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Effect of *aroeira* (*Schinus terebinthifolius* Raddi) fruit against polyunsaturated fatty acids and cholesterol thermo-oxidation in model systems containing sardine oil (*Sardinella brasiliensis*)

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ABSTRACT

This study evaluated the protective effect of ground *aroeira* (*Schinus terebinthifolius* Raddi) fruit addition against fatty acids and cholesterol oxidation in model systems containing sardine oil (*Sardinella brasiliensis*) during heating (150 and 180 °C). High temperatures reduced the amount of essential polyunsaturated fatty acids and caused the formation of oxidized products. Total cholesterol oxides content increased from 58.9 ± 0.26 to 577.5 ± 2.14 μg/g oil, after heating at 180 °C. However, *aroeira* significantly protected lipids from oxidation. Although the synthetic antioxidant applied as standard (butylated hydroxytoluene) showed greater results, it was used in the maximum concentration permitted by Brazilian legislation (0.01%), suggesting that *aroeira* fruit could be used as a natural antioxidant for the food industry. The protective effect of *aroeira* may be correlated to its antioxidant capacity and the presence of bioactive compounds which were identified by UHPLC-ESI-MS in the *aroeira* extract.

1. Introduction

Marine oils contain high levels of *n*-3 polyunsaturated fatty acids (PUFAs), principally eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), which provide many benefits for human health, such as protection against cardiovascular diseases, depression and rheumatoid arthritis (Abdelhamid et al., 2018; Ghasemi Fard, Wang, Sinclair, Elliott, & Turchini, 2019). These unsaturated compounds are readily susceptible to oxidation when exposed to high temperatures and may be oxidized, affecting food quality (Ferreira et al., 2017; Leal-Castañeda, Hernández-Becerra, Rodríguez-Estrada, & García, 2017; Lira et al., 2017; Saldanha, Benassi, & Bragagnolo, 2008). In addition, fish oil presents great amounts of cholesterol (Saldanha et al., 2008).

High temperatures may induce lipid oxidation, leading to the formation of radicals by reducing the activation energy for the hydrogen abstraction (Barriuso, Ansorena, & Astiasarán, 2017). This oxidation process compromises not only sensorial characteristics but also the nutritional value, resulting in loss of functionality and increase toxicity, mainly due to the formation of cholesterol oxidation products (COPs) (Leal-Castañeda et al., 2017; Zardetto, Barbanti, & Dalla Rosa, 2014). Exogenous COPs obtained from the diet can be harmful due to their cytotoxic, atherogenic, mutagenic, neurodegenerative, and carcinogenic activities (Maldonado-Pereira, Schweiss, Barnaba, & Medina-Meza, 2018).

Regarding the health-compromising effects of COPs, it is crucial to limit the extent of cholesterol oxidation in foods, thus, antioxidants are extensively applied by the food industry. However, due to concerns about the safety and toxicity of synthetic additives such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), GP (propyl gallate), and TBHQ (tert-butylhydroquinone), the use of natural antioxidants is being evaluated as an alternative to control lipid oxidation (de Oliveira et al., 2018; Ferreira et al., 2017; Lorenzo et al., 2018).

Natural sources of antioxidant compounds such as herbs, spices, and fruits have been studied to assess their effects on lipid oxidation and possible use in food processing. *Schinus terebinthifolius* Raddi is a native plant of South America, commonly known as *aroeira* or pink pepper. It is widely found in Brazilian flora and presents a great potential of use as ornamental, culinary and medicinal specie (Carvalho, Melo, Aragão,

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Raffin, & Moura, 2013; Ennigrou, Casabianca, Laarif, Hanchi, & Hosni, 2017). Numerous studies have highlighted the antioxidant properties of *aroeira*, attributed to the presence of bioactive compounds such as tannins, anthocyanins, flavonoids, phenolic acids, and terpenes (Carvalho et al., 2013; Ennigrou et al., 2017; Feuereisen, Zimmermann, Schulze-Kaysers, & Schieber, 2017). However, there is no available study on the protective effect of *S. terebinthifolius* Raddi fruit against oxidation during heating of sardine oil.

Thus, the purpose of this study was to evaluate the inhibitory effect of *aroeira* fruit addition on cholesterol and PUFAs oxidation during heating in model systems containing sardine oil. The oil containing *aroeira* was heated at 150 °C and 180 °C for 7 min, BHT was also employed for comparison. To investigate this effect, thiobarbituric acid reactive substances (TBARs), fatty acids degradation, and cholesterol oxides formation were considered. Furthermore, the total phenolic content of *S. terebinthifolius* Raddi was determined and its extract was analyzed by Ultra-High Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (UHPLC-ESI-MS) aiming to identify the bioactive compounds that may present antioxidant properties.

2. Materials and methods

2.1. Standards, reagents, and solvents

All solvents and reagents were purchased from Vetec (Sigma, São Paulo, Brazil) and from Merck (Darmstadt, Germany). 7-ketocholesterol (7-keto), 7β-hydroxycholesterol (7β-OH), 5,6α-epoxycholesterol (5,6α-Ep), 5,6β-epoxycholesterol (5,6β-Ep), 20α-hydroxycholesterol (20α-OH), 22S-hydroxycholestrol (22S-OH), and 25-hydroxycholesterol (25-OH) were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Cholesterol and 7α-hydroxycholesterol (7α-OH) were acquired from Steraloids (Wilton, N.H., U.S.A.). Undecanoic methyl ester was purchased from Sigma and the standard mixtures of fatty acids were purchased from Supelco TM 37 (FAME Mix 18919, Bellefonte, Pa., U.S.A.). The purity of the standards was at least 95%. High-performance liquid chromatography (HPLC) grade *n*-hexane and 2-propanol were obtained from Vetec (Sigma, São Paulo, Brazil). Formic acid was purchased from Synth (São Paulo, Brazil) and HPLC grade methanol from Merck (Darmstadt, Germany). Deionized water was obtained with a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Aroeira fruit

Schinus terebinthifolius Raddi fruit was collected at Embrapa Agroindústria de Alimentos in Guaratiba, Rio de Janeiro, Brazil, at latitude 23°00′00.3″S and longitude 43°34′56.7″W, in June 2017. The plant material was authenticated and a voucher specimen (RBR 45484) was deposited at the Herbarium of the University Federal Rural of Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil.

The ripe fruit was manually harvested and carefully selected considering its round shape, pink color, firmness and the absence of injuries. Then, it was washed and sanitized with 0.96% sodium hypochlorite. The fresh fruit was dried in an oven with air circulation (Solab, SP, Brazil) for 48 h at 40 °C and ground in a domestic processor (Cadence, Brazil); then packaged and stored at room temperature until analyses.

2.3. Bioactive compounds and antioxidant capacity

2.3.1. Preparation of aroeira fruit extract

Around 2 g of ground *aroeira* were homogenized with 20 mL of methanol/water solution (80:20% v/v). The mixture was sonicated for 30 min in a sonic bath (40 Hz) (Elmasonic P, Elma Schmidbauer GmbH), then centrifuged (NI 1813, Nova Instruments) at 3500 rpm for 5 min at room temperature. The supernatant was transferred to a 50 mL volumetric amber flask and made up to volume with the extracting solution.

