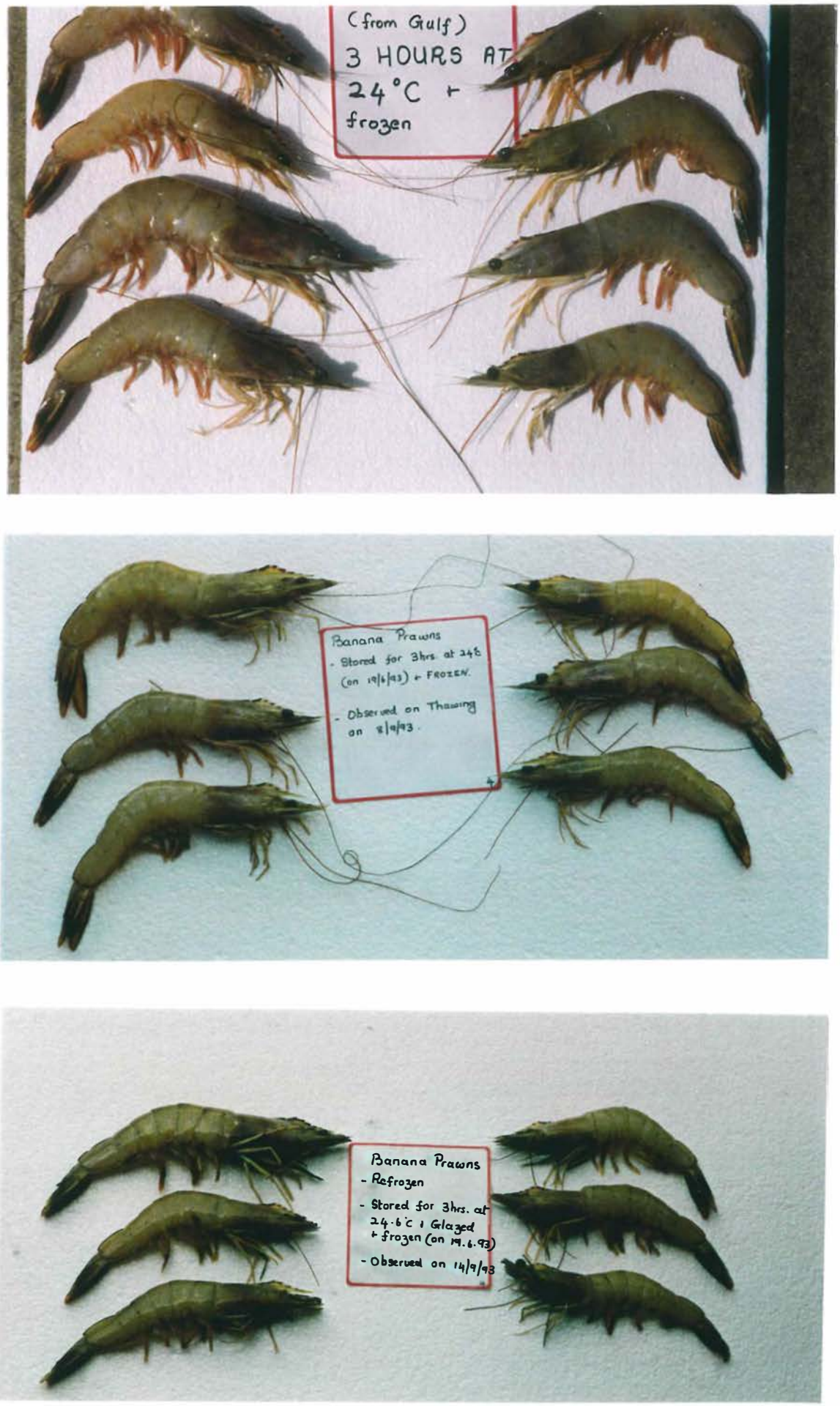


Figure 7.3.4 Banana prawns held at ambient temperature on deck then frozen stored (a) for 46 days (b) for 81 days and (c) sample (b) refrozen for a further 41 days.

