ELSEVIER

International Journal of Food Microbiology 42 (1998) 45–55

International Journal of Food Microbiology

Volatile components associated with bacterial spoilage of tropical prawns

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Received 12 August 1997; received in revised form 23 February 1998; accepted 31 March 1998

Abstract

Analysis of headspace volatiles by gas chromatography/mass spectrometry from king (*Penaeus plebejus*), banana (*P. merguiensis*), tiger (*P. esculentus/semisulcatus*) and greasy (*Metapenaeus bennettae*) prawns stored in ice or ice slurry, which is effectively an environment of low oxygen tension, 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 more prevalent in prawns stored on ice (normal oxygen conditions) at the latter stages of storage (more than 8 days) and were only produced by *Pseudomonas fragi*, whereas sulphides and amines occurred whether the predominant spoilage organism was *Ps.fragi* or *Shewanella putrefaciens*.

The free amino acid profiles of banana and king prawns were high in arginine (12–14%) and low in cysteine (0.1–0.17%) and methionine (0.1–0.2%). Filter sterilised raw banana prawn broth inoculated with a total of 15 cultures of *Ps. fragi* and *S. putrefaciens* and incubated for two weeks at 5°C, showed the presence of 17 major compounds in the headspace volatiles analysed using gas chromatography/mass spectrometry (GC/MS). These were mainly amines, sulphides, ketones and esters.

Principal Component Analysis of the results for the comparative levels of the volatiles produced by pure cultures, inoculated into sterile prawn broth, indicated three subgroupings of the organisms; I, *Ps. fragi* from a particular geographic location; II, *S. putrefaciens* from another geographic location; and III, a mixture of *Ps. fragi* and *S. putrefaciens* from different geographic locations.

The sensory impression created by the cultures was strongly related to the chemical profile as determined by GC/MS. Organisms, even within the same subgrouping classified as identical by the usual tests, produced a different range of volatiles in the same uniform substrate.

Keywords: Headspace volatiles; Prawn spoilage; *Pseudomonas fragi*; *Shewanella putrefaciens*

1. Introduction

The initial microflora on sub-tropical prawns depends on their environment, but subsequently the major spoilage organism is selected by the means of chilled storage (Chinivasagam et al., 1996). Storage in ice, where oxygen is not limiting, results in a flora

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0168-1605/98/$19.00
PII: S0168-1605(98)00057-9

of *Pseudomonas fragi* that produce odours reminiscent of spoiling tropical fruit which gradually increase in intensity throughout most of the storage period. Storage in ice slurry, where oxygen is limiting, results in a spoilage flora of *Shewanella putrefaciens* but here the sulphurous odours are not detected until close to the point where the prawns are rejected (Chinivasagam et al., 1996). *S. putrefaciens* is well known as a spoiler of cold and temperate water marine fish, whereas *Ps. fragi* is more prevalent as a spoiler in marine and freshwater species in tropical and subtropical areas (Gram et al., 1989; Gram, 1993; Liston, 1992; Shewan, 1977).

Gillespie and Macrae (1975) reported that *Pseudomonas* were the most important genus causing fish spoilage in the sub-tropical waters of South East Queensland and that 43% of these isolates could produce sulphydryl odours when incubated in a trypsin treated fish muscle homogenate. In a subsequent numerical taxonomic study, 11 groupings of the *Pseudomonas* spp. were found, two of which contained the majority of those able to cause overt spoilage (Gillespie, 1981). The first group was established to be *S. putrefaciens*, most of which could produce sulphydryls but only eight of 35 isolates could produce H_2S on Peptone Iron Agar. The second group was *Ps. fragi*, many of which produced both fruity and sulphydryl odours.

