Distribution of Bromophenols in Species of Marine Algae from Eastern Australia

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Forty-nine species (87 samples) of marine macroalgae from eastern Australia were analyzed by GC/MS for the key seafood flavor components 2- and 4-bromophenol, 2,4- and 2,6-dibromophenol, and 2,4,6-tribromophenol. All five bromophenols were found in 62% of samples, four in 32% of samples, and three in the remaining 6% of samples. 2,4,6-Tribromophenol was found in all samples and, with few exceptions, was present in the highest concentrations. The total bromophenol content determined on a wet-weight basis varied widely across species from 0.9 ng/g in the green alga *Codium fragile* to 2590 ng/g in the red alga *Pterocladiella capillacea*. Species with the highest concentrations of bromophenols were all collected from sites exposed at low tide. The study demonstrates the wide occurrence of bromophenols in marine algae and provides a possible source of such compounds in fish that feed predominantly on ocean plants. The possible effect that dietary marine algae has on the flavor of omnivorous ocean fish is discussed.

Keywords: *Marine macroalgae; bromophenols; GC/MS analysis; flavor; fish diet*

INTRODUCTION

The simple bromophenols, particularly 2,6-dibromophenol (2,6-DBP) and 2-bromophenol (2-BP), have strong flavors that have been described as iodoform- or iodine-like (Whitfield et al., 1988; Boyle et al., 1992a). 2,6-Dibromophenol has a flavor threshold concentration in water of 0.0005 ng/g and 2-BP of 0.03 ng/g; however, in seafoods, particularly prawn meat, these values are higher, 0.06 and 2 ng/g, respectively (Whitfield et al., 1988). 2,4,6-Tribromophenol (2,4,6-TBP) also has an iodoform-like flavor but is less intense than 2,6-DBP and has flavor threshold concentrations of 0.6 ng/g in water and of 50 ng/g in prawn meat (Whitfield et al., 1996). These three bromophenols, together with 4-bromophenol (4-BP) and 2,4-dibromophenol (2,4-DBP), have been identified as key flavor components in Australian ocean prawns (Whitfield et al., 1988, 1997), Australian ocean fish (Whitfield et al., 1998), North American crustaceans, North American molluscs, Pacific salmon (Boyle et al., 1992b), and North Atlantic shrimp (Anthoni et al., 1990). In all of these animals the bromophenols were considered to be derived from natural sources. The occurrence of such bromophenols in the marine environment has been reviewed (Boyle et al., 1993). Recently it has been claimed that the major dietary source of bromophenols in Australian ocean prawns is marine polychaetes (Whitfield et al., 1997). Polychaetes are also considered to be a source of bromophenols in Australian ocean fish that are benthic carnivores (Whitfield et al., 1998). However, the same authors have claimed that marine algae are a major source of these compounds in herbivorous fish and a contributing source in fish that are diverse omnivores (Whitfield et al., 1998).

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In a survey of the dietary habits of Australian ocean fish, at least seven genera of red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta) algae were identified in the stomach contents of 15 species of fish that were either diverse omnivores or restricted omnivores (Anonymous, 1981). The most commonly identified algal genera were *Enteromorpha*, *Gracilaria*, and *Ulva*, but in most gut samples it was not possible to identify algal material beyond division. However, anecdotal information supplied by amateur fishermen and spear fishermen indicates that omnivorous fish feed on a wide variety of algae and particularly on those algae that have a "soft" texture. Species most favored are those found growing on rocky outcrops in shallow (1−2 m), highly oxygenated inshore (upper sublittoral) waters. Such algae are readily detected at low tide as those that have been cropped most heavily. The current survey was undertaken to assess the distribution of bromophenols in species of algae frequently eaten by ocean fish, together with a range of other species that grow in the same intertidal (littoral) and upper sublittoral zones.

