




# Impact of antioxidant herbal salts on the lipid fraction, acceptability and consumption intent of roasted Dolphinfish

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## Abstract

The impact of herbal salt as a natural antioxidant on the lipids of roasted Dolphinfish (*Coryphaena hippurus* Linnaeus, 1758), as well as in the sensory characteristics of this food was evaluated after using refined or herbal salts, by analyzing the differences in its fatty acid contents, thiobarbituric acid reactive substances (TBARS), product acceptability and consumption intent. Centesimal composition, total phenols and characterization of phenolic compounds were also determined. The cooking caused a significant increase ( $p < 0.05$ ) in the protein, lipid, ash, whose percentages were higher in fish salt roasted with herbs, compared to fish roasted with refined salt, except for ash. Rutin phenolic compound presented higher concentrations in fish treated with the three herbs. After cooking, the total monounsaturated and polyunsaturated fatty acids of herbal salt-roasted Dolphinfish increased by 128% and 109% compared to refined salt-roasted. TBARS values for salt-roasted gold were 558% higher than *in natura* and for herbal salt-roasted gold they corresponded to 174%. These results show that the phenolic compounds detected in herbs exerted an antioxidant effect on the preservation of monounsaturated and polyunsaturated fatty acids from fish roasted with herbal salt. Sensory analysis resulted in good acceptability and purchase intent for herbal salt-roasted Dolphinfish.

**Keywords:** fatty acids; centesimal composition; TBARS; phenols; *Coryphaena hippurus*; sensory analysis.

**Practical Application:** This study indicates that herbal salt had an antioxidant effect on the fatty acids of roasted Dolphinfish, especially on the unsaturated ones. It also improved sensory attributes and can be recommended as a substitute of sodium chloride salt, minimizing its consumption. Such results may encourage the preparation of meals and fish products using herbal salt.

## 1 Introduction

Cardiovascular diseases (CVDs) are important causes of death worldwide (Sociedade Brasileira de Cardiologia, 2010), especially the systemic arterial hypertension (SAH) because of its higher prevalence, affecting about 30 to 45% of the population (European Society of Hypertension, 2013). Excessive intake of salt and fat, especially saturated fat, increases the risk of its development (Nilson et al., 2012), and dietary habits are crucial for its prevention (Martelli, 2014).

The World Health Organization (WHO) recommend the consume of 5 g salt or 2 g sodium per day (World Health Organization, 2013), and the herbal salt, *i.e.*, a mixture of refined cooking salt with dehydrated herbs such as oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.) and basil (*Ocimum basilicum* L.), can be used as a salt substitute in food preparation (Ghawi et al., 2014).

Fish are traditionally considered very good for a balanced diet to achieve a healthy life, and based on the fact that they are source of n-3 and n-6 series of polyunsaturated fatty acids (PUFA), especially docosahexaenoic (DHA) and eicosapentaenoic

(EPA) fatty acids (Figueiredo et al., 2015), as well as of high biological value proteins, it is recommended to consume two portions of 140 g of fish per week (Ruxton, 2011). The presence of polyunsaturated products represents health benefits (Santos et al., 2013), however, it makes fish susceptible to lipid oxidation (Brewer, 2011), being still accentuated by cooking, which can develop substances harmful to consumer health (Paglarini & Pollonio, 2015).

Herbal salt may also be an interesting strategy to inhibit the occurrence of lipid oxidation in foods, as they contain many phenolic compounds of antioxidant capacity, favoring consumer health (Elosta et al., 2012).

The dolphinfish, *Coryphaena hippurus* (Linnaeus, 1758), also called “Dorado” in some countries, and “Dourado do Mar” in Brazil, is an oceanic epipelagic species with circum-tropical distribution, and an important fishery resource around the world, with an excellent flavor, suitable for fillet, and widely marketed in Maceio-Alagoas, Brazil. Given the complete lack of information in the literature about the use of herbal salt in

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Dolphinfish culinary preparations, this work aimed to investigate the impact of its addition on lipid oxidation of this baked food, and its acceptability and purchase intention.

## 2 Methodology

### 2.1 Material

Six samples of dorado (*C. hippurus* Linnaeus, 1758) were acquired shortly after fishing in the coast of Alagoas, Brazil, between the geographical coordinates 8°8'12" S and 10°29'12" S, and where the sea water reaches a temperature above 20 °C and presents high and constant salinity (Correia, & Sovierzost, 2005). The samples were packed in plastic bags, kept in polystyrene with ice and immediately taken to the laboratory.

The herbs used for cooking (oregano, rosemary and basil) were purchased at a health food store in Maceió, Alagoas, Brazil. The herbal salt was prepared according to the "Good Practice Guide" of the Brazilian National Agency for Sanitary Vigilance (Brasil, 2014) and consisted of a mixture of each mentioned herb and refined salt, all at a rate of 25% each.

