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Bromophenol concentrations in fish from Salvador, BA, Brazil

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ABSTRACT

The main objective of this work is to evaluate the occurrence of bromophenols (2-bromophenol, 4-bromophenol, 2, dibromophenol, 2,6-dibromophenol and 2,4,6-tribromophenol), in the flesh and guts in two species of the Lutjanid Family: *Lutjanus synagris* and *Ocyurus chrysurus*. The bromophenols were extracted by steam distillation with pentan ether (7:3 v/v), identified by reverse phase High Performance Liquid Chromatography (HPLC-UV), and quantified the external-standard method. Total bromophenol concentrations were similar in the muscle of both species, rangi from 36 ng g^{-1} to 349 ng g^{-1}. The total bromophenol concentrations in stomach (ranging from 12 ng g^{-1} to 586 ng g^{-} were slightly higher than in muscle. The presence of bromophenol in the muscles of the species under study may occ as a result of their diet. The results of this work are therefore expected to contribute toward a better understanding the path of bromophenol absorption from the fish's stomach to the rest of its body.

Key words: bromophenols, flavor, marine fishes, *Lutjanus* , *Ocyurus* .

INTRODUCTION

Flavor, or the consumer's perception thereof, is an important attribute of the quality of marine fishes and other seafoods (Lindsay 1990, Stansby 1962), and is the first and principal discriminative factor in his evaluation, acceptance, rejection or preference for the product (Boyle et al. 1992a). This fact has led to extensive research in several areas, including agriculture and the food and beverage industry, aimed at putting on the market products of excellent nutritional quality and especially of pleasant flavor (Lindsay 1990).

The success of aquaculture products has been ham-

since many consumers can clearly distinguish the ence between the flavor of cultivated and wild seafoods (Boyle et al. 1992a). Knowledge of the and chemical substances that determine flavor ca tribute significantly to the improvement and exp of aquaculture and to the preservation, storage, and improved quality of seafoods.

However, there is still a paucity of infor about the specific substances that give fishes an seafoods their widely diverse flavors and other differences. In the last few decades, a group of compounds called simple bromophenols has bee sidered the main component of the flavor of

phenol (4-BP), 2,4-dibromophenol (2,4-DBP), 2,6-dibromophenol (2,6-DBP) and 2,4,6-tribromophenol (2,4,6-TBP), (Fig. 1) in water, have very low sensory threshold concentrations in the ng g^{-1} range (Whitfield 1988).

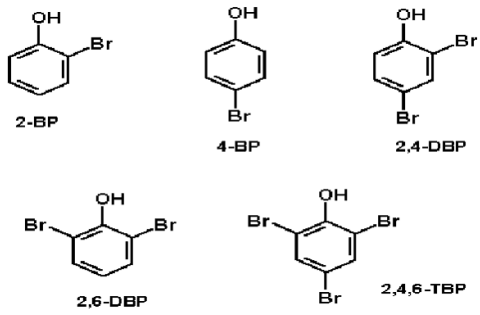


Fig. 1 – Simple bromophenols: 2-bromophenol (2-BP); 4-bromophenol (4-BP); 2,4-dibromophenol (2,4-DP); 2,6-dibromophenol (2,6-DP) and 2,4,6-tribromophenol (2,4,6-TBP).

Bromophenols, which have been found in marine fishes (Boyle et al. 1992a, Whitfield et al. 1998), crustaceans (Chung et al. 2003a, Whitfield et al. 1997, 2002) and mollusks (Boyle et al. 1992a), are strongly associated with pleasant (marine- or ocean-like) or unpleasant (plastic-, medicinal-, disinfectant-, iodoform or iodine-like) flavors, alone or in different combinations and concentrations. Marine food gourmets describe the flavor of some fish species as mildly candy-like and others as marine or oceanic-like, which is characteristic of the presence of bromophenols in different concentrations (Whitfield et al. 1998). Boyle et al. (1992a), who compared four Pacific salmon species (*Oncorhynchus spp*) from marine and freshwater environments, found that the marine species contained 6.1 to 34.8 ng.g^{-1} of total bromophenols while the freshwater species not only contained none of the five bromophenols investigated but also had none of the characteristic oceanic-like flavor. Although 2,4-DBP and especially 2,4,6-TBP have been considered important anthropic pollutants (playing an important role as industrially produced flame retardant and pesticides) (Polo et al. 2006), the presence of bromophenols in these marine organisms has been attributed to their natural diets; in other words, these compounds