EXPERIMENTAL PROCEDURES

Materials. Samples of marine algae were collected from Bateau Bay NSW (February 1991−1993 and November 1991), Great Barrier Reef QLD (December 1992), Botany Bay NSW (February 1993−1994), Garie Beach NSW (March 1993), Turimetta Head NSW (February 1994−1995), and Batemans Bay (April 1995). The samples were immediately stored on ice and transported to the laboratory in North Ryde, NSW. Sand and other loose material were gently washed from the algae with seawater and finally, purified water. Excess water was allowed to drain from the algae before they were sorted into individual species. Small samples of individual species from each collection were frozen and were subsequently identified by either Dr. P. Ferrant or Dr. A. J. K. Millar of the Royal Botanic Gardens, Sydney NSW. The authorities for these identifications can be found in the following references: for Rhodophyta: Millar, 1990; Millar and Kraft, 1993; for Phaeo-

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Table 1. Distribution of Bromophenols in Eastern Australian Red, Brown, and Green Marine Algae[a]

species	source (year)	bromophenol content[b] (ng/g) 2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total	growth range[c] (m)
			Red Algae					
Amphiroa anceps	Bateau Bay (91)	1.6	5.1	26	6.4	1300	1339	1-10[d]
Callophycus tridentifer	Bateau Bay (91)	7.6	tr[e]	0.3	1.1	2.7	12	1-10
Cheilosporum sagittatum	Bateau Bay (91)	7.1	0.8	27	23	220	278	1-6
Chondria succulenta	Batemans Bay (95)	64	2.2	12	36	150	264	1-6
Corallina berteri	Bateau Bay (91)	0.1	0.7	9.6	0.1	16	27	0.5-7
Corallina officinalis	Bateau Bay (91)	0.8	0.5	5.4	0.4	6.6	14	2-19
Delisea pulchra (N)[f]	Bateau Bay (91)	10	ND[g]	1.4	68	81	160	1-20
Delisea pulchra (S)[f]	Bateau Bay (91)	2.0	ND	5.0	49	96	152	1-20
Delisea pulchra	Botany Bay (93)	1.3	ND	24	8.5	47	81	1-20
Delisea pulchra	Botany Bay (94)	9.3	ND	1.6	1.6	160	173	1-20
Delisea pulchra	Batemans Bay (95)	11	ND	21	14	230	276	1-20
Galaxaura marginata	Bateau Bay (92)	ND	ND	19	3.3	18	40	2-8
Galaxaura marginata	Bateau Bay (93)	0.02	ND	5.2	2.2	22	29	2-8
Galaxaura marginata	Batemans Bay (95)	ND	6.6	11	4.7	41	63	2-8
Galaxaura obtusata	Bateau Bay (91)	2.8	ND	18	9.0	96	126	2-20
Gracilaria edulis (S/E)[h]	Botany Bay (93)	3.1	17	18	4.2	78	120	2-10
Gracilaria edulis (S/W)[h]	Botany Bay (93)	0.6	1.4	15	13	37	67	2-10
Gracilaria edulis (S/E)	Botany Bay (94)	0.5	29	65	1.1	28	124	2-10
Gracilaria edulis (S/W)	Botany Bay (94)	0.3	0.1	7.3	10	56	73	2-10
Gracilaria secundata	Batemans Bay (95)	2.2	2.5	5.2	4.3	3.0	17	0.5-13
Haliptilon roseum	Bateau Bay (91)	7.2	35	105	26	190	363	2-12
Halymenia floresia	Barrier Reef (92)	6.3	ND	6.2	10	72	95	3-14
Lomentaria catenata	Batemans Bay (95)	2.1	1.8	9.0	2.9	110	126	1-10
Plocamium angustatum	Batemans Bay (95)	ND	2.9	61	23	250	337	1-15
Porphyra columbina	Turimetta Head (95)	0.1	0.4	6.2	0.3	1.2	8	0.5-1
Pterocladiella capillacea (N)	Bateau Bay (91)	6.0	ND	41	17	1000	1064	1-10