#### *Preparation of fish samples*

Each sample was divided into three 500 g rectangular portions. The raw portions constituted group I. Group II corresponded to the raw portions added with 2.5 g of refined salt, and group III were the portions of raw fish that received 2.5 g of herbal salt. Group II and III samples were roasted in dry heat, with no added fat, for 35 minutes at 220°C. The preparation conditions of the fish samples were standardized through preliminary tests, based on the sensory characteristics of the food. After cooking, the samples were crushed and aliquots were weighed for chemical analysis.

### 2.2 Chemical analysis

#### *Determination of total phenolic compounds in herbs, herbal mixture (rosemary, basil and oregano) and herbal salt*

The samples of oregano, basil and rosemary were macerated, according to Elfalleh et al. (2012) and homogenized at 80% methanol, at a ratio of 1:10 (m: V). The total phenolic contents were measured by spectrophotometry, according to Singleton et al. (1999), modified by Meda et al. (2005). The obtained dry extract was diluted in 80% methanol, obtaining a concentration of 2000 µg/mL, homogenized with 250 µL of the Folin-Ciocalteu reagent, 2 mL of 7.5% calcium carbonate and 6 mL of distilled water and allowed to stand, at room temperature for 2 hours. The absorbance was measured at 750 nm and the results were expressed as gallic acid equivalent (mg gallic acid/100 g sample extract).

#### *High-performance liquid chromatography analysis of phenolic compounds in herbs, herbal mixture (rosemary, basil and oregano) and herbal salt*

The standards used for the chromatographic analysis of phenolic compounds were purchased or from Sigma-Aldrich® either from AcrosOrganics®, respectively: gallic acid, catechol,

vanillic acid, salicylic acid, vanillin, syringaldehyde, coumaric acid, chlorogenic acid, coumarin, rutin, quercetin, kaempferol, caffeic acid. All solvents used for chromatography were of analytical grade; methanol (Panreac®), formic acid (Dinamica®) and ultrapure water obtained from a Milli-Q® system.

The quantitative analysis of phenolic compounds was carried out in a Shimadzu® (Kyoto, Japan) high-performance liquid chromatography (HPLC) system, equipped with quaternary pump model LC-20AT, degasser model DGU-20A 5R, interface model CBM-20A, automatic injector model SIL-20A HT, and detector model SPD-20A. The baseline resolution was obtained using a C18 reverse phase packed column (Agilent - Zorbax Eclipse XDB, 4.6 mm x 250 mm, 5 µm), with the mobile phase for elution of the compounds analyzed using 1% formic acid solution in milli-Q water (Solvent A) and methanol (Solvent B). The method used for quantification was external standardization. The chromatograms were recorded at 290 nm at 33 °C, with a constant flow rate of 0.6 mL min<sup>-1</sup> and 20 µL of injection volume. For the standards, stock solutions using a concentration of 40 mg L<sup>-1</sup> in an aqueous-ethanolic solution (70%), were prepared. For the construction of the analytical curves, dilutions of an intermediate solution were made, containing a mixture of all standards obtained by diluting the previously prepared stock solutions. In this intermediate solution, all standards were at a concentration of 10 mg L<sup>-1</sup>. Samples/standards solutions were filtered through 0.45 µm polyethylene membrane (Milipore®) and injected directly into the chromatographic system, eluting according to the gradient shown in Table 1, totaling a run of 80 min. Each sample/standard was injected three times into the HPLC system to obtain the mean concentrations and retention times. The quantification was performed with the interpolation of the areas of the chromatographic peaks of the samples/standards through the linear function (linear regression of the calibration curve). Thus, the identity of the analytes was confirmed by retention time and peak profile.

The validation of analytical procedures was performed according to Cass & Degani (2011) and the ICH guidelines (International Conference on Harmonization, 2005). The validated parameters were specificity, linearity, accuracy, precision (repeatability and intermediate precision), limit of quantification (LOQ) and limit of detection optical (LOD).

#### *Centesimal composition of the dolphinfish preparations*

The moisture, protein and ash contents were determined in triplicate using the methodologies of the Manual of the Association of Official Analytical Chemists (1997). Total lipid was extracted by Folch et al. (1957) and the lipid content was gravimetrically determined. The caloric value was calculated from the caloric coefficients corresponding to the proteins and lipids (Livesey, 1990).

