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Fishing Industry Research Trust Account

Final Report - 30 June 1983

THE BIOLOGICAL ORIGIN OF COMPOUNDS RESPONSIBLE FOR DISTINCTIVE OFF-FLAVOURS IN PRAWNS AND OTHER EDIBLE CRUSTACEANS.

CSIRO, Division of Food Research

1. SUMMARY

Past studies have shown that garlic-like off-flavours in deep sea prawns and sand lobsters are due to the presence of bis-(methylthio)-methane and an unidentified compound. This compound has now been identified as trimethylarsine. Both of these compounds in deep sea prawns are believed to be produced by bacterial action before the catch is landed. However, production of bis-(methylthio)-methane in the male sand lobster is produced by bacterial action during the handling and transportation of the catch. Studies have shown that γ-irradiation of freshly killed male sand lobsters prevents the formation of bis-(methylthio)-methane. As a consequence of this study it was demonstrated that bis-(methylthio)-methane can be removed from off-flavoured deep sea prawns and sand lobsters by short term irradiation with γ-rays.

The metallic off-flavour encountered in deep sea prawns is due to (5Z)-octa-1,5-dien-3-ol and oct-1-en-3-ol. These compounds are formed by the oxidation of polyunsaturated fatty acids. This off-flavour is not, however, a major problem in the sand lobster. Studies have shown that the sand lobster contains an additional polyunsaturated fatty acid, but the role, if any, that this acid plays in reducing the effect of the metallic off-flavour in this crustacean is unknown.

The kerosene-like off-flavour found in some estuarine prawns has been traced to petrochemical hydrocarbons present as a result of environmental pollution.

The endeavour prawn (Metapeneaus endeavouri) frequently possesses an idoform-like off-flavour. Two compounds with flavours described as iodoform-like have now been isolated from off-flavoured endeavour prawns. The major component is 2,4,6-tribromophenol and the other has been tentatively identified as a dibromophenol. The compounds are believed to be derived from the breakdown of precursor compounds present in the crustacea's diet.

An effort to locate specific areas within crustacea where off-flavour compounds are synthesised was in the main unsuccessful. However, this study has shown that the compounds responsible for garlic-like and iodoform-like off-flavours in prawns are initially concentrated in the region of the digestive organs.

On the basis of the above studies a number of recommendations are made to the Industry which could reduce the severity of off-flavour problems in crustacea.

2. GARLIC-LIKE FLAVOURS

The observation that a garlic flavour appeared in both deep sea prawns, such as the commercially important royal red prawn (Hymenopenaeus sibogae), and the shallow water sand lobster (Ibacus peronii) led to the discovery that two quite chemically discrete compounds were responsible for this off-flavour. Earlier work showed that this off-flavour was attributable to the sulphur containing compound bis-(methylthio)-methane together with an unidentified component[1]. A refinement of experimental technique led to the structural illucidation of this other garlic component, which has been identified as trimethylarsine[2].

(a) Isolation and identification of trimethylarsine

Examination of headspace volatiles of prawns, with an intense garlic flavour, by gas liquid chromatography (g.l.c.) combined with sniffing of the effluent gas led to the detection of an extremely volatile compound with a sweet-garlic aroma. This compound could not be identified by combined gas liquid chromatography-mass spectrometry (g.l.c.-m.s.) using trap and transfer techniques[3]. However examination of the total headspace volatiles by g.l.c.-m.s., a compound tentatively identified as trimethylarsine, $(CH_3)_3As$ was detected. The naturally occurring compound had mass spectral features with prominent and diagnostic ions as follows:

m/z (R.I.) 120 M^+ (66), 105 (78), 103 (100), 102 (8),
101 (16), 91 (10), 90 (15), 89 (40),
88 (13), 77 (4), 75 (5).

The high resolution mass spectral examination gave a molecular weight of 119.9931 which was consistent only with the molecular formula C_3H_9As (Calc. 119.9920). Synthesis of trimethylarsine gave a material identical in retention time and mass spectrum to the natural compound[4].

(b) Quantitative analyses

For the estimation of trimethylarsine in crustacea, standard solutions of the synthetic compound were prepared in tetrahydrofuran and their total and non-volatile arsenic content were determined by neutron activation analysis[5]. Headspace volatiles and reference solutions were chromatographed separately using helium (1 cm^3min^{-1}) on a glass SCOT column (50m, 0.5 mm i.d.) coated with Silicone OV-101 heated isothermically at 40°C and coupled to a Varian MAT 311A mass spectrometer. Ionization was by electron impact at 70 ev with an ion source temperature of 250°C. The m.s. was repetitively scanned over a mass range of 34-150 with a cyclic time of 3.5 sec and the data was stored in a MAT SS-100 data system for subsequent processing. Trimethylarsine was located in the chromatogram by retrieval of the mass chromatograms of ions (m/z 103, 105 and 120 and quantitated using the molecular ion m/z 120.