2.3.2. Determination of total phenolic

Total phenolics were quantified by the Folin-Ciocalteu method (Swain & Hillis, 1959). The extract (1 mL) was mixed with 10 mL of distilled water and 1 mL of Folin–Ciocalteu reagent. The solution was allowed to react for 3 min, then, 1.5 mL of 10% sodium carbonate solution was added. The mixture was homogenized and incubated for 2 h at room temperature in the dark. The absorbance was determined at 725 nm using a spectrophotometer (Model NOVA 2000 UV). A gallic acid calibration curve was prepared (0-25 μg/mL, $r = 0.9975$) and the results were expressed as mg gallic acid equivalent (GAE) per g of sample.

2.3.3. Identification of bioactive compounds by UHPLC-ESI-MS

Chromatographic analyses were conducted with a UPHLC Acquity chromatographer coupled with a TQD Acquity mass spectrometer (Micromass-Waters), with electrospray ionization (ESI) in the negative and positive ion mode. The conditions were: cone ± 30 V, capillary ± 3.00 kV, desolvation temperature 350 °C, source temperature 150 °C, with data acquisition between *m/z* 100 and 900. MS/MS of selected peaks were acquired via CID with collision energy of 30 V. The chromatographic conditions were: a C18 BEH Waters Acquity (2.1 mm × 50 mm × 1.7 μm) column at 30 °C; solvent A (0.1% formic acid) and solvent B (HPLC grade methanol) at a flow rate of 0.2 mL/min with a linear gradient starting at 5% B and increasing to up 100% methanol in 7.5 min, before holding until 9 min, and returning to the initial conditions, followed by column re-equilibration until 10 min; injection of 5 μL. Compounds were putatively identified by comparison of their product ions with data from literature.

2.4. Fish oil

Sardines (*Sardinella brasilliensis*) were obtained from a store in Rio de Janeiro city, Brazil, in April 2017. The whole sardines were washed and immediately processed in a grinder (Becker, Santa Catarina, Brazil) to obtain a homogeneous mass, which was dried in an oven with air circulation (Solab, São Paulo, Brazil) at 40 °C for 72 h. The oil was obtained from dried sardines by cold extraction, using a mixture of chloroform-methanol-water according to Bligh and Dyer (1959). The oil was stored in amber flasks under nitrogen atmosphere and maintained at room temperature in the dark until the preparation of the model systems.

2.5. Model system: samples preparation and heating

Samples were: fish oil (Control), fish oil with ground *aroeira* fruit 0.2% (GAF0.2), fish oil with ground *aroeira* fruit 0.5% (GAF0.5), and fish oil with BHT 0.01% (BHT). The concentration of BHT (0.01%) was determined according to the maximum content permitted by Brazilian Health Regulatory Agency for oil and fat (ANVISA, 2019). The natural antioxidant addition was based on a study performed by Sancho, de Lima, Costa, Mariutti, and Bragagnolo (2011). There are no studies applying *aroeira* fruit directly in food systems such as fish oil, thus, 0.5% was used as the highest level considering the sensory acceptance for further studies.

Aliquots of 7 mL of oil were homogenized with the additives in a ultrasonic bath (Elmasonic P, Elma Schmidbauer GmbH), according to each treatment, in open glass vials which were then placed in an oven (Quimis, São Paulo, Brazil) previously stabilized at 150 °C and 180 °C. After 7 min, the vials were taken out from the oven and cooled down. The temperatures and heating time were defined in order to model thermal culinary preparations of fish.

2.6. Thiobarbituric acid reactive substances (TBARs) assay

Quantification of the substances reactive with thiobarbituric acid was performed according to the methodology described by Vyncke (1975), with some modifications. Samples (0.15 g) were mixed with 5 mL of 7.5% trichloroacetic acid solution and 5 mL of 2-thiobarbituric acid solution (0.02 M). The mixtures were heated in a boiling water bath for 15 min. Then, the samples were filtered on filter paper and the absorbance measured in a spectrophotometer (model NOVA 2000 UV) at 532 nm. A standard curve was prepared using TEP (1,1,3,3-tetraethoxypropane) and results were expressed as g malonaldehyde/100 g of oil (g MDA/100 g of oil).

2.7. Fatty acids composition

An aliquot of samples (0.05 g) was submitted to transesterification and converted into methyl ester (Zhu, Svendsen, Jaepelt, Moughan, & Rutherfurd, 2011). Methyl esters were analyzed using a gas chromatographer (Shimadzu GC 2010, Tokio, Japan), injection using split mode (1:50), fused silica CP-SIL 88 capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness) (Chrompack, Middelburg, The Netherlands), flame ionization detector, and workstation computer. The chromatographic conditions were: initial temperature of 140 °C for 5 min, then, the temperature increased in a rate of 4 °C/min up to 230 °C (0 min), then 5 °C/min up to 240 °C (18 min). The injector and detector temperatures were stabilized at 250 °C and 280 °C, respectively. Hydrogen was the carrier gas (1 mL/min), and nitrogen was used as the make-up gas (30 mL/min). Identification of the chromatographic peaks of the samples was done by evaluating the retention times of fatty acids methyl esters standards and the quantification was performed by internal standardization, applying the undecanoic methyl ester as standard.

2.8. Cholesterol and cholesterol oxides

The cholesterol and cholesterol oxides were extracted simultaneously by direct saponification of samples (Dionisi, Golay, Aeschlimann, & Fay, 1998). In tubes, 10 mL of potassium hydroxide ethanolic solution (1 M) were added to 0.15 g of sample to perform saponification at room temperature for 22 h in the dark. For the extraction of the unsaponifiable matter, 5 mL of distilled water and 10 mL of hexane were added, then, the mixture was shaken and the hexane fraction was separated. The extraction with 10 mL of hexane was repeated three times (Saldanha, Sawaya, Eberlin, & Bragagnolo, 2006).

Identification and quantification were performed by High-Performance Liquid Chromatography-Mass Spectrometry on a HPLC-MS-PDA (Shimadzu LCMS-2020, Tokyo, Japan), equipped with an autoinjector (SIL-10AF, Shimadzu, Tokyo, Japan), Hypersil Cyano column (250 mm × 4.6 mm) (Thermo Scientific, Massachusetts, EUA), and Photodiode Array Detector (PDA) (SPD-M20A, Shimadzu, Tokyo, Japan). The pump flow was 1 mL/min and the column temperature was 35 °C. MS detection was performed in the positive ion mode with Atmospheric Pressure Chemical Ionization (APCI) ionization. The mobile-phase was *n*-hexane:2-propanol (97:3, v/v) and the chromatograms were obtained in the selective ion monitoring (SIM) mode. The selected ions were: *m/z* 367, 369, 385, 401, and 403. The compounds were determined by comparison of retention times of peaks in samples with those of reference standards and by *m/z*. The PDA detector was used for quantification by external standardization using calibration curves (Saldanha et al., 2006).

2.9. Statistical analyses

All analyses were conducted in triplicate and results were expressed as mean ± standard deviation. One way variance analysis (ANOVA) was used, as well as Tukey test for post hoc means comparison. The statistical analyses were performed using 5% of significance. The relation among variables was evaluated by Pearsońs correlation test as proposed by Teles et al. (2019). Principal Component Analysis (PCA) was carried out to understand Pearsońs correlations and similarities between variables and/or samples. PCA was performed after variable standardization to avoid the influences of the different units. Additionally, Hierarchical Clustering of Principal Components (HCPC) was performed in order to group samples with similar characteristics. All statistical analyses were performed using software R language.