#### *Fatty acid profile of the dolphinfish preparations*

Aliquots of lipid extracts obtained according to Folch et al. (1957) were converted to methyl esters (Hartman and Lago, 1973) and injected into a SP-2560 fused silica chromatographic column (biscynopropyl polysiloxane) with 100m in length and 0.25 mm

**Table 1.** Solvent injection gradient in the mobile phase of HPLC-system for phenol separation and characterization of the studied samples of herbal products.

TIME	MODULE	ACTION	VALUE
0.01	Pump	B. conc.	7
5.00	Pump	B. conc.	11
10.00	Pump	B. conc.	16
15.00	Pump	B. conc.	25
30.00	Pump	B. conc.	25
34.00	Pump	B. conc.	38
38.00	Pump	B. conc.	50
42.00	Pump	B. conc.	60
46.00	Pump	B. conc.	65
50.00	Pump	B. conc.	70
54.00	Pump	B. conc.	75
58.00	Pump	B. conc.	85
62.00	Pump	B. conc.	25
63.00	Pump	B. conc.	7
66.00	Pump	B. conc.	7
80.00	Controller	Stop	-

in diameter, of a gas chromatograph (GC) (Chromatograph GC Shimadzu® 2010-plus/ software SP-2560 GC solution). The column temperature programming was isothermal at 140 °C for 5 min and then heating at 4 °C/min to 240 °C, remaining at this for 20 min. The vaporizer temperature was 250 °C and the detector temperature was 260 °C, using Helium (1 mL/min) as gas carrier. The sample split ratio was 1/50. The retention time of methyl esters of the samples was compared with the retention time of standard fatty acid methyl esters. The quantification of the fatty acids was done by normalizing the area, expressing the result in mg/100g.

#### *Thiobarbituric Acid Reactive Substances (TBARS) in dolphinfish preparations*

The determination of TBARS was performed by the method of Tarladgis et al. (1964), modified by Gonçalves & Jorge (1998). Absorbance was measured at 538nm by spectrophotometer. Results were expressed in mg malonaldehyde (MDA) per kg TBA reacted sample (Angelo, 1996). The six-point standard curve was performed with concentrations of 1,1,3,3 Tetramethoxypropane (TMP) ranging from  $4,85 \times 10^{-5}$  to  $9,70 \times 10^{-5}$  M.

### 2.3 Sensory analysis of the dolphinfish preparations

#### *Population and ethical aspects*

The profile of the tasters was established through a questionnaire specially developed for the research. Sensory analysis was performed with adolescents, excluding those who were allergic or intolerant to any component used in sample preparation. Those responsible for the adolescents agreed to participate through the

Informed Consent Form (ICF). The study was approved by the Research Ethics Committee (protocol: 51191715.6.0000.5013).

#### *Sample presentation for the participants*

Roasted salt and herbal samples were prepared as set out in methodology. They were then stored in an oven at 70°C until analyzes were performed. 30 g portions of the samples were presented to judges in individual chambers provided with white light (Instituto Adolfo Lutz, 2008).

The acceptability test was performed using a 9-point hedonic scale (Stone & Bleinbaun, 2012), using the attributes appearance, odor, taste, texture and overall quality. The Acceptability Index (AI) % was calculated as:  $AI (\%) = A \times 100/B$ , where, A = average grade obtained for the product, and B = maximum grade given to the product (Fernandes & Salas-Mellado, 2017). The AI% with good repercussion was that  $\geq 70\%$  (Gularte, 2009).

The judges indicated their intention to buy the product through a 5-point hedonic scale, according to Garcia et al. (2009), with extreme limits of: 1 - "certainly would not buy" and 5 - for "certainly would buy". Purchase intent was considered the sum of the "would certainly buy" and "probably buy" points, and the absence of purchase intent was considered the sum of the "certainly would not buy" and "probably would not buy" points.

### 2.4 Statistical analysis

It was planned a completely randomized experimental design with three treatments - *in natura* Dolphinfish (DIn), roasted Dolphinfish treated with herbal salt (DRHS) or with refined salt (DRRS). Data were subjected to analysis of variance (ANOVA), with a significance level of 5%, with subsequent performance of the Tukey parametric test when necessary. Tabulation and data analysis were performed using SPSS® Statistics software, version 17.

## 3 Results and discussions

### *3.1 Total content and types of phenolic compounds in herbs and herbal salt*

Table 2 shows the total phenolic compounds contents of oregano, basil, rosemary and herbal salt. Oregano had a higher content of total phenolic compounds and significant difference ( $p < 0.05$ ) compared to rosemary and basil. Research conducted by Alezandro et al. (2011) also found that oregano contains more phenolic compounds than rosemary and basil. López-Córdoba et al. (2017) found higher values of total phenols in rosemary (197 mg/g) compared to the present study.