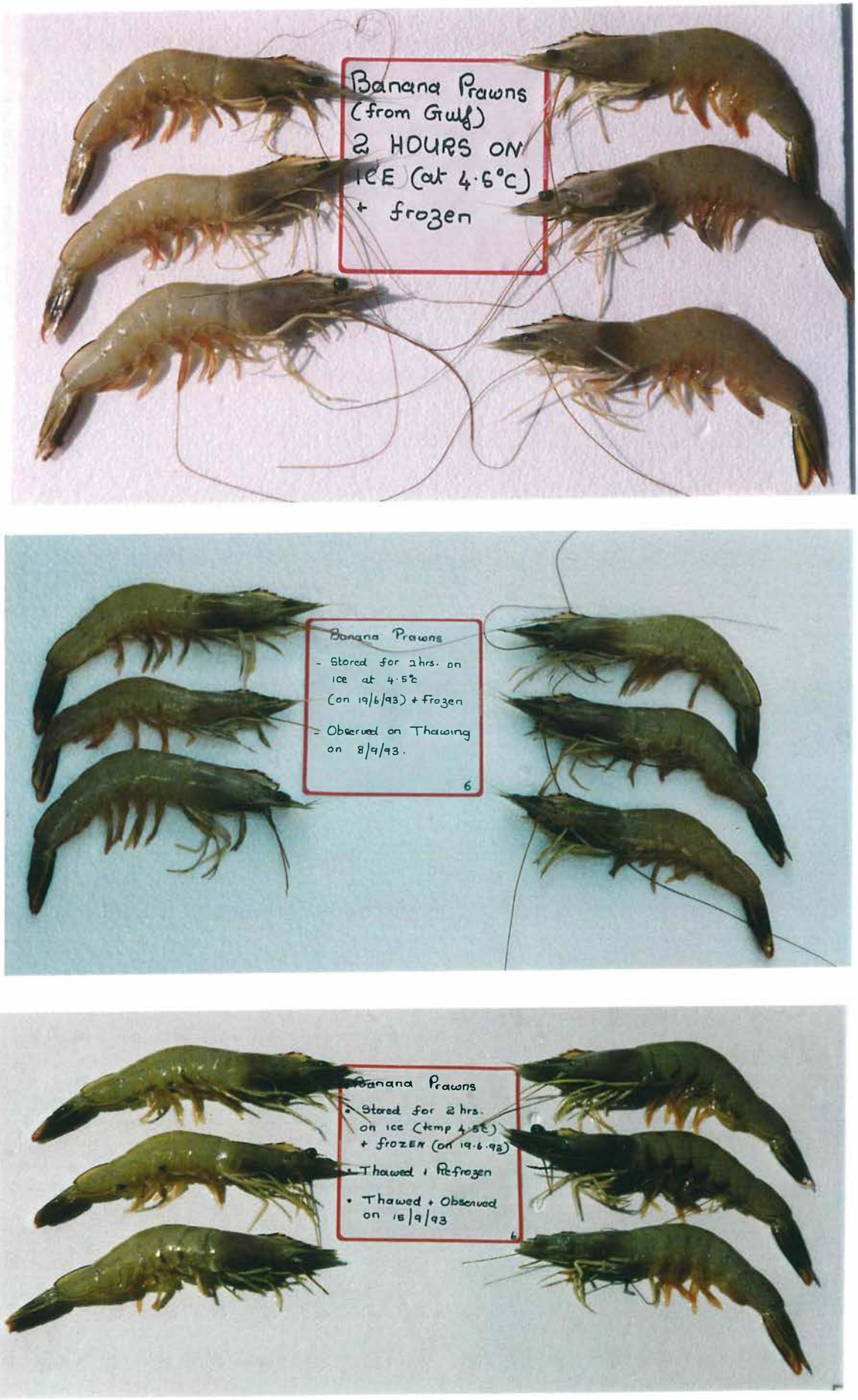


Figure 7.3.5 Banana prawns stored in ice for two hours then frozen stored (a) for 46 days (b) for 81 days and (c) sample (b) refrozen for a further 41 days.

SECTION 8 PRODUCT DEVELOPMENT INVESTIGATIONS

8.1 Value added packs

8.2 Procedures for producing smoked prawns

8.3 Utilisation of prawn head and shell as meal

8.1 Value added packs

Vacuum skin packaging provides an excellent means of displaying prawn products to advantage. The pack has visual appeal and the features of the product are readily visible. The presentation can be enhanced by the incorporation of other additives and ingredients to form the basis of a ready to prepare dish.

Several types of display packs containing prawns and other ingredients were prepared for various functions. These were well received and were found to be quite stable when frozen.