Pterocladiella capillacea (S)	Bateau Bay (91)	15	27	170	180	1200	1592	1-10
Pterocladiella capillacea	Bateau Bay (92)	7.3	39	260	126	1300	1726	1-10
Pterocladiella capillacea	Bateau Bay (93)	2.3	4.0	25	160	1900	2091	1-10
Pterocladiella capillacea	Turimetta Head (94)	ND	30	320	440	1800	2590	1-10
Pterocladia lucida	Bateau Bay (91)	0.2	0.2	1.2	5.9	39	47	2-3
Pterocladia lucida	Batemans Bay (95)	30	ND	29	9.6	110	179	2-3
Solieria robusta	Batemans Bay (95)	0.7	2.1	5.2	10	35	53	
			Brown Algae					
Cladostephus spongiosus	Bateau Bay (91)	0.02	1.2	41	0.1	6.9	49	2-3
Cladostephus spongiosus	Bateau Bay (92)	0.7	8.6	12	2.0	19	42	2-3
Cladostephus spongiosus	Bateau Bay (93)	0.3	4.5	5.3	0.9	14	25	2-3
Colpomenia sinuosa	Bateau Bay (92)	0.6	6.5	22	ND	16	45	1-10
Colpomenia sinuosa	Bateau Bay (93)	3.5	3.4	8.1	2.1	26	43	1-10
Cystophora intermedia (N)	Bateau Bay (91)	0.2	ND	0.5	0.1	3.2	4	0.5-2
Cystophora intermedia (S)	Bateau Bay (91)	1.6	0.7	8.8	1.2	1.0	13	0.5-2
Cystophora intermedia	Bateau Bay (93)	1.0	ND	2.8	1.0	7.8	13	0.5-2
Cystophora intermedia	Batemans Bay (95)	2.7	1.5	4.9	1.0	14	24	0.5-2
Cystophora moniformis	Bateau Bay (93)	ND	tr	1.2	0.3	7.4	9	1-28
Cystoseira trinodis	Bateau Bay (93)	tr	0.2	4.7	0.1	3.1	8	0.5-1
Ecklonia radiata	Bateau Bay (92)	1.6	13	26	1.5	190	232	1-20
Ecklonia radiata	Bateau Bay (93)	ND	ND	0.2	0.02	13	13	1-20
Ecklonia radiata	Bateau Bay (94)	ND	0.1	1.2	ND	18	19	1-20
Endarachne binghamiale	Turimetta Head (95)	0.5	ND	0.2	0.2	0.6	2	
Halopteris paniculata	Bateau Bay (91)	0.02	1.8	6.0	0.4	14	22	0.5-13
Halopteris paniculata	Bateau Bay (92)	0.3	3.7	62	0.2	2.8	69	0.5-13
Halopteris platycena	Bateau Bay (92)	1.8	8.7	17	5.5	26	59	0.5-20
Homoeostrichus sinclairii (N)	Bateau Bay (91)	8.4	ND	0.8	71	22	102	0.5-35
Homoeostrichus sinclairii (N)	Bateau Bay (91)	58	8.1	16	2.6	6.4	91	0.5-35
Homoeostrichus sinclairii (S)	Bateau Bay (91)	16	9.0	35	29	63	152	0.5-35
Homoeostrichus sinclairii	Bateau Bay (93)	6.3	ND	8.1	9.1	27	51	0.5-35
Hormosira banksii	Bateau Bay (92)	2.9	3.2	1.8	0.3	32	40	0.5-1
Lobophora variegata	Bateau Bay (93)	0.4	ND	2.1	3.3	15	21	0.5-36
Phyllospora comosa	Bateau Bay (91)	ND	98	55	0.2	280	433	1-12
Phyllospora comosa (N)	Bateau Bay (92)	0.3	40	35	29	130	234	1-12
Phyllospora comosa (S)	Bateau Bay (92)	0.1	3.9	3.5	0.7	230	298	1-12
Phyllospora comosa	Garie Beach (93)	3.0	140	52	9.0	250	454	1-12
Sargassum erosum[i]	Bateau Bay (91)	0.04	ND	0.9	0.3	2.8	4	1-20
Sargassum erosum	Bateau Bay (92)	0.02	ND	0.7	0.1	7.7	9	1-20
Sargassum globulariaefolium	Bateau Bay (91)	0.3	ND	1.6	0.5	82	84	1-20
Sargassum globulariaefolium	Bateau Bay (92)	2.2	9.7	14	0.5	56	82	1-20
Sargassum lophocarpum	Bateau Bay (92)	0.1	2.0	30	0.05	0.8	33	1-20
Sargassum neurophorum	Bateau Bay (91)	0.2	ND	0.7	0.1	18	19	1-20
Sargassum polyacanthum	Bateau Bay (91)	1.7	1.2	7.4	0.4	22	33	1-20
Sargassum polyacanthum	Bateau Bay (92)	0.6	4.4	12	0.2	18	35	1-20
Sporochnus comosus	Bateau Bay (92)	1.8	2.1	8.7	1.2	18	32	1-41