On beef the compounds of major sensory significance produced by *Pseudomonas* were ethyl methyl esters of C_2–C_8 fatty acids, sulphur containing compounds, thiols, sulphides and thio esters (Edwards et al., 1987). Volatile sulphydryls, alcohols, ketones and pyrazines were produced on sterile fish muscle by *P. percolens* including a pyrazine derivative responsible for a musty potato odour (Miller et al., 1973). Herbert et al. (1971) recommended the use of sterile muscle as the appropriate medium for inoculation of pure cultures to establish the odours produced by them. This technique has been used on fish pieces (Herbert, 1970; Fletcher and Statham, 1988a,b; Fletcher and Hodgson, 1988) but is not practical with prawns because of their small size and the difficulties of sterilizing the outer surface.

The results reported here were obtained as part of a project investigating the storage and packaging of value added prawn products (Chinivasagam et al., 1995). In order to design packaged products with the appropriate storage characteristics it is necessary to know the pattern of spoilage. Results obtained during the course of the project indicated that different odours were produced on prawns stored in different ways (Chinivasagam et al., 1996). This was probably due to different volatiles being produced by different species and strains of bacteria but it was not known whether they were associated with the species of prawn or the location of catch. This paper reports the results of studies in which the volatiles generated during chill storage from four prawn species were subjected to headspace gas analysis using GC/MS. The volatiles produced by bacterial isolates inoculated into filter-sterilised prawn extract were also analysed and Principal Component Analysis was used to seek underlying relationships in the results.

2. Materials and methods

2.1. Experimental outline including storage of prawns

Four species of prawns from the initial and spoilage stages of storage (Chinivasagam et al., 1996), king (*Penœus plebejus*), greasy (*Metapenœus bennettae*), tiger (*P. esculentus*/*semiculcatus*) and banana prawns (*P. merguiensis*), were stored in ice and ice slurry. Samples were taken immediately after catch and at irregular intervals for (a) GC/MS analysis of the volatiles and (b) estimation of numbers of H_2S producers. Bacterial isolates used in this study were dominant at the time of rejection of the four species of prawns stored in either ice or ice slurry (Chinivasagam et al., 1996). Several cultures were isolated and identified and eight strains of *Ps. fragi* and seven of *S. putrefaciens* were randomly selected.

2.2. Preparation of prawns for analysis of volatile compounds

The volatiles produced by the spoiling prawns were examined by headspace gas chromatography coupled to mass spectrometry. Prawns were taken from the storage trials (Chinivasagam et al., 1996) after 2 and 8 days and at the end. Eight days was arbitrarily chosen as a suitable interval since, at this stage, there was substantial bacterial growth but the prawns were not beyond their marketable shelflife.

2.2.1. Estimation of volatiles in prawn flesh

The prawns from the initial and spoilage stages were stored frozen (−40°C) before analysis of the headspace volatiles by the method of Wood et al. (1994). This method uses cryogenic collection of the headspace volatiles; the sample in a closed flask is swept with helium through a water trap into a cold trap immersed in liquid nitrogen (−196°C). When collection was completed, the trapped volatiles were swept with warmed helium into a Shimadzu GC 9 gas liquid chromatograph coupled to a Finnigan-MAT Model 700 ion-trap detector. Peak identification was achieved by matching the mass spectral data to those in the National Bureau of Standards Mass-Spectral Library. This system has been shown to be reproducible for standard compounds at the low concentrations encountered, exhibiting a linear relationship between log peak area and log concentration, although response slopes were different (Wood et al., 1994). For analysis of whole prawns, 10 g macerated prawn was placed in a 100 ml Erlenmeyer flask fitted with a septum. The flask was then evacuated with high purity helium and allowed to equilibrate at room temperature (21°C) for 1 h; 50 ml was then purged from the flask for analysis. For analysis of broths a 2 ml sample was placed in a 10 ml sample vial fitted with a septum. After incubation of the inoculates, a 10 ml sample of headspace gas was removed for analysis. It was not necessary to purge with helium since neither sufficient water nor air was present to cause problems with the GC/MS.

2.2.2. Estimation of volatiles produced by bacterial isolates

Bacterial isolates from spoiling prawns were inoculated into sterile prawn broth which was then incubated and the volatiles examined by GC/MS as above.