3. Results and discussion

3.1. Phenolic compounds and identification of bioactive compounds by UHPLC-ESI-MS

The total phenolic content of the *aroeira* extract was 3.11 ± 0.26 mg GAE/g. Previous studies have reported varied results. D'Sousa'Costa et al. (2015) evaluated ethanolic extracts of *aroeira* fruit obtained by using a soxhlet apparatus and maceration and found approximately 5.0 and 108 mg GAE/g extract, respectively. Different methods (soxhlet, ultrasound-assisted extraction, supercritical fluid extraction) and solvents (hexane, ethanol, ethyl acetate) were applied by Andrade, Poncelet, and Ferreira (2017), who reported values varying from 2.9 to 60 mg GAE/g extract. Methanolic extract of *aroeira* presented the highest levels, around 270 mg GAE/g extract (Ennigrou et al., 2017).

Although phenolic compounds are the main bioactive components of natural extracts responsible for the antioxidant properties of these materials, plants are food matrixes of diversified and complex composition. Several compounds that may contribute to *aroeira* fruit nutritional value and antioxidant potential were putatively identified by UHPLC-ESI-MS (Table 1) in relation to other studies using ESI-MS/MS. Triterpenes previously found in *aroeira* fruit were identified in this study: masticadienoic acid ($[M-H]^-$ *m/z* 453) and 3*α*-hydroxymasticadienolic (schinol, $[M-H]^-$ *m/z* 455) (da Silva et al., 2018; de Araujo Gomes et al., 2019). In addition, three other compounds (*m/z* 371, 373 and 469) were putatively identified as terpenes in the negative ion mode, in agreement with prior studies (Carneiro et al., 2016; de Araujo Gomes et al., 2019). Linoleic and stearic acids were detected at *m/z* 279 and 283, respectively, both in the negative mode, as previously reported (da Silva et al., 2018; de Araujo Gomes et al., 2019).

Biflavonoids were determined at *m/z* 537, 539 and 541, corresponding to agathisflavone, hinokiflavone and tetrahydroamentoflavone, respectively. The literature describes the identification of tetrahydroamentoflavone and agathisflavone in *S. terebinthifolius* Raddi (da Silva et al., 2018; de Araujo Gomes et al., 2019; Feuereisen et al., 2014). Hinokiflavone was confirmed in the positive ionization mode by Feuereisen et al. (2017) in the fruit exocarp. The anthocyanin, 7-O-methylpelargonidin 3-O-galactoside, also found previously in *aroeira* fruit (Feuereisen et al., 2017), was detected in the positive ion mode. In the negative ion mode, the extract showed the presence of ethyl digallate (*m/z* 349) and a gallotanin (*m/z* 539), as related by other authors (de Araujo Gomes et al., 2019; Feuereisen et al., 2014, 2017). UHPLC-MS/MS of selected ions of ground *aroeira* fruit in the negative and in the positive ion modes are shown in Fig. 1 and Fig. 2, respectively.

Besides the presence of functional fatty acids and terpenoids, phenolic compounds were also detected as *aroeira* constituents. Polyphenols are abundantly distributed in the plant kingdom and easily found in natural extracts, contributing to their preventive and therapeutic effects against several diseases (Shahidi & Ambigaipalan, 2015; Silva, Pinheiro-Castro, Novaes, Pascoal, & Ong, 2019).

Biflavonoids exhibit a large range of pharmacological activities (Andrade et al., 2018; Zhou et al., 2018). According to Zhou et al. (2018), hinokiflavone presented anti-tumor activity against colorectal tumor cell *in vitro* and in mice model. Andrade et al. (2018) isolated

Table 1
Proposed identification of bioactive compounds of *aroeira* fruit by comparison of ESI-MS/MS product ions with data from literature.

RT (min)	Mode	Precursor ion (*m/z*)	Product ions (*m/z*)	Proposed identification	Reference
3.222	−	349	347.7; 319.2; 294.8; 250; 197.2; 128	Ethyl digallate	de Araujo Gomes et al. (2019)
3.862	−	541	415; 389.3	Tetrahydroamentoflavone	da Silva et al. (2018)de Araujo Gomes et al. (2019)
4.201	−	539	539.2; 413.1; 387.3; 193.9	Gallotanin	Feuereisen et al. (2017); Feuereisen et al. (2014)
4.237	−	537	537.1	Agathisflavone	da Silva et al. (2018)de Araujo Gomes et al. (2019)Feuereisen et al. (2014)
4.452	+	447	447.0; 341.2; 284.6; 129.0	7-O-methylpelargonidin 3-O-galactoside	Feuereisen et al. (2017)
4.478	+	539	497.1; 419.2; 403; 305.2; 239.2; 118.8	Hinokiflavone	Feuereisen et al. (2017)
7.293	−	469	469.5	Triterpene acid	de Araujo Gomes et al. (2019)
7.520	−	279	279.3	Linoleic acid	de Araujo Gomes et al. (2019)
7.536	−	453	453.4	Masticadienoic acid	de Araujo Gomes et al. (2019)
7.731	−	455	455.6	3α-hydroxymasticadienolic (schinol)	da Silva et al. (2018)de Araujo Gomes et al. (2019)
7.967	−	371	371.5; 327.3	Terpene acid	Carneiro et al. (2016)
8.152	−	283	283.6	Stearic acid	da Silva et al. (2018)de Araujo Gomes et al. (2019)
8.195	−	373	373.4; 329.6	Terpene acid	Carneiro et al. (2016)

agathisflavone from *Caesalpinia pyramidalis* Tull. leaves and demonstrated its antioxidant capacity, reducing potential and inhibitory effect against lipid peroxidation. The anti-inflammatory properties of gallotanins from medicinal plants were summarized in a recent review (Kiss & Piwowarski, 2018).

As demonstrated, these polyphenols play an important role in human diet and health due their biological activities generally associated to their antioxidant properties. Besides, synthetic additives applied by the food industry are based on the same mechanisms of action of phenolic antioxidants, where they act as free radical scavengers, delaying or inhibiting the initiation or interrupting the propagation of lipid oxidation processes (Shahidi & Ambigaipalan, 2015). Thus, natural antioxidants obtained from plants such as *aroeira* have been of increasing relevance as functional food ingredients and as an alternative to substitute synthetic antioxidants.

3.2. Effects on formation of reactive substances of thiobarbituric acid

The TBARs value of unheated fish oil was 0.0026 ± 0.41 g MDA/100 g oil. After heating at 150 °C, the TBARs value increased to 0.0081 ± 0.45 g MDA/100 g in the control samples. Higher values for TBARs were observed after heating at 180 °C (0.0116 ± 0.50 g MDA/100 g oil); however, the addition of *aroeira* and BHT reduced the formation of malonaldehyde. Fig. 3 shows the protective effect of the antioxidants against TBARs formation at 150 °C and 180 °C.

The addition of *aroeira* fruit inhibited the formation of thiobarbituric acid reactive substances after heat treatment at 180 °C in 18.97% (GAF0.2%) and 26.73% (GAF0.5%). BHT exhibited a higher percentage of inhibition (27.59%), however, the effect of BHT and GAF (0.5%) addition, did not present significant differences at both applied temperatures ($p > 0.05$).

