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Enhancement of Bromophenol Levels in Aquacultured Silver Seabream (*Sparus sarba*)

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The effect of the addition of marine algae in fish feed on the levels of bromophenols in fish flesh was studied. These bromophenols include 2-bromophenol, 4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol, and 2,4,6-tribromophenol. Two types of algae-containing fish feeds with 30% *Padina arborescens* and 30% *Sargassum siliquastrum* were developed. The total bromophenol contents of these feeds were 132 and 340 ng/g respectively, which were significantly higher than that of the control feed (8.9 ng/g) (ANOVA, $p < 0.05$). Silver seabream was used as the model fish for the feeding experiment. Bromophenol contents of both fish gut and flesh were monitored at 2-week intervals throughout the 8-week period. Two-way ANOVA showed that only the 30% *S. siliquastrum*-containing feed significantly ($p < 0.05$) increased the total bromophenol content in the fish flesh with time. This also produced sensorial differences in the fish flesh.

KEYWORDS: Bromophenols; seafood; fish; aquaculture; fish feed

INTRODUCTION

The world demand for fishery products increases every year (*1*). To relieve this pressure, many countries utilize aquaculture as an alternative way to increase the productivity and meet the demand of the consumers. Indeed, fresh fish is one of the most popular foods consumed in Hong Kong SAR, China. To increase the fishery production, the local government has put in much effort to help the aquaculture sector by providing technical services and credit supports (*2*). In 2000, production from the aquaculture sector was 1770 tonnes valued at about US $13 million (*3*). The organoleptic quality of these aquacultural products is generally acceptable, but some consumers have suggested that there is an obvious difference in flavor between the cultivated and the wild harvested seafood such as fishes (*4*, *5*). Recently, bromophenols, including 2-bromophenol (**1**), 4-bromophenol (**2**), 2,4-dibromophenol (**3**), 2,6-dibromophenol (**4**), and 2,4,6-tribromophenol (**5**), were suggested to be a group of compounds responsible for such flavor differences (*6*, *7*). In a study on Australian prawns, it was found that the concentrations of bromophenols detected in wild-harvested prawns were much higher than those in the cultivated ones (*6*, *7*). Sensory evaluations showed that the meat of wild-harvested prawns possessed briny, ocean-like, and prawn-like flavors, whereas the cultivated ones were described as bland (*6*). It was believed that the subtle differences between the wild-harvested and the cultivated seafood were caused by the quantitative differences in their bromophenol contents (*6*).

As bromophenols originated from the diets of seafood (*6*−*8*), differences in the bromophenol contents between wild-harvested and cultivated prawns were likely to be related to the difference in the bromophenol contents in their diets (*6*, *7*). In the wild-harvested prawns, their dietary components probably consist of bromophenols-producing species such as polychaetes, with high bromophenol contents (*6*, *9*−*11*). In the cultivated seafood, commercial feeds usually have a relatively low amount of bromophenols present (1.4−40 ng/g in prawn feeds) (*6*, *12*). Apparently, the bromophenol contents in the seafood are affected by their amount present in the animal's diet.

Previous studies showed that bromophenols were commonly detected in a variety of marine organisms including marine fish, crustaceans, mollusks, algae, polychaetes, etc. (*6*, *8*, *11*, *13*−*17*). Our study also showed that bromphenols were present in high concentrations in several marine algae collected in Hong Kong during winter (*15*). Such marine algae could be utilized as a possible dietary source of bromophenols for the cultivation of many aquacultured animals (*18*).

To enhance the organoleptic property of the aquacultured animals, their flavor quality should be an important aspect for consideration. A feed that is rich in bromophenol may enhance the bromophenol contents in the seafood and contribute to their

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desirability. Our objectives were to evaluate the possibility of utilizing the algae-containing feed to increase the bromophenol content in an aquacultured fish and to evaluate the effect of such feeds on the flavor quality of the fish.

MATERIALS AND METHODS

Preparation of Traditional Fish Feed. The fish feed was formulated according to the method of Woo and Kelly (*19*). The ingredients were purchased from local suppliers in Hong Kong SAR, China. Briefly, white fishmeal (82.66%), dextrin (1.54%), oils (8.5%), vitamins (2%), minerals (3.8%), and carboxymethyl cellulose (1.5%) were mixed well in a plastic container. Water was added to make the dough soft, which was then extruded with a Kenwood large mincer (A940, PK001/W, Kenwood Limited, Havant, U.K.). The extruded feeds (1 cm in length and 0.5 cm in diameter) were packed and stored at −80 °C before freeze-drying. The dried fish feeds were temporarily stored at 4 °C.