(c) Concentration of trimethylarsine in different species of crustacea

Of the eight crustacea examined (see Table 1) only the royal red, carid and red prawns and the Balmain Bug possessed aromas described as distinctively garlic, intensely so with the last two species[2]. Both these species on occasions contain relatively high concentrations of bis-(methylthio)-methane, up to 10 $\mu g\ kg^{-1}$ in red prawns and 100 $\mu g\ kg^{-1}$ in Balmain Bugs[2]. In the

current studies the red prawn also contained the highest concentration of trimethylarsine. The Balmain Bug contained only 0.002 μg kg^{-1}, indicating that its garlic aroma was due essentially to bis-(methylthio)-methane.

Of the deep sea species examined, only the royal red prawn is currently of commercial importance. Cooking this species whole does not appreciably decrease the level of bis-(methylthio)-methane[6]. In the present studies, cooking also had little effect on the concentration of trimethylarsine: in one sample concentrations were 0.180 μg kg^{-1} and 0.160 μg kg^{-1} in the uncooked prawns and cooked respectively.

Crustacea accumulate arsenic in their tissues principally in the form of complex organometallic compounds such as the non-toxic arsenobetaine[7]. Recent studies of Eastern Australian crustacea showed the concentration of inorganic arsenic in school and royal red prawns is about 0.1 mg kg^{-1} but that of total organic arsenic can be as high as 14.6 mg kg^{-1} [8]. From our studies the contribution of trimethylarsine to this total is neglegible.

(d) Biological origin of trimethylarsine

Scopulariopsis brevicaulis[9], a soil mould, Candida humicola[10], a yeast from soil and sewerage sludge, and a strain of Methanobacterium[11] isolated from ocean mud all convert arsenic derivatives into alkylarsines by reductive methylation. It is likely, therefore, that methanogenic microorganisms in the digestive systems of crustacea could convert either inorganic or organic arsenic compounds into trimethylarsine. Whether this conversion takes place as a normal part of the animals metabolic process or during handling and storage is unknown.

It has been shown that the principal source of bis-(methylthio)-methane was the head section of the prawn[6]. We have now found that the concentration of trimethylarsine in the head is up to eight times that in the tail. Thus, until the biological process leading to the formation of these compounds are

fully understood the current practice of heading the catch prior to cooking appears to remain the most suitable method for reducing the level of the garlic-metallic off-flavours in susceptible species of prawns.

(e) Biological origin of bis-(methylthio)-methane

In samples of crustacea containing bis-(methylthio)-methane we have always detected trace quantitites of methanethiol, dimethyl sulphide and dimethyl disulphide. The presence of these compounds in the off-flavoured crustacea suggests microbial involvement and in particular Pseudomonas species. It is well established that these bacteria, which have been isolated from spoiling cod, convert methionine into methanethiol and dimethyl disulphide and cyst(e)ine into hdyrogen sulphide[12]. Specifically, even at 0°C Pseudomonas putrefaciens and Pseudomonas perolens produce methanethiol, dimethyl disulphide and dimethyl trisulphide on inoculation of sterile fish muscle[13,14]. Critical examination of the digestive organs and flesh below the shell of crustacea affected by the garlic off-flavour indicated the presence of numerous and diverse bacteria.

Formaldehyde is reported to be a common component of marine organisms, formed by enzymic degradation of trimethylamine oxide, and is present in substantial amounts in salt water fish and some species of arthropoda and invertebrates[15]. It has recently been suggested that bis-(methylthio)-methane in fish oil is derived from either methanethiol or hydrogen sulphide and a formaldehyde equivalent[16]. It is possible that a similar process is involved in the formation of bis-(methylthio)-methane in off-flavoured deep sea prawns.

Earlier studies showed that only the adult male Balmain Bug was affected by the garlic off-flavour, and consistent with this was the finding that bis-(methylthio)-methane could only be detected in the male. Formaldehyde analysis of tail flesh of female and garlic flavoured male sand lobsters was

carried out, estimating the formaldehyde as its dinitrophenylhydrazone derivative by HPLC[17]. The male sand lobsters had five times the concentration of this precursor as did the females (1104 mg·kg^{-1} versus 230 mg kg^{-1}). No significant difference between males and females in sulphur containing amino acids could be found.

