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Role of feed ingredients in the bromophenol content of cultured prawns

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Abstract

Commercial and experimental pelleted feeds were fed to prawns (*Penaeus monodon*) in aquaculture ponds and laboratory tanks to assess the effect of different ingredients on the bromophenol content of cultivated animals. Ingredients with bromophenols typically used in commercial feeds were fish, shrimp head, krill and squid meals and those additionally used in experimental feeds were dried marine algae, dried polychaetes, free bromophenols and bromophenol sulphate esters. After harvest, prawns and feed were analysed by GC/MS for 2- and 4- bromophenol, 2,4- and 2,6-dibromophenol and 2,4,6-tribromophenol. The total bromophenol content of commercial feeds ranged from 1.4 to 153 ng/g and those of experimental feeds from 2.9 ng to 18.9 μg/g. Retention of bromophenols by prawns was low for all ingredients; prawns fed commercial feeds ranged from 0.3 to 9.7 ng/g and those fed experimental feeds ranged from trace to 22.2 ng/g. Sensory analysis showed that the flavour of most prawns was bland and lacked the characteristic ocean flavour of wild crustaceans. Possible reasons for the low retention of bromophenols in prawns fed pelleted feeds are discussed.

Keywords: Shrimp; *Penaeus monodon*; Aquaculture; GC/MS analysis; Flavour; Processed feeds

1. Introduction

Simple bromophenols, 2- and 4- bromophenol (2-BP, 4-BP), 2,4- and 2,6-dibromophenol (2,4-DBP, 2,6-DBP) and 2,4,6-tribromophenol (2,4,6-TBP), have been identified as key flavour components in seafoods (Boyle, Lindsay, & Stuiber, 1992a, 1992b; Whitfield, Helidoniotis, Shaw, & Svoronos, 1997, 1998; Whitfield, Last, Shaw, & Tindale, 1988). In water, the most strongly flavoured of these compounds, 2,6-DBP, 2-BP and 2,4,6-TBP, have flavour threshold concentrations (FTC) of 5×10^{-4}, and 3×10^{-2} and 0.6 ng/g respectively (Whitfield et al., 1988). At these concentrations, the flavours of 2,6-DBP and 2,4,6-TBP are described as iodoform-like and that of 2-BP as phenolic/iodine like. However, at levels below their FTC these bromophenols contribute recognisable marine- or ocean-like flavours to seafoods as well as enhance the intensities of existing seafood flavours (Boyle et al., 1992a; Whitfield et al., 1997, 1998).

Whole wild ocean prawns have a total bromophenol content (TBC) that ranges from 9.5 to 1114 ng/g, depending on species, location and time of year of catch (Whitfield et al., 1997). By comparison, cultivated or farmed prawns have a much lower TBC that ranges from 0.31 to 1.3 ng/g (Whitfield et al., 1997). As a consequence, the flavour of cultivated prawns is frequently described as bland and lacking the natural flavours of wild crustaceans. This difference in bromophenol content has been attributed to the respective diets of wild and cultivated prawns (Whitfield et al., 1997). Polychaetes, marine worms of the phyllum Annelida, are a major dietary component of wild prawns and these worms are known to produce high concentrations (>100 μg/g) of bromophenols (Whitfield, Drew, Helidoniotis, & Svoronos, 1999). In contrast to the natural diet of prawns, commercial feeds are generally very low (1.4–40 ng/g) in bromophenols (Whitfield et al., 1997). The major sources of bromophenols in such products are typically prawn (or shrimp) head meal or other crustacean processing by-product meals; these ingredients usually account for 10% or more of the final feed mixture (Tacon & Akiyama, 1997). The bromophenol

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content of such ingredients are, however, extremely variable and are frequently <1 μg/g (Whitfield et al., 1997). The low bromophenol content of cultivated prawns can be attributed to the low levels of these compounds in commercial feeds (Whitfield et al., 1997). In addition, it has been suggested that the availability of bromophenols in formulated feeds is further reduced by the significant bonding of these compounds to other components of the feeds (Whitfield et al., 1997). As a consequence, the current study was undertaken in an effort to modify the bromophenol content of cultivated prawns by the use of a range of ingredients as alternative sources of bromophenols in artificial feeds.

2. Materials and methods

2.1. Samples of raw materials

Samples of commercial prawn feeds were obtained from international suppliers, local producers and from commercial prawn farms in southern Queensland and northern New South Wales. Samples of imported ingredients such as fish, shrimp head, krill and squid meals were obtained from local suppliers. Experimental ingredients, such as freeze-dried marine algae (*Ulva lactuca*) and freeze dried polychaete (*Australonuphis teres* and *Marphysa sanguinea*), were prepared in the laboratory's processing area. *U. lactuca* was harvested from the intertidal zone at Turimetta Head, NSW and samples of *A. teres* and *M. sanguinea* were provided by Mr. J Park (Seal Rocks, NSW) and Dr. J Patterson (Wynnum, QLD), respectively. The freeze drying of these materials was performed in a laboratory designed apparatus. During the course of the drying process the maximum temperature of the product was <20 °C and the minimum pressure was <0.2 mm. Prawns (*Penaeus monodon*) used in the feeding trials, were either purchased from commercial hatcheries as fifteen day old postlarvae and were raised over a 15-week period to juveniles (~ 5 g) in 10,000 l outdoor tanks or were purchased as juveniles from a prawn farm and held at the laboratory for at least 2 weeks before use. Harvested (15–25 g) cultivated prawns (*P. monodon*) were purchased either from growers in New South Wales and Queensland or from the Sydney Fish Marketing Authority. All samples required for sensory and instrumental analysis were obtained raw and were refrigerated at -20 °C until used.