Preparation of Experimental Fish Feeds. Two experimental fish feeds were prepared, which contained 30% *Padina arborescens* and 30% *Sargassum siliquastrum* (w/w), respectively. These two species of algae were collected from the water near a remote island, Tung Ping Chau, in Hong Kong SAR, China, in winter when their bromophenol content was highest (*15*). Fresh algal samples were packed in plastic bags and immediately transported to the laboratory at the Chinese University of Hong Kong and stored in a cold room (4 °C). Within 24 h, they were gently cleaned and rinsed with distilled water to remove sands and living organisms and to have their holdfast cut. They were repacked and frozen at −80 °C for freeze-drying. After 1 week of freeze-drying, the algae were ground into powder with a National blender (MX-T2GM, Matsushita Electric Co. Ltd., Taipei, Taiwan). Both algae and the aforementioned fish feed ingredients were mixed to form a dough. The feed preparation procedures were the same as those of the traditional fish feed described above. The feeds were temporarily stored at 4 °C.

Feeding Experiment. Silver seabream (*Sparus sarba*) were obtained from a local sea cage in Sai Kung, Hong Kong SAR, China. The average initial weight of an individual fish was ∼80 g. They were acclimatized and grown in a closed seawater circulating system at the Marine Science Laboratory, the Chinese University of Hong Kong. They were fed with traditional fish feed before the experiment started. Fish were then divided into a control (traditional feed) and two experimental groups (feeds containing 30% *P. arborescens* and 30% *S. siliquastrum*) and were grown in three indoor 1-tonne tanks. About thirty fish were cultivated in each group. When the experiment started, traditional fish feed and algae-containing fish feeds (weight of feed = 2−3% of body weight) were manually fed, respectively, to the control and experimental groups daily. Three fish were then randomly picked every 2 weeks from each group to evaluate their bromophenol contents. Their flesh and gut were separately collected for bromophenols analyses.

Solvents and Chemicals. Standard chemicals of **1**, **3**, and **5** were purchased from Aldrich Chemical Co. (Milwaukee, WI), whereas **2** and **5** were purchased from Acros Organics (Geel, Belgium) with purities ranging from 97 to 99%. Organic solvents, pentane and diethyl ether, were bought from Lab-scan Ltd. (Dublin, Ireland) with purities of 99 and 99.5% respectively.

Simultaneous Steam Distillation−Solvent Extraction (SDE). The bromophenol extraction method was adapted from that of Whitfield et al. (*6*, *8*, *13*−*15*, *20*). The flesh (∼50 g) or the gut (∼6 g) was collected from each fish, and each was homogenized by a National blender (MX-T2GM, Matsushita Electric Co. Ltd., Taipei, Taiwan) for 2 min. Homogenized samples and 500 mL of boiled double-distilled water were transferred to a 5-L round-bottom flask, mixed, and acidified to pH 1 with 96% sulfuric acid. Then 1 mL of internal standard (1,3,5-trimethylbenzene, concentration at 84.7 μg/mL) was added, and the extraction with 40 mL of pentane/diethyl ether (9:1 v/v) for 2.5 h was carried out in a Likens−Nickerson type SDE apparatus (catalog no. K-523010-0000, Kontes, Vineland, NJ). The extract collected was concentrated to 0.25 mL with a stream of ultrahigh-purity (99.999%) nitrogen and dried by 2.85 g of anhydrous sodium sulfate. The concentrated extract was temporarily stored in a 15-mL conical tube at −80 °C for further analyses.

Gas Chromatography−Mass Spectrometry (GC-MS). A GC-MS system [Hewlett-Packard 6890 GC coupled with an HP 5973 mass selective detector (MSD), Hewlett-Packard Co., Palo Alto, CA] was used for qualitative and quantitative analyses. Five microliters of each extract was injected, in splitless mode with an injector temperature at 200 °C, into a fused silica open tubular column (Supelcowax-10, 60 m length × 0.25 mm i.d. × 0.25 μm film thickness; Supelco, Inc., Bellefonte, PA). Helium gas (ultrahigh-purity grade, 99.999%) was used as the carrier gas at constant linear velocity of 30 cm/s. Oven temperature was programmed from 100 to 200 °C at a ramp rate of 10 °C/min. The initial and final hold times were 5 and 75 min, respectively. MS interface, ion source, and MS quadrupole temperatures were set at 250, 230, and 106 °C, respectively. Ionization voltage was 70 eV, and electron multiplier voltage was 1200 V. Selected ion monitoring (SIM) GC-MS procedure was used. Ions were monitored for **1** and **2** at *m*/*z* 172 and 174, for **3** and **4** at *m*/*z* 250 and 252, for **5** at *m*/*z* 330 and 332, and for internal standard 1,3,5-trimethylbenzene at *m*/*z* 105 and 120 (*6*, *8*, *13*−*15*, *20*, *21*).