Table 1 (Continued)

species	source (year)	bromophenol content[b] (ng/g) 2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	total	growth range[c] (m)
				Green Algae				
Caulerpa cactoides	Bateau Bay (93)	2.8	0.6	94	2.8	19	119	0.5-38
Chlorodesmis major	Barrier Reef (92)	0.8	1.7	150	3.0	7.0	163	2-6
Cladophoropsis herpestica	Barrier Reef (92)	0.2	6.8	5.4	1.3	32	46	0.5-1
Codium fragile	Bateau Bay (91)	ND	0.2	0.2	0.03	0.5	0.9	0.5-2
Codium galeatum	Bateau Bay (92)	0.1	0.2	20	3.1	62	85	0.5-37
Codium galeatum	Bateau Bay (93)	2.2	1.2	12	13	39	67	0.5-37
Codium lucasii	Bateau Bay (91)	0.02	ND	34	0.06	1.5	36	1-5
Enteromorpha intestinalis	Bateau Bay (93)	18	260	640	75	1400	2393	0.5-7
Enteromorpha intestinalis	Botany Bay (93)	tr	2.7	11	1.4	520	535	0.5-7
Enteromorpha intestinalis	Turimetta Head (94)	0.5	ND	35	5.6	1300	1341	0.5-7
Halimeda cuneata	Barrier Reef (92)	13	1.0	15	1.1	41	71	0.5-6
Halimeda discoidea	Barrier Reef (92)	0.03	6.6	8.6	2.0	31	48	0.5-6
Halimeda opuntia	Barrier Reef (92)	0.04	1.7	11	3.0	17	33	0.5-6
Ulva lactuca	Bateau Bay (91)	1.5	6.6	130	1.1	400	539	0.5-6
Ulva lactuca	Bateau Bay (93)	0.1	2.2	25	1.2	1200	1229	0.5-6
Ulva lactuca	Botany Bay (93)	0.1	ND	6.2	1.2	400	428	0.5-6
Ulva lactuca	Turimetta Head (94)	0.2	0.4	47	3.3	390	441	0.5-6

[a] Values are expressed as wet weight. [b] The average percentage recoveries for individual bromophenols are as follows: 2-BP, 76% (RSD = 3%); 4-BP, 40% (RSD = 2%); 2,4-DBP, 74% (RSD = 74%); 2,6-DBP, 94% (RSD = 1%); and 2,4,6-TBP, 79% (RSD = 4%). [c] Womersly (1984); Millar, A. J. K. Royal Botanic Gardens, Sydney, NSW, Australia, personal communication, 1998. [d] Algae growing along the NSW coast line at depth between 0.5 and 2 m are routinely exposed to air at low tide. [e] Trace, tr = 0.01−0.02 ng/g. [f] (N), (S), northern or southern side of bay. [g] ND, not detected at a detection level of 0.01 ng/g. [h] (S/E), (S/W), eastern or western end of southern side of bay. [i] Australian species of the family Sargassaceae is currently under review.

phyta: Millar and Kraft, 1994a; and for Chlorophyta: Millar and Kraft, 1994b. The bulk of the samples were surface dried by gently patting the algae between cotton tea towels. Individual specimens were labeled, packaged in polyethylene bags, frozen at −20 °C, and stored at this temperature until required for extraction and analysis.

Reference samples of the five target bromophenols were purchased from Aldrich Chemical Co. Inc., Milwaukee, WI, and 2,6-dibromophenol-d_3 was obtained from C/D/N 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 Corporation, Bedford, MA). All inorganic chemicals and organic solvents were analytical reagent grade (>98% pure). The solvents were further purified by distillation through a packed fractionating column.