2.2. Chemicals and reagents

Reference samples of the five target bromophenols were purchased from Aldrich Chemicals Co. Inc. Milwaukee, WI, and 2,6-dibromophenol-d_3 was obtained from CDN Isotopes Inc., Pointe Claire, PQ, Canada. The purity of each compound ($>98\%$) was confirmed by gas chromatography/mass spectrometry (GC/MS) analysis. Distilled water was purified through a Milli-Q purification system (Millipore Corp., Bedford, MA). All organic chemicals and organic solvents were of analytical reagent grade ($>98\%$ pure). The solvents were further purified by distillation through a packed fractionating column. The organic reagent sulphur trioxide pyridine was purchased from Aldrich Chemical Co. Inc.

2.3. Preparation of basal and experimental prawn feeds

The basal feed used in the feeding trials was similar to that described by Glencross, Smith, Tonks, Tabrett, and Williams (1999). The major ingredients in the diets were fish meal (30%), pre-gelatinised starch (30%), wheat gluten (6%), solvent extracted soybean meal (10%), sodium alginate, (Manucol, Kelco) (6%), diatomaceous earth (5%), squid oil (4%) and supplements (3%). The bromophenol contents of some of these ingredients are recorded in Table 1. The supplements, such as vitamins, minerals and chemical modifiers, were added in the same proportions as previously reported by Glencross et al. (1999). In the experimental feeds, where shrimp head meal, krill meal, freeze-dried algae and freeze-dried polychaete were added to modify the bromophenol content of these feeds, the proportions of fish meal, squid oil, starch and diatomaceous earth were adjusted to maintain the same ash, crude protein and total lipid contents across diets. The inclusion level of ingredients of marine origin in the experimental diets is shown in Table 2.

The basal and experimental feeds were prepared as follows: in the laboratory, batches of feed made from 100 g of dry ingredients were generally prepared but this was scaled up to 1 kg when required. The tetrasodiumpyrophosphate (TSPP, 1.5 g) was dissolved in 230 ml of water and sodium alginate (Manucol, 6 g) was slowly mixed into it to form a gel. The remaining dry ingredients and squid oil were thoroughly mixed before being added to the alginate/TSPP solution. The combined ingredients were mixed and extruded through a 60-ml plastic syringe with a 3-mm outlet hole into a bath of 10% aqueous calcium chloride. With larger batches, a sausage extruder with a multiple-hole die was

Table 1
Concentrations of bromophenols in some commercial ingredients used in prawn feed formulations

Ingredients	Bromophenol content (ng/g)					
	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Fish meal	0.6	11.0	ND[a]	ND	tr[b]	11.6
Shrimp head meal	5.3	4.4	370	17.0	460	857
Krill meal	3.9	4.0	5.3	20.0	48.0	81.2
Squid meal	1.3	8.0	ND	1.2	ND	10.5

[a] ND, not detected at a detection limit of 0.01 ng/g.
[b] Trace, tr = 0.01–0.1 ng/g.

Table 2
Concentrations of bromophenols in experimental prawn feeds containing commercial ingredients

Code	Ingredients[a]	Processing method	Bromophenol content (ng/g)						
			2-BP	4-BP	2,4-DBP	2,5-DBP	2,4,6-TBP	Total	Expected[b]
A	15% shrimp; 20% fish	Extruded	0.2	0.5	29	0.7	32.0	62.4	118
B	15% shrimp; 15% fish	Extruded	0.6	ND[c]	41	2	52.0	95.6	117
C	15% shrimp; 20% fish	Pelletized	ND	0.5	29	0.5	33.0	63.0	118
D	15% shrimp; 15% fish	Pelletized	0.3	tr[d]	24	0.5	23.0	47.9	117
E	10% krill; 20% fish	Pelletized	0.3	0.3	0.3	0.9	1.1	2.9	9.4
F	10% krill; 5% squid; 20% fish	Pelletized	0.5	1.6	0.4	1.6	2.7	6.8	9.8
G	15% krill; 20% fish	Pelletized	0.4	1.9	0.5	1.9	4.5	9.2	13.1

[a] As reported in Table 1.
[b] Based on an average moisture content of 10%.
[c] ND, not detected at a detection limit of 0.01 ng/g.
[d] Trace, tr = 0.01–0.1 ng/g.

used. The noodle-like product was left in the calcium chloride bath for 5 min before being removed, drained and air-dried in a fume cupboard for 12 h. It was then broken up into approximately 10-mm lengths and stored in sealed plastic bags at 4 °C until used.

For the experimental feeds containing either free bromophenols or bromophenol sulphate esters, solutions of these compounds in methanol were added to the basal feed immediately after the addition of the dry ingredients to the alginate solution. The quantities of the bromophenols added either as the free compounds or as their sulphate esters, are recorded in Table 3. The bromophenol sulphate esters, as their dipotassium salts, were prepared according to the method of Hodgkin, Craigie and McInnes (1966).

In some feeding trials, larger quantities of feeds were required. For these preparations the mixed ingredients (10–15 kg) were either processed by steam pelletization or by extrusion. The pelletization was performed in a Sprout-W-Junior ACE 500B steam pellet press (Sprout, Melbourne, Australia) and the extrusions in an APV Baker MPF 40 twin screw extruder (APV Baker, Peterborough, UK). The die size for both machines was 2.5 mm. Water was added as steam, during the course of both processes, to produce products of desired consistency and texture. The products were stored in sealed plastic bags at 4 °C. Before use, the water stability (particulate loss and leached solubles) and moisture content of all feeds (commercial and experimental) were determined according to international standards (ASAE, 1996).