**Table 2.** Total phenolic compounds (mg/g) in samples of individual and mixture of herbs.

Samples	Phenolic Compounds
Rosemary	128.19 ( $\pm 5.20$ ) <sup>a</sup>
Oregano	156.08 ( $\pm 5.72$ ) <sup>b</sup>
Basil	134.91 ( $\pm 8.70$ ) <sup>a</sup>
Herbal Mixture (Rosemary, Oregano, Basil)	164.95 ( $\pm 8.80$ ) <sup>b</sup>

Averages of 3 samples in triplicate with their respective standard deviations. Equal letters in the same column do not differ statistically at the 5% probability level by the Tuckey test.

**Table 3.** Determination and quantification of phenolic compounds (mg/L) in samples of individual herbs (rosemary, basil and oregano), their mixture (herbal mix) as well as in herbal salt.

Sample	Vanillic Acid	Caffeic Acid	Coumaric Acid	Coumarin	Salicylic Acid	Rutin	Quercetin	Kaempferol
<b>Rosemary (Average)</b>	*	*	2.638a	*	8.768 <sup>a</sup>	587.041 <sup>a</sup>	6.924 <sup>a</sup>	9.065 <sup>a</sup>
<b>DIn</b>	*	*	± 0.156	*	± 0.310	± 0.880	± 0.308	± 0.102
<b>DR(%)</b>	*	*	5.903	*	3.532	0.150	4.445	1.127
<b>Basil (Average)</b>	1.168 <sup>a</sup>	1.281 <sup>a</sup>	0.978 <sup>b</sup>	0.443 <sup>a</sup>	2.594 <sup>a</sup>	21.355 <sup>b</sup>	0.940 <sup>b</sup>	0.693 <sup>b</sup>
<b>DIn</b>	± 0.086	± 0.048	± 0.084	± 0.005	± 0.637	± 1.754	± 0.614	± 0.047
<b>RDF(%)</b>	7.403	3.758	8.632	1.194	24.553	8.215	65.305	6.836
<b>Oregano (Average)</b>	7.809 <sup>b</sup>	*	1.284 <sup>c</sup>	30.592 <sup>b</sup>	36.649 <sup>b</sup>	506.711 <sup>c</sup>	*	*
<b>DIn</b>	± 0.157	*	± 0.005	± 0.040	± 7.288	± 0.462	*	*
<b>DR(%)</b>	2.013	*	0.384	0.130	19.885	0.091	*	*
<b>Herbs Mix (Average)</b>	9.533 <sup>c</sup>	*	1.191 <sup>c</sup>	20.544 <sup>c</sup>	26.308 <sup>c</sup>	565.967 <sup>d</sup>	*	*
<b>DIn</b>	± 0.921	*	± 0.008	± 0.099	± 4.051	± 7.688	*	*
<b>DR(%)</b>	9.662	*	0.630	0.484	15.398	1.358	*	*
<b>Herbal Salt (Average)</b>	2.436 <sup>d</sup>	1.318 <sup>a</sup>	*	4.898 <sup>d</sup>	6.068 <sup>a</sup>	128.917 <sup>e</sup>	4.375 <sup>c</sup>	*
<b>DIn</b>	± 0.075	± 0.032	*	± 0.014	± 0.077	± 0.244	± 0.485	*
<b>DR(%)</b>	3.070	2.419	*	0.276	1.261	0.189	11.091	*

Averages with respective standard deviations followed by equal letters in the same column do not differ statistically at a 5% probability level, according to Tukey test. Din = *in natura* Dolphinfish; DR = roasted Dolphinfish. \*Not detected.

Alezandro et al. (2011) found in oregano, rosemary and basil contents of phenolic compounds much lower than in the present study.

The content of phenolic compounds present in herbs is related to their antioxidant activity, and there is evidence of a proportional correlation between the amount of total phenolic compounds with antioxidant potential (Embuscado, 2015). The use of natural antioxidants is an important measure to alleviate lipid oxidation in foods because of their ability to sequester or prevent free radical formation (Kulawik et al., 2013). Gök et al. (2011) and Uçák et al. (2011) state that the use of rosemary reduced lipid oxidation in fish and chicken.

Table 3 shows the profile of phenolic compounds of rosemary, basil, oregano, herbal mix and herbal salt. Six phenolic acids and two flavonoid acids were separated, identified and quantified. Coumaric acid, salicylic acid and rutin were identified in rosemary, basil and oregano, and rutin was the phenolic compound with the highest concentrations in all groups.

The values of coumaric acid were detected at low concentrations in the three herbs analyzed, with significant difference ( $p < 0.05$ ) between the samples. These values were lower than those found by Deschamps & Ramos (2002), where the levels for elephant grass samples were 5.87mg/g; and higher than those found by Salgueiro & Castro (2016) for green propolis extract samples, and their findings ranged from 0.31mg/g to 1.92mg/g.