Elevated temperatures cause the degradation of hydroperoxides leading to an increase in TBARs value that indicates the formation of secondary oxidation products, which are directly associated with rancidity in food. Similar effects were reported in other fish samples as Russian sturgeon (*Acipenser gueldenstaedtii*) using different thermal processes (boiling, steaming, microwaving-cooking, oven-cooking, and frying) (Hu et al., 2017). These results highlight the relevance of natural antioxidants as a tool to prevent or to reduce lipids oxidation, as demonstrated in this study.

3.3. Effects on fatty acids

Twenty-nine fatty acids were identified in unheated samples. The main fatty acids determined were palmitic (C16:0, 21.66 ± 1.44 g/100 g oil), eicosapentaenoic EPA (C20:5 *n*3, 15.04 ± 0.17 g/100 g oil), docosahexaenoic DHA (C22:6 *n*3, 12.61 ± 0.26 g/100 g oil), oleic (C18:1 *n*9c, 8.55 ± 0.67 g/100 g oil), palmitoleic (C16:1 *n*7, 8.50 ± 0.52 g/100 g oil), and myristic (C14:0, 8.41 ± 0.41 g/100 g oil) (Table 2). Comparable fatty acids profiles were reported for fish oil (Bahurmiz, Adzitey, & Ng, 2017) and sardines (Bandarra, Marçalo, Cordeiro, & Pousão-Ferreira, 2018; Ferreira et al., 2017; Saldanha et al., 2008).

Heating caused a decreased in saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) levels of 2.92 and 9.43% (180 °C), respectively. Similar reductions were found in previous studies in grilled sardines (Saldanha et al., 2008) and Atlantic hake fillets (*Merluccius hubbsi*) (Saldanha & Bragagnolo, 2007, 2008), while varied effects were observed in fish oil samples submitted to different microwave heating conditions (Leal-Castañeda et al., 2017).

Although SFAs are more stable than MUFAs and PUFAs, they may undergo oxidation when exposed to temperatures higher than 150 °C and air, preferentially at the positions close to the ester carbonyl group (Nawar, 1984). Thus, losses of PUFAs were more representative; their amounts decreased approximately 7% (150 °C) and 23% (180 °C). The *n*-3 fatty acids EPA and DHA contents reduced 9.58% and 32.84%, respectively, after heating at 180 °C.

PUFAs oxidation is mainly carried out by an autocatalytic mechanism of free radicals induced by oxygen in the presence of initiators such as heat. Leal-Castañeda et al. (2017) found a drop of approximately 10% in fish oil PUFAs content after 10 min of heating using a conventional oven at 180 °C.

Fatty acid degradation was much higher in control samples than in the presence of BHT and *aroeira* fruit, demonstrating that both antioxidants provided a protective effect. The effectiveness of antioxidants decreased in the following order: GAF0.5% > BHT > GAF0.2%, showing no significant differences among the three treatments ($p > 0.05$). The additives were able to maintain 92.11% (GAF0.2%), 93.86% (GAF0.5%) and 93.24% (BHT) of the total content of PUFAs in the treatment at 180° C (Fig. 3). Similar protection was verified for SFA and MUFA.

The protective effect of *aroeira* may be due to the presence of bioactive compounds such as phenolic components identified by the chromatographic analyses, as discussed above. Phenolic compounds present in natural extracts show different mechanisms of action, acting both individually and synergistically, inhibiting free radical chain reactions or protecting lipids from initiators of these reactions (Shahidi & Ambigaipalan, 2015).

3.4. Effects on cholesterol oxides formation

The cholesterol and cholesterol oxides contents are shown in

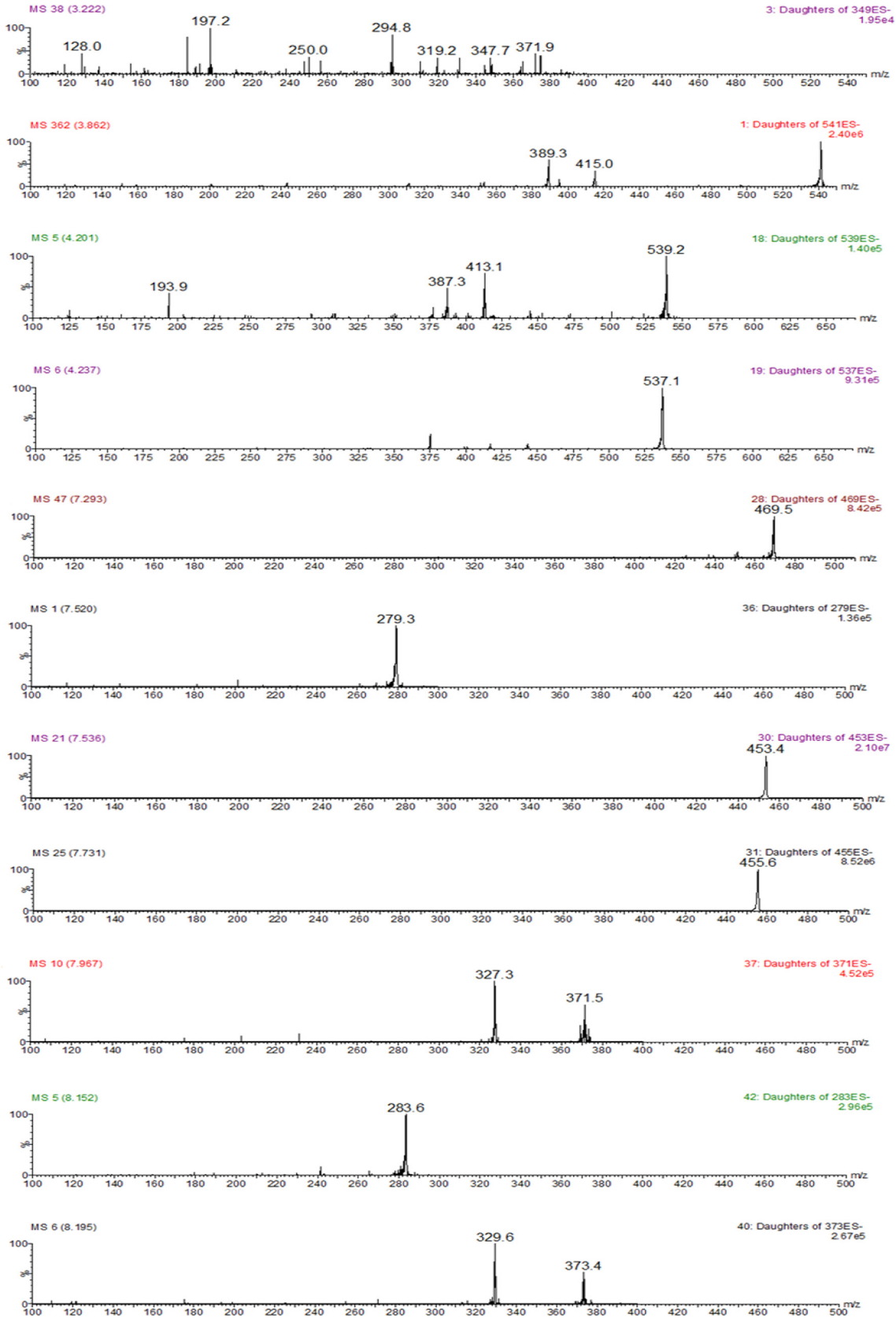

Fig. 1. UHPLC-MS/MS of selected ions of ground *aroeira* fruit in the negative ion mode.