2.4. Feeding trials

Mesh cages (1×1×1 m), deployed in an outdoor raceway pond (60×10^3 l) and fibreglass aquarium tanks (90 l) in an indoor laboratory, were used for the trials. Seawater was pumped into the raceway pond for 3 h every 24 h from a nearby grow-out pond that was fully stocked with prawns, resulting in about a 50%/day exchange of water in the raceway. The water temperature varied between 23 and 32 °C (mean ± S.D. of 28 ± 2 °C). The aquarium tanks were supplied with filtered, heated, flowing seawater (>300 ml/min) and were individually aerated. The temperature of the tanks was maintained at 28 ± 1 °C and the salinity ranged between 34 and 35 ppt. Each pond cage was stocked with 25×prawns (5–6 g)

Table 3
Concentrations of bromophenols in experimental prawn feeds containing free bromophenols or their sulphate esters

Product	Condition	Bromophenol content (ng/g)						
		2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Expected[a]
Free bromophenols[b]	Unprocessed[c]	7.1	8.9	17	0.5	9.6	43.1	560
Free bromophenols	Pelletized[d]	3.7	4.7	14	0.3	7.1	29.8	1680
Bromophenol salts[b]	Unprocessed[c]	117	77	126	18	40	378	560
Bromophenol salts	Pelletized[d]	56	ND[e]	11	6	13	86	1680
Control feed	Pelletized[d]	3.3	3.6	11	1.6	8.3	27.8	

[a] Calculated for a pelletized product with 10% water.
[b] Amount of bromophenols added to each mixture: 2-BP 170 ng/g; 4-BP 110 ng/g; 2,4-DBP 180 ng/g; 2,6-DBP 40 ng/g; and 2,4,6-TBP 60 ng/g; (total 560 ng/g).
[c] Contains 70% water.
[d] Contains 10% water.
[e] ND, not detected at a detection limit of 0.01 ng/g.

with five replicate cages for each treatment. The aquarium tanks were stocked with six prawns (10–12 g) with six replicate tanks allocated to each treatment. Feeding trials held in ponds were of 8 weeks duration whereas those held in tanks were of 2 weeks duration. In the pond trials, the prawns were fed to excess twice daily with the diet allocated to that particular cage. However, in the tank trials, the prawns were fed at the above rate with the basal diet for one week to adapt to the type of diet. This was followed by 2 weeks of feeding with the experimental diet allocated to that particular tank. At the end of each trial, the prawns were killed 3 h after the morning feed time by immersion in an ice/seawater slurry. The average weight (±S.D.) of prawns at the end of the pond trial was 18±3 g.

Prawns from each cage or tank were divided into two sets, one set for bromophenol analyses and the other set for sensory analyses. The prawns were packaged in polyethylene bags and, after sealing the bags, the prawns were frozen and stored at −20 °C until required for instrumental or sensory analyses.

2.5. Extraction of bromophenols from feeds, feed ingredients and prawns

Samples of frozen uncooked prawns (100–500 g) were carefully dissected into heads and tails, the weight of each section was recorded, and they were then treated as separate samples in the extraction process. Samples of the feed and feed ingredients (25–100 g) were taken for extraction. The samples were placed in purified water (1.5 l) and homogenized for 5 min in a Panasonic Super Blender. The homogenates were acidified to pH 1 with 10 M sulphuric acid and were then left to stand at 20 °C overnight to confirm that sufficient acid had been added to achieve the required pH. The volatile components were isolated by combined steam distillation-solvent extraction (SDE) with 30 ml of pentane/diethyl ether (9:1) as solvent (Whitfield et al., 1988). After 3 h, the pH of the residues was again measured to confirm that the homogenate had remained acidic during the isolation procedure. The internal standard, 2,6-dibromophenol-d_3 (100 ng in 100 μl iso-octane) was added to the solvent extract, which was then dried by cooling to −15 °C and the solvent fraction decanted. The extracts were concentrated by the careful removal of the pentane/ether by fractional distillation, and the concentrates in iso-octane (about 100 μl) were stored in 2-ml glass autosampler vials at −15 °C until required for analysis by gas chromatography/mass spectrometry GC/MS.

2.6. Analysis of bromophenols by GC/MS

The bromophenols in the various extracts were analysed using a Hewlett-Packard HP5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) interfaced to a Hewlett Packard HP5971A mass selective detector, operated in the multiple ion detection mode (MID). The GC oven was fitted with a 25 m×0.25 mm i.d. fused silica column, coated with a 0.33 μm film of bonded siloxane HP-5 and a precolumn retention gap, 5 m×0.25 mm i.d., uncoated but deactivated. The retention gap was necessary to protect the column from the large quantity of steam-volatile fatty acids present in some extracts. As a consequence, it was necessary to replace the retention gap frequently when linearity of the calibration curve no longer applied; this usually occurred suddenly. Aliquots (1 μl) of the sample extracts or calibration solutions were injected automatically by a Hewlett-Packard HP7673 autosampler. For all analyses, the injections were split 1:20. The GC oven was temperature programmed as follows: the temperature was initially held at 60 °C for 1 min, programmed from 60 to 225 °C at 15 °C/min, then from 225 to 280 °C at 40 °C/min and finally held at 280 °C for 37 min. The helium flow was 0.48 ml/min, the injector temperature was 280 °C and the GC/MS transfer line was 300 °C. The MS was operated in electron ionization mode with an energy of 70 eV and an ion source temperature of 180 °C.

Quantitative analysis by MID was performed under software control by a Hewlett-Packard Vectra 386/25 computer running a Hewlett-Packard MS ChemStation data system. In the bromophenol analyses, ions were monitored for 2-BP and 4-BP at m/z 172 and 174, for 2,4-DBP and 2,6-DBP at m/z 250 and 252, for 2,4,6-TBP at m/z 330 and 332, and for the internal standard 2,6-dibromophenol-d_3 at m/z 255 and 257. The retention times for these compounds were 2-BP, 6.60 min; 4-BP, 9.01 min; 2,4-DBP, 9.67 min; 2,6-DBP, 9.99 min; 2,4,6-TBP, 12.45 min and 2,6-dibromophenol-d_3, 9.97 min. The GC/MS was calibrated by the analysis of three different concentrations of each of the five bromophenols (0.5, 5, and 25 μg/ml in iso-octane) with a constant concentration of internal standard (1 μg/ml). Disappearance of the lowest calibration level and loss of peak shape indicated the need to replace the retention gap and occasionally the column. Response factors for each compound, with respect to the internal standard, were calculated by the data system software, and these were used to determine the concentration of the target compounds in the extracts. The calibrations were performed on the day of analysis, and each analysis was carried out in duplicate. If a sample contained analytes outside the calibration range, a diluted subsample was analysed after addition of more internal standard. The detection limit for individual bromophenols in the prawns, feeds and feed ingredients was 0.01 ng/g, based on a factor of three times background noise.