Isolation of Bromophenols. Composite samples of individual species of algae were allowed to thaw and were cut into small pieces. A portion of this material (50−100 g) was blended in purified water (1.5 L) for 5 min using either a Sunbeam food processor or a Panasonic Super Blender. The homogenates were acidified to pH 1 with 10 M sulfuric acid and were left to stand for 2 h 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 residual material was measured again to confirm that the homogenates had remained acidic during the isolation procedure. The internal standard, 2,6-dibromophenol-d_3 (100 ng in 100 μL isooctane), was added to the solvent extracts, which were dried by cooling to −15 °C and decanting the solvent fraction. The extracts were concentrated by the careful removal of the solvent by fractional distillation, and the concentrates in isooctane (about 100 μL) were stored in glass autosampler vials at −15 °C until required for analysis by GC/MS.

Analysis by GC/MS. The bromophenols in the algal extracts were analyzed by a Hewlett-Packard HP5890 gas chromatograph interfaced to a Hewlett-Packard HP5971A mass selective detector and operated in the multiple ion detection (MID) mode. The GC oven was fitted with a 25 m × 0.25 mm i.d. fused silica column coated with methyl phenylsilicone HP5 of 0.33 μm film thickness (Hewlett-Packard, Palo Alto, CA) 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. However, it was necessary to replace the retention gap frequently when linearity of the calibration curve no longer applied, which 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 programmed by holding the temperature initially at 60 °C for 1 min, programming from 60 to 225 °C at 15 °C/min and then from 225 to 280 °C at 40 °C/min, and finally holding 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 analysis, ions were monitored for 2-BP and 4-BP at *m*/*z* 172, 174; for 2,4-DBP and 2,6-DBP at *m*/*z* 250, 252; for 2,4,6-TBP at *m*/*z* 330, 332; and for the internal standard, 2,6-dibromophenol-d_3, at *m*/*z* 255, 257. The retention times of these compounds were 2-BP, 6.60 min; 4-BP, 9.02 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 isooctane) with a constant concentration of the 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 performed in duplicate. If a sample contained analytes outside of the calibration range, a diluted subsample was analyzed after addition of more internal standard. Reported data has been corrected for losses during extraction and concentration (see below). The detection limit for individual bromophenols in the algae was 0.01 ng/g based on a factor of 3 times the 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 reten-

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

Extraction Efficiencies. The extraction efficiencies of the SDE technique for the recovery of individual bromophenols from algae were determined as follows. Samples (100 g) of the alga *Codium fragile*, of low bromophenol content, was 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 were as follows: 2-BP, 76% (RSD = 3%); 4-BP, 40% (RSD = 2%); 2,4-DBP, 74% (RSD = 4%); 2,6-DBP, 94% (RSD = 1%); and 2,4,6-TBP, 79% (RSD = 4%). The lower recovery of 4-BP was attributed to the greater solubility of this compound in water compared to the other bromophenols.

RESULTS AND DISCUSSION

Bromophenol Content of Australian Marine Algae. The data obtained from the bromophenol analyses of 87 samples of marine algae, representing 49 species, are recorded in Table 1. Species have been grouped according to the division to which individual species belong, namely Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae). Of the 49 species studied, the data for 27 species were obtained from single collections, whereas the data for the remaining 22 species were obtained from two or more collections. When more than one collection was analyzed, the samples had been taken either from different geographical locations or were collected from the same site over several years. The selection of a species for multiple collection was determined either by the dominant nature of that species at a particular site or because it was known to be a major food source for herbivorous and omnivorous species of fish. Importantly, the majority of samples were collected during summer (December to February) when the bromophenol content of marine algae is highest (Flodin et al., 1998).