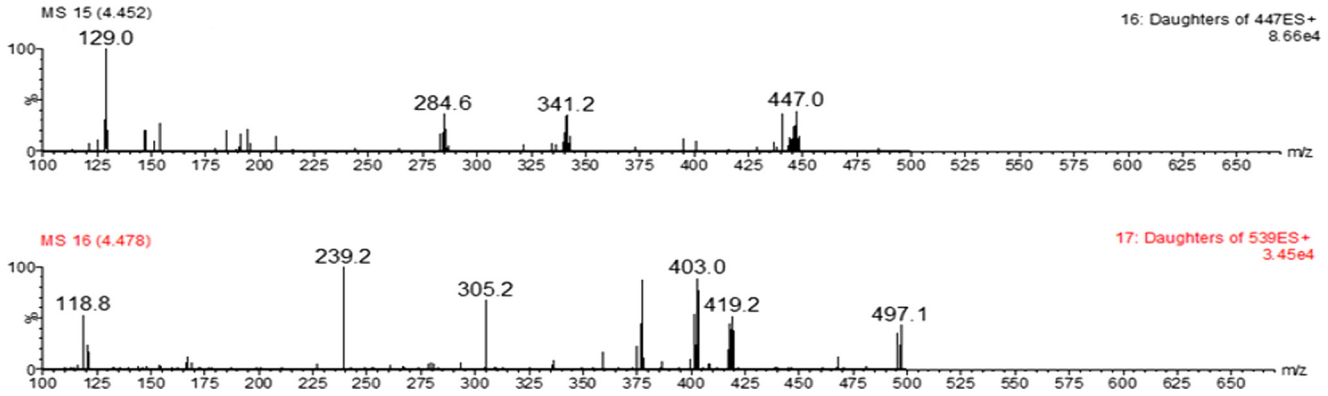

Fig. 2. UHPLC-MS/MS of selected ions of ground *aroeira* fruit in the positive ion mode.

Table 3. Unheated samples showed a cholesterol level of 553.04 ± 7.9 mg/100 g oil. Lower amounts were determined by other authors in raw *Sardinella brasiliensis* (Ferreira et al., 2017; Saldanha et al., 2008). However, after heating, reductions around 44.76% (150 °C) and 66.67% (180 °C) were observed in control samples.

The degradative effect of high temperatures was observed in model systems containing standard cholesterol solutions. Barriuso, Ansorena, Calvo, Cavero, and Astiasarán (2015) reported degradation around 34% in pure cholesterol standard solutions heated at 180 °C for 10 min. According to Derewiaka and Molińska (2015), heating caused reductions of 51.4% (150 °C/30 min) and 59.3% (180 °C/30 min) in pure cholesterol standard.

Regarding the presence of cholesterol oxides (COPs) in samples, five oxides were determined in unheated samples: 5,6α-epoxycholesterol, 5,6β-epoxycholesterol, 7-ketocholesterol, 7β-hydroxycholesterol, and 7α-hydroxycholesterol; being attributed to the fish metabolic process (Osada, Kodama, Cui, Yamada, & Sugano, 1993). However, heated samples presented higher content of COPs. The total content of COPs increased from 58.9 ± 0.26 μg/g oil to 478.2 ± 0.14 and 577.5 ± 2.14 μg/g oil, after heating of control samples at 150 °C and 180 °C, respectively.

The liquid state of cholesterol promotes a higher contact with oxidizing agents and it has been shown that, in the absence of other promoters, cholesterol is relatively stable up to temperatures below the melting point (148.5 °C), thus higher formation of oxidized products may be expected at 180 °C (Derewiaka & Molińska, 2015).

In this study, COPs formation was much lower than cholesterol degradation ratio, revealing the decomposition of cholesterol by other routes. Hence, hydrocarbons, aldehydes, ketones, and alcohols can also be formed during thermal degradation of cholesterol (Smith, 1987).

The major COPs identified in heated samples were 7-ketocholesterol, 7β- and 7α-hydroxycholesterol. COPs profile and amount can be affected by factors such as temperature, oxidation time, substrate form, oxygen, light, medium composition, initial cholesterol concentration, and others, leading to a set of different mechanisms that characterize the complexity of this process (Ansorena et al., 2013; Barriuso et al., 2017; Chien, Wang, & Chen, 1998; Medina-Meza & Barnaba, 2013; Smith, 1987).

The levels of 7-ketocholesterol in the heated samples ranged from 111.9 ± 0.27 to 250.6 ± 0.09 μg/g oil, values higher than those determined for 7β-hydroxycholesterol (from 70.5 ± 0.03 to 146.3 ± 0.28 μg/g oil) and 5,6β-epoxycholesterol (from 30.7 ± 0.03 to 102.8 ± 0.09 μg/g oil). 7-keto is recognized as the predominant oxide regarding thermo-oxidation, being characterized as the main COP derived from oxidation process in carbon 7, such as dehydration of 7α- and 7β-hydroperoxides and / or dehydrogenation of 7α- and 7β-hydroxycholesterol (Smith, 1987). Ansorena et al. (2013) noticed COPs formation when heating a triacylglycerol:cholesterol(100:1) solution at 180 °C, determining a total of 9100 μg/g cholesterol after 10 min, with 7-ketocholesterol being the predominant oxide.

5,6α- and 5,6β-epoxycholesterol amounts also increased with the thermal treatment varying from 3.7 ± 0.03 to 30.3 ± 0.01 and from 14.3 ± 0.08 to 102.8 ± 0.09 μg/g oil, respectively; which could be justified by the exposure of samples to atmospheric oxygen leading to air oxidation (Chien et al., 1998). 20α-hydroxycholesterol and 25R-hydroxycholesterol were only detected in expressively lower concentrations after heating at 180 °C. Oxides derived from the oxidation of the cholesterol side chain are commonly minorities (Barriuso et al.,

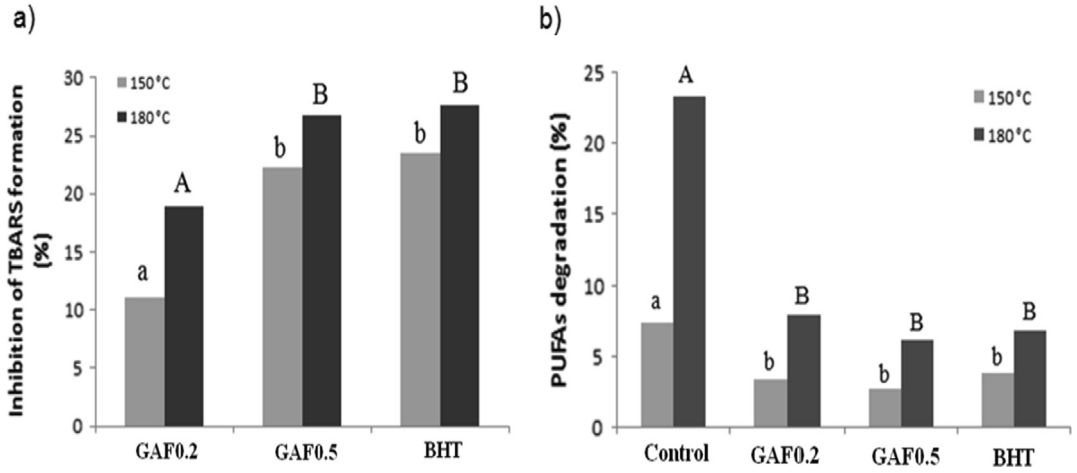

Fig. 3. (a) Inhibition of TBARS formation. (b) Percentage of PUFAs degradation of heated samples. Different capital and different lowercase letters mean statistically different results ($p < 0.05$).