According to previous studies, bromophenols have been detected in a variety of other marine organisms such as macroalgae (Chung et al. 2003b, Lee et al. 2007, Pedersén et al. 1974, Phillips and Towers 1981, Whitfield et al. 1992b, 1999a, Xu et al. 2003), polychaetes (Goerke and Weber 1990, 1991, Steward and Lovell 1997, Whitfield et al. 1999b), sponges (Hattori et al. 2001, Unson et al. 1994, Vetter and Janussen 2005) and bryozoans (Whitfield et al. 1999b), which are a major dietary source for many marine organisms including fish (Whitfield et al. 1997, 1998, 1999a, 1999b, Ma et al. 2005).

The flavor of marine fishes varies depending on the location and time of year when they were caught (Whitfield et al. 1995), and the diet of certain fish species can vary considerably according to the availability of alimentary components, which depends on seasonal variations (Whitfield et al. 1998). It is believed that the bromophenols in marine fish come from their natural diet (Whitfield et al. 1998). This hypothesis is strongly supported by the fact that bromophenols have been detected in the stomach content and the flesh, with higher concentrations in the former (Chung et al. 2003a, Whitfield et al. 1995), allied to the fact that benthic carnivorous fishes feeding on polychaetes and herbivorous fishes feeding on macroalgae have a strong flavor while piscivorous fishes feeding primarily on other fish do not contain these bromophenols (Whitfield et al. 1994, 1995, 1996).

However, the identification of specific organisms that may introduce bromophenols into the diet of marine fish requires a broader investigation (Whitfield et al. 1996, 1998). Some reports suggest that fish do not accumulate bromophenols, but gradually metabolize or excrete them (Whitfield et al. 1992b, Anthoni et al. 1990). Therefore, studies are needed to establish the route whereby bromophenols are transferred to different fish species.

Fish is a staple food among coastal populations in the state of Bahia. Standing out among the most popular fish species are the members of the Lutjanidae family (popularly known as "red"), which are highly valued and widely accepted by the consumer market of the city of

species their particular flavor (Santos et al. 2001, Veloso et al. 2001). The purpose of this work was therefore to evaluate the occurrence of bromophenols in the flesh and guts (stomach content and pyloric ceca or appendices) of two species of the Lutjanidae family, *Lutjanus synagris* and *Ocyurus chrysurus*, and to identify the probable alimentary source of these bromophenols in these species.

MATERIALS AND METHODS

Solvents, Reagents and Standards

The bromophenols standards were obtained from Aldrich (Milwaukee, WI), in purities ranging from 97 to 99%. Purified water was obtained by distillation and filtration through an E-pure Alltech system (Deerfield, IL). Acetonitrile (HPLC grade) was obtained from Aldrich and filtered through a 0.45μm membrane. The other reagents (pentane, diethyl ether, sodium chloride and sulfuric acid) were of analytical grade produced by Merck (Darmstadt, Germany).

Collection and Preparation of Samples

Two fish species of the Lutjanidae family were studied: *Lutjanus synagris* and *Ocyurus chrysurus*. Fresh fish caught in the coastal waters of Bahia, Brazil (13°01′S and 38°31′W), were purchased from commercial fishermen. Three specimens of each species were purchased, with an average weight of 1.0 kg and 30 cm length. In the laboratory, the fish were washed in distilled water, gutted, and the flesh was separated from the heads, tails, and backbone.

The flesh was washed in a saturated NaCl solution and then blended into a smooth purée in a food processor (Triton-Arno). Samples of puréed flesh (in portions of 250 g) were stored in sealed polyethylene bags at −15°C prior to their analysis.