Bromophenols were found above their detection limit of 0.01 ng/g in all 87 samples analyzed. All 5 bromophenols were found in 54 of the 87 samples studied, 4 bromophenols in 30 samples, and 3 bromophenols in the remaining three samples. Only two species, the red alga *Delisea pulchra* and the brown alga *Sargassum erosum*, consistently had four bromophenols when two or more samples were analyzed. In most multisampled species, all five bromophenols were found in at least one of the samples. Of the five target bromophenols, 2,4,6-TBP and 2,4-DBP were found in all samples, 2,6-DBP in 98% of samples, 2-BP in 91% of samples, and 4-BP in 72% of samples. In 71 of the 87 samples, 2,4,6-TBP was present in the highest concentrations, followed by 2,4-DBP in 13 samples, 2-BP in two samples, and 2,6-DBP in one sample. When 2,4,6-TBP was the dominant bromophenol, 2,4-DBP was present in the next highest concentrations in 52 samples. When 2,4-DBP was dominant, 2,4,6-TBP was next highest in nine samples. By comparison, a similar relationship between the concentrations of 2,4,6-TBP and 2,6-DBP was only observed in 10 samples. As 2,4-DBP was present in higher concentrations than 2,6-DBP in most samples, this result would suggest that when bromophenols are being synthesized in the majority of marine algae, bromination of the 2 and 4 positions of the phenolic ring is preferred. It could also be interpreted that 2,4-DBP is the likely immediate precursor of 2,4,6-TBP in most algae.

Among the 87 samples of algae analyzed, the red and green algae contained greater quantities of bromophenols than the brown algae. The total bromophenol content (TBC) of the 33 samples of red algae varied between 8 and 2590 ng/g (19 samples had TBCs > 100 ng/g), and the TBC of the 17 samples of green algae varied between 0.9 and 2393 ng/g (nine samples had TBCs > 100 ng/g). By comparison, the TBC of the 37 samples of brown algae varied between 2 and 454 ng/g (seven samples had TBCs > 100 ng/g). Importantly, all species that had TBCs > 100 ng/g were collected either from the intertidal zone or from highly oxygenated waters besides submerged rocks and reefs. However, not all species growing in these environments had TBCs > 100 ng/g, and no species growing in deeper waters (4−10 m) exceeded this figure. The growth range for the majority of algal species studied is presented in Table 1. In the current survey, most samples of brown algae were collected from the upper sublittoral zone. Species of red and green algae with the highest TBCs (> 1000 ng/g) were all taken from the littoral zone that would normally be exposed to air and sunlight for extensive periods. These species are the red algae *Amphiroa anceps* (1339 ng/g) and *Pterocladiella capillacea* (1064−2590 ng/g) and the green algae *Enteromorpha intestinalis* (535−2393 ng/g) and *Ulva lactuca* (428−1229 ng/g).

Of the 18 species of algae collected on more than one occasion either from different locations or from different years, 12 species showed >50% variation in bromophenol content. The largest difference was shown by samples of the green alga *Enteromorpha intestinalis* collected from Bateau Bay (TBC 2393 ng/g) and from Botany Bay (TBC 535 ng/g) in 1993 (Table 1). These bays are about 150 km apart with Botany Bay the more southerly. A similar effect was observed for the green alga *Ulva lactuca* collected from the same two bays during 1993. Algae collected from the same site but during different years also varied in bromophenol content. The largest difference was shown by samples of the brown alga *Ecklonia radiata* collected from Bateau Bay in February 1992 (TBC 232 ng/g) and February 1993 (TBC 13 ng/g). TBC also varied in samples of the same species collected from nearby sites. The largest difference was shown by samples of the red alga *Gracilaria edulis* collected from the eastern (TBC 120 ng/g) and western ends (67 ng/g) of Botany Bay in 1991. In contrast, the red alga *Delisea pulchra* obtained from different locations of Bateau Bay in 1991 showed no appreciable difference.