During the GC/MS analyses the presence of individual bromophenols was confirmed by the appearance of a single peak in the total ion chromatogram at the appropriate retention time, by the presence of the two

characteristic ions listed above, and by the appearance of the correct isotopic ratios for these ions.

2.7. Extraction efficiencies

The extraction efficiencies of the SDE technique for the recovery of individual bromophenols from prawns were determined as follows. Tails (100 g) from samples of cultivated *P. monodon* (9594) of low bromophenol content were homogenized in water (1.5 l). To this mixture was added an aliquot (1 ml) of a solution containing each of the five bromophenols (1 μg/ml) in ethanol. As previously described, the mixture was extracted by SDE after acidification to pH 1. The extractions were performed in duplicate. The average percentage recoveries (±S.D.) were as follows:

2-BP, 94±6%; 4-BP, 41±1%; 2,4-DBP, 74±0%; 2,6-DBP, 81±0% and 2,4,6-TBP, 74±4%.

2.8. Sensory panel assessment of prawn tails

A sensory panel, consisting of six staff from the Division of Marine Research, Cleveland Laboratory, was trained for sensory evaluation. The panel was initially trained to discriminate between sweetness and blandness, and to recognize four flavours: prawn, brine, ocean/iodine and iodoform-like, and to assess for overall acceptability. A 150-mm intensity scale was used to evaluate the above criteria. Training was carried out 1 week before the formal analysis, and was achieved by requesting the panel to assess samples of freshly caught ocean prawns and commercial cultivated prawns. In addition, on the day before assessment of the experimental material, the panel was again requested to evaluate samples of ocean and cultivated prawns.

Prawns were prepared for assessment as follows. Each batch of prawns was thawed for 1 h at room temperature before cooking. They were then cooked by immersion in boiling fresh water for 3 min. The cooked prawns were removed from the water, drained, cooled rapidly under a stream of tap water, peeled, and the tails cut into 1–2 g portions. The sensory assessments were carried out within 15 min of cooking. Each batch of experimental prawns was assessed in duplicate on two separate days. The evaluations were arranged so that, in each sitting, there would be samples from three batches of prawns. These evaluations were spread across two sittings per day, morning and afternoon. The accumulated data for each sensory criteria were subjected to analysis of variance (ANOVA) and the results were expressed as means±standard error.

3. Results and discussion

3.1. Studies involving commercial feeds

Data obtained from the analysis of 14 samples of commercial prawn feed are presented in Table 4. Of these materials, five had been manufactured in Australia, three each in Taiwan and Japan, two in Thailand and one in Indonesia. The feeds were all manufactured between the years 1994 and 1998. The majority of the feeds, Aquafeed(AF), Charoen Pokphand (CP), Chin Da (CD), Growbest (GB), Lucky Star (LS) and President (PR), were produced by steam pelletization. Of the feeds studied, only those produced by Higashimaru

Table 4
Concentrations of bromophenols in commercial prawn feeds

Product	Diet	Country of origin	Bromophenol content (ng/g)					
			2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total
Aquafeed (1994)[a]	AF94	Australia	0.3	1.2	0.9	0.8	0.7	3.9
Aquafeed (1995A)[a]	AF95A	Australia	0.4	ND[b]	0.4	0.4	0.2	1.4
Aquafeed (1995B)[a]	AF95B	Australia	17.0	10.0	3.4	3.5	5.8	39.7
Aquafeed (1997)[a]	AF97	Australia	2.0	37.0	36.0	23.0	5.0	153
Aquafeed (1998)[a]	AF98	Australia	0.8	0.4	1.5	1.4	0.9	5.0
Charoen Pokphand (1994)[a]	CP94	Thailand	0.5	0.1	15.0	2.0	3.0	20.6
Charoen Pokphand (1995)[a]	CP95	Thailand	0.4	ND	14.0	2.0	20.0	36.4
Chin Da (1994)[a]	CD94	Taiwan	ND	6.3	5.1	1.7	2.3	15.4
Growbest (1994)[a]	GB94	Indonesia	1.0	13.0	8.4	3.4	6.3	32.1
Higashimaru (1995)[c]	HM95	Japan	ND	23.0	32.0	3.8	25.0	83.8
Higashimaru (1996)	HM96	Japan	0.4	ND	1.7	2.5	2.4	7.0
Higashimaru (1998)[c]	HM98	Japan	1.1	0.7	7.5	3.2	3.8	16.3
Lucky Star (1998)[a]	LS98	Taiwan	0.1	0.7	0.1	0.5	0.2	1.6
President (1994)[a]	PR94	Taiwan	ND	ND	ND	1.2	1.8	3.0

[a] Product was steam pelletized.
[b] ND, not detected at a detection limig of 0.01 ng/g.
[c] Product was extruded.

(HM) were extruded. The analytical data for the 14 feeds showed that the total bromophenol content (TBC) of these materials ranged from 1.4 ng/g (AF 1995A) to 153 ng/g (AF 1997); however, the majority of these feeds had TBCs <40 ng/g. These TBC values are far less than those predicted for the natural diets of some species of wild prawns (Whitfield et al., 1999). Here the TBC of such diets was considered to be >2000 ng/g.

