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, 2,4-dibromophenol, 2,6-dibromophenol and 2,4,6-tribromophenol), in the flesh and guts in two species of the Lutjanidae Family: Lutjanus synagris and Ocyurus chrysurus. The bromophenols were extracted by steam distillation with pentane-ether (7:3 v/v), identified by reverse phase High Performance Liquid Chromatography (HPLC-UV), and quantified by the external-standard method. Total bromophenol concentrations were similar in the muscle of both species, ranging 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⁻¹) were slightly higher than in muscle. The presence of bromophenol in the muscles of the species under study may occur as a result of their diet. The results of this work are therefore expected to contribute toward a better understanding of 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 hampered by problems relating to the quality of their flavor, since many consumers can clearly distinguish the difference between the flavor of cultivated and wild harvest seafoods (Boyle et al. 1992a). Knowledge of the factors and chemical substances that determine flavor can contribute significantly to the improvement and expansion of aquaculture and to the preservation, storage, control and improved quality of seafoods.

However, there is still a paucity of information about the specific substances that give fishes and other seafoods their widely diverse flavors and other subtle differences. In the last few decades, a group of organic compounds called simple bromophenols has been considered the main component of the flavor of several seafoods (Boyle et al. 1992b, 1993, Silva et al. 2007, Whitfield et al. 1992a, Whitfield 1988). These compounds, including 2-bromophenol (2-BP), 4-bromo-
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-bromo-
phenol (4-BP); 2,4-dibromophenol (2,4-DBP); 2,6-dibromophenol (2,6-
DBP) 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), crus-
and mollusks (Boyle et al. 1992a), are strongly associ-
ated with pleasant (marine- or ocean-like) or unpleasant
(plastic-, medicinal-, disinfectant-, iodoform or iodine-
like) flavors, alone or in different combinations and con-
centrations. 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 com-
pared 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 to-
tal bromophenols while the freshwater species not only
contained none of the five bromophenols investigated
but also had none of the characteristic oceanic-like fla-
vor. 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 bro-
mophenols in these marine organisms has been attributed
to their natural diets; in other words, these compounds
may come from other species in the food chain. De-
spite of their importance does not exists limit value to
the bromophenol content in marine species.

According to previous studies, bromophenols have
been detected in a variety of other marine organ-
isms such as macroalgae (Chung et al. 2003b, Lee et al.
2007, Pedersen et al. 1974, Phillips and Towers 1981,
Whitfield et al. 1992b, 1999a, Xu et al. 2003), poly-
chaetes (Goerke and Weber 1990, 1991, Steward and
Lovell 1997, Whitfield et al. 1999b), sponges (Hattori et
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 de-
tected in the stomach content and the flesh, with higher
concentrations in the former (Chung et al. 2003a, Whit-
field 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 piscivo-
rorous fishes feeding primarily on other fish do not contain
However, the identification of specific organisms
that may introduce bromophenols into the diet of ma-
rine fish requires a broader investigation (Whitfield et
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 popu-
lar 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
Salvador. Nevertheless, few studies have focused on the
chemistry of these species, particularly with regard to
the volatile organic compounds (VOCs) that give these

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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 bromophenol 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 0.45 μm 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 until required for analysis.

**Preparation of Bromophenol Standards and Calibration Solutions**

Stock solutions (100 mg mL⁻¹) were prepared by first weighing each bromophenol and then dissolving it in acetonitrile. The standard calibration solutions were prepared by diluting the bromophenol stock solutions in acetonitrile, in concentrations of 200 to 1000 ng mL⁻¹. The resulting solutions were stored at 4°C in dark glass flasks. The standard solutions were prepared at least once a week. More detailed information is available elsewhere (Silva et al. 2005).

**Extraction of Bromophenols**

Representative samples of flesh (250 g) or guts (30 g) were homogenized separately in purified water (1000 mL) and the homogenates, acidified to pH 1 with 10 mol L⁻¹ sulfuric acid, were left to stand at ambient temperature (26 ± 3°C) for about 12 h. The volatile components were isolated by combined continuous hydrodistillation-solvent extraction with 2 mL of pentane/diethyl ether (6:4) using a modified Clevenger apparatus (Vidrosel Ltda, Brazil) adapted for this study (Silva et al. 2005). The hydrodistillation process was completed after 4 hours, and the pH of the residues was measured. The collected extract was concentrated under a gentle stream of ultrahigh purity (99.999%) nitrogen. The concentrated extract was then dissolved in acetonitrile (500 μL) and stored in 2 mL dark glass vials at −15°C until it was analyzed.

**Separation of Compounds**

A PerkinElmer series 200 liquid chromatograph equipped with a Rheodyne (Cotati, California, USA) injector valve with a 20 μL sample loop and a PerkinElmer UV-visible detector were used. Chromatographic separation of bromophenols was performed in a LiChroChrom 100 Rp-18 (244 mm × 4.4 mm I.D., 5 μm; Merck) column coupled to a LiChrospher guard column with similar characteristics (14 mm × 4 mm I.D.; Merck). The mobile phase vacuum-degassed in a sonicator was a mixture of water:acetonitrile pumped in gradient mode (Table I) at a flow rate of 1.0 mL min⁻¹ at ambient temp-
The detection was performed at 286 nm, where the 2-BP, 4-BP, 2,4- and 2,6-DBP show significant absorptive values and at 297 nm for 2,4,6-TBP.

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

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution with 45% CH$_3$CN : 55% H$_2$O</th>
<th>% CH$_3$CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>22</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>27</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

### RESULTS AND DISCUSSION

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 associated with the growth cycle of marine species (Chung et al. 2003a, b). The abundance and weight, as well the bromophenol content, decrease in summer.

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TABLE II
Range of bromophenol concentrations (ng g⁻¹) found in muscle.

<table>
<thead>
<tr>
<th>Fish</th>
<th>2-BF</th>
<th>4-BF</th>
<th>2,4-DBF</th>
<th>2,6-DBF</th>
<th>2,4,6-TBF</th>
<th>TBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ocyurus chrysurus</em></td>
<td>0.20–19</td>
<td>nd–14</td>
<td>7–158</td>
<td>nd–28</td>
<td>6–119</td>
<td>36–299</td>
</tr>
</tbody>
</table>

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 the species *L. synagris* and *O. chrysurus* during the period of this study. With the exception of 2,4-BF, the bromophenol pattern found for *Lutjanus synagris* was similar in the muscle and stomach (Fig. 3). In contrast, *O. chrysurus* showed substantial differences, especially in 2,4-BF, 2,6-BF and TBF (Fig. 4), which were found predominantly in the stomach.

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

Fig. 4 – Average bromophenol concentrations and TBCs in the muscle and stomach of *O. chrysurus*.
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, 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 e 2,4,6-tribromofenol), no músculo e estômagos de duas espécies de peixes da Família 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 ambos as espécies foram similares e estiveram na faixa de 36 ng g⁻¹ à 349 ng g⁻¹. As concentrações totais de bromofenóis no estômagos (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 absorção de bromofenóis nos peixes.


REFERENCES


LA MORINIÈRE EC, PULLUX JIA, NAGELKERKEN I AND VAN DER YELDE G. 2003. Diet shifts of Caribbean grunts (Haemulidae) and snappers (Lutjanidae) and the relation with nursery-to-coral reef migrations. Estuar Coast Shelf S 57: 1–11.
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