The guts (full stomach contents and pyloric ceca) were dissected, weighed and stored in a refrigerator (0°C). Subsequently, an incision was made in each stomach to check the types of food items and classify them according to the highest taxon. This material and the pyloric ceca were then blended together into a purée, stored in sealed polyethylene bags at −15°C u
quired for analysis.

Preparation of Bromophenol Standards and Calibration Solutions

Stock solutions (100 mg mL^{-1}) were prepared
weighing each bromophenol and then dissolvi
acetonitrile. The standard calibration solutions we
pared by diluting the bromophenol stock solut
acetonitrile, in concentrations of 200 to 1000 ng
The resulting solutions were stored at 4°C in dar
flasks. The standard solutions were prepared
once a week. More detailed information is av
elsewhere (Silva et al. 2005).

Extraction of Bromophenols

Representative samples of flesh (250 g) o
(30 g) were homogenized separately in purifi
ter (1000 mL) and the homogenates, acidified
1 with 10 mol L^{-1} sulfuric acid, were left to s
ambient temperature (26 ± 3°C) for about 12 h
volatile components were isolated by combined
uous hydrodistillation-solvent extraction with 2
pentane/diethyl ether (6:4) using a modified Cle
apparatus (Vidrosel Ltda, Brazil) adapted for thi
(Silva et al. 2005). The hydrodistillation proce
completed after 4 hours, and the pH of the residu
measured. The collected extract was concentra
der a gentle stream of ultrahigh purity (99.999
trogen. The concentrated extract was then disso
acetonitrile (500μL) and stored in 2 mL dark gla
at −15°C until it was analyzed.

Separation of Compounds

A PerkinElmer series 200 liquid chromatograph
ped with a Rheodyne (Cotati, California, USA)
tor valve with a 20μL sample loop and a Perki
UV-visible detector were used. Chromatographi
ration of bromophenols was performed in a LiChr
100 Rp-18 (244 mm × 4.4 mm I.D., 5μm; Merc
umn coupled to a LiChrospher guard column wi
ilar characteristics (14 mm × 4 mm I.D.; Merck

perature. The detection was performed at 286nm, where the 2-BP, 4-BP, 2,4- and 2,6-DBP show significant absorptive values and at 297nm for 2,4,6-TBP.

TABLE I
RP-HPLC solvent gradient varying the mobile phase water-acetonitrile composition used in the bromophenols separation.

Time (min)	Solution with 45% CH_3CN : 55% H_2O	% CH_3CN
0	100	0
10	100	0
20	45	55
22	45	55
27	100	0

Analytical Calibration Curve and Quantification

Analytical calibration curves were built by plotting the observed peak height against the amount of injected bromophenol (200 to 1000 ng mL^{-1}). Quantification of the bromophenols was performed using an external standard ($n = 5$), by measuring the peak height at each retention time calculated from the calibration curve. Spikes of each bromophenol were produced in the samples to ascertain the exact retention times. The total bromophenol content (TBC), used in the literature to reflect the impact of flavor produced by all the bromophenols contained in a food (Whitfield et al. 1995, 1996, 1998), was calculated by incrementing the concentration (ng g^{-1}) of all the simple bromophenols detected in a sample.

RESULTS AND DISCUSSION

The items found in the stomach contents of the specimens indicated that the main taxonomic categories were Crustacea (Decapoda) and Teleostei, with a smaller proportion of representatives of the taxon Mollusca. When full, the pyloric appendices or pyloric ceca presented a stomach fluid, probably due to a previous digestion. The five simple bromophenols were found to occur in the muscle and stomach of the species under study. Tables II and III list the concentration of each of the five

The highest bromophenol concentrations in the two fish species involved 2,4-DBP and 2,4,6-TBP, which were present in concentrations exceeding 110 ng g^{-1} in muscle and stomach (Tables II and III). 2-BF and 4-BF showed the lowest concentrations.