Compound Identification and Quantification. The presence of each bromophenol was confirmed by the detection of a single peak in the selected ion chromatogram at corresponding retention time and by the presence of the two characteristic ions listed above with particular isotope ratios (*6*, *8*). For quantification, three-point calibration curves for each bromophenol were established. Details can be found in our previous investigations (*14*, *15*).

Recoveries. The recovery of each bromophenol from the SDE technique was calculated by the ratio of concentration of bromophenol detected to the concentration of bromophenol added to the grass carp meat (*14*, *15*). Briefly, it was determined by extracting the known amount of each bromophenol with the SDE method and quantified with the same GC-MS system under the same experimental conditions described above. The average recovery percentages for compounds **1**, **2**, **3**, **4**, and **5** were 99.7 ± 0.4, 38.0 ± 3.2, 99.3 ± 0.9, 93.1 ± 0.3, and 62.5 ± 7.8%, respectively (*14*, *15*). These values were then used to calculate the original amount of bromophenols in the samples.

Moisture Determination. The percentage moisture of each of the samples was determined with a Mettler LJ16 moisture analyzer (Mettler-Toledo, Nanikon, Switzerland). Dry weights of the samples were calculated. Concentrations of the bromophenols in the samples were expressed on a dry weight basis.

Flavor Value (FV). The individual FV of each bromophenol was calculated by dividing the bromophenol concentration by its evaluation flavor concentration determined by Boyle et al. (*14*, *22*, *23*). The evaluation flavor concentrations determined in marinated whitefish were 10, 20, 50, 0.1, and 10 ng/g for compounds **1**, **2**, **3**, **4**, and **5**, respectively (*14*, *22*). Total FV was calculated by the summation of the five individual FVs (*14*).

Statistical Analysis. Total bromophenol contents (TBC) were compared between the gut and the flesh of the fish by Student's *t* tests at $p = 0.05$ level of significance. TBCs in different fish feeds were analyzed by one-way analysis of variance (ANOVA) and compared by the Tukey test at $p = 0.05$ level of significance. Besides, the TBCs in the fish flesh fed with different fish feeds for the 8-week feeding period were analyzed by two-way ANOVA at $p = 0.05$ level of significance.

Sensory Tests. Sensory differences between the fishes in the control group (fed with traditional fish feed) and the experimental groups (fed with either 30% *P. arborescens* or 30% *S. siliquastrum* modified fish feed for 8 weeks, respectively) were determined. The flesh were collected, filleted into 1-cm^3 cubes, and stored at −80 °C until evaluation by triangle tests was ready. Twenty-two subjects participated in the sensory evaluation. Orientation and training were provided to the subjects before the fish samples were tested to allow them to familiarize themselves with the flavor of bromophenols and the procedures involved in the triangle test. On the day before the tests, subjects were provided with different test solutions containing different concentrations of bromophenols and were asked to sniff and differentiate among the various sets of solutions on the basis of the procedures of the triangle tests (*24*).

During the actual sample evaluation, subjects were physically separated from each other by partitions in a clean and cooled room

Table 1. Distribution of Bromophenols in Fish Feeds

bromophenol concentration (ng/g of dry wt)					
compound **1**[a]	compound **2**[a]	compound **3**[a]	compound **4**[a]	compound **5**[a]	TBC[b]
		Traditional Fish Feed			
ND[c]	ND	3.01 ± 1.58[d]	2.02 ± 1.03	3.87 ± 0.91	8.90 ± 3.10 A
		Modified Fish Feed (30% *P. arborescens*)			
10.8 ± 3.7	37.0 ± 17.7	44.5 ± 24.9	12.1 ± 2.5	27.8 ± 13.5	132 ± 62 B
		Modified Fish Feed (30% *S. siliquastrum*)			
3.88 ± 1.23	9.17 ± 1.82	67.6 ± 13.9	7.39 ± 0.59	252 ± 37	340 ± 42 C

[a] Compound **1**, 2-bromophenol; compound **2**, 4-bromophenol; compound **3**, 2,4-dibromophenol; compound **4**, 2,6-dibromophenol; compound **5**, 2,4,6-tribromophenol. [b] Total bromophenol content. Mean values of TBCs in different types of fish feeds marked with different letters (A–C) in a column are significantly different (Tukey, $p < 0.05$). [c] Not detected. [d] Mean bromophenol concentration (ng/g of dry wt) ± standard deviation (SD) of three replicates each from different samples.