(f) Elimination of garlic-like off-flavours

To demonstrate the role of bacteria in the formation of bis-(methylthio)-methane in crustacea, male sand lobsters were subjected to a period of γ-irradiation to destroy all non-spore-forming bacteria. Freshly caught creatures were snap frozen in liquid nitrogen, then cut longitudinally while frozen. It was noticed at this stage that four of these had some evidence of garlic aroma. While still frozen they were irradiated at two different strengths. Those halves treated with 2.5 M rads showed no sign of garlic odour. Those treated with only 0.5 M rads still had a slight garlic aroma. On leaving these to thaw, no increase in garlic aroma was noticed after 16 h.

A 10^{-4}M solution of bis-(methylthio)-methane was irradiated with 2.5 M rads. All garlic aroma disappeared and the solution chromatographically showed none of the starting material. Sulphurous acid was produced as a product of irradiation, and presumably methanol, however the latter was not chemically substantiated.

Irradiation of garlic flavoured royal red prawns at both 2.5 and 0.5 M rads removed all traces of the garlic off-flavour. However a slight burnt off-flavour, more noticeable in those irradiated at the higher dose made these prawns unacceptable to some taste panelists. Nevertheless, irradiation may in the future offer both a method of sterilization and a means of removing bis-(methylthio)-methane and presumably trimethylarsine from contaminated samples.

3. METALLIC OFF-FLAVOURS

Biological origin of the metallic components of the garlic-metallic off-flavour.

The metallic off-flavour frequently encountered in species of deep-sea prawns affected by the garlic off-flavour is due to the presence of the unsaturated alcohols (5Z)-octa-1,5-dien-3-ol and oct-1-en-3-ol which are established oxidation products of unsaturated fatty acids. Oct-1-en-3-ol is an autoxidation product of linoleic acid[18] and (5Z)-octa-1,5-dien-3-ol is a product of copper catalysed oxidation of eicosapentaenoic acid and docosahexaenoic acid[19].

These polyunsaturated fatty acids are known components of the lipid fraction of crustacea[20] and copper is present in crustacea as the oxygen carrier haemocyanin. In our studies we have shown that the production of the alcohols oct-1-en-3-ol and (5Z)-octa-1,5-dien-3-ol is facilitated when homogenates of royal red prawns are heated at 40° with oxygen, α-tocopherol and copper palmitate. It is therefore most likely that these unsaturated alcohols are products of in vivo lipid oxidation.

The lipid contents of the royal red prawn and the Balmain Bug, a species not usually affected by this off-flavour, were analysed[20] and there were no highly significant differences in the fatty acid content of these creatures except that the sand lobster contained an additional polyunsaturated fatty acid, docosapentaenoic acid. Unless this compound blocked the pathway for off-flavour production the lipid content does not account for the difference in concentration of the unsaturated alcohols in these species of crustacea.

4. KEROSENE OFF-FLAVOUR

Identification and origin of compounds responsible for kerosene-like off-flavour

Complaints of kerosene or petrochemical like flavours in some species of Australian prawns prompted an investigation of common pollutants accumulated in these creatures. A variety of prawns were investigated from the main prawning outlets close to Sydney, these included school prawns (Metapenaeus macleayi), king prawns (Penaeus plebejus), royal red prawns (Hymenopenaeus sibogae) and carids (Plesionika sp.).

Volatile components were collected from prawn homogenates using headspace techniques and these were analysed by combined g.l.c.-m.s.. Results indicated that a large number of aromatic hydrocarbons, following the same pattern as atmospheric hydrocarbon pollutants[21], as well as some halogenated compounds occurred in prawns regardless of whether they were caught in shallow estuaries close to industrial development or in depths of 300 fathoms 40 miles out to sea. A list of components found in these prawns is presented in Table 2.

The biological origin of these compounds in prawns is directly through the food chain. As prawns are filter feeders, they present a good mechanism for bioaccumulation of pollutants. Surface runoff, fresh water discharge from sewage and industrial waste discharged directly into marine environment are potential sources of pollution, as is aerial precipitation which is an important input route for chlorinated hydrocarbons.