2.3. Isolation and identification of bacterial isolates

Total bacterial counts were estimated by surface plating duplicate serial dilutions of prawn samples and incubating at 30°C for 2 days. Ten grams of flesh from 3–5 macerated prawns was homogenised in a Colworth Stomacher 400 (model BA 6021, Seward Medical, London, UK) for 30 s in 90 ml of sterile peptone (0.1% w/v) diluent. Total counts and total H_2S producers were tested according to the method described by Gram et al. (1987). All colonies from a sector of the plate were re-streaked onto nutrient agar (BBL). Once pure colonies were obtained, they were transferred to peptone yeast extract agar slopes (Oxoid) and stored at 4°C for biochemical identification. All colonies were tentatively identified as outlined in Chinivasagam et al. (1996) and further confirmed using the API 20NE (France) test kit and the BIOLOG system (microlog™ 3 release 3.01A) for Gram-negative bacteria.

2.4. Preparation and inoculation of sterile muscle broth

Freshly caught prawns (2–3 h) were transported to the laboratory in sterile plastic bags held in ice. On arrival the prawns were rinsed in sterile distilled water and aseptically cut at the anterior and posterior region through the flesh without cutting into the gut and the shell was removed. The muscle was chopped and blended with chilled (4°C) sterile distilled water (1:3 flesh/water) in a high speed blender (Waring Blender, model 32 BL 79, Dynamics Corporation, USA) for 2 min. The chilled contents were centrifuged at 20 000 rpm (Sorvall Instruments, RC5C, rotor SS 34, Du Pont Clinical and Instruments, Newtown, CT, USA) for half an hour. The supernatant was collected, chilled again and filtered first using a sterile Millipore (Millipore Corporation, Bedford, Mass., USA) filtration system through a 0.45 μ filter and then through a 0.22 μ filter. The resultant broth was checked for sterility by streaking on nutrient agar and checking for any growth after 2 days incubation, prior to dispensing in 2 ml lots into sterile bottles (5 ml size with septum on lids), after which individual bottles were also checked for sterility prior to inoculation. All operations were performed in a laminar flow cabinet and aseptic conditions were maintained at all stages of sterile muscle production. The sterile broth was used within three days of preparation and was held at a temperature of 0–1°C. One microliter of a turbid (previously identified) rapidly growing culture (10^7–10^8 ml^{-1}) incubated for 18 h at 30°C was introduced into the bottles by micropipette, which were then incubated at 5°C for 14 days. The volatiles were analysed on the fourteenth day.

2.5. Characterisation of odour

The odour of the volatiles in the bottles was evaluated at the time of test by one of the experimenters (H.N.C.), experienced in judging odours of spoiling prawns, as being neutral or one of three intensities, slight, present or strong, for the attributes of sulphidy and fruity.

2.6. Data analysis

Peak areas from the GC/MS spectrographs of the volatiles were transformed to $\log_{10}$ values and then subjected to Principal Component Analysis (PCA) using the UNSCRAMBLER Version 5.5 package (CAMO A/S, Trondheim, Norway).

2.7. Amino acid analyses

Free amino acids in the fresh flesh of king and banana prawns were determined at the Deakin Research Laboratory of the CSIRO Division of Food Science and Technology, North Ryde, New South Wales, Australia. An homogenised flesh sample was weighed and a measured quantity of water added and the mass placed in an Ultraspin 5000 MW membrane and then centrifuged. An aliquot of the extract was dried and then derivatised with phenylisothiocyanate (PITC) using the Waters picotag reagent kit (Waters Chromatography Division, Milford, MA, USA) according to the standard method of the Waters Picotag Handbook. These PITC derivatives were analysed on a Waters HPLC system using a 3.9×300 mm Waters free amino acid column.