(20 °C) to minimize disturbances. Red lighting was used to reduce the interference from the sample color (*24*). Sample cubes were steamed for 15 min, transferred to portion cups marked with a three-digit random code, and served immediately to subjects. Each subject was asked to perform four sets of triangle test by evaluating the samples in their mouth. Two triangle tests evaluated the overall flavor between the control group and the experimental group fed with 30% *P. arborescens*-containing feed, whereas the other two tests were between the control feed and the 30% *S. siliquastrum* feed. Subjects were asked to differentiate the odd one in each set, and results were marked in each score sheet provided. Open space was provided for the subjects to comment on the flavor of the samples they evaluated. Results were evaluated by the number of correct answers obtained and the total number of responses. A minimum number of correct answers was required to signify a difference among samples at the 95% confidence level (*24*, *25*).

RESULTS AND DISCUSSION

Development of Bromophenol-Rich Fish Feed. A possible means to increase both the acceptance and the value of the aquacultured products could be to increase their flavor quality by elevating their bromophenol contents. In this study, marine algae were added to the fish diet as natural sources of bromophenols (*6*, *7*, *15*, *18*, *26*). Two species of marine algae (*P. arborescens* and *S. siliquastrum*) were used in this experiment because they were growing abundantly and were readily available at the collection site in Hong Kong SAR, China. *Padina* species were reported to be potential dietary sources of protein and lipid for fish (*27*). Other studies also showed that these two local species contained relatively higher amounts of bromophenols, particularly during the winter season (*15*). Although another species, *Lobophora variegata*, was shown to have the highest content of bromophenols among the three local species studied (*15*), it was not used in the current feeding experiment due to its low availability at the collection site. During the feed preparation, a marine alga was mixed with other feed ingredients at a proportion of 30% (w/w) because preliminary data showed that a lower level of marine algae (10%) could not significantly increase the bromophenol content in the fish flesh. To reduce the loss of bromophenols throughout the feed preparation process, collected algae were temporarily kept at −80 °C, freeze-dried, and stored at 4 °C. The bromophenol contents in various fish feeds were analyzed and are shown in **Table 1**.

The total bromophenol content (TBC) of the traditional fish feed was 8.90 ng/g in this experiment, whereas those in the modified feeds containing 30% *P. arborescens* and 30% *S. siliquastrum* were 132 and 340 ng/g, respectively. Statistical analysis (one-way ANOVA) on TBCs showed that the three types of feeds were significantly different from each other ($p < 0.05$). The total bromophenol contents of the modified feed with 30% *S. siliquastrum* were higher than those with 30% *P. arborescens*. All five individual bromophenols were detected in the two modified feeds. For the two modified feeds, the concentrations of compounds **1**, **2**, and **4** were higher in the 30% *P. arborescens* feed, whereas those of compounds **3** and **5** were higher in the 30% *S. siliquastrum* feed. The concentrations of both compounds **3** and **5** in each feed were the highest among the other bromophenols. Such high concentrations were consistent with our previous findings that they were present at higher amount in marine algae (*15*).

Feeding Experiment Using Traditional or Algae-Containing Fish Feeds. In this experiment, silver seabream (*Sparus sarba*) was chosen as the experimental fish because it is one of the fish species commonly cultured and consumed in Hong Kong, and it is easily obtained from a local sea cage. Besides, techniques and formulation of the feed for seabream were well developed (*19*). Therefore, these current conditions facilitate this investigation.

The distributions of the bromophenols in the fish gut and flesh throughout the experiment are shown in **Tables 2**−**4**, and changes in the TBC in the fish flesh are shown in **Figure 1**. To represent the accumulation of the five bromophenols in the fish flesh, TBCs were calculated (*6*, *14*, *15*) and were used to evaluate the overall effects of the fish feeds. Generally, the TBCs in gut were significantly higher than those in the flesh (Student's *t* test, $p < 0.05$). In the control group (**Table 2**; **Figure 1**), the TBCs of the fish flesh remain at constant levels (23.6−39.2 ng/g). However, when feed containing *P. arborescens* was used, the TBCs between the experimental (30% *P. arborescens*) and the control groups were significantly different ($p < 0.001$). The average TBC in the experimental group was higher than that in the control group (**Figure 1**). This difference, however, was not obvious at different times, nor was it affected by time (two-way ANOVA, $p = 0.165$). Therefore, feed containing 30% *P. arborescens* did not significantly increase the bromophenol contents in the fish flesh with time throughout the 8-week period.