5. IODOFORM-LIKE OFF-FLAVOURS

The endeavour prawn (Metapenaeus endeavouri) caught in Exmouth Gulf, Western Australia has long been known to possess an iodine or iodoform-like off-flavour. However, chemical tests for the presence of both iodine and iodoform in these prawns proved negative.

Trapping the volatile components using headspace techniques yielded no data on this particular off-flavour. It was therefore presumed to be only slightly volatile, and as an alternative a chemical extraction procedure was devised. Because the aftertaste of an iodoform flavoured prawn had similar characteristics to those of other phenolic off-flavoured foods, an extraction designed to isolate phenols was employed.

(a) Isolation and identification

Whole prawns (5 kg) were homogenized with water and the final pH adjusted to 12 with NaOH solution (5 M). Continuous extraction with diethyl ether as solvent was carried out to remove neutral and basic compounds. The aqueous phase was then acidified with sulphuric acid (5 M) to pH 2, and continuous extraction with ether resumed for 2 x 24 hours. The organic phase was washed with $NaHCO_3$ (5%), brine, dried over anhydrous sodium sulphate and evaporated. Preparative chromatography on silica using 2.5% acetic acid in ether as developing solvent yielded four major fractions. One of these (r.f. 0.67) was recrystallized from absolute ethanol to give a white waxy substance.* The supernatant was reduced, and the residue repeatedly recrystallized from aqueous ethanol to give white needles of 2,4,6 tribromophenol (2.1 mg m.p. 95-96, lit[22] 95°). Subequently an, as yet, unidentified dibromophenol was isolated from this species and was shown to possess a flavour not detectably different from the off-flavour present in contaminated prawns.

(b) Origin of bromophenols

Bromophenols are known to occur in sea weeds and marine algae as their glycoside and sulphate derivatives[23]. As the endeavour prawn feeds on weed and algae, at certain times of the year, these glycosides and sulphates will be present for short periods in the crustacean's digestive system. Upon death

* This was later shown to be the ubiquitous ß-sitosterol.

these glycosides and sulphates will be hydrolysed by bacterial or endogenous enzymic activity to yield the bromphenols and either a sugar fraction or an inorganic sulphate.

6. DISSECTION OF OFF-FLAVOURED CRUSTACEA

Attempts to locate the site of production of the garlic off-flavour within the Balmain Bug yielded no conclusive results.

Tissue examined included stomach and digestive system, gills, reproductive tract, head and tail flesh. Any creature possessing a garlic off-flavour had all tissues contaminated, the greatest concentration of bis-(methylthio)-methane was found in the gills and head flesh.

Furthermore, all sections of tissue removed from an apparently uncontaminated creature developed the off-flavour in isolation within 24 h of standing at room temperature.

Problems in experimental techniques were encountered when dealing with small organs having a large surface area - e.g. vas deferens, through which much of the highly volatile bis-(methylthio)-methane could escape.

Similarly, attempts to pinpoint the origin of off-flavour within the royal red prawn and the endeavour prawn were not successful. However, it was observed that the compounds responsible for garlic-like and iodoform-like off-flavours were always initially concentrated in the region of the digestive organs.

7. RECOMMENDATIONS TO THE INDUSTRY

This study has led to the identification of three new compounds responsible for off-flavours in crustacea and has provided additional data on the biological origin of compounds responsible for off-flavours in prawns and sand lobsters. The data obtained from the biological studies has provided supporting evidence for earlier statements that correct handling and

processing of crustacea can greatly reduce the severity of contamination by naturally occurring off-flavours.

In order to reduce the severity of these off-flavours it is recommended that:

For royal red prawns -

Trawl times should be kept to a minimum.

The catch should be sorted and washed immediately upon landing and be either frozen or placed in refrigerated sea water held at -2°C.

For processing, the prawns should be headed and sold as frozen tails.

For endeavour prawns -

The catch should be sorted and washed immediately upon landing and snap frozen.

For processing, the prawns should be headed and sold as frozen tails.

For sand lobsters (Ibacus peronii) -

The catch should be shorted and washed immediately upon landing.

Several alternatives are then available.

The catch may be marketed alive.

The catch boiled for 10 min immediately after cleaning and marketed as a cooked product.

Where Ibacus peronii is to be transported long distances for resale uncooked, the tails should be removed and snap frozen and the product marketed as frozen tails.

8. ACKNOWLEDGEMENTS

We wish to thank Mr T. Gorman, New South Wales Department of Fisheries, and the Sydney Fish Markets for the gifts of prawns and sand lobsters and Mr A. Ward from Lakes' Entrance Fishermans Coop for providing us with samples of sand lobsters. The award of a Fishing Industry Research Grant (to D.J.F.) is gratefully acknowledged.