No shelflife, microbiology or safety studies were done on these products since the microbiology of mixed seafood products incorporating vegetables, fruits, herbs or other condiments is of a complexity beyond the scope of this study. It is an area that needs basic investigation if packaged fresh seafood products are to become common in supermarkets.

Illustrations of these packs can be seen in Sections 4.2 and 8.2 and other examples are included here.

Figure 8.1.1 Banana prawns in vacuum skin packs garnished with vegetables

Figure 8.1.2 Banana prawns vacuum skin packed with orange and herbs

Figure 8.1.3 Barbecue pack, satay style banana prawns, vacuum skin packed

8.2 Procedures for producing smoked prawns

Aim

To establish methods for producing smoked prawns.

Introduction

There are often prawns available which are not suited for prime products or for further chilled storage. These prawns may be wholesale and microbiologically sound but may be small, physically damaged about the head or legs or be showing signs of melanosis or other imperfections. If the meat from these prawns is sound, then smoking is one avenue of converting them into a value added product.

From discussions with staff and industry contacts it was established that the preference was for smoked prawns that were not tough in texture and had only a mild smoked flavour.

Investigations therefore centred on the correct cooking times and smoking times to achieve this desired result.

Materials and methods

Frozen banana prawns were used from the same batch to achieve uniformity of raw material. Preliminary trials indicated that steaming rather than boiling gave the best results.

A trial was conducted in which prawns were thawed, weighed, deheaded and peeled, brined then steamed for either 70 seconds or 130 seconds, then each group was smoked for 25 minutes (Figure 8.2.1).

Following the results of this trial a second batch was prepared using the one cooking time (130 seconds). This batch was split into two groups one of which was smoked for 15 minutes, the other for 25 minutes.

These two groups were evaluated by a sensory panel of 30 people who judged the acceptability of the colour, texture, moisture, smoked flavour and overall acceptability on 0-100 scales with 0 representing dislike extremely to 100 like extremely. These samples were presented in a random balanced order.

Samples were taken for measurement of water activity, total bacterial counts and measurement of texture by using peak force shear measurements obtained on an Instron texture measuring instrument. Samples of prawns cooked for either 70 seconds or 130 seconds and smoked for 25 minutes were evaluated by sensory taste panel using the triangle test in which 29 tasters had to pick which one of three prawns was different to the other two.

Results

After cooking and smoking only 40-50% of the original prawn weight remained (Table 1). The cooking and steaming decreases the water activity (from 1.0) slightly (Table 2) but it still remains high enough for spoilage organisms to grow on the prawn as a substrate if it were recontaminated before packaging.

The initial total bacterial counts were quite low and, after steaming, and through the cooking process no bacteria were detectable (Table 3) under the conditions used.

No significant difference was detected between the two cooking times. Fifteen panellists selected as being different prawns cooked for one time and 14 the other. However, in the comments, those cooked for 70 seconds were considered by some to be mushy. Therefore for the second trial a cooking time of 2 minutes 10 seconds was chosen.

The Instron measurements (Table 4) also indicated that the shorter cooking time resulted in a softer texture.

The second taste panel results are presented in Table 5.

Overall acceptability scores showed a preference for the 25 minutes smoking treatment, but difference in scores was not statistically significant (p>05).

The main differences between the two treatments were in colour and intensity of smoke flavour. In both these attributes there was a significant (p>0.01) difference between mean panel scores, and the scores for the 25 minute treatment were very close to "just right". The 15 minute treatment resulted in a prawn which was considered too pale and lacking in smokiness:

Both treatments were considered very close to "just right" for tenderness/toughness and moistness. The prawns which had been smoked 25 minutes were rated slightly tougher and drier than the others, as might be expected, but the difference in scores was not statistically significant (p>0.05).

Comments from the panellists were also recorded. Generally those smoked for 15 minutes were thought to be not quite smoky enough and a little light in colour. However some commented that those smoked for 25 minutes were a little tough and a bit chewy, sometimes dry.
A vacuum skin pack of the smoked prawns is shown in Figure 8.2.2

Conclusion

Cooking by steaming for about 2 minutes and smoking for between 15 and 25 minutes can result in a very acceptable attractive product. This product as it comes out of the smoker is virtually sterile and provided it is packed in a manner that does not recontaminate it, the product should have a significantly long shelf life. Such product would make an excellent snack, entree or component in a main meal.

Table 1 Recovery rates of smoked banana prawns using different cooking and smoking times.