With 30% *S. siliquastrum* in the feed, the difference in the values of TBCs between the experimental and the control groups was significant ($p < 0.001$). The TBCs in the fish fed with modified feed were higher than those fed with traditional feed. Moreover, the difference in the TBC values among the two groups was also significant with time or affected by time (two-way ANOVA, $p = 0.037$). The TBCs in the experiment group increased significantly along the 8-week period with reference to the control group. In short, modified feed containing 30% *S. siliquastrum* could increase the bromophenol contents in the aquacultured fish, and such results were probably due to the accumulation of these compounds (*13*), especially compound **5**, in the flesh during the 8-week feeding period. In fact, TBC

Table 2. Distribution of Bromophenols in the Gut and Flesh of Silver Seabream Fed with Traditional Fish Feed (Control) for 8 Weeks

			bromophenol concentration (ng/g of dry wt)					
week	sample	moisture (%)	compound **1**[a]	compound **2**[a]	compound **3**[a]	compound **4**[a]	compound **5**[a]	*p* value[b]
0	gut	69.10	ND[c]	ND	14.1 ± 8.2[d]	ND	88.9 ± 8.0	<0.001
	flesh	70.64	ND	ND	5.60 ± 1.29	ND	33.4 ± 7.4	
2	gut	68.87	ND	ND	32.6 ± 8.6	ND	188 ± 47	0.004
	flesh	70.98	ND	ND	3.56 ± 0.46	ND	21.3 ± 6.4	
4	gut	65.33	ND	ND	10.5 ± 8.9	ND	28.9 ± 17.6	0.372
	flesh	68.48	ND	ND	3.61 ± 1.07	ND	20.0 ± 5.2	
6	gut	62.97	ND	ND	9.23 ± 2.30	ND	38.4 ± 12.5	0.477
	flesh	64.59	ND	ND	4.30 ± 2.61	ND	31.0 ± 21.1	
8	gut	65.60	ND	ND	15.5 ± 6.0	31.1 ± 7.4	110 ± 44	0.018
	flesh	69.50	ND	ND	13.2 ± 5.4	ND	26.0 ± 4.9	

[a] Compound **1**, 2-bromophenol; compound **2**, 4-bromophenol; compound **3**, 2,4-dibromophenol; compound **4**, 2,6-dibromophenol; compound **5**, 2,4,6-tribromophenol. [b] *p* value of total bromophenol content (TBC) in gut and in flesh compared by Student's *t* test. [c] Not detected. [d] Mean bromophenol concentration (ng/g of dry wt) ± standard deviation (SD) of three replicates each from different fish samples.

Table 3. Distribution of Bromophenols in the Gut and Flesh of Silver Seabream Fed with Fish Feed Containing 30% *P. arborescens* for 8 Weeks

			bromophenol concentration (ng/g of dry wt)					
week	sample	moisture (%)	compound **1**[a]	compound **2**[a]	compound **3**[a]	compound **4**[a]	compound **5**[a]	*p* value[b]
0			same values as shown in **Table 2**, week 0					
2	gut	65.67	23.6 ± 17.7[d]	370 ± 139	92.6 ± 31.1	ND[c]	423 ± 236	<0.001
	flesh	69.11	3.19 ± 2.06	ND	7.77 ± 2.87	ND	36.1 ± 19.8	
4	gut	68.10	56.6 ± 29.6	ND	286 ± 106	ND	895 ± 443	0.028
	flesh	69.91	9.60 ± 8.57	ND	21.4 ± 12.4	ND	75.1 ± 45.7	
6	gut	68.77	37.8 ± 24.9	ND	101 ± 29	ND	289 ± 115	0.025
	flesh	69.88	6.02 ± 2.78	ND	11.1 ± 6.4	ND	69.7 ± 35.8	
8	gut	71.43	12.6 ± 4.3	ND	37.9 ± 7.5	ND	123 ± 12	0.001
	flesh	72.49	8.06 ± 0.66	ND	10.7 ± 3.1	ND	57.5 ± 8.8	

[a] Compound **1**, 2-bromophenol; **2**, 4-bromophenol; **3**, 2,4-dibromophenol; **4**, 2,6-dibromophenol; **5**, 2,4,6-tribromophenol. [b] *p* value of total bromophenol content (TBC) in gut and flesh compared by Student's *t* test. [c] Not detected. [d] Mean bromophenol concentration (ng/g of dry wt) ± standard deviation (SD) of three replicates each from different fish samples.