Table 1

Occurrence of trimethylarsine in Australian crustacea

Common name	Zoological name	Source	Average Concentration ($\mu g\ kg^{-1}$)
Prawns			
Royal red "A"	Hymenopenaeus sibogae	NSW, ocean, 275-685 m.	0.040
Royal red "B"	Hymenopenaeus sibogae	NSW, ocean. 275-685 m.	0.180
Carid	Plesionika sp.	NSW, ocean, 275-685 m.	0.120
Red	Aristeomorpha foliacea	NSW, ocean, 275-685 m.	0.980
Endeavour	Metapenaeus endeavouri	Western Australia ocean, zero-50 m.	0.070
School "C"	Metapenaeus macleayi	NSW, estuarine.	0.080
School "D"	Metapenaeus macleayi	NSW, estuarine.	0.030
Red spot king	Penaeus longistylus	NSW, ocean, 35-55 m.	0.040
Sand lobsters			
Balmain bug	Ibacus peronii	Victoria, ocean, 70-140 m.	0.002
Moreton Bay Bug	Thenus orientalis	Queensland, ocean, 70-140 m.	0.014

Suffixes "A,B,C,D" indicate species caught in different localities.

Table 2

Some volatile pollutants present in Crustacea

Aromatic compounds	Halogenated compounds	Sulphur containing compounds
Benzaldehyde	bromoform	carbon disulphide
Benzene	chloroform	dimethyl disulphide
	dibromochloromethane	dimethyl trisulphide
C_2 alkylbenzenes:	dichlorobenzene	methanethiol
ethylbenzene	dichlorobromomethane	
o-xylene	tetrachloroethylene	
m-xylene	1,1,2-trichloroethane	
p-xylene	trichlorethylene	
C_3 alkylbenzenes:		
isopropylbenzene		
propyl benzene		
1-methyl-4-ethylbenzene		
1-methyl-3-ethylbenzene		
1-methyl-2-ethylbenzene		
1,3,5-trimethylbenzene		
1,2,4-trimethylbenzene		
1,2,3-trimethylbenzene		
C_4 alkylbenzenes:		
isobutylbenzene		
1,2-diethylbenzene *		
dimethylethylbenzene x 3		
1-methyl-3-isopropylbenzene		
methyl propyl benzene		
naphthalene		
dihydronaphthalene		

* Three of these compounds were detected, however assignment of substituents was not possible on g.l.c.-m.s. data alone.

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- ☐ NEW PROPOSAL
- ☐ CONTINUING PROJECT
- ☒ FINAL REPORT
- ☐ PROGRESS REPORT

OUT-OF-SESSION -- December 1983

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL/PROJECT: Biological Origin of Compounds Responsible for Distinctive Off-flavours in Prawns and Other Edible Crustaceans

ORGANISATION: CSIRO Division of Food Research

PERSON(S) RESPONSIBLE:

FUNDS SOUGHT/GRANTED

YEAR	SOUGHT	GRANTED
1981/82		$23,300
1982/83		26,500

RELATED APPLICATIONS:

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.....Judith La.....
for Secretary
Fishing Industry Research Council

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current studies the red prawn also contained the highest concentration of trimethylarsine. The Balmain Bug contained only 0.002 μg kg^{-1}, indicating that its garlic aroma was due essentially to bis-(methylthio)-methane.

Of the deep sea species examined, only the royal red prawn is currently of commercial importance. Cooking this species whole does not appreciably decrease the level of bis-(methylthio)-methane[6]. In the present studies, cooking also had little effect on the concentration of trimethylarsine: in one sample concentrations were 0.180 μg kg^{-1} and 0.160 μg kg^{-1} in the uncooked prawns and cooked respectively.

Crustacea accumulate arsenic in their tissues principally in the form of complex organometallic compounds such as the non-toxic arsenobetaine[7]. Recent studies of Eastern Australian crustacea showed the concentration of inorganic arsenic in school and royal red prawns is about 0.1 mg kg^{-1} but that of total organic arsenic can be as high as 14.6 mg kg^{-1} [8]. From our studies the contribution of trimethylarsine to this total is neglegible.