Table 2
Fatty acid composition of samples (g/100 g of oil).

	Unheated	Control 150 °C	GAF0.2	GAF0.5	BHT	Control 180 °C	GAF0.2	GAF0.5	BHT
C12:0	0.09 ± 0.00	0.08 ± 0.00^{a}	0.09 ± 0.00^{a}	0.09 ± 0.00^{a}	0.09 ± 0.01^{a}	0.08 ± 0.02^{A}	0.08 ± 0.00^{A}	0.09 ± 0.01^{A}	0.09 ± 0.01^{A}
C14:0	8.41 ± 0.41	8.33 ± 0.04^{a}	8.3 ± 0.156^{a}	8.35 ± 0.09^{a}	8.35 ± 0.08^{a}	8.26 ± 0.06^{A}	8.39 ± 0.05^{A}	8.40 ± 0.16^{A}	8.40 ± 0.06^{A}
C15:0	0.96 ± 0.04	0.90 ± 0.05^{a}	0.95 ± 0.02^{a}	0.96 ± 0.00^{a}	0.91 ± 0.01^{a}	0.89 ± 0.02^{A}	0.91 ± 0.03^{A}	0.94 ± 0.01^{A}	0.91 ± 0.01^{A}
C16:0	21.66 ± 1.44	21.54 ± 0.05^{a}	21.55 ± 0.02^{a}	21.61 ± 0.15^{a}	21.61 ± 0.24^{a}	21.39 ± 0.23^{A}	21.42 ± 0.4^{A}	21.52 ± 0.26^{A}	21.57 ± 0.26^{A}
C17:0	0.84 ± 0.05	0.81 ± 0.01^{a}	0.84 ± 0.02^{a}	0.83 ± 0.01^{a}	0.82 ± 0.00^{a}	0.78 ± 0.07^{A}	0.79 ± 0.01^{A}	0.81 ± 0.01^{A}	0.82 ± 0.07^{A}
C18:0	5.08 ± 0.23	4.86 ± 0.03^{a}	4.90 ± 0.03^{a}	4.90 ± 0.03^{a}	4.88 ± 0.09^{a}	4.78 ± 0.04^{AB}	4.75 ± 0.03^{B}	5.04 ± 0.07^{A}	5.00 ± 0.06^{AB}
C20:0	0.62 ± 0.02	0.58 ± 0.02^{a}	0.61 ± 0.01^{a}	0.61 ± 0.01^{a}	0.61 ± 0.01^{a}	0.56 ± 0.01^{A}	0.61 ± 0.02^{A}	0.62 ± 0.04^{A}	0.61 ± 0.01^{A}
C21:0	0.04 ± 0.04	0.04 ± 0.00^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.02 ± 0.01^{A}	0.04 ± 0.01^{A}	0.05 ± 0.02^{A}	0.05 ± 0.02^{A}
C22:0	0.20 ± 0.01	0.17 ± 0.01^{a}	0.19 ± 0.00^{ab}	0.20 ± 0.01^{b}	0.19 ± 0.01^{ab}	0.16 ± 0.00^{A}	0.19 ± 0.00^{A}	0.20 ± 0.01^{A}	0.19 ± 0.00^{A}
C24:0	0.12 ± 0.00	0.11 ± 0.01^{a}	0.11 ± 0.00^{a}	0.11 ± 0.00^{a}	0.11 ± 0.02^{a}	–	–	–	–
C14:1	0.34 ± 0.02	0.33 ± 0.02^{a}	0.33 ± 0.01^{a}	0.34 ± 0.00^{a}	0.33 ± 0.01^{a}	0.32 ± 0.00^{A}	0.33 ± 0.01^{A}	0.34 ± 0.01^{A}	0.34 ± 0.00^{A}
C15:1	0.11 ± 0.02	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	0.12 ± 0.01^{a}	0.08 ± 0.01^{A}	0.08 ± 0.01^{A}	0.11 ± 0.03^{A}	0.09 ± 0.04^{A}
C16:1	8.5 ± 0.52	8.45 ± 0.02^{a}	8.35 ± 0.08^{a}	8.43 ± 0.08^{a}	8.41 ± 0.30^{a}	8.41 ± 0.12^{A}	8.49 ± 0.21^{A}	8.45 ± 0.11^{A}	8.47 ± 0.13^{A}
C17:1	0.99 ± 0.02	0.97 ± 0.03^{a}	0.97 ± 0.01^{a}	0.99 ± 0.01^{a}	0.99 ± 0.04^{a}	0.93 ± 0.03^{A}	0.98 ± 0.01^{A}	0.98 ± 0.04^{A}	0.97 ± 0.02^{A}
C18:1 *n9t*	0.13 ± 0.02	0.11 ± 0.01^{a}	0.13 ± 0.01^{a}	0.13 ± 0.00^{a}	0.11 ± 0.00^{a}	0.08 ± 0.01^{A}	0.11 ± 0.01^{A}	0.12 ± 0.01^{A}	0.11 ± 0.01^{A}
C18:1 *n9c*	8.55 ± 0.67	7.82 ± 0.09^{a}	8.49 ± 0.11^{a}	8.48 ± 0.06^{a}	8.40 ± 0.10^{a}	7.67 ± 0.55^{A}	8.42 ± 0.05^{A}	8.52 ± 0.23^{A}	8.52 ± 0.13^{A}
C20:1 *n9*	1.50 ± 0.12	1.42 ± 0.04^{a}	1.43 ± 0.02^{a}	1.44 ± 0.01^{a}	1.44 ± 0.05^{a}	0.78 ± 0.07^{A}	1.10 ± 0.05^{B}	1.33 ± 0.08^{C}	1.34 ± 0.01^{C}
C22:1 *n9*	1.38 ± 0.15	1.24 ± 0.04^{a}	1.33 ± 0.01^{a}	1.35 ± 0.03^{a}	1.34 ± 0.03^{a}	1.20 ± 0.03^{A}	1.33 ± 0.03^{AB}	1.37 ± 0.02^{AB}	1.38 ± 0.03^{B}
C24:1 *n9*	0.56 ± 0.03	0.53 ± 0.01^{a}	0.53 ± 0.01^{a}	0.53 ± 0.02^{a}	0.53 ± 0.03^{a}	0.52 ± 0.02^{A}	0.55 ± 0.00^{A}	0.56 ± 0.02^{A}	0.55 ± 0.02^{A}
C18:2 *n6t*	0.15 ± 0.06	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	0.15 ± 0.01^{a}	0.15 ± 0.03^{a}	0.04 ± 0.01^{A}	0.15 ± 0.01^{A}	0.14 ± 0.06^{A}	0.13 ± 0.07^{A}
C18:2 *n6c*	3.06 ± 0.01	2.37 ± 0.12^{a}	2.91 ± 0.06^{b}	2.95 ± 0.06^{b}	2.90 ± 0.01^{b}	1.57 ± 0.02^{A}	1.66 ± 0.07^{A}	1.76 ± 0.07^{A}	1.77 ± 0.04^{A}
C18:3 *n6*	0.24 ± 0.01	0.17 ± 0.02^{a}	0.24 ± 0.01^{a}	0.24 ± 0.01^{a}	0.24 ± 0.01^{a}	0.13 ± 0.01^{A}	0.23 ± 0.02^{A}	0.24 ± 0.01^{A}	0.24 ± 0.02^{A}
C18:3 *n3*	1.47 ± 0.02	1.37 ± 0.02^{a}	1.41 ± 0.04^{a}	1.45 ± 0.01^{a}	1.40 ± 0.01^{a}	1.09 ± 0.06^{AB}	1.22 ± 0.07^{B}	1.44 ± 0.05^{B}	0.27 ± 0.15^{AB}
C20:2 *n6*	2.50 ± 0.07	2.42 ± 0.03^{a}	2.50 ± 0.02^{a}	2.5 ± 0.02^{a}	2.47 ± 0.03^{a}	1.97 ± 0.15^{A}	2.37 ± 0.12^{B}	2.46 ± 0.05^{B}	2.45 ± 0.05^{B}
C20:3 *n6*	0.12 ± 0.02	0.09 ± 001^{a}	0.12 ± 0.00^{a}	0.12 ± 0.00^{a}	0.12 ± 0.00^{a}	0.07 ± 0.01^{A}	0.12 ± 0.01^{B}	0.12 ± 0.01^{B}	0.13 ± 0.00^{B}
C20:3 *n3*	0.19 ± 0.01	0.15 ± 0.01^{a}	0.18 ± 0.01^{a}	0.18 ± 0.01^{a}	0.17 ± 0.01^{a}	0.15 ± 0.00^{A}	0.15 ± 0.01^{A}	0.16 ± 0.01^{A}	0.15 ± 0.03^{A}
C20:4 *n6*	1.14 ± 0.06	1.10 ± 0.02^{a}	1.11 ± 0.00^{a}	1.11 ± 0.05^{a}	1.12 ± 0.04^{a}	0.96 ± 0.08^{A}	1.04 ± 0.01^{A}	1.06 ± 0.02^{A}	1.06 ± 0.01^{A}
C20:5 *n3*	15.04 ± 0.17	13.82 ± 0.38^{a}	14.32 ± 0.02^{b}	14.31 ± 0.02^{b}	14.10 ± 0.11^{ab}	13.60 ± 0.22^{A}	14.22 ± 0.09^{B}	14.34 ± 0.24^{B}	14.30 ± 0.13^{B}
C22:6 *n3*	12.61 ± 0.26	12.27 ± 0.37^{a}	12.38 ± 0.11^{a}	12.52 ± 0.09^{a}	12.46 ± 0.16^{a}	8.47 ± 0.16^{A}	12.49 ± 0.03^{B}	12.57 ± 0.13^{B}	12.56 ± 0.03^{B}
ΣSFA	38.02 ± 2.08	37.47 ± 0.14^{a}	37.60 ± 0.24^{a}	37.72 ± 0.16^{a}	37.66 ± 0.33^{a}	36.91 ± 0.18^{A}	37.18 ± 0.45^{A}	37.70 ± 0.17^{A}	37.65 ± 0.24^{A}
ΣMUFA	22.06 ± 1.26	20.93 ± 0.10^{a}	21.65 ± 0.15^{a}	21.79 ± 0.16^{a}	21.69 ± 0.45^{a}	19.98 ± 0.63^{A}	21.40 ± 0.17^{AB}	21.79 ± 0.06^{B}	21.78 ± 0.18^{B}
ΣPUFA	36.54 ± 0.68	33.86 ± 0.14^{a}	35.30 ± 0.11^{b}	35.55 ± 0.05^{b}	35.13 ± 0.18^{b}	8.06 ± 0.28^{A}	33.66 ± 0.22^{B}	34.30 ± 0.18^{B}	34.07 ± 0.27^{B}
Σn3	29.31 ± 0.46	27.63 ± 0.25^{a}	28.31 ± 0.15^{ba}	28.47 ± 0.07^{b}	28.29 ± 0.30^{ba}	3.32 ± 0.40^{A}	28.08 ± 0.07^{B}	28.51 ± 0.11^{B}	28.30 ± 0.30^{B}
Σn6	7.22 ± 0.21	6.23 ± 0.11^{a}	6.99 ± 0.07^{b}	7.08 ± 0.03^{b}	7.00 ± 0.04^{b}	4.74 ± 0.14^{A}	7.94 ± 0.27^{B}	5.78 ± 0.13^{C}	7.60 ± 0.11^{B}
Σn3/Σn6	4.06 ± 0.32	4.34 ± 0.21^{a}	4.05 ± 0.09^{b}	3.96 ± 0.03^{c}	4.04 ± 0.24^{b}	4.91 ± 0.4^{A}	3.53 ± 0.2^{B}	4.95 ± 0.11^{A}	3.72 ± 0.21^{B}