Table 4. Distribution of Bromophenols in the Gut and Flesh of Silver Seabream Fed with Fish Feed Containing 30% *S. siliquastrum* for 8 Weeks

			bromophenol concentration (ng/g of dry wt)					
week	sample	moisture (%)	compound **1**[a]	compound **2**[a]	compound **3**[a]	compound **4**[a]	compound **5**[a]	*p* value[b]
0			same values as shown in **Table 2**, week 0					
2	gut	66.90	11.9 ± 6.3[c]	ND[d]	63.4 ± 19.2	ND	502 ± 267	0.044
	flesh	69.52	ND	ND	13.8 ± 3.2	ND	68.5 ± 47.6	
4	gut	73.83	ND	ND	219 ± 39	ND	310 ± 38	0.001
	flesh	75.77	ND	ND	8.40 ± 3.69	ND	51.7 ± 19.6	
6	gut	67.63	19.3 ± 4.7	ND	31.3 ± 4.5	32.7 ± 12.5	126 ± 16	0.020
	flesh	69.91	ND	ND	32.3 ± 23.4	ND	74.5 ± 15.4	
8	gut	67.10	25.9 ± 9.9	ND	74.8 ± 49.0	75.4 ± 72.5	330 ± 115	0.057
	flesh	71.37	11.9 ± 6.2	ND	16.9 ± 2.9	ND	105 ± 28	

[a] Compound **1**, 2-bromophenol; **2**, 4-bromophenol; **3**, 2,4-dibromophenol; **4**, 2,6-dibromophenol; **5**, 2,4,6-tribromophenol. [b] *p* value of total bromophenol content (TBC) in gut and flesh compared by Student's *t* test. [c] Mean bromophenol concentration (ng/g of dry wt) ± standard deviation (SD) of three replicates each from different fish samples. [d] Not detected.

detected in the fish flesh in this group increased from 39.0 ng/g at week 0 to 134 ng/g at week 8.

The TBC in the fish feed containing 30% *S. siliquastrum* was 340 ng/g, whereas that of the fish feed containing 30% *P. arborescens* was 132 ng/g. The TBC in the former was 2.58 times higher than that of the latter. Relatively higher levels of bromophenols were provided by the feed containing 30% *S. siliquastrum* than by the *P. arborescens* feed, and apparently these were more efficiently absorbed by the fish. As a result, the effect of feeding cultivated fish with feed containing high bromophenol contents would be more significant to the amount of TBC found at the end of the feeding period.

Both compounds **3** and **5** were detected in all of the samples (**Tables 2**−**4**), and the latter one was present in the highest concentrations among the five bromophenols in most of the samples. These results were consistent with the bromophenol contents found in other seafood previously analyzed (*14*, *15*). On the other hand, compound **4**, which could be found in most of the seafood samples in the previous survey (*14*), was not accumulated in the current fish sample. Such a phenomenon could be explained by the octanol/water partition coefficients (i.e., Log *P* values) of the bromophenols (*13*). According to Poels et al., compounds with Log $P \geq 3.0$ would favor bioconcentrations in the marine animal (*13*, *28*). Because the

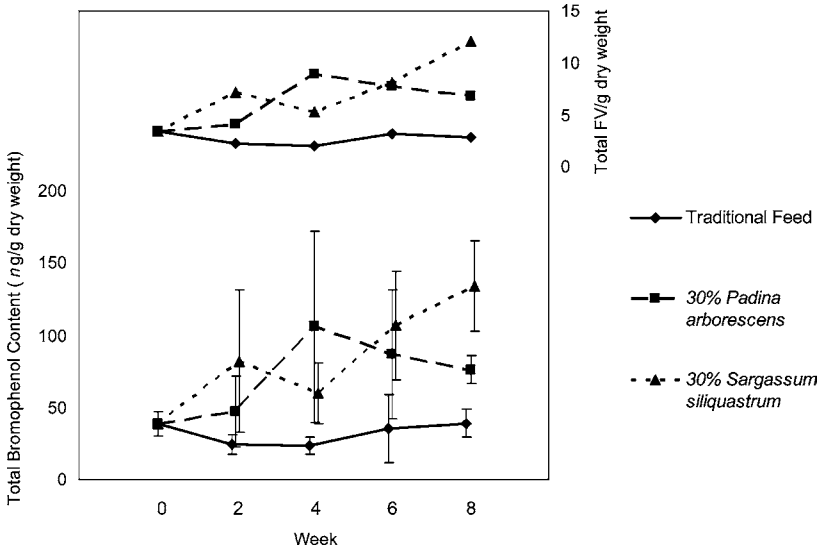

Figure 1. Total flavor values (total FVs) and mean (± SD) total bromophenol contents (TBCs) of the flesh of silver seabream fed with different fish feeds for 8 weeks.