Steaming (100°C) & smoking times (75°C)	Loss in weight (%) during deheading	Loss in weight after cooking and smoking
Cook 70 sec. Smoke 15 min.	33	56
Cook 4 min. Smoke 15 mins	35	51
Cook 3 min. Smoke 10 min.	37	60
Cook 2.1 min. Smoke 15 min.	40	52

Table 2 Water activity of smoked prawns using different processing treatments.

Treatment	Water activity
Steamed 70 seconds	0.975
Steamed 130 seconds	0.982
Steamed 130 seconds & smoked 15 mins	0.965
Steamed 130 seconds & smoked 25 mins	0.970

Table 3 Total bacterial counts in smoked prawns using different processing techniques.

Total Bacterial Count cfu/g	Incubation temperature 30°C	Incubation temperature 5°C
Raw	$1.8x10^4$	$3.4X10^3$
Steam 70 seconds	ND	ND
Steam 70 seconds & smoke 25 min	ND	ND
Steam 130 seconds & smoke 25 min	ND	ND

ND - none detected

Table 4 Instrumental results for the peak shear force required to break the texture of smoked prawns.

Treatment	Peak shear force measurement (joule)
Cooked 70 seconds & smoked 25 min	0.288
Cooked 130 seconds & smoked for 25 min	0.526

Table 5 Mean panel scores for product acceptability tests (30 tasters)

Smoking treatment @ 75°C	Colour Pale/Dark	Texture Tender/ Tough	Moisture Moist/Dry	Flavour (Smokiness)	Overall Accept*
15 mins	32.39[a]	49.72	56.89	32.72[a]	62.11
25 mins	48.50[b]	52.78	59.22	49.33[b]	69.94

[a,b] Means in same column with different suffices are significantly ($p<0.01$) different

Scales 0-50-100 (e.g. too pale - just right - too dark)
*(Overall dislike extremely →like extremely)