Qualitatively, individual bromophenols were well represented in 13 of the feeds studied; all five were found in eight feeds and four in five feeds. However, only two of these compounds were found in the feed manufactured by PR. Quantitatively, 4-BP and 2,4,6-TBP were present in highest concentrations in four feeds each and 2,4-DBP was the dominant bromophenol in three feeds.

Results from the bromophenol analyses of cultivated prawns commercially raised on six of these feeds (AF 1994, 95B, 97, 98 and CP 1994, 95) are recorded in Table 5. A sample of wild *P. monodon*, caught in 1996, was also analysed for these compounds. The TBC of whole cultivated prawns ranged from 0.3 ng/g (5495 and 17595) to 9.7 ng/g (8497) whereas the TBC for the wild prawns (23596) was 180 ng/g. Of the cultivated prawns, the highest TBC (9.7 ng/g) was obtained for those animals raised on AF 1997. This feed had the highest TBC (153 ng/g) of the 14 feeds analysed (Table 4). However, no further correlation between the TBC of the feed and cultivated prawns was observed (Tables 4 and 5). The two feeds with TBCs of 36.4 and 39.7 ng/g yielded prawns with only 0.3 ng/g of bromophenols, whereas the feed with a TBC of 5 ng/g gave prawns with 0.8 ng/g. Accordingly, the TBC of the tails of five of the six samples ranged from ND to 0.3 ng/g, values comparable with those previously reported for other samples of commercial cultivated prawns. (Whitfield et al., 1997). The exception was sample 8497 in which the TBC of the tails (8.5 ng/g) was very similar to that of the wild *P. monodon*, sample 23596 (13.1 ng/g).

Sensory analyses of the cultivated prawns showed that, while all samples were acceptably sweet, their flavour, with the exception of sample 8497, was bland and lacked the characteristic flavour of wild prawns. The flavour of sample 8497 was described as slightly briny and iodine-like.

3.2. Studies involving experimental feeds containing commercial ingredients

Data from the analyses of samples of commercial fish meal, shrimp head meal, krill meal, and squid meal (ingredients believed to be the major source of bromophenols in prawn feeds) are presented in Table 1. Of these samples, the shrimp head meal had the highest TBC (857 ng/g), and fish meal and krill meal the lowest (11.6 and 10.5 ng/g, respectively). Shrimp head meal

Table 5
Concentrations of bromophenols in uncooked prawns fed commercial feeds

Diet	Code	Sample	Ratio heads/tails	Bromophenol content (ng/g)						
				2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Total[a] whole prawns
AF94	9594[b]	Head	0.76	ND[c]	ND	0.2	0.6	0.7	1.5	0.6
		Tails		ND	ND	ND	ND	ND	ND	
CP94	25894[b]	Head	0.88	ND	ND	0.2	0.4	0.5	1.1	0.6
		Tails		ND	0.1	ND	0.1	ND	0.2	
AF95B	5495[b]	Head	0.62	tr[d]	ND	0.1	0.1	0.2	0.4	0.3
		Tails		tr	tr	tr	0.1	0.1	0.2	
CP95	17595[b]	Head	0.50	tr	ND	0.1	0.1	0.2	0.4	0.3
		Tails		tr	ND	0.1	0.1	0.1	0.3	
AF97	8497[e]	Head	0.73	0.4	3.2	2.9	0.8	4.1	11.4	9.7
		Tails		0.4	3.7	3.7	0.7	tr	8.5	
AF98	16298[e]	Head	0.71	0.1	0.1	0.1	1.0	0.5	1.8	0.8
		Tails		tr	tr	tr	0.1	tr	0.1	
Natural	23596[f]	Heads	0.57	tr	3.0	5.0	6.0	460	474	180
		Tails		tr	0.3	0.6	0.2	12.0	13.1	

[a] Calculated from the weight of individual batches of heads, tails and total prawns extracted.
[b] Prawns were obtained from commercial growers in northern New South Wales and southern Queensland and were fed on either Charoen Pokphand or Aquafeed.
[c] ND, not detected at a detection limit of 0.01 ng/g.
[d] Trace, tr = 0.01–0.1 ng/g.
[e] Prawns were fed Aquafeed (1997, 1998) in laboratory supervised ponds.
[f] Wild prawns caught off Cairns, Queensland.

and krill meal were the only ingredients that contained all five bromophenols, and in these materials 2,4,6-TBP was present at the highest concentrations.

Experimental feeds were prepared with different combinations of these ingredients (see Table 2) to establish what effect, if any, such ingredients had on the retention of bromophenol by cultivated prawns. All experimental feeds were processed by steam pelletization; however, two of these were also produced by extrusion, to evaluate the effect that processing conditions could have on bromophenol retention. Data obtained from the analysis of these experimental feeds are presented in Table 2. The four feeds containing shrimp head meal (feeds A, B, C, D) had the highest TBCs. These values ranged from 47.9 (feed D) to 95.6 ng/g (feed B). The other two feeds (feeds A, C) had almost identical TBCs (62.4 and 63 ng/g, respectively).

Retention of bromophenols in the steam pelletized feeds (feeds C and D) was 54 and 41%, respectively and, in the extruded feeds (feeds A and B), 54 and 81%, respectively. The other three feeds (E, F and G), that contained either 10 or 15% krill meal, had TBCs that ranged from 2.9 to 9.2 ng/g. Retention of bromophenols during processing ranged from 31% for feed E to 70% for feed G. Overall, these experimental feeds covered a similar range of TBCs, as was previously observed for the majority of commercial feeds (Table 4).

Experiments to assess the effect of processing methods on the retention of bromophenols in prawn feeds were inconclusive. A difference between extrusion and pelletization was obtained for feeds B and D, containing 15% fish meal. However, no difference was observed with feeds A and C, containing 20% fish meal (Table 2).