(d) Biological origin of trimethylarsine

Scopulariopsis brevicaulis[9], a soil mould, Candida humicola[10], a yeast from soil and sewerage sludge, and a strain of Methanobacterium[11] isolated from ocean mud all convert arsenic derivatives into alkylarsines by reductive methylation. It is likely, therefore, that methanogenic microorganisms in the digestive systems of crustacea could convert either inorganic or organic arsenic compounds into trimethylarsine. Whether this conversion takes place as a normal part of the animals metabolic process or during handling and storage is unknown.

It has been shown that the principal source of bis-(methylthio)-methane was the head section of the prawn[6]. We have now found that the concentration of trimethylarsine in the head is up to eight times that in the tail. Thus, until the biological process leading to the formation of these compounds are

fully understood the current practice of heading the catch prior to cooking appears to remain the most suitable method for reducing the level of the garlic-metallic off-flavours in susceptible species of prawns.

(e) Biological origin of bis-(methylthio)-methane

In samples of crustacea containing bis-(methylthio)-methane we have always detected trace quantitites of methanethiol, dimethyl sulphide and dimethyl disulphide. The presence of these compounds in the off-flavoured crustacea suggests microbial involvement and in particular Pseudomonas species. It is well established that these bacteria, which have been isolated from spoiling cod, convert methionine into methanethiol and dimethyl disulphide and cyst(e)ine into hdyrogen sulphide[12]. Specifically, even at 0°C Pseudomonas putrefaciens and Pseudomonas perolens produce methanethiol, dimethyl disulphide and dimethyl trisulphide on inoculation of sterile fish muscle[13,14]. Critical examination of the digestive organs and flesh below the shell of crustacea affected by the garlic off-flavour indicated the presence of numerous and diverse bacteria.

Formaldehyde is reported to be a common component of marine organisms, formed by enzymic degradation of trimethylamine oxide, and is present in substantial amounts in salt water fish and some species of arthropoda and invertebrates[15]. It has recently been suggested that bis-(methylthio)-methane in fish oil is derived from either methanethiol or hydrogen sulphide and a formaldehyde equivalent[16]. It is possible that a similar process is involved in the formation of bis-(methylthio)-methane in off-flavoured deep sea prawns.

Earlier studies showed that only the adult male Balmain Bug was affected by the garlic off-flavour, and consistent with this was the finding that bis-(methylthio)-methane could only be detected in the male. Formaldehyde analysis of tail flesh of female and garlic flavoured male sand lobsters was

carried out, estimating the formaldehyde as its dinitrophenylhydrazone derivative by HPLC[17]. The male sand lobsters had five times the concentration of this precursor as did the females (1104 mg kg^{-1} versus 230 mg kg^{-1}). No significant difference between males and females in sulphur containing amino acids could be found.

(f) Elimination of garlic-like off-flavours

To demonstrate the role of bacteria in the formation of bis-(methylthio)-methane in crustacea, male sand lobsters were subjected to a period of γ-irradiation to destroy all non-spore-forming bacteria. Freshly caught creatures were snap frozen in liquid nitrogen, then cut longitudinally while frozen. It was noticed at this stage that four of these had some evidence of garlic aroma. While still frozen they were irradiated at two different strengths. Those halves treated with 2.5 M rads showed no sign of garlic odour. Those treated with only 0.5 M rads still had a slight garlic aroma. On leaving these to thaw, no increase in garlic aroma was noticed after 16 h.

A 10^{-4}M solution of bis-(methylthio)-methane was irradiated with 2.5 M rads. All garlic aroma disappeared and the solution chromatographically showed none of the starting material. Sulphurous acid was produced as a product of irradiation, and presumably methanol, however the latter was not chemically substantiated.

Irradiation of garlic flavoured royal red prawns at both 2.5 and 0.5 M rads removed all traces of the garlic off-flavour. However a slight burnt off-flavour, more noticeable in those irradiated at the higher dose made these prawns unacceptable to some taste panelists. Nevertheless, irradiation may in the future offer both a method of sterilization and a means of removing bis-(methylthio)-methane and presumably trimethylarsine from contaminated samples.

3. METALLIC OFF-FLAVOURS

Biological origin of the metallic components of the garlic-metallic off-flavour.

The metallic off-flavour frequently encountered in species of deep-sea prawns affected by the garlic off-flavour is due to the presence of the unsaturated alcohols (5Z)-octa-1,5-dien-3-ol and oct-1-en-3-ol which are established oxidation products of unsaturated fatty acids. Oct-1-en-3-ol is an autoxidation product of linoleic acid[18] and (5Z)-octa-1,5-dien-3-ol is a product of copper catalysed oxidation of eicosapentaenoic acid and docosahexaenoic acid[19].