3. Results

3.1. Bacterial growth

The development of the H_2S producing bacteria depended on whether prawns were stored in ice slurry or ice as indicated in Fig. 1. There was a gradual increase in the percentage of H_2S producing organisms over a period of 2 days for king, 5 days for tiger and 7 days for banana prawns stored in ice slurry, but after this the increase was rapid. With prawns stored on ice, the percentage of the H_2S producers was around 10% or less of the total flora

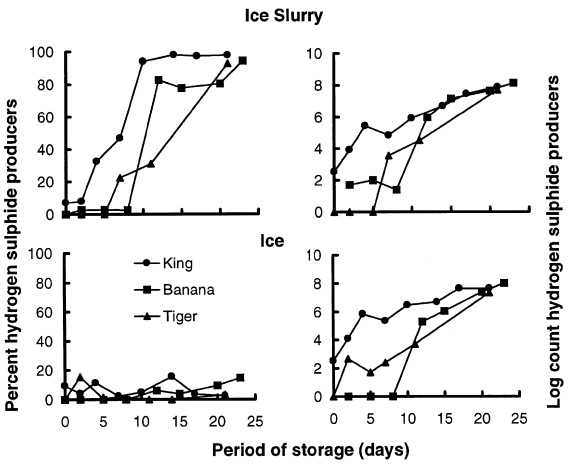

Fig. 1. Percentage and total count of H_2S producing bacteria detected on black colony agar found in three prawn species stored in ice slurry or ice for up to 23 days.

during the entire course of the trial. Higher counts of H_2S producing organisms were found on king prawns than the other two species of prawn for the majority of the storage period.

3.2. Amino acids

The free amino acid profile of king and banana prawns showed low levels of cysteine (0.17 and 0.07 mg g^{-1}) and methionine (0.23 and 0.11 mg g^{-1}), with high levels of arginine (14.38 and 11.93 mg g^{-1}) and glycine (5.68 and 9.61 mg g^{-1}), respectively (Table 1).

3.3. Volatiles produced from stored prawns

A large range of volatiles was detected and only the major components of interest are listed in Table 2. Compounds produced from banana and greasy prawns in less than 8 days of storage in ice slurry were mainly amines. Volatiles from king prawns were not determined before 8 days of storage. Sulphides mainly developed after 8 days of storage in ice slurry for the three prawn species.

For banana and greasy prawns stored on ice for less than 8 days, amines were the major type of volatile, whereas after 8 days, sulphides and esters were predominant. The presence of sulphides was evident from prawns stored on ice for less than

Table 1
Free amino acid content of fresh king and banana prawns

Amino acid	Prawn type	
	King (mg g^{-1})	Banana (mg g^{-1})
Asparagine	0.46	0.25
Aspartic acid	0.04	0.01
Arginine	14.38	11.93
Alanine	1.10	0.75
Cysteine	0.17	0.07
Glycine	5.68	9.61
Glutamic acid	0.26	0.11
Glutamine	2.11	2.16
Histidine	0.17	0.13
Isoleucine	0.23	0.11
Leucine	0.42	0.59
Lysine	0.50	0.17
Methionine	0.23	0.11
Proline	5.71	4.21
Phenylalanine	0.15	0.10
Serine	0.97	0.35
Tyrosine	0.20	0.14
Threonine	0.54	0.13
Valine	0.41	0.20

8 days but to a lesser extent than in the later stages of storage.

Amines from banana and greasy prawns were more prevalent during the initial storage whether the prawns were stored in ice slurry or on ice. They were mainly trimethylamine (TMA), *O*-(2-methylpropyl) hydroxylamine, and *O*-(3-methylbutyl) hydroxylamine. Esters were the major volatiles from all these species of prawns stored on ice after 8 days of storage. Also detected were ethyl butyrate, 2-methyl-2-butanoic acid ethyl ester and ethanoic-*S*-ethyl ester.