There was a significant difference ( $p < 0.05$ ) between the rutin contents of the three isolated herb samples. Rosemary presented the highest concentrations, contrary to what was observed in the basil sample.

The rutin and quercetin flavonoids are the most important functional components of the phenolic compound class, presenting high pharmacological activities with health beneficial characteristics, such as: antihyperlipidemic, antitumor, antioxidant,

hepatoprotective, antidiabetic, antimicrobial and antiulcer action (Abdel-Raouf et al., 2011; Chua, 2013; Sharma et al., 2013). In the present study, quercetin was detected at low concentrations in rosemary and basil.

Rutin, being one of the most potent flavonoids in the prevention of oxidative damage, has a more significant antilipoperoxidant role, with consequent elevation of HDL-cholesterol and reduction of risk factors for atherosclerosis and CVD (Metodiewa et al., 1997; Rodrigues et al., 2003).

Salicylic acid presented higher concentrations in oregano, differing significantly ( $p < 0.05$ ) compared to basil and rosemary. However, there was no significant difference ( $p < 0.05$ ) between basil and rosemary.

Basil was the only herb in which the presence of all phenolic compounds found in the present study was detected. However, at significantly lower concentrations ( $p < 0.05$ ), compared to the values detected in rosemary and oregano. Güez et al. (2017) found seven phenolic compounds in basil: Caffeic acid, rutin, quercetin, kaempferol, gallic acid, rosmarinic acid and the acid found in the highest concentration, chlorogenic acid. In the present study, chlorogenic acid was not identified in basil, and a distinct behavior was observed, with higher concentration of rutin.

The content of phenolic compounds detected in the herbal mixture (vanillic acid, coumarin, salicylic acid and rutin) was higher than in the herbal salt, differing significantly ( $p < 0.05$ ). This could be explained by the higher oxidizing effect of refined salt (Mariutti & Bragagnolo, 2017) present in the herbal salt composition.

The types of phenolic compounds present in herbs is of paramount importance due to their contribution to human health. Thus, herbs are regarded as functional foods (Elosta et al., 2012; Mendes et al., 2015), especially if added to foods inversely rich in phenolic compounds (flavonoids), reducing the incidence of cardiovascular disease and their associated risk factors (Toh et al., 2013).

### 3.2 Centesimal composition and caloric value of the dolphinfish preparations

The results of the centesimal composition and caloric value of the *in natura* and roasted Dolphinfish treated with herbal salt or refined salt, are shown in Table 4.

Moisture content of fresh Dolphinfish decreased significantly ( $p < 0.05$ ) after roasting with herbal salt and roasting with refined salt, due to the reduction of water in the food by heat treatment (Campo et al., 2013). This change caused a significant increase ( $p < 0.05$ ) in the protein, lipid, ash and caloric values in roast fish compared to the fresh sample. The percentage of ashes was higher ( $p > 0.05$ ) in refined salt-roasted Dolphinfish due

to the higher salt content used in this preparation, as sodium is one of the minerals present in food ashes (Traficante et al., 2010). Dolphinfish is a source of high biological value protein even after roasting and according to Jacquot (1961), it can be classified as low lipid meat since it has less than 2% of this component.

### 3.3 Fatty acid profile of the dolphinfish preparations

Table 5 shows the fatty acid composition of the *in natura* (Din) and roasted Dolphinfish treated with herbal salt (DRHS) or with refined salt (DRRS). Twenty fatty acids were separated, identified and quantified. The predominant fatty acids (FA) found

**Table 4.** Centesimal composition (g/100 g) and caloric value (kcal/100 g) of fresh (*in natura*) and roasted Dolphinfish (treated with herbal salt or with refined salt).

Analytes *	<i>In natura</i>	Roasted with herbal salt	Roasted with refined salt
<b>Moisture</b>	77.52 ( $\pm 0.36$ )a	68.39 ( $\pm 0.27$ )b	69.82 ( $\pm 0.25$ )c
<b>Proteins</b>	20.87 ( $\pm 0.53$ )a	28.94 ( $\pm 0.52$ )b	27.73 ( $\pm 0.19$ )c
<b>Lipids</b>	1.05 ( $\pm 0.07$ )a	1.69 ( $\pm 0.11$ )b	1.27 ( $\pm 0.15$ )c
<b>Ashes</b>	1.28 ( $\pm 0.09$ )a	1.49 ( $\pm 0.14$ )b	1.72 ( $\pm 0.12$ )c
<b>Caloric value (kcal/100 g)</b>	92.95 ( $\pm 2.12$ )a	130.99 ( $\pm 1.79$ )b	122.30 ( $\pm 1.18$ )c

\*Average and standard deviation of 6 samples analyzed in triplicate. Averages followed by the same letter on the same line do not differ statistically at 5% probability by Tukey test.