Values represent means ± standard deviation ($n = 3$). Different capital letters and different lowercase letters mean statistically different results by the *Tukey* test ($P < 0.05$) among control, GAF 0.2, GAF0.5, and BHT.

2015; Smith, 1987).

The addition of both natural and synthetic antioxidants minimized cholesterol degradation and thermo-oxidation, with consequent reduction of COPs formation. In samples containing ground *aroeira* fruit, the cholesterol levels after heating varied from 395.78 ± 0.33 to 418.51 ± 0.88 mg/100 g (150 °C) and from 349.81 ± 1.92 to 370.28 ± 0.61 mg/100 g (180 °C). These values were higher than the ones found in control samples after heating, 305.54 ± 3.95 mg/100 g (150 °C) and 184.36 ± 2.13 mg/100 g (180 °C).

The effectiveness of antioxidants addition on COPs formation decreased in the following order: BHT > GAF0.5% > GAF0.2% (Fig. 4). After heating at 180 °C, protective effects of 15.51% and 23.22% were determined for GAF0.2% and GAF0.5%, respectively.

The protective capacity of the synthetic antioxidant (BHT) against COPs formation was higher than that presented by the ground fruit ($p < 0.05$). The addition of BHT reduced total COPs content from 577.5 ± 2.14 μg/g (control) to 326.2 ± 0.23 μg/g (180 °C), while ground *aroeira* fruit at 0.5% reduced to 441.9 ± 0.61 μg/g. Although BHT had a greater protective effect against COPs formation, *aroeira* fruit had a significant protective effect at both concentrations. In addition, higher concentrations of *aroeira* can be evaluated to obtain similar or even better results against cholesterol thermo-oxidation whereas BHT was evaluated at the highest permitted concentration.

It is well known that exogenous COPs formed during thermo-oxidation cause deleterious health effects, such as inflammation, cytotoxicity, atherogenesis, carcinogenesis and alterations in cell membrane properties, as well as the development of degenerative and chronic diseases (Maldonado-Pereira et al., 2018). Thus, it is crucial to consider the use of natural antioxidants to minimize damages from COPs ingestion. Antioxidant compounds can retard or/and prevent cholesterol oxidation, since they control pro-oxidants concentration and inactivate free radicals formed in carbons such as C7 and C 25 (7α-hydroperoxycholesterol, 7β-hydroperoxycholesterol, and 25-hydroperoxycholesterol) (Medina-Meza & Barnaba, 2013).