Log *P* values of compounds **3** and **5** were 3.00 and 3.74, respectively (*13*), they were likely to accumulate in the flesh of the animal. However, the rest of the bromophenols having Log $P < 3.0$ were less likely to have similar accumulation (*13*, *28*). The accumulation of these compounds might require some dietary sources that contained relatively high concentrations of them. In the current study, the concentrations of compounds **3** and **5** in the experimental feeds were much higher than the rest of the bromophenols. These two compounds could be readily absorbed from the feeds. As a result, compounds **3** and **5** were often the most abundant bromophenols found in a fish.

In the current study, it was found that the bromophenol content in the fish flesh could be increased by the algae-containing fish feed, and the amount of TBC was relatively high. In a study by Whitfield et al. (*7*), prawns fed with feed containing 10% freeze-dried *Ulva lactuca* for 2 weeks had a trace amount of TBC in their bodies. Such a quantitative difference in TBC might be due to the different animal models used in the feeding experiment. Silver seabream (*Sparus sarba*) was used in the current study, whereas prawns (*Penaeus monodon*) were used in the other study. Also, *S. siliquastrum* was used in this study, but *U. lactuca* was used in the other study to produce the algae-containing feeds. Finally, the length of feeding periods was not the same in the two studies (8 vs 2 weeks). It seems that the accumulation of bromophenols in the animal flesh was a rather slow process. A 2-week feeding period might not be enough for the bromophenols to accumulate to a significant level. However, a long feeding period of the algae-containing feeds would increase the average production cost of the aquaculture products as the production of such feeds requires some costly and complicated procedures (such as algae collection and freeze-drying).

Flavor Value (FV) and Impact of Bromophenols. Increase in the individual bromophenols in the experiment group might affect the flavor of the fish flesh. For example, the concentrations of compounds **1**, **3**, and **5** in the fish flesh in the 30% *S. siliquastrum* group at week 8 (sample with the highest TBC) were 3.41, 4.84, and 30.1 ng/g of fresh weight, respectively. These concentrations were higher than the reference threshold values in water, which were 3×10^{-2}, 4, and 6×10^{-1} ng/g for compounds **1**, **3**, and **5**, respectively (*6*, *7*, *20*). Because compound **4** was often not detected in the fish flesh, an iodine-like flavor would not have much perceptual impact (*20*). On the other hand, when considering the evaluation flavor concentration reported in marinated whitefish (*22*), to produce the sea salt-like and fish-like flavor, compound **5** alone should have a concentration of at least 10 ng/g (*22*). Compound **5**, being the major bromophenol accumulated in the fish flesh of the experimental group, might dominantly affect the flavor of the cultivated fish with its persistent increase in concentration throughout the feeding period. When compound **5** (0.5 ng/g) was mixed with compound **1** (0.5 ng/g) in marinated whitefish, a richer sea-like and slight iodine-like flavor was perceived (*22*). Therefore, fish samples with bromophenols having concentrations higher than their evaluation flavor concentrations should give a sea-like, sea salt-like, and fish-like flavor (*6*, *8*, *13*, *22*).

To estimate the general effects of changes in bromophenol concentrations on the flavor of the fish samples, FV was calculated using the evaluation flavor concentrations (EFC) found in the literature (*14*, *22*, *23*). The total FVs of the bromophenols in the fish samples are shown in **Figure 1**. Changes in both the total FVs and the TBCs showed similar patterns as the feeding time increased. The total FVs of the control group remained at a relatively constant level that ranged from 2.07 to 3.45. For the 30% *P. arborescens* and 30% *S. siliquastrum* groups, the total FVs fluctuated from 3.45 at week 0 to 6.77 and 12.0 at week 8, respectively. Apparently, as the feeding time increases, the total FVs also increase. This may imply a stronger impact at the end of a longer feeding period.

Sensory Evaluation on the Flesh of the Fish Fed with Different Fish Feeds. Triangle tests were carried out to evaluate the difference in the flavor perception of the fish cultivated with the traditional and modified feeds (**Table 5**). Difference in flavor between the control group and the experimental group fed with feed containing 30% *P. arborescens* for 8 weeks was not found ($p > 0.05$), but similar triangle tests using feed containing 30% *S. siliquastrum* showed a significant difference ($p < 0.05$) (*24*, *25*). This was indicative of a flavor difference between fishes fed with the traditional and the modified (containing 30% *S. siliquastrum*) feeds cultivated for 8 weeks.