Results from the bromophenol analysis of prawns raised on these experimental feeds in pond cages for 8 weeks, are recorded in Table 6. The TBC of the different batches of whole prawns ranged from 0.4 to 12 ng/g, a similar range to that observed for prawns raised on commercial feeds (Table 5). The highest TBC (12 ng/g) was obtained for those animals raised on diets B and C. These feeds had two of the highest TBCs (95.6 and 63 ng/g) of the seven experimental feeds (Table 2). By comparison, the feeds E, F and G with the lowest TBCs (2.9, 6.8 and 9.2 ng/g, respectively) gave prawns with the lowest TBCs (0.7, 0.7 and 0.4 ng/g, respectively). The TBC of the tails of three of these samples (E, F and G) ranged from 0.2 to 0.3 ng/g, whereas the other four ranged from 2.3 to 10.5 ng/g (Table 6). These four values are in agreement with those reported for the TBCs of the tails of species of wild prawns (Whitfield et al., 1997). Such results would suggest that, provided ingredients contain sufficient bromophenols that would yield prawn feeds with TBCs of 100 ng/g or greater, it should be possible to obtain cultivated prawns with bromophenol contents comparable with those of the wild animals. The major source of bromophenols in such feeds is likely to be shrimp head meal or some other product derived from crustacean processing

Table 6
Concentrations of bromophenols in uncooked prawns fed experimental feeds containing commercial ingredients[a]

Diet[b]	Sample	Ratio heads/tails	Bromophenol content (ng/g)						
			2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Total[c] whole prawns
A	Heads	0.97	0.1	0.1	1.1	0.4	12	13.7	8.6
	Tails		0.1	0.1	0.5	0.1	2.9	3.7	
B	Heads	0.79	0.1	0.1	0.8	0.3	13	14.3	12
	Tails		0.1	0.1	1.5	0.4	8.4	10.5	
C	Heads	0.86	0.1	0.2	2.0	0.9	20	23.2	12
	Tails		tr[d]	tr	0.3	ND[e]	2	2.3	
D	Heads	0.77	0.1	0.3	1	0.3	3.8	5.5	4.5
	Tails		0.1	0.2	0.6	0.1	2.8	3.8	
E	Heads	0.66	0.1	ND	0.2	0.8	0.4	1.5	0.7
	Tails		tr	tr	tr	0.2	tr	0.2	
F	Heads	0.71	0.1	tr	0.1	0.6	0.4	1.2	0.7
	Tails		tr	tr	tr	0.2	0.1	0.3	
G	Heads	0.68	tr	0.1	0.1	0.3	0.2	0.7	0.4
	Tails		tr	tr	0.1	0.1	tr	0.2	

[a] Prawns were fed on experimental diets in laboratory tanks and ponds for 8 weeks.
[b] As reported in Table 2.
[c] Calculated from weight of individual batches of heads, tails and total prawns extracted.
[d] Trace, tr = 0.01–0.1 ng/g.
[e] ND, not detected at a detection limit of 0.01 ng/g.

waste. Over a period of 5 years, some 10 batches of meals derived from shrimp processing waste were analysed and, in the majority of samples, were found to have TBCs <50 ng/g. The batch used in the preparation of the above experimental feed (Table 2) was the only shrimp head meal to have TBC >100 ng/g. Accordingly, this ingredient is extremely variable in its bromophenol content. Such variability is to be expected as it has been reported that the TBC of wild whole prawns can range from 9.5 to 1114 ng/g (Whitfield et al., 1997). By comparison, the analyses of a similar number of batches of fish meal and squid meal showed that the TBC of these ingredients were consistent and were always <20 ng/g. From these results, it would appear that shrimp head meal is the key ingredient for the addition of bromophenols to prawn feeds and for the retention of these compounds in cultivated prawns.

Sensory analyses of the experimental prawns showed that all of the prawns were acceptably sweet. However, the flavours of those fed on diets C, E, F and G were described as bland whereas those fed on diets A, B and D had a flavour described as briny and ocean/iodine like.

Results from the above feeding trials, involving commercial feed and experimental feeds containing commercial ingredients, highlight the difficulty in controlling the levels of bromophenols in prawn feed and cultivated prawns. A possible solution, to overcome the variability in bromophenol content in cultivated prawns, was to supplement their diet in the finishing stage of their growth with a feed high in bromophenols. The results of some of these experiments are described and discussed below.

3.3. Studies with experimental feeds containing free bromophenols or their sulphate esters

The concentrations of bromophenols in experimental feed containing either free bromophenols or their sulphate salts are recorded in Table 3. The TBC of the pelletised feed containing free bromophenols was 29.8 ng/g (1.8% retention) whereas the TBC of the feed containing bromophenol salts was 86 ng/g (5.1% retention). Contribution of fish meal to the level of bromophenols in these experimental feeds was calculated as 3.5 ng/g. Major losses of bromophenols were experienced during the preparation of these experimental feeds, both during the mixing of the ingredients and pelletisation (Table 3). Some losses of the free bromophenols were expected, due to the volatility of these compounds. However, for the bromophenols to be lost from the feed containing the bromophenol salts, hydrolysis of the sulphate esters would have been necessary. The control feed containing shrimp head meal had a similar TBC (27.8 ng/g) to that of the feed containing free bromophenols.