These polyunsaturated fatty acids are known components of the lipid fraction of crustacea[20] and copper is present in crustacea as the oxygen carrier haemocyanin. In our studies we have shown that the production of the alcohols oct-1-en-3-ol and (5Z)-octa-1,5-dien-3-ol is facilitated when homogenates of royal red prawns are heated at 40° with oxygen, α-tocopherol and copper palmitate. It is therefore most likely that these unsaturated alcohols are products of in vivo lipid oxidation.

The lipid contents of the royal red prawn and the Balmain Bug, a species not usually affected by this off-flavour, were analysed[20] and there were no highly significant differences in the fatty acid content of these creatures except that the sand lobster contained an additional polyunsaturated fatty acid, docosapentaenoic acid. Unless this compound blocked the pathway for off-flavour production the lipid content does not account for the difference in concentration of the unsaturated alcohols in these species of crustacea.

4. KEROSENE OFF-FLAVOUR

Identification and origin of compounds responsible for kerosene-like off-flavour

Complaints of kerosene or petrochemical like flavours in some species of Australian prawns prompted an investigation of common pollutants accumulated in these creatures. A variety of prawns were investigated from the main prawning outlets close to Sydney, these included school prawns (Metapenaeus macleayi), king prawns (Penaeus plebejus), royal red prawns (Hymenopenaeus sibogae) and carids (Plesionika sp.).

Volatile components were collected from prawn homogenates using headspace techniques and these were analysed by combined g.l.c.-m.s.. Results indicated that a large number of aromatic hydrocarbons, following the same pattern as atmospheric hydrocarbon pollutants[21], as well as some halogenated compounds occurred in prawns regardless of whether they were caught in shallow estuaries close to industrial development or in depths of 300 fathoms 40 miles out to sea. A list of components found in these prawns is presented in Table 2.

The biological origin of these compounds in prawns is directly through the food chain. As prawns are filter feeders, they present a good mechanism for bioaccumulation of pollutants. Surface runoff, fresh water discharge from sewage and industrial waste discharged directly into marine environment are potential sources of pollution, as is aerial precipitation which is an important input route for chlorinated hydrocarbons.

5. IODOFORM-LIKE OFF-FLAVOURS

The endeavour prawn (Metapenaeus endeavouri) caught in Exmouth Gulf, Western Australia has long been known to possess an iodine or iodoform-like off-flavour. However, chemical tests for the presence of both iodine and iodoform in these prawns proved negative.

Trapping the volatile components using headspace techniques yielded no data on this particular off-flavour. It was therefore presumed to be only slightly volatile, and as an alternative a chemical extraction procedure was devised. Because the aftertaste of an iodoform flavoured prawn had similar characteristics to those of other phenolic off-flavoured foods, an extraction designed to isolate phenols was employed.

(a) Isolation and identification

Whole prawns (5 kg) were homogenized with water and the final pH adjusted to 12 with NaOH solution (5 M). Continuous extraction with diethyl ether as solvent was carried out to remove neutral and basic compounds. The aqueous phase was then acidified with sulphuric acid (5 M) to pH 2, and continuous extraction with ether resumed for 2 x 24 hours. The organic phase was washed with $NaHCO_3$ (5%), brine, dried over anhydrous sodium sulphate and evaporated. Preparative chromatography on silica using 2.5% acetic acid in ether as developing solvent yielded four major fractions. One of these (r.f. 0.67) was recrystallized from absolute ethanol to give a white waxy substance.* The supernatant was reduced, and the residue repeatedly recrystallized from aqueous ethanol to give white needles of 2,4,6 tribromophenol (2.1 mg m.p. 95-96, lit[22] 95°). Subequently an, as yet, unidentified dibromophenol was isolated from this species and was shown to possess a flavour not detectably different from the off-flavour present in contaminated prawns.

(b) Origin of bromophenols

Bromophenols are known to occur in sea weeds and marine algae as their glycoside and sulphate derivatives[23]. As the endeavour prawn feeds on weed and algae, at certain times of the year, these glycosides and sulphates will be present for short periods in the crustacean's digestive system. Upon death

* This was later shown to be the ubiquitous ß-sitosterol.

these glycosides and sulphates will be hydrolysed by bacterial or endogenous enzymic activity to yield the bromphenols and either a sugar fraction or an inorganic sulphate.

6. DISSECTION OF OFF-FLAVOURED CRUSTACEA

Attempts to locate the site of production of the garlic off-flavour within the Balmain Bug yielded no conclusive results.