Biosynthesis of Bromophenols in Marine Algae. Many species of red, green, and brown algae have been shown to contain bromoperoxidases, enzymes capable of brominating organic substrates in the presence of bromide and hydrogen peroxide (Niedleman and Geigert, 1986; Moore and Okuda, 1996). By comparison, only one chloroperoxidase has been found in these plants, and it can only oxidize chloride at low pH (Manthey and Hager, 1989). Bromoperoxidases isolated from red algae have been shown to catalyze the bromination of phenol and 2-hydroxybenzyl alcohol to 2,4,6-TBP (Yamada et al., 1985), while similar enzymes from the green alga *Ulva lactuca* have been shown to brominate 4-hydroxybenzoic acid and 4-hydroxybenzyl alcohol to mixtures of 2-BP, 4-BP, 2,4-DBP, 2,6-DBP and 2,4,6-TBP (Flodin and Whitfield, 1999a). Both 4-hydroxybenzoic acid and 4-hydroxybenzyl alcohol are

products of the catabolism of tyrosine by marine phytoplankton (Landymore et al., 1978). Consequently, they have been proposed as likely precursors of bromophenols in marine algae (Flodin and Whitfield, 1999a). The biosynthetic pathway from tyrosine to the bromophenols is thought to be similar to that of the synthesis of lanosol (2,3-dibromo-4,5-dihydroxybenzyl alcohol) by the red alga *Odonthalia floccosa* (Manley and Chapman, 1978) and the synthesis of 3-bromo-4-hydroxybenzoic acid by the phytoplanktons *Isochrysis galbana* and *Navicula incerta* (Landymore et al., 1978). In these pathways, tyrosine is deaminated and successively oxidized and decarboxylated to yield 4-hydroxybenzaldehyde which can be either oxidized to 4-hydroxybenzoic acid or reduced to 4-hydroxybenzyl alcohol. The identification of these three compounds in *Ulva lactuca*, together with several intermediary compounds and a bromoperoxidase, strongly supports this pathway to bromophenols in marine algae (Flodin and Whitfield, 1999b).

The bromination reaction requires, in addition to suitable precursors, a bromoperoxidase, bromide, and hydrogen peroxide. The marine environment is a limitless source of bromide, with a concentration of 0.65 mg/kg in seawater and about the same concentration in marine algae. As a consequence, the production of hydrogen peroxide is the key factor in the formation of halometabolites in algae (Collén et al., 1994; Mtolera et al., 1996). Hydrogen peroxide is produced in the Mehler reaction when oxygen is consumed in photosynthesis (Mehler, 1951) and in photorespiration during the conversion of glycolate to glyoxylate (Asada and Takahashi, 1987). It can also be produced in the absence of light in some plants (Peng and Kue, 1992; Palenick and Morel, 1991). However, in marine algae the formation of halometabolites is dependent on photosynthesis as a source of hydrogen peroxide (Pedersén et al., 1996). Hydrogen peroxide can be toxic to algal cells; thus, the formation of halometabolites may be a response to oxidative stress within the plant caused by its exposure to air at low tide (Pedersén et al., 1996). Such conditions could also favor the formation of the more highly brominated phenol. The observation that some of the species most frequently exposed to air and light, such as *Amphiroa anceps*, *Enteromorpha intestinalis*, *Pterocladiella capillacea*, and *Ulva lactuca*, contain the highest concentrations of 2,4,6-TBP (Table 1) appears to support this opinion. However, although three of these species, *E. intestinalis*, *P. capillacea*, and *U. lactuca*, have "fragile" structures, there does not appear to be a relationship between algal morphology and bromophenol content. Other species with "fragile" structures such as *Galaxaura marginata* and *Pterocladia lucida* contain relatively low concentrations of these compounds (Table 1).

Accordingly, the formation of halogenated compounds by haloperoxidases may provide a means of scavenging hydrogen peroxide before it reaches toxic concentrations in some species of algae (Pedersén et al., 1996). Such halometabolites could therefore be regarded as waste products either accumulating within the plant as it ages or being excreted into the surrounding seawater (Pedersén et al., 1996).