Total bromophenol concentrations showed a similar predominance in the muscle of both species, ranging from 36 ng g^{-1} to 349 ng g^{-1} (Table II). The total concentrations in the stomach (ranging from 12 ng g^{-1} to 586 ng g^{-1}) (Table III) were slightly higher than in the muscle.

The results presented in Table II are consistent with those found by Whitfield (1998, 1999), Chung et al. (2003a) and Silva et al. (2005) in marine fish. Those authors reported high total bromophenol concentrations (2.72 to 462 ng g^{-1}), especially for 2,4,6-TBP, which was the most frequent and abundant bromophenol (Chung et al. 2003a, Silva et al. 2005). The concentrations of 2,6-DBP, 2,4-DBP and 2,4,6-TBP determined in seven fish species (*Branchiostegus wardi*, *Girella tricuspidata*, *Nemadactylus douglassi*, *Rhabdosargus sarba*, *Acanthopagrus australis*, *Meuschenia trachylepis*, and *Pseudorhombus jenynsii*) (Parejo et al. 2004) fell within the range of 0.4-18 ng g^{-1}, 112-150 ng g^{-1} and 5.7-170 ng g^{-1}, respectively. The concentrations reported here (Table II) show a similar predominance of these three bromophenols in the two fish species studied.

In the muscle of the species *Lutjanus synagris*, 2,4-DBF (110 ng g^{-1}) and 2,4,6-TBF (171 ng g^{-1}) stood out, particularly in the specimens collected in winter, as indicated in Table II and showed in the Figure 2. The muscle of the species *Ocyurus chrysurus* showed similar results, i.e., 2,4-DBF (158 ng g^{-1}) and 2,4,6-TBF (119 ng g^{-1}) (Table II). The analysis of bromophenol in the stomach also showed a predominance of 2,4-DBF and 2,4,6-TBF in both species, although the species *O. chrysurus* showed significant concentrations of 2,6-DBF as well (Table III). The higher bromophenol concentrations found in the specimens collected in winter were consistent with the greater abundance and weight of *L. synagris* and *O. chrysurus* (Costa et al. 2002) in autumn and winter. Low temperature seasons are asso-

TABLE II
Range of bromophenol concentrations (ng g^{-1}) found in muscle.

Fish (N=15)	2-BF	4-BF	2,4-DBF	2,6-DBF	2,4,6-TBF	TBC
Lutjanus synagris	1–35	nd–20	11–110	3–29	15–171	41–349
Ocyurus chrysurus	0.20–19	nd–14	7–158	nd–28	6–119	36–299

2-BF: 2-bromophenol, 4-BF: 4-bromophenol, 2,4-DBF: 2,4-dibromophenol, 2,6-DBF: 2,6-dibromopheno; 2,4,6-TBF: 2,4,6-tribromophenol; TBC = Total Bromophenol Concentration; nd = not detected.

TABLE III
Range of bromophenol concentrations (ng g^{-1}) in stomach.

Fish (N=9)	2-BF	4-BF	2,4-DBF	2,6-DBF	2,4,6-TBF	TBC
Lutjanus synagris	nd–6	nd–11	7–247	nd–27	3–104	12–396
Ocyurus chrysurus	nd–14	nd–8	98–372	nd–214	nd–55	179–586

2-BF: 2-bromophenol, 4-BF: 4-bromophenol, 2,4-DBF: 2,4-dibromophenol, 2,6-DBF: 2,6-dibromophenol; 2,4,6-TBF: 2,4,6-tribromophenol; TBC = Total Bromophenol Concentration; nd = not detected.