Sample 1 average weight 13.33 g

Sample 2 average weight 13.31 g

Figure 8.2.1

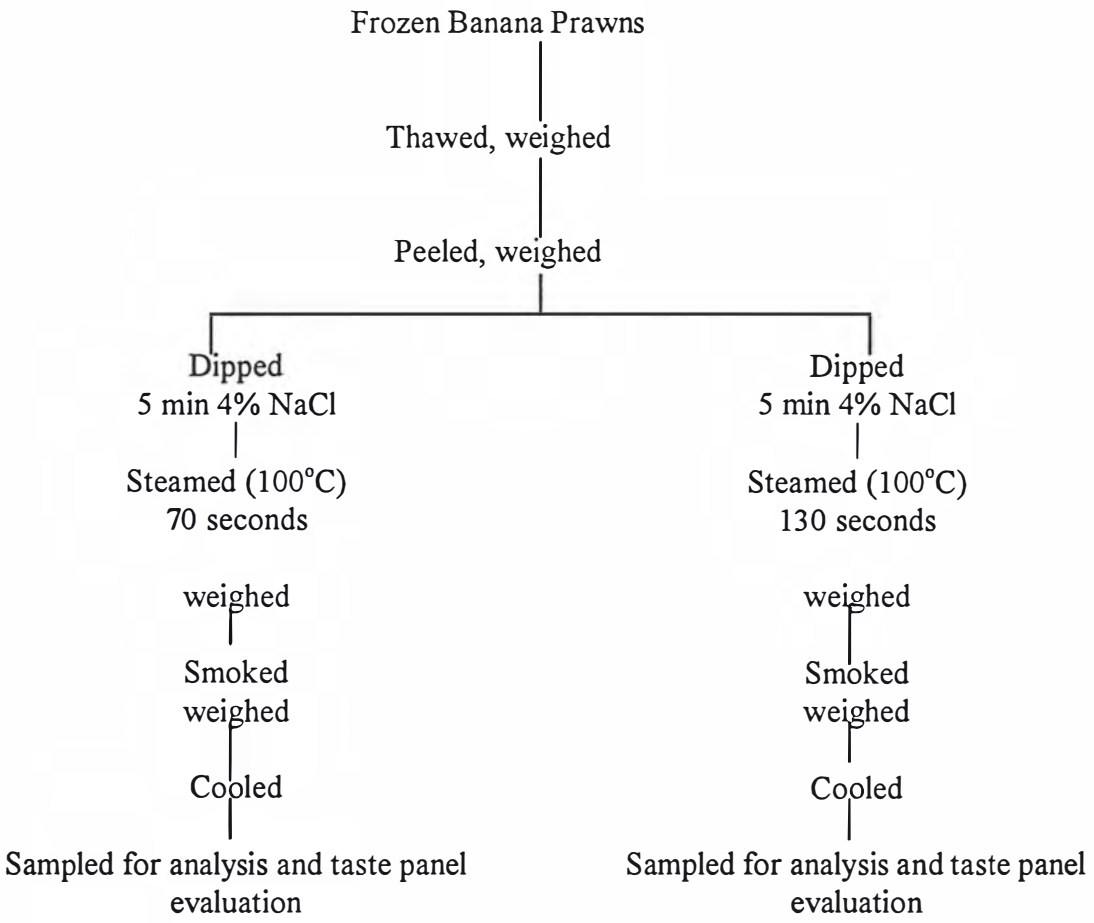

Frozen Banana Prawns
Thawed, weighed
Peeled, weighed
Dipped
5 min 4% NaCl
Steamed (100°C)
70 seconds
weighed
Smoked
weighed
Cooled
Sampled for analysis and taste panel evaluation
Dipped
5 min 4% NaCl
Steamed (100°C)
130 seconds
weighed
Smoked
weighed
Cooled
Sampled for analysis and taste panel evaluation

Figure 8.2.2 A vacuum skin pack of smoked prawns

8.3 Utilisation of prawn head and shell waste as prawn meal for aquaculture

Introduction

The application of the total utilisation concept *through processing of secondary raw material to prawn head and shell meal* would underwrite the processing cost in developing value added prawn products for either domestic or export markets. Reduction in processing cost of both domestic and export products would provide an incentive for processing companies to explore production of convenience products

At present, prawn secondary raw material is not utilised in any useful form, but it is dumped as a land-fill creating environmental pollution. Australia produces more than 2,000 tonnes of fresh prawn secondary raw material a year (Walsh, 1994). In gross terms, *this would be sufficient to produce* 500 tonnes of prawn head and shell meal.. The estimated requirement of the prawn aquaculture industry for prawn head and shell meal was about 200 tonne for the 1993/94 production season. The aquaculture feed industry imports this feed ingredient from South East Asia at a cost of about A$900/ton. In fact, the amount of prawn secondary raw material is not only enough to meet the requirement of the prawn aquaculture industry, but also creates an excellent export opportunity for Australia.

Prawn head and shell meal can be used in fish feeds but is mainly recommended for diets of prawn species (New, 1987) due to its contribution their nutrition, since they lose significant amounts of nutrient by the regular moulting process.

Prawn head and shell meal has a high crude protein content which varies between 35% and 50%. However, the true protein level is around 50% to 70% of the crude protein content (New, 1987). It is because some nitrogen is in the form of non-protein nitrogen which is the constituent of chitin. Chitin is a polysaccharide and is poorly digested by prawns.

Prawn head and shell meal is rich in minerals (particularly calcium), choline, pigments, cholesterol, phospholipids and fatty acids (especially 22:5ω3 and 22:6ω3). The ash constituent of prawn shell contains up to 70% calcium. Due to regular loss of calcium through moulting, prawns require calcium at a level of 1.0% dry diet when they are reared under intensive aquaculture conditions. In nature, prawns regularly consume their moult in order to compensate for the loss of minerals. The meal contains significant amounts of astaxanthin, the pigment responsible for the desirable colouration in prawns. Although it could be used between 5% and 15% inclusion levels it has to be finely ground before being incorporated into diets. It results in poor water stability due to its fibrous texture. Generally, prawn head and shell meal has a dry matter digestibility of 56.8% and protein digestibility of 74.6%

Preliminary work

A preliminary process was developed which resulted in a prawn meal with better characteristics than any available imported product in every aspect. (*Table 1*). *This production process gave a yield of 24% prawn head and shell meal.*

Table 1 Characteristics of IFIQ and imported prawn head and shell meal.

	IFIQ product	Imported product
Dry matter (%)	94.2	90.0
Crude protein (% dry matter, DM)	52.2	40.0
Crude fat (% DM)	6.5	5.0
Ash (% DM)	26.5	20.0
Colouring	Bright reddish-orange	Light orange

References

New, M B 1987. Feed and feeding of fish and shrimp. FAO publications, ADCP/REP/87/26, FAO, Rome, pp 275.