Traces of aliphatic and aromatic hydrocarbons such as hexane and ethyl benzene and various substituted phenols were also detected but they did not increase during chilled storage. In addition, a variety of terpenes α- and β-pinene, camphene, α-phellandrene, citral and limonene as well as other unidentified terpenes were present in nearly all the samples (results not shown).

Table 2
Major volatile compounds in the headspace from banana, greasy and king prawns detected by GC/MS after storage of the prawns on ice or in ice slurry for less than or greater than 8 days

	Ice slurry						Ice					
	Stored less than 8 days			Stored more than 8 days			Stored less than 8 days			Stored more than 8 days		
	Banana	Greasy	King	Banana	Greasy	King	Banana	Greasy	King	Banana	Greasy	King
Amines												
Trimethylamine	+	+	na	+	+	−	+	+	na	+	+	−
2-Methyl-2-propanamine	−	+	na	−	−	−	+	+	na	−	−	−
O-(2-Methylpropyl) hydroxylamine	+	+	na	+	+	−	+	+	na	−	−	−
O-(3-Methylbutyl) hydroxylamine	+	+	na	−	+	−	+	+	na	−	+	−
N-Methyl methanamine	−	−	na	−	−	+	−	−	na	−	−	−
Sulphides												
Methyl disulphide	+[1]	+	na	+	+	−	+[1]	+	na	+	+	−
Carbon disulphide	−	−	na	+	+	−	+[1]	+	na	+	−	−
Dimethyl disulphide	−	−	na	+	+	−	−	−	na	+	+	+
Methyl (methyl thio) methyl sulphide	+[1]	+	na	+	+	−	+[1]	+	na	+	+	−
Methyl propyl disulphide	−	−	na	+	+	+	−	−	na	+	+	+
Dimethyl trisulphide	−	−	na	+	−	−	−	−	na	+	−	−
2-Methyl thio propane	−	−	na	−	−	−	−	−	na	−	−	+
Esters												
2-Methyl propanoic acid ethyl ester	−	+	na	−	+	−	−	−	na	+	+	+
Ethyl butyrate	+	−	na	−	−	−	+	−	na	+	+	−
2-Methyl-butanoic acid ethyl ester	−	−	na	−	−	−	−	−	na	−	+	+
Ethanoic-*S*-ethyl ester	−	−	na	−	−	−	−	−	na	−	−	+

+, Present; −, absent; na, not analysed; +[1]present on day 1 of experiment.

3.4. Bacterial characteristics

All 15 cultures used in the trial were present in large numbers on spoiling prawns and it was assumed they had spoilage potential; eight cultures were *Ps. fragi* and seven cultures were *S. putrefaciens*. The distinct difference between the species was the ability of *S. putrefaciens* to reduce TMAO and produce H_2S in the media of Gram et al. (1987), whereas none of the *Pseudomonas* strains had this ability. The colonies of *S. putrefaciens* were light pink to darker pink, while those of the *Pseudomonas* spp. were white.

3.5. Volatiles from sterile broth

The nature of the odours produced by the various isolates in sterile banana prawn broth is listed in Table 3. The *Ps. fragi* isolates produced odours that were generally fruity and sulphydryl in nature, whereas those from *S. putrefaciens* were either sulphide-like or had no distinctive odours and were designated 'neutral'. Hydrogen sulphide, if present, would have co-eluted with the initial major water/air peak and was thus not detected by this headspace system.

A total of 21 major volatile compounds was detected from the different cultures (Table 4) with obvious differences occurring in the range of volatiles produced individually. Results are listed as peak area/ml of headspace (Wood et al., 1994). Eleven of the volatiles contain some form of sulphur and were produced by most isolates. However, those from *S. putrefaciens*, e.g. methanethiol, dimethyl sulphide and dimethyl trisulphide, were detected in much higher relative amounts. On the other hand, *Ps. fragi* isolates produced 1- and 2-propanethiol, whereas *S. putrefaciens* isolates did not, with the exception of one which showed a trace of 2-propanethiol. One isolate of *Ps. fragi* produced a very wide range of sulphides, several esters and ketones and a relatively large amount of pyrrolidine and it was noted that the broth had strong fruity odours.