Data from the bromophenol analyses of prawns fed these feeds, as finishing feeds, for 2 weeks are recorded in Table 7. The TBC of whole prawns, fed material containing free bromophenols, ranged from 7.4 to 9.1 ng/g, whereas those fed on material containing the bromophenol salts ranged from 2.2 to 2.3 ng/g. Prawns fed the control feed had a TBC that ranged from 0.9 to 1.2 ng/g. An increase in TBC was observed for the prawns fed material containing free bromophenols, compared with those fed the control feed. Both of these feeds had

Table 7
Concentrations of bromophenols in uncooked prawns fed experimental feeds containing free bromophenols or their sulphate esters[a]

Diet[b]	Sample	Ratio heads/tails	Bromophenol content (ng/g)						
			2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Total[c] whole prawns
Free bromophenols (tank 1)	Heads	0.88	0.4	2	6.1	1.1	6.2	15.8	7.4
	Tails		ND[d]	ND	ND	ND	ND	ND	
Free bromophenols (tank 2)	Heads	0.81	0.6	1.3	10.0	ND	8.2	20.1	9.1
	Tails		ND	ND	0.2	ND	ND	0.2	
Bromophenol salts (tank 3)	Heads	1.00	ND	ND	1.1	1	1.4	3.5	2.3
	Tails		0.5	ND	0.5	ND	ND	1.0	
Bromophenol salts (tank 4)	Heads	0.82	0.4	ND	1.2	0.5	2.7	4.8	2.2
	Tails		ND	ND	ND	ND	ND	ND	
Control feed (tank 5)	Heads	0.94	ND	ND	0.6	0.6	1.3	2.5	1.2
	Tails		ND	ND	ND	ND	ND	ND	
Control feed (tank 6)	Heads	0.90	ND	ND	1.6	ND	ND	1.6	0.9
	Tails		ND	ND	0.3	ND	ND	ND	

[a] Prawns were fed on experimental diets in laboratory tanks for 2 weeks.
[b] As reported in Table 3.
[c] Calculated from weight of individual batches of heads, tails and total prawns extracted.
[d] ND, not detected at a detection limit of 0.01 ng/g.

comparable TBCs (Table 3). By comparison, little increase in bromophenol content was seen in those prawns fed material containing the bromophenol salts. Notably, the concentration of bromophenols in the tails of all prawn samples was low and ranged from ND to 1 ng/g (Table 7). This result would indicate that the addition of either free bromophenols or their salts as supplements, in finishing feeds, does not increase the retention of these compounds in cultivated prawns.

Sensory analyses of the experimental prawns showed that they were acceptably sweet but their flavour was bland. As such, no increase in flavour had been achieved by the use of free bromophenol or bromophenol ester supplements in the finishing feeds.

3.4. Studies with experimental feeds containing freeze dried algae or polychaetes

The concentration of bromophenols in raw materials (algae and polychaetes), freeze dried products and experimental feeds are recorded in Table 8. These results show that the bromophenols were readily lost during the freeze drying of the algae (*U. lactuca*) with only 26% retention of these compounds in the dry product. By comparison, the loss of bromophenols from the polychaetes, *A. teres* and *M. sanguinea* was far less, with 70–84% retention of these compounds (Table 8). Bromophenols were also lost during the preparation of the feeds containing freeze-dried *U. lactuca* and *A. teres*, with only 36 and 25% retention, respectively, in the finished products. However, with the feeds containing freeze dried *M. sanguinea,* minimum losses occurred, with over 90% retention of bromophenols, (Table 8). The loss of bromophenols, during both freeze-drying and pelletisation of the feeds, would suggest that a proportion of these compounds is present, in such materials, in a free state. The retained bromophenols are possibly chemically bound. Whether these compounds were initially bound in the raw materials or became bound during processing is open to speculation. Current techniques for the analysis of bromophenols requires the addition of sulphuric acid during extraction. This procedure would most probably hydrolyse any bound bromophenols in plant or animal matrices.

The two feeds containing the freeze-dried polychaete, *M. sanguinea* (I and II) had the highest TBCs, with values 9409 and 18932 ng/g, respectively. Much lower TBCs were found in the feeds containing *A. teres* (43.1 ng/g) and that containing the freeze-dried alga, *U. lactuca* (10.9 ng/g). Accordingly, the feeds containing *M. sanguinea* had TBCs that approximated the levels of bromophenols believed to be found in the natural diet of some ocean prawns (>2000 ng/g). Those containing *A. teres* and *U. lactuca* had TBCs that were comparable with commercial feeds.

Data from the bromophenol analyses of prawns fed experimental feeds containing either algae or polychaetes are recorded in Table 9. These feeds were again used as finishing feeds and were fed to prawns only for 2 weeks (see Section 3.3). Whole prawns fed material

Table 8
Concentrations of bromophenols in experimental prawn feed ingredients and experimental feeds[a]

Ingredient/product	Bromophenol content (ng/g)						
	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Calculated
Ulva lactuca	0.4	6	20	4	74	104	
Freeze-dried *U. lactuca*	3	12	24	13	217	2697	1040[b]
U. lactuca feed	ND[c]	0.6	0.2	0.1	10	10.9	30[d]
Australonuphis teres	13	6	34	61	220	334	
Freeze-dried *A. teres*	48	24	170	110	1400	1752	2088[e]
A. teres feed	3	1	7	5	27	43	175[d]
Marphysa sanguinea I	10	12	55	16	21000	21093	
Freeze dried *M. sanguinea I*	120	340	2200	190	92000	92870	131831[e]
M. sanguinea I feed	6	11	71	21	9300	9409	10319[d]
M. sanguinea II	68	77	1600	88	36000	37833	
Freeze-dried *M. sanguinea II*	230	260	3300	760	180000	184550	236456[e]
M. sanguinea II feed	100	12	520	300	18000	18932	20506[d]
Basal feed[f]	ND	tr[g]	0.1	tr	2.2	2.3	
Commercial feed	0.7	6.5	1.2	3.2	2.4	14	

[a] Contains 10% freeze dried ingredients.
[b] Average moisture content of algae 90%.
[c] ND, not detected at a detection limit of 0.01 ng/g.
[d] Calculated from freeze-dried ingredients. Average moisture content of 10%.
[e] Average moisture content of polychaetes 84%.
[f] Contains 30% fish meal.
[g] Trace, tr = 0.01–0.1 ng/g.