Walsh, P 1994. An analysis of the Western Australian fishing and seafood processing industry to determine the major source and tonnage of by-catch and by-product waste. Project Report (project no 8), "Fish meal production using by-products of commercial fisheries (pilot study)", pp 17.

(IX) BENEFITS

The sector of the industry that can benefit most from this research is the prawn fishing and processing industry. Quantification of the benefits in terms of price is beyond the scope of this project, which was to ascertain some baseline technical information on the properties of Australian prawns. Processors that have access to good quality chilled or frozen product potentially stand to gain most from these results. Aquaculture farmers and processors can also gain when they wish to diversify.

If the results are taken up by industry better standard product could enter both domestic and export markets.

(X) INTELLECTUAL PROPERTY

No intellectual property arises directly from this research.

(XI) FURTHER DEVELOPMENTS

An enormous amount of work has been covered in this project. It was intended to follow with a parallel project on value adding which would have retained core staff familiar with the intricacies of the project who would have been ideal to advise industry and extend these results to the industry as well as to use them as a springboard for applied developments. This project was not supported.

Elements of these results that require development, not research, can be formed into project applications to the NSC with one of the industry partners in the proposal, Other elements that are research, such as the storage of vacuum skin packages in CO2 and the extension of its application to a range of seafoods, are incorporated in a proposal to be put to FRDC in the annual call this December.

Work on other aspects such as shelflife and safety of prawn products, particularly involving mixtures with other commodities is not being done. Further work on packaging materials and storage systems for prawns is not being done either.

It is anticipated that, as time permits, sections of this work will be prepared for articles in journals like Queensland Fisherman and Professional Fisherman. Some parts of the results will also be offered for presentation at the next Preseason Prawn Workshop.

Scientific publications will be prepared also and it is anticipated the work will be presented at "Making the most of the catch", a seafood conference to be run by IFIQ in late July 1996 in Brisbane prior to the 2nd World Fisheries Congress.

(XII) STAFF

H N Chinivasagam	Microbiologist	100%
H A Bremner	Senior Principal Scientist	15%
S J Thrower	Principal Scientist	15%
R Reeves	Senior technician	30%
R Naidoo	Technician	50%
L Tan	Technician	50%

(XIII) FINAL COSTS

The statement is attached.

(XIV) DISTRIBUTION LIST

ASIC	AUSEAS
AFMA	U of Q GATTON
CSIRO FISHERIES	
IFIQ	DPI
QCFO	

Fisheries Research and Development Corporation

Statement of Receipts and Expenditure for the period ending 30 June 1995

Name of Research Organisation	FRDC Project Number	Title of Project
Department of Primary Industries	91/100 95 52 012 AF5	Prawn Presentation and Product Development

Budget Summary	1991/1992	1992/1993	1993/1994	1994/95[1]
Original Budget	106,200.00	115,250.00	115,384.00	
Current Budget [2]				

Summary Receipts and Expenditure for the Project since commencement

	1991/1992	1992/93	1993/94	1994/95
B/F	-	84,208.31	29,292.50	15,661.68
FRDC Funds (Plus)	106,200.00	115,250.00	122,191.84	-
Expenditure (Minus)	21,991.69	134,511.97	135,822.66	44,507.68
Refunds[3]		35,653.84	-	-
Balance C/F	84,208.31	29,292.50	15,661.68	O/D 28,846.00

Details Financial Year to 30 June 1995

Funds Available

	Balance brought forward from previous year	15,661.68
	Total Funds received from FRDC during Financial Year 1994/95	-
	Funds Available for FY 1994 - 95 [4]	15,661.68

Allocation FY[5]			
	Less Expenditure		
$	Salaries	41,436.99	
$	Travel	80.22	
$	Operating	2,990.47	
.....	Capital	-	44,507.68
TOTAL $ -	Balance as at 30 June		O/D 28,846.00

Notes:

(1) Use the column for the final ONLY regardless of the length of the project.
(2) Total current budget shall not exceed Total original budget without approval in writing from the FRDC.
(3) Refunds should only be paid at completion of the project together with the final audited statement.
(4) ACTUAL EXPENDITURE (whether cash or accrual) ONLY. Commitment shall not be included.
(5) Show allocation for the current financial year. Transfers between budget heads allowed under 9(f) of the project Agreement, or the approved in writing by the FRDC shall be listed in the comments.

Comments

Certified by:

S. Hallam

(Signature)

Sue Hallam

(Printed Name)