**Table 5.** Fatty acid profile (mg/100 g) of fresh (*in natura*) and roasted Dolphinfish (treated with herbal salt or with refined salt).

Fatty Acids*	<i>In natura</i>	Roasted with herbal salt	Roasted with refined salt
Mystical (C14: 0)	17.61 ( $\pm 2.87$ )a	30.89 ( $\pm 2.78$ )b	25.69 ( $\pm 4.42$ )b
Pentadecanoic (C15: 0)	51.05 ( $\pm 15.26$ )a	51.85 ( $\pm 27.06$ )a	45.62 ( $\pm 9.48$ )a
Palmitic (C16: 0)	204.87 ( $\pm 26.32$ )a	339.25 ( $\pm 21.46$ )b	253.29 ( $\pm 30.62$ )c
Heptadecanoic (C17: 0)	13.71 ( $\pm 1.58$ )a	20.54 ( $\pm 2.71$ )b	17.82 ( $\pm 1.44$ )b
Stearic (C18: 0)	109.39 ( $\pm 12.71$ )a	180.03 ( $\pm 14.21$ )b	130.54 ( $\pm 11.50$ )c
Archaic (C20: 0)	3.53 ( $\pm 2.05$ )a	6.07 ( $\pm 3.10$ )a	4.64 ( $\pm 2.68$ )a
Palmitoleic (C16: 1n-7)	13.44 ( $\pm 9.18$ )a	19.65 ( $\pm 2.16$ )a	28.01 ( $\pm 4.72$ )a
Cis-10-Heptadecanoic (C 17: 1)	4.57 ( $\pm 1.69$ )a	8.05 ( $\pm 0.96$ )b	6.22 ( $\pm 0.53$ )a
Oleic (C18: 1n-9)	132.63 ( $\pm 17.98$ )a	234.25 ( $\pm 23.80$ )b	156.68 ( $\pm 16.18$ )a
Gadoleic (C20: 1n-11)	6.06 ( $\pm 1.13$ )a	9.67 ( $\pm 1.90$ )b	7.21 ( $\pm 0.66$ )a
Nervonic (C24: 1)	8.02 ( $\pm 1.60$ )a	9.58 ( $\pm 5.01$ )a	12.30 ( $\pm 6.44$ )a
Linoleic (C18: 2n-6)	15.16 ( $\pm 2.56$ )a	40.53 ( $\pm 15.00$ )b	16.42 ( $\pm 1.76$ )a
Linolenic (C 18: 3 n 3)	0.81 ( $\pm 1.42$ )a	4.04 ( $\pm 4.66$ )a	0.89 ( $\pm 2.18$ )a
Eicosadienoic (C20: 2n-6)	6.45 ( $\pm 1.81$ )a	13.05 ( $\pm 4.06$ )a	11.77 ( $\pm 11.97$ )a
Arachidonic (C20: 4n-6)	46.87 ( $\pm 7.99$ )a	55.12 ( $\pm 9.60$ )a	42.41 ( $\pm 20.26$ )a
Eicosapentaenoic Acid (EPA) (C20: 5n-3)	28.90 ( $\pm 4.24$ )a	35.60 ( $\pm 2.65$ )a	28.81 ( $\pm 9.26$ )a
Docosapentaenoic (C22: 5n-3)	15.23 ( $\pm 2.24$ )a	21.64 ( $\pm 4.40$ )a	14.53 ( $\pm 7.61$ )a
Docosahexaenoic (DHA) (C22: 6n3)	270.52 ( $\pm 34.92$ )a	358.89 ( $\pm 31.46$ )b	300.92 ( $\pm 33.92$ )a
Docosatetranoic acid (OTD) (C 22: 4n6)	2.82 ( $\pm 2.45$ )a	1.70 ( $\pm 2.67$ )a	6.61 ( $\pm 6.60$ )a
Elaidic (C18: 1n-9t)	4.44 ( $\pm 1.11$ )a	6.88 ( $\pm 0.91$ )b	5.42 ( $\pm 0.56$ )a
<b>UNIDENTIFIED</b>	83.01 ( $\pm 20.01$ )a	140.99 ( $\pm 36.81$ )a	138.13 ( $\pm 101.93$ )a
ΣSaturated	400 ( $\pm 10.13$ )a	628 ( $\pm 11.8$ )b	482.52 ( $\pm 10.22$ )c
ΣMonounsaturated	164.9 ( $\pm 6.03$ )a	282.1 ( $\pm 10.66$ )b	210.4 ( $\pm 8.1$ )c
ΣPolyunsaturated	386.76 ( $\pm 8.03$ )a	533.65 ( $\pm 9.97$ )b	422.3 ( $\pm 13.07$ )c
Polyunsaturated/ Saturated	0.96	0.84	0.87
Σn-3	315.47 ( $\pm 10.7$ )a	420.17 ( $\pm 10.79$ )b	345.15 ( $\pm 13.24$ )b
Σn-6	71.30 ( $\pm 2.27$ )a	113.48 ( $\pm 16.46$ )a	90.30 ( $\pm 13.16$ )b
Relation n-6/n-3	1:4.42 ( $\pm 0.33$ )a	1:3.70 ( $\pm 0.71$ )a	1:3.82 ( $\pm 0.59$ )a
EPA + DHA	299.42 ( $\pm 19.58$ )a	394.50 ( $\pm 17.05$ )a	329.73 ( $\pm 21.59$ )b