Bromophenol contents in the fish flesh fed with modified feed containing 30% *S. siliquastrum* increased to levels sufficient to provide desirable sea salt-like and fish-like flavor (e.g., compound **5** concentration > 10 ng/g of fresh weight) at week 8. Thus, most subjects could differentiate the experimental

Table 5. Results of Triangle Tests on the Flesh of Fish Fed with Different Fish Feeds

group compared	control vs 30% *P. arborescens*[a]	control vs 30% *S. siliquastrum*[b]
correct answer	17	24
incorrect answer	27	20
total	44	44
sig[c]	–	*

[a] Evaluation on the flesh of fish fed with traditional fish feed and that of feed containing 30% *P. arborescens*. [b] Evaluation on the flesh of fish fed with traditional fish feed and that of feed containing 30% *S. siliquastrum*. [c] *, significant difference between groups ($p < 0.05$); –, no significant difference between groups ($p > 0.05$).

from the control fish. Besides, some subjects described the perception as "sweet" and "seafood-like flavor" for the fish flesh in the experimental group. In fact, compound **5** had the highest FV among all of the other bromophenols. The sensorial difference found in the experimental group might be contributed mainly by the increase in the concentration of compound **5**. These results suggest that the effect of the feed containing 30% *S. siliquastrum* on the flavor of aquacultured fish was significant. The flavor quality in the cultivated fish could be improved by feeds containing higher amounts of bromophenols.

Toxicology of Bromophenols. The toxicity of the bromophenols is one of the major concerns when bromophenol-containing fish feed is applied in the aquaculture industry. This issue has been discussed in detail in a review paper written by Boyle et al. (*29*). The authors stated that the oral administrattion toxic levels of bromophenols reported in rodent LD_{50} tests were 2000−5000 times higher than the levels naturally present in the Australian crustaceans (*20*, *29*). Some recent studies were reported to have examined the neurotoxicity, immunotoxicity (*30*), estrogen-like activities (*31*), and differentiation of human neuroblastoma cells in the presence of bromophenols (*32*). Concentrations of bromophenols that cause toxic effects are much higher than that either detected in the fish flesh in the present study or required to produce desirable flavor in seafood. It is known that bromophenols are produced by some marine organisms such as the marine algae and accumulate in many different marine animals such as fish, crustaceans, and mollusks along the food chain (*6*, *8*, *11*−*17*, *18*, *20*). In this study, marine algae were chosen as a fish feed ingredients and as a natural source of bromophenols for improving the fish flavor. It is unlikely that these naturally occurring compounds, which have been found in many different seafoods at levels only sufficient to provide desirable flavor, would cause serious health problems in humans. Moreover, excessively high amounts of bromophenols in seafood would produce an unpleasant iodine-like off-flavor and would be rejected by the consumers long before the toxic level is reached. Nevertheless, acute and subchronic toxicity tests can be carried out by future researchers to confirm the safety of the seafood product containing natural bromophenols.

Conclusion. Low bromophenol concentrations were believed to cause the lack of sea-like flavor in aquacultured animals. Methods were developed and evaluated to enhance the concentrations of bromophenols by increasing the bromophenol content in their diet. Marine algae were chosen as the source of such compounds. Two types of modified fish feeds were developed including (1) fish feed containing 30% *P. arborescens* and 70% traditional fish feed powder and (2) fish feed containing 30% *S. siliquastrum* and 70% traditional fish feed powder. Both types of the modified feeds possessed significantly higher bromophenol concentrations than the traditional feed. Feeds developed were used to cultivate the model fish, *Sparus sarba*. Changes in the bromophenol concentrations were investigated at different times. Only the feed containing 30% *S. siliquastrum* could significantly increase the TBCs in the experimental fish during the 8-week cultivation ($p < 0.05$). Sensory evaluations on the flesh from the experimental fishes indicated that the flavor of the flesh from fish fed with 30% *S. siliquastrum* feed was significantly different when compared with that of fish fed with traditional feed. This study was carried out in limited scope, yet it showed that fish feed containing marine algae could gradually increase the bromophenol contents of the experimental fish. This study could provide a basis for further investigations on the various effects of time, concentration, etc. on the accumulation of bromophenols in cultured fishes, and eventually could benefit the aquaculture industry and satisfy the desire of most consumers to have cultured fish with desirable sea salt-, seafood-, fish-like flavor.

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