3.6. Principal component analysis

The initial analysis using results from 16 broths and 21 peaks (volatiles) provided little information regarding peak groupings and object separation. It confirmed that culture 830 was entirely 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 six peaks were required to describe the cultures (Fig. 2). The first component explained 56% of the variance in the data and was described by a combination of 1-propanethiol, bis(methylethyl) disulphide and 2-propanethiol in one direction and

Table 3
Description of the odour of the volatile compounds produced by 15 different cultures of spoilage bacteria (*Ps. fragi* and *S. putrefaciens*) in sterile banana prawn broth

Culture number	Bacteria	Isolation source	Location	Odour	PCA subgroup
813	*Ps. fragi*	Banana	Moreton Bay	Slightly fruity	III
827	*Ps. fragi*	Banana	Moreton Bay	Neutral	III
348	*Ps. fragi*	King	Mooloolaba	Fruity/sulphide	I
395	*Ps. fragi*	King	Mooloolaba	Fruity/sulphide	I
363	*Ps. fragi*	King	Mooloolaba	Fruity/sulphide	I
397	*Ps. fragi*	King	Mooloolaba	Neutral	III
830	*Ps. fragi*	Tiger	Cairns	Strong fruity	
824	*Ps. fragi*	Tiger	Cairns	Slightly fruity	III
749	*S. putrefaciens*	Banana	Moreton Bay	Sulphidy	II
755	*S. putrefaciens*	Banana	Moreton Bay	Sulphidy	II
771	*S. putrefaciens*	Banana	Moreton Bay	Sulphidy	II
777	*S. putrefaciens*	Banana	Moreton Bay	Sulphidy	II
437	*S. putrefaciens*	King	Mooloolaba	Neutral	III
858	*S. putrefaciens*	Tiger	Cairns	Neutral	III
864	*S. putrefaciens*	Tiger	Cairns	Neutral	II

Table 4
Headspace volatiles (peak area ml^{-1}) produced by 15 different cultures of *Ps. fragi* and *S. putrefaciens* in sterile prawn broth

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

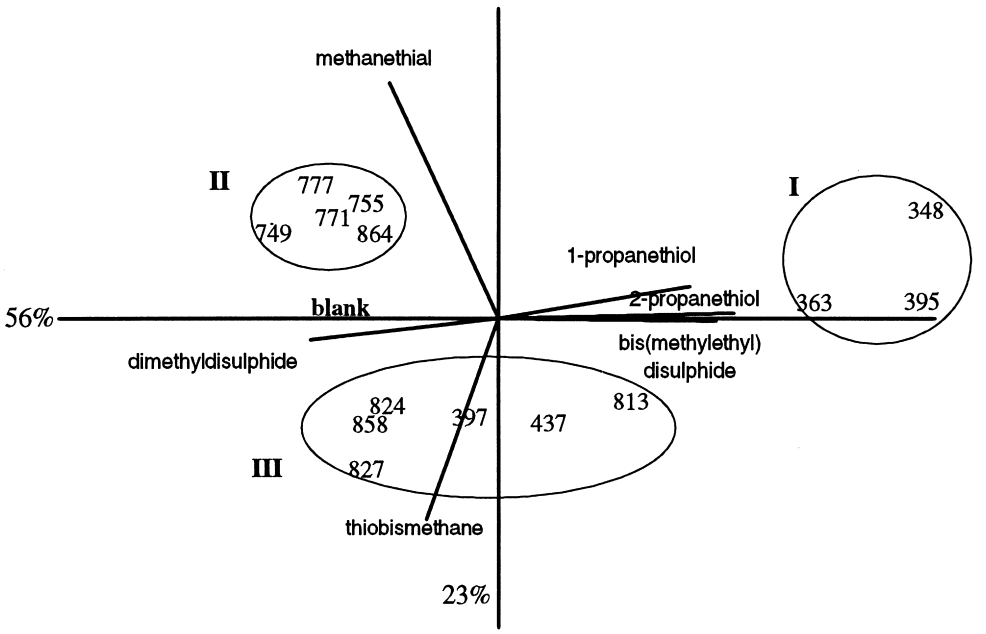

Fig. 2. Biplot of the principal component scores for 15 cultures defined by six headspace volatiles. Subgroupings (I, II and III) were determined by visual inspection.