Function of Bromophenols in Marine Algae. Within algae, halogenated compounds have been found to be localized in the chloroplasts, in vesicles in the cytosol, in the middle lamella, and in the outer cell walls (Pedersén, 1979, 1980; Pedersén et al., 1981, 1983). In these sections of the plant, such compounds are readily accessible to competitors, parasites, and grazers. Accordingly, some authors have linked the survival of certain algae to the presence of halometabolites through their involvement in interalgal competition (Mtolera et al., 1996), as a defense against bacterial and fungal infections (Reichelt and Borowitzka, 1984; Hornsey and Hide, 1985) and as deterrents to grazers (Moore, 1977; Gibson et al., 1979). Thus, as well as being sinks for hydrogen peroxide, the halometabolites probably contribute to the chemical defenses of the algae. However, the exact role of bromophenols in algal survival is still to be determined. Of interest, not all grazers are discouraged by the presence of halometabolites, as recent studies have shown that some 15 species of Australian omnivorous fish feed on algae with high bromophenol content (Whitfield et al., 1998). Thus, as with any biological defensive system, there would appear to be some limits to the effectiveness of halometabolites to protect marine algae.

Role of Marine Algae in the Flavor of Omnivorous Ocean Fish. Recent studies have shown that the edible flesh of many species of Australian ocean fish contain significant concentrations (1.2−58 ng/g) of total bromophenols (Whitfield et al., 1998). The flesh of such fish are frequently described as possessing ocean-, brine-, or iodine-like flavors attributable to the presence of bromophenols. The same studies also showed that the bromophenols were totally derived from the individual diets of the fish and that marine algae were major dietary components of most species of omnivorous ocean fish (Whitfield et al., 1998). Furthermore, in the current study it has been shown that many species of Australian marine algae contain relatively high concentrations of bromophenols (433−2590 ng/g). Accordingly, marine algae could be regarded as a major source of bromophenols in those fish that feed predominantly on marine plants. Species of Australian ocean fish that are known to feed largely on marine algae (Anonymous, 1981) include the diverse omnivores *Acanthapogrus australis* (up to 40% diet), *Meuschenia freycineti* (up to 60% diet), *Meuschenia trachylepis* (up to 78% diet), and *Rhabdosargus sarba* (up to 55% diet), and the restricted omnivore *Girella tricuspidata* (25−97% diet). All of these species have been shown to contain significant concentrations (1.2−5.3 ng/g) of total bromophenols in their edible flesh and even greater concentrations (3.4−150 ng/g) in their guts (Whitfield et al., 1998). The algae most commonly identified in the guts of these omnivorous fish were principally from the genera *Enteromorpha*, *Gracilaria*, and *Ulva* (Anonymous, 1981). As reported in the current studies, species from two of these genera, *Enteromorpha* and *Ulva*, contained relatively high concentrations of bromophenols (441−2393 ng/g), while in species from the genera *Gracilaria* the concentrations of these compounds (17−126 ng/g) were somewhat lower (Table 1). Accordingly, these species of algae found in the gut of such animals, together with those algae that could only be identified to division, are a major source of bromophenols in omnivorous ocean fish. Consequently, marine algae can be regarded as principal contributors of bromophenol related ocean-, brine-, or iodine-like flavors in fish that feed extensively on such plants.

CONCLUSION

It has been suggested that the flavor of cultivated (farmed) fish and prawns could be modified to have a

"marine-like" flavor by the use of a feedstock containing a natural supplement that is high in bromophenols (Boyle et al., 1992a; Whitfield et al., 1997). Marine algae were considered potential sources of these compounds (Whitfield et al., 1997). On the basis of the current study, two species, *Ulva lactuca* and *Pterocladiella capillacea*, could be considered suitable for this purpose. On a dry weight basis these algae would contain between 10 000 and 20 000 ng/g of total bromophenols. Since many manufactured fish and prawn feeds contain up to 25% cereal, the replacement of the cereal with dried marine algae would provide a feed with between 2500 and 5000 ng/g of bromophenols. The TBC of commercial prawn feed currently available in Australia varies between 1.4 and 40 ng/g (Whitfield et al., 1997). The flesh of prawns fed on these low bromophenol containing feeds are described as sweet but bland. Thus it could be expected that an increase in bromophenol concentration in the feed would impact on the flavor of the recipient. However, the bromophenols in the supplemented feed would need to be available in the free form when digested (Whitfield et al., 1997). Accordingly, further research is necessary to establish the range of bromophenol concentrations necessary in feeds to achieve a desirable flavor effect. Too high a concentration in the animal's flesh could lead to an undesirable iodoform-like flavor.

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