containing either the alga, *U. lactuca*, or the polychaete, *A. teres,* had a TBC of trace and 0.2 ng/g, respectively, whereas those fed material containing *M. sanguinea* ranged from 10.3 to 22.2 ng/g. By comparison, prawns fed either the basal or commercial feed had a TBC of trace and 1.1 ng/g, respectively. Accordingly, prawns fed material containing *U. lactuca* or *A. teres* had a similar TBC as those fed the basal or commercial feeds, respectively, even though the experimental feeds had significantly higher concentrations of bromophenols (Table 8). Furthermore, prawns fed *M. sanguinea* I had a similar TBC (10.3 ng/g) to that found in prawns (9.7 ng/g) fed the commercial feed AF97 (Table 5) even though the commercial feed had a TBC of 153 ng/g, whereas that of the experimental feed was 9409 ng/g (Table 8). In addition, prawns fed *M. sanguinea* II feed had a TBC of only 22.2 ng/g, although the feed itself had a TBC of 18932 ng/g (Table 8). These results indicate that the bromophenols present in experimental feeds containing either algae or polychaetes, are not readily retained by prawns under the conditions of cultivation described in this study. Furthermore, total bromophenols in the tails of prawns fed such feeds were very low, ranging from trace to 0.7 ng/g (Table 9). This range was less than that obtained for prawns fed either commercial feeds (Table 5) or feeds containing commercial ingredients (Table 6). It was, however, comparable with that obtained with prawns fed feeds containing either free bromophenols or their sulphate esters (Table 7).

Sensory analyses of the prawns fed the experimental feeds or commercial feeds showed that they were acceptably sweet but devoid of any characteristic flavour. As such, no increase in flavour had been achieved by the use of algal and polychaete supplements in the finishing feeds, although some of these feeds contained relatively high concentrations (>10 000 ng/g) of total bromophenols.

3.5. Role of bromophenols in ocean prawns

Studies by Boyle et al. (1992b) have shown that the presence of low (~1 ng/g) concentrations of the target bromophenols in fish, shrimp and vegetable oil matrices impart flavour notes reminiscent of marine fish and crustaceans. Sea-, iodine-, sea salt-, sea fish-like flavours were detected when 2-BP, 2,6-DBP or 2,4,6-TBP were individually incorporated in marinated whitefish at concentrations ranging from 0.25 ng/g for 2,6-DBP to 10 ng/g for 2,4,6-TBP (Boyle et al., 1992a). However, when these three bromophenols were combined in marinated whitefish at lower concentrations (2-BP, 0.5 ng/g; 2,6-DBP, 0.1 ng/g: 2,4,6-TBP, 0.5 ng/g) they produced flavours described as crab-, iodine- and sea fish-like (Boyle et al., 1992a). In the current study, no sample of prawn tails had all three bromophenols present at concentrations that exceeded these values. But four samples, including the wild prawn 23596 (Table 5) and the cultivated prawns fed diets A, B and D (Table 6), had two bromophenols (2,6-DBP and 2,4,6-TBP) that exceeded their target concentrations. The three prawns fed the experimental feeds all had flavours described as briny, iodine/ocean-like (Section 3.2). The flavour of

Table 9
Concentrations of bromophenols in uncooked prawns fed experimental feeds containing freeze dried algae or freeze dried polychaetes[a]

Diet[b]	Sample	Ratio heads/tails	Bromophenol content (ng/g)						
			2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	Total	Total[c] whole prawns
Ulva lactuca feed	Head	1	tr[d]	tr	tr	ND[e]	tr	tr	tr
	Tail		tr	tr	ND	ND	ND	tr	
Australonuphis teres feed	Head	1	tr	tr	tr	ND	tr	tr	0.2
	Tail		tr	ND	tr	ND	0.3	0.3	
Marphysa sanguinea I feed	Head	0.87	0.7	1.0	0.2	ND	20.0	21.9	10.3
	Tail		tr	ND	ND	ND	0.2	0.2	
M. sanguinea II feed	Head	1.1	1.0	ND	1.8	1.0	38.0	41.8	22.2
	Tail		tr	ND	tr	tr	0.7	0.7	
Basal feed	Head	1.1	ND	ND	ND	ND	tr	tr	tr
	Tail		tr	tr	ND	ND	ND	tr	
Commercial feed	Head	1.2	ND	0.5	0.3	0.4	1.8	3.0	1.1
	Tail		ND	tr	tr	tr	0.1	0.1	

[a] Prawns were fed on experimental diets in laboratory tanks for 2 weeks.
[b] As reported in Table 8.
[c] Calculated from weight of individual batches of heads, tails and total prawns extracted.
[d] Trace, tr = 0.01–0.1.
[e] ND, not detected at a detection limit of 0.01 ng/g.

prawns 8497, fed a commercial feed (Table 5) was also described as slightly briny and iodine-like (Section 3.1). In these prawns, the concentrations of 2-BP and 2,6-DBP in the tails were 0.4 and 0.7 ng/g, respectively (Table 5). Accordingly, a relationship appears to exists between the flavour of cultivated prawns and the presence of at least two of these nominated bromophenols. This relationship had been previously observed in some species of wild prawns (Whitfield et al., 1997).

4. Conclusions

This study has shown that some experimental feeds containing commercial ingredients can increase the levels of bromophenols retained by cultivated prawns. The key commercial ingredient would appear to be shrimp head meal with a TBC > 800 ng/g. However, to achieve such results, the shrimp head meal had to be added to the feed at a high inclusion rate (15%) and the prawns were given the feeds for a period of 8 weeks. Attempts to produce a finishing feed, containing either freeze dried polychaete or freeze dried algae that would achieve the same result, were unsuccessful. The reason for this failure remains unclear, although it is possible that the bromophenols present in these ingredients were in a form that precluded their retention by the cultivated prawns. Accordingly, the chemical form of bromophenols in grow-out and finishing feeds (free or bound) could be the key factor that determines the level at which such compounds are retained by prawns. Future studies should attempt to identify the form or forms in which bromophenols are present in processed feed ingredients and natural dietary components. This information would provide an insight into the best practice for the control of bromophenol concentrations in cultivated prawns.

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