dimethyl disulphide in the other. The second component explained a further 23% of the variance but was less defined and included methanethial and thiobismethane.

4. Discussion

The reason that *S. putrefaciens* and not *Ps. fragi* was the dominant organism isolated from prawns stored in ice slurry (where oxygen was limiting), in contrast to those stored in ice, is that unlike *Ps. fragi*, *S. putrefaciens* cannot use TMAO as an electron acceptor (Castell and Greenough, 1959). In general, Pseudomonads grow well in oxygen concentrations down to 2% but are progressively inhibited at lower concentrations (Clark and Burki, 1972). On iced fish from temperate waters *S. putrefaciens* can grow well and cause spoilage but some *Pseudomonas* spp. may outgrow and inhibit *S. putrefaciens* due to siderophore-mediated competition for iron (Gram, 1993; Gram and Melchiorsen, 1996).

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

In the early stages of spoilage, before tissue invasion and decomposition starts, the free amino acids are readily available substrates for bacterial growth (Cobb et al., 1976). Levels of free amino acids in prawns depend on species and circumstance, e.g. salinity (Finne, 1992), and the present results show some similarities in levels to those reported for other species (Matsumoto et al., 1991; Hirano et al., 1992). There were low levels of cysteine and methionine, high levels of arginine and of glycine which imparts a sweet flavour to prawns (Finne, 1992).

It must be assumed that the cysteine and methionine were the source of the volatile sulphur compounds (Herbert and Shewan, 1976), although other sources such as glutathione and taurine were not estimated. The only comparative data in the literature is based on fish not prawns. Castell and Greenough (1959) inoculated cooked fish flesh with isolates of *Ps. fragi* and described the progression of fruity, vegetable and onion-like odours. They also placed these isolates in media containing individual

amino acids but none appeared to utilize cysteine, methionine, taurine or glutathione even though they produced onion-like odours from the fish. In contrast, in the present study, sulphides were produced by all isolates including *Ps. fragi*, whereas ketones and aldehydes were produced mostly by *Ps. fragi*. Proteolysis only occurs when spoilage is very well advanced (Castell and Greenough, 1959) and while the breakdown products of fish protein formed from proteolysis will support growth of spoilage bacteria, the normal odorous spoilage products do not arise (Lerke et al., 1967). However, the protein free extract of fish flesh will rapidly spoil under the same circumstances (Lerke et al., 1967). The esters and the wide variety of sulphides that comprised the major spoilage volatiles are likely to have arisen from the metabolism of the free amino acids by decarboxylation and interesterification. *Ps. fragi* produces fruity odours by acting on mono-carboxylic acids and onion-like odours from monoamino- and diamino-heterocyclic amino acids (Castell and Greenough, 1959).

It is quite remarkable that TMA and other 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 where mixed cultures would have occurred. Formation of TMA from TMAO is commonly regarded as an indicator of spoilage but the TMAO levels of the extracts were not determined. Yamagata and Low (1995) reported a level of 18.6 mg/100 g TMAO-N in the flesh of aquacultured banana prawns (*P. merguiensis*), which is quite low since the reported range is up to 80 mg TMAO-N/100 g. Assuming a similar low level of TMAO in the banana prawns used to make the sterile broth, then the concentration of TMAO would be 0.025% or about 3.3 mmol (4.7 mg TMAO-N/100 g).