\*Average and standard deviation of 6 samples analyzed in duplicate. Averages followed by the same letter on the same line do not differ statistically at 5% probability by Tukey test.

in the *in* Dolphinfish were docosahexaenoic (DHA), palmitic, oleic, stearic, pentadecanoic, arachidonic, eicosapentaenoic (EPA) and linoleic acids.

After roast, there were significant increases ( $p < 0.05$ ) of lipids in both preparations when compared to the fresh samples, mainly in the content of palmitic and stearic saturated fatty acids (SFA), being significantly higher levels ( $p < 0.05$ ) in the samples of DRHS in comparison with the ones of DRRF. Stearic SFA had a 64% increase in DRHS, while in the DRRS the increase in comparison to the fresh fish was only 19%.

The content of oleic monounsaturated fatty acid (MUFA), DHA and linoleic polyunsaturated fatty acids (PUFA) were significantly higher ( $p < 0.05$ ) in fish roasted with herbal salt than *in natura* and roasted with refined salt. There was no significant difference ( $p < 0.05$ ) between fresh and roasted samples with refined salt. An increase of 77% was observed in oleic MUFA roasting with herbal salt compared to fresh fish, whereas in refined salt roasting it was 18%. Oleic MUFA is referred to as hypolipidemic, as it reduces plasma cholesterol (LDL) levels as well as decreasing the LDL/HDL ratio (Food Ingredients Brasil, 2014).

DHA fatty acid increased by 33% in herbal salt-roasted Dolphinfish, while in refined salt-roasted Dolphinfish, the increase was 11% compared to fresh fish. As fish is a major source of DHA, it has important beneficial effects on the body in preventing atherosclerosis, depression, heart attack (Daley et al., 2010); improvement of retinal and neuronal tissues (Merdzhanova et al., 2012), reduction in blood pressure and glycemic control (Vaz et al., 2014), this result should be valued.

The heat treatment can increase the fatty acid contents by causing a concentration of them (Koubaa et al., 2012). However, in fish salted with herbs salt there was a greater preservation of fatty acids, compared to roast with refined salt. It is inferred that the natural antioxidants present in the herbs were not degraded during heat treatment and exerted an antioxidant effect that minimized the degradation and/or loss of fatty acids, especially unsaturated ones.

The use of salt can be pointed as a factor that contributed to not preserving monounsaturated and polyunsaturated fatty acids in samples roasted with refined salt, due to the catalytic action of the salt induced lipid oxidation reaction (Mariutti & Bragagnolo, 2017).

A significant increase ( $p < 0.05$ ) of 171% in total MUFA was found in DRHS samples compared to the ones of fresh Dolphinfish, and an increase of 75% compared to the DRRS samples. There was a significant ( $p < 0.05$ ) elevation of 79% in total PUFA from the DRHS compared to the DRRS and 109% relative to DIn samples.

In the total of EPA + EPA, there was a significant increase ( $p < 0.05$ ) of 32% and 10%, respectively, in the samples of DRHS and DRRS, when compared to the fresh fish. Between the two forms of preparation, fish baked with herbal salt had 16% higher values.

Lower EPA + DHA results were reported in the lengua 235 mg/100 g and chick fish at 142.98 mg/100 g (Castro González et al., 2013). In grilled and cooked fish, inferior results were also detected (Maulvault, 2009).

The significant ( $p < 0.05$ ) increase of the n-6 linoleic PUFA content in roasted Dolphinfish with herbal salt compared to *in natura* (167%) and roasted Dolphinfish treated with refined salt (59%) may be due to the incorporation of this component, present in high quantities in aromatic herbs (Elosta et al., 2012).