Tissue examined included stomach and digestive system, gills, reproductive tract, head and tail flesh. Any creature possessing a garlic off-flavour had all tissues contaminated, the greatest concentration of bis-(methylthio)-methane was found in the gills and head flesh.

Furthermore, all sections of tissue removed from an apparently uncontaminated creature developed the off-flavour in isolation within 24 h of standing at room temperature.

Problems in experimental techniques were encountered when dealing with small organs having a large surface area - e.g. vas deferens, through which much of the highly volatile bis-(methylthio)-methane could escape.

Similarly, attempts to pinpoint the origin of off-flavour within the royal red prawn and the endeavour prawn were not successful. However, it was observed that the compounds responsible for garlic-like and iodoform-like off-flavours were always initially concentrated in the region of the digestive organs.

7. RECOMMENDATIONS TO THE INDUSTRY

This study has led to the identification of three new compounds responsible for off-flavours in crustacea and has provided additional data on the biological origin of compounds responsible for off-flavours in prawns and sand lobsters. The data obtained from the biological studies has provided supporting evidence for earlier statements that correct handling and

processing of crustacea can greatly reduce the severity of contamination by naturally occurring off-flavours.

In order to reduce the severity of these off-flavours it is recommended that:

For royal red prawns -

Trawl times should be kept to a minimum.

The catch should be sorted and washed immediately upon landing and be either frozen or placed in refrigerated sea water held at -2°C.

For processing, the prawns should be headed and sold as frozen tails.

For endeavour prawns -

The catch should be sorted and washed immediately upon landing and snap frozen.

For processing, the prawns should be headed and sold as frozen tails.

For sand lobsters (Ibacus peronii) -

The catch should be shorted and washed immediately upon landing.

Several alternatives are then available.

The catch may be marketed alive.

The catch boiled for 10 min immediately after cleaning and marketed as a cooked product.

Where Ibacus peronii is to be transported long distances for resale uncooked, the tails should be removed and snap frozen and the product marketed as frozen tails.

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Table 1

Occurrence of trimethylarsine in Australian crustacea

Common name	Zoological name	Source	Average Concentration ($\mu g\ kg^{-1}$)
Prawns			
Royal red "A"	Hymenopenaeus sibogae	NSW, ocean, 275-685 m.	0.040
Royal red "B"	Hymenopenaeus sibogae	NSW, ocean. 275-685 m.	0.180
Carid	Plesionika sp.	NSW, ocean, 275-685 m.	0.120
Red	Aristeomorpha foliacea	NSW, ocean, 275-685 m.	0.980
Endeavour	Metapenaeus endeavouri	Western Australia ocean, zero-50 m.	0.070
School "C"	Metapenaeus macleayi	NSW, estuarine.	0.080
School "D"	Metapenaeus macleayi	NSW, estuarine.	0.030
Red spot king	Penaeus longistylus	NSW, ocean, 35-55 m.	0.040
Sand lobsters			
Balmain bug	Ibacus peronii	Victoria, ocean, 70-140 m.	0.002
Moreton Bay Bug	Thenus orientalis	Queensland, ocean, 70-140 m.	0.014

Suffixes "A,B,C,D" indicate species caught in different localities.

Table 2

Some volatile pollutants present in Crustacea

Aromatic compounds	Halogenated compounds	Sulphur containing compounds
Benzaldehyde	bromoform	carbon disulphide
Benzene	chloroform	dimethyl disulphide
	dibromochloromethane	dimethyl trisulphide
C_2 alkylbenzenes:	dichlorobenzene	methanethiol
ethylbenzene	dichlorobromomethane	
o-xylene	tetrachloroethylene	
m-xylene	1,1,2-trichloroethane	
p-xylene	trichlorethylene	
C_3 alkylbenzenes:		
isopropylbenzene		
propyl benzene		
1-methyl-4-ethylbenzene		
1-methyl-3-ethylbenzene		
1-methyl-2-ethylbenzene		
1,3,5-trimethylbenzene		
1,2,4-trimethylbenzene		
1,2,3-trimethylbenzene		
C_4 alkylbenzenes:		
isobutylbenzene		
1,2-diethylbenzene*		
dimethylethylbenzene x 3		
1-methyl-3-isopropylbenzene		
methyl propyl benzene		
naphthalene		
dihydronaphthalene		

* Three of these compounds were detected, however assignment of substituents was not possible on g.l.c.-m.s. data alone.

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