One isolate produced copious amounts of pyrollidine. This compound is a well known metabolite of the intestinal *Bacteroides fragilis* and *Clostridium perfringens* (Allison and Macfarlane, 1989) and is probably formed from proline. It has been detected in freshwater carp (Shimakura et al., 1991) and in cooked crab meat, where it imparts an alkaline/rancid meal/yolk flavour (Chung et al., 1995) and is likely to have formed through Strecker degradation.

It is noticeable that some compounds were detected at low relative levels in the headspace of the uninoculated broth that either were not detected or were present at lower levels in the test cultures. None of the compounds were important as discriminators loading onto the first principal component and hence their presence does not detract from the analysis and the conclusions concerning the subgroupings. It is also possible that some of the volatiles produced in the chill stored prawns could be of enzymic or oxidative origin since breakdown of the n-3 fatty acids results in volatile aldehydes and ketones (Kawai, 1996) but, in the author's opinion, it is more likely their formation is bacterial.

This work agrees with Gillespie (1981) that closely related strains of the same species, which have identical biochemical profiles in the test regimes, can produce a very different range of volatiles on a natural substrate. Different strains of the same species occur in different geographic locations and Gram et al. (1990) found that strains of *Pseudomonas* which were identical in standard taxonomic tests did not produce the same off odours.

The hydrocarbons may be an environmental contaminant or a natural constituent of the prawns' metabolism or feed. The terpenes are most likely to have arisen from the food chain. The seeds of the seagrass *Zostera capricorni* are a preferred feed for juvenile tiger prawns (*Penaeus esculentus*) who select the ripe from the unripe seeds from the spathe (Wassenberg, 1990; Dall et al., 1992). It is likely that the terpenes are an attractant to the prawns since they perform this role in many terrestrial feeds. As a relatively stable set of compounds they may also contribute to the subtlety and desirability of prawn flavour.

Based on the PCA it is sulphide compounds that differentiate between the cultures and they could be distributed into three major subgroupings (Fig. 2). Cultures 363, 348 and 395 (all *Ps. fragi*) formed one subgrouping and all originated from one geographic area (Mooloolaba; 23°S, 153°E). Cultures 749, 777, 771, 755 and 864 formed another subgroup (all *S. putrefaciens*) and with the exception of 864 were from a different geographic location (Moreton Bay; 27°S, 153°E). In contrast, cultures 824, 858, 397, 827, 437 and 813 were either *S. putrefaciens* or *Ps. fragi* and all were from either Mooloolaba, Moreton Bay or Cairns (15°S, 145°E) and thus formed a third subgroup of variable nature. Although culture 830 (*Ps. fragi*) was not included in the PCA final model,

it came from the Cairns region. Thus strains from different environments produce different spoilage products and patterns.

Furthermore, the subgroupings contained organisms which produced similar odours! Subgroup I contained organisms which produced fruity sulphide odours, subgroup II contained those that produced sulphydryl odours and subgroup III contained those that were classed as neutral, with one organism (813) classed as slightly fruity. This organism produced relatively lower levels of specific volatiles than other isolates (Table 4). Taxonomically identical organisms produced different volatiles from the same substrate.

5. Conclusion

This work has confirmed the conclusions of Chinivasagam et al. (1996) that *Ps. fragi* is a major spoilage organism in tropical prawns. 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.

The nature of spoilage volatiles will depend on the particular strain of bacteria in the capture environment, which is then selected on the basis of availability of oxygen and nutrients and storage conditions.

Volatiles accumulate in the plastic packages used for seafood and the flora is selected by residual oxygen concentrations within the pack. Since *S. putrefaciens* and *Ps. fragi* are major spoilers of tropical seafood then a knowledge of the relationship of the interactions between them is critical in understanding the spoilage of chilled tropical seafood in order to design packaging to minimise spoilage. To help, for example, to make the choice between an oxygen permeable and an oxygen impermeable pack.

Acknowledgements

This work was supported by the Fisheries Research and Development Corporation Project 91/100.

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