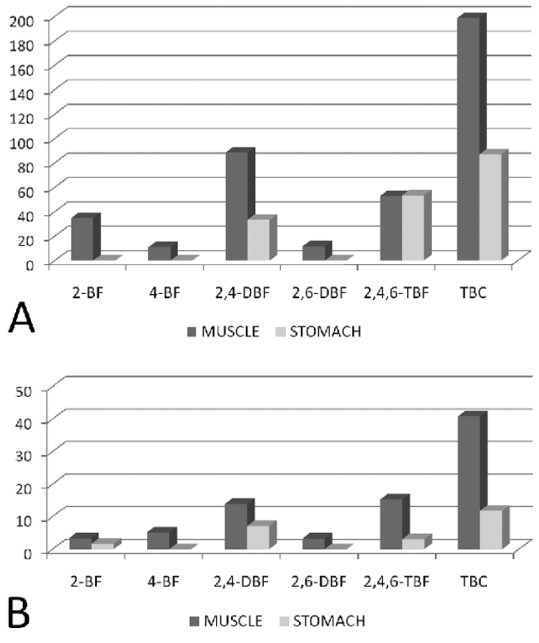


Fig. 2 – Bromophenol concentrations and TBCs in the muscle and stomach of *L. synagris* collected in winter (A) and summer time (B).

Figures 3 and 4 indicate, respectively, the variation in average bromophenol concentrations and TBCs in the muscle (15 samples) and stomach (9 samples) of

similar in the muscle and stomach (Fig. 3). In c
O. chrysurus showed substantial differences, esp
in 2,4-BF, 2,6-BF and TBF (Fig. 4), which were
predominantly in the stomach.

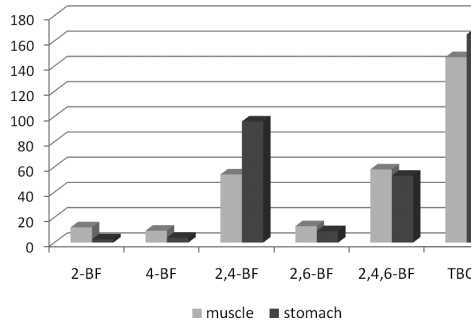
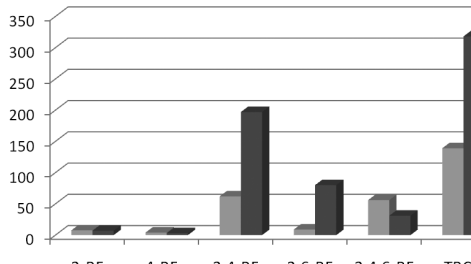


Fig. 3 – Average bromophenol concentrations and TBCs in th
and stomach of *L. synagris*.



The predominant items in the diet of *L. synagris* and *O. chrysurus* are crustaceans (mainly Decapoda) and fish, and a smaller proportion of mollusks and polychaetes (Costa et al. 2002, Druzhinin 1970, Szpilman 1991, Filho 1994, La Morinière et al. 2003). With the exception of polychaetes, the stomach contents of the specimens collected were compatible with those reported in the literature. Thus, the bromophenol found in the muscle of the species under study may come from their diets.

The route of bromophenol absorption from the fish's stomach to the rest of its body, as well as its physiological roles, is still unknown (Boyle et al. 1992b, Chung et al. 2003b). Additional research is therefore necessary to clarify such questions, whose answers are of paramount importance for aquaculturists.

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RESUMO

O principal objetivo do presente trabalho foi o estudo de bromofenóis (2-bromofenol, 4-bromofenol, 2,4-dibromofenol, 2,6-dibromofenol and 2,4,6-tribromofenol), no músculo e estômago de duas espécies de peixes da Familia Lutjanidae: *Lutjanus synagris* e *Ocyurus chrysurus*. Os bromofenóis foram extraídos através de destilação por arraste a vapor com pentano-éter (7:3 v/v), analisados por Cromatografia Líquida de Alta Eficiência e quantificados por padronização externa. As concentrações totais de bromofenóis no músculo de ambas as espécies foram similares e estiveram na faixa de 36 ng g^{-1} a 349 ng g^{-1}. As concentrações totais de bromofenóis no estômago (na faixa de 12 ng g^{-1} a 586 ng g^{-1}) foram mais altas que no músculo. A presença de bromofenóis no músculo das espécies estudadas pode ter origem na dieta. Os resultados deste trabalho contribuirão para o melhor entendimento das rotas de

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