Natural antioxidants have been widely applied against cholesterol thermo-oxidation. The protective effects of *Melissa officinalis* and manacubiu (*Solanum sessiliflorum*) were evaluated in standard cholesterol solutions in model systems by Barriuso et al. (2015) and Barriuso, Mariutti, Ansorena, Astiasarán, and Bragagnolo (2016), respectively. Parsley (*Petroselinum crispum*), chives (*Allium schoenoprasum* L.), and the mixture of the two herbs presented a protective effect against cholesterol oxidation in air-fried sardine (*Sardinella brasiliensis*) fillets (Ferreira et al., 2017).

3.5. Correlation and PCA

The two first components in PCA explain 76% of the data variability (Dim 1: 62.5% and Dim 2: 13.5%), which can be considered a good percentage of explanation. The control was characterized by greater amounts of SFAs, PUFAs, MUFAs, and cholesterol (Tables 2 and 3). The amount of COPs increased with heating, which is observed in the Dim 1, thus, the samples demonstrated an increase in cholesterol oxides production (from left to right, Fig. 5a and b), in this sense, the protective effect showed the increasing order: BHT (150 °C) < GAF0.5 (150 °C) < GAF0.2 (150 °C) < BHT (180 °C) < GAF0.5 (180 °C) < GAF0.2 (180 °C), control (150 °C), and control (180 °C).

Table 3
Cholesterol (mg/100 g of oil) and cholesterol oxides levels (μg/g of oil) of samples.

Cholesterol oxides (μg/g)	Unheated	Control 150 °C	GAF0.2	GAF0.5	BHT	Control 180 °C	GAF0.2	GAF0.5	BHT
5,6α-EP	3.7 ± 0.03	22.2 ± 0.07^{a}	19.4 ± 0.02^{b}	18.6 ± 0.01^{b}	11.1 ± 0.01^{c}	30.3 ± 0.01^{A}	25.5 ± 0.11^{B}	24.2 ± 0.04^{B}	28.1 ± 0.06^{C}
5,6β-EP	14.3 ± 0.08	81.7 ± 0.08^{a}	58.7 ± 0.08^{b}	52.8 ± 0.04^{c}	37.2 ± 0.03^{d}	102.8 ± 0.09^{A}	83.6 ± 0.04^{B}	70.3 ± 0.13^{C}	30.7 ± 0.03^{D}
7-keto	5.2 ± 0.17	212.6 ± 0.04^{a}	160.0 ± 0.15^{b}	147.0 ± 0.02^{c}	111.9 ± 0,27^{d}	250.6 ± 0.09^{A}	207.2 ± 0.13^{B}	190.2 ± 0.18^{C}	142.2 ± 0.19^{D}
7β-OH	6.4 ± 0.03	123.1 ± 0.06^{ab}	102.4 ± 0.10^{bc}	83.1 ± 2.87^{cd}	75.8 ± 0,07^{cd}	146.3 ± 0.28^{A}	120.3 ± 0.22^{AB}	111.3 ± 0.17^{B}	70.5 ± 0.03^{C}
7α-OH	29.3 ± 0.03	38.6 ± 0.05^{a}	27.7 ± 0.02^{b}	27.7 ± 0.05^{b}	12.2 ± 0.02^{c}	45.3 ± 0.12^{A}	45.5 ± 0.06^{A}	42.8 ± 0.06^{B}	38.1 ± 0.05^{C}
20α-OH	–	–	–	–	–	0.4 ± 0.01^{A}	4.5 ± 0.13^{B}	3.4 ± 0.02^{B}	3.4 ± 0.02^{B}
25R-OH	–	–	–	–	–	2.2 ± 0.04^{A}	3.3 ± 0.05^{A}	3.11 ± 0.05^{A}	16.5 ± 0.29^{B}
Total COPs	58.9 ± 0.26	478.2 ± 0.14^{a}	368.1 ± 0.31^{b}	329.2 ± 2.91^{c}	248.2 ± 0.24^{d}	577.5 ± 2.14^{A}	485.5 ± 1.92^{B}	441.9 ± 0.61^{C}	326.2 ± 0.23^{D}
Cholesterol (mg/100 g)	553.04 ± 7.90	305.54 ± 3.95^{a}	395.78 ± 0.33^{b}	418.51 ± 0.88^{c}	487.15 ± 3.41^{d}	184.36 ± 2.13^{A}	349.81 ± 1.92^{B}	370.28 ± 0.61^{C}	431.60 ± 1.07^{D}

Values represent means ± standard deviation (n = 3). Different capital letters and different lowercase letters mean statistically different results by the *Tukey* test ($P < 0.05$) among control, GAF 0.2, GAF0.5 and BHT.

Fig. 4. Total COPs content (μg/g) of heated samples. Different capital letters and different lowercase letters mean statistically different results ($p < 0.05$).

Cholesterol presented a strong positive and significant correlation with Σ*n*3, ΣPUFA, ΣSFA, and ΣMUFA with r values of 0.84, 0.88, 0.89, and 0.90, respectively (Fig. 5c). The main COPs (5,6α-Ep, 5,6β-Ep, 7-keto, 7α-OH, 7β-OH) and total COPs presented significant strong and negative correlations (r values from −0.70 to −0.90) with unsaturated fatty acids, suggesting that radicals and oxygenated species derived from lipid oxidation can exert a pro-oxidant effect towards cholesterol (Barriuso et al., 2017).

In Fig. 5b, it is possible to obtain some groups of samples. One group is just composed by the control without heating sample, while the samples heated at the same temperature trend to be in the same group. The formation of the groups may be improved by the use of HCPC, thus, it can be observed four groups (Fig. 5d): a group formed by the control without heating, a group formed by the GAF0.2, GAF0.5, and BHT heated at 150 °C, a group formed just by the control heated at 180 °C, and other group formed by BHT, GAF0.2, and GAF0.5 (heated at 180 °C) and control (heated at 150 °C). These groups suggest the protective effect of ground *aroeira* fruit, which results demonstrated the same action of BHT inside each group.

4. Conclusions

This study demonstrated the negative impact of high temperatures on PUFAs degradation and COPs formation and the effectiveness of *aroeira* fruit against lipid oxidation in model systems containing fish oil. The COPs formation was much lower than cholesterol degradation ratio, revealing the decomposition of cholesterol by other routes. The fruit presented antioxidant effect for most samples, showing a protective effect lower or similar to the synthetic antioxidant BHT; however, BHT was used at the maximum concentration allowed by the Brazilian legislation (0.01%). Thus, this

research indicates the potential application of *aroeira* fruit as a natural inhibitor of cholesterol and PUFAs thermo-oxidation by the food industry. Moreover, aroeira may provide additional benefits due to its nutritional and health promoting attributes.

5. Authors' contribution

Vanessa S. de Oliveira undertook almost most of the experimental work presented in this paper. Tatiana Saldanha designed, supervised, and organized the study. Davy W.H. Chávez did the statistical analysis. Alexandra C.H.F. Sawaya was responsible for the mass spectrometry analyses. Ormindo D. Gamallo designed the models system and helped with experimental works. Rosane N. Castro helped with cholesterol and cholesterol oxides analysis. Geni R. Sampaio and Elizabeth A.F.S. Torres supervised and organized the study. Vanessa S. de Oliveira and Tatiana Saldanha predominantly interpreted the results and drafted the