In this study, the ratio values of n-6/n-3 in all samples are in accordance with the recommendation (1:4) (Simopoulos, 2008). The ratio of these nutrients, in fresh fish such as carp and catfish, was 1:2 and 1:1, respectively (Stancheva et al., 2012). The proper relationship between n-6 and n-3 is necessary to avoid an imbalance in eicosanoid synthesis at risk of n-3 deficiency (Merdzhanova et al., 2012). The n-6/n-3 ratio also influences the development of obesity and cardiovascular disease (Vaz et al., 2014).

The PUFA/SFA ratio in fresh, herbal salt and refined salt samples is in accordance with the England Department of Health (England, 1994) recommendation 0.45.

### 3.4 Thiobarbituric Acid Reactive Substances (TBARS) on dolphinfish preparations

Results of TBARS analysis are shown in Table 6. Malonaldehyde was detected in all samples. Fresh fish may undergo oxidative changes soon after death because to the action of bacteria and oxygen, which leads to lipid oxidation (Huss, 1997). This fact was found in the fresh samples of the present study, but the TBARS values detected in Din and DRHS samples are within the limits of fish quality indication (5-8mg MDA/kg) (Ozogul et al., 2011).

There was a significant increase ( $p < 0.05$ ) in TBARS values of 558% and 139% respectively to DIn and DRHS samples in comparison to DRRS, demonstrating an increase in lipid oxidation due to the addition of salt. An increase of 174% in DRHS was found, compared with Din samples. Since malonaldehyde formation in the TBARS test is associated with lipid oxidation (Ganhão et al., 2011), the results show the antioxidant potential of herbs by decreasing the occurrence of high TBARS levels.

**Table 6.** Content of TBARS (mg/kg) of fresh (*in natura*) and roasted Dolphinfish (treated with herbal salt or with refined salt).

Dolphinfish samples	TBARS
<i>In natura</i>	1.90 ( $\pm 0.86$ )a
Roasted with herbal salt	5.22 ( $\pm 2.09$ )a
Roasted with refined salt	12.51 ( $\pm 1.66$ )b

Averages with respective standard deviations of 6 samples in triplicate. Equal letters in the same column do not differ statistically. at 5% probability level by Tukey test. TBARS - Thiobarbituric Acid Reactive Substances.

### 3.5 Sensory analysis of dolphinfish preparations

Eighty judges with an average age of 16.4 ( $\pm$  1.96) years participated in the sensory analysis, most of them female (52.5%). Of these adolescents, 1.3% reported being diagnosed with SAH, 68.8% reported high salt intake, and 46.3% were from processed foods, 17.5% from food preparations and 35% from both. The shapes. 37.5% knew the “herbal salt” and of these 70% already consumed preparations with it. The frequency of fish consumption was 2.5% for daily consumption, 40% 1-2 times a week, 30% once a week and 28.8% occasionally.

The AI% of roasted preparations of Dolphinfish treated with herbal salt (DRHS) and with refined salt (DRRS) were respectively 86.3% and 82.4% for appearance, 86% and 82.2% for odor, 84% and 86% for taste, 79% and 83% for texture and 83% and 84% for overall quality. There was no statistical difference ( $p > 0.05$ ) between the AI% for the several parameters between the tested preparations. Also, DRHS and DRRS preparations triggered purchase intention in 67.5% and 71.7% of the participants who consumed them, respectively, while 28.8% and 21.7% of them were neutral and 3.8% and 6.7% of those reported no intention to acquire the product.

Despite the low frequency of hypertension among adolescents, the high salt intake reported is a matter of concern, due to the need to reduce their intake in early age to prevent the development of hypertension in adulthood (Bibbins-Domingo et al., 2010), as the pathology is strongly related to the excessive consumption of the mineral (Sociedade Portuguesa de Hipertensão, 2014). Aromatic herbs, in addition to improving sensory attributes, can be recommended as important salt substitutes, contributing to a reduction in their consumption.

## 4 Conclusion

Fresh Dolphinfish can be considered a source of high biological value proteins, n-3 (EPA and DHA) and n-6 (arachidonic and linoleic) polyunsaturated fatty acids, n-9 monounsaturated fatty acid (oleic) and saturated fatty acids. The cooking altered the fatty acid profile, being detected higher values of DHA, oleic, linoleic, palmitic and stearic in the fish roasted with herbs salts, showing that the herbal salt was able to inhibit the lipid oxidation process. There was a positive effect of the phenolic compounds of the herbs in the preservation of the fatty acids, as well as the lower TBARS contents of this preparation. Herbal salt-roasted Dolphinfish also had good acceptability and triggered purchase intent. Therefore, it is a healthier preparation, rich in natural antioxidants, with a pleasant taste, with consumer health benefits, preserving the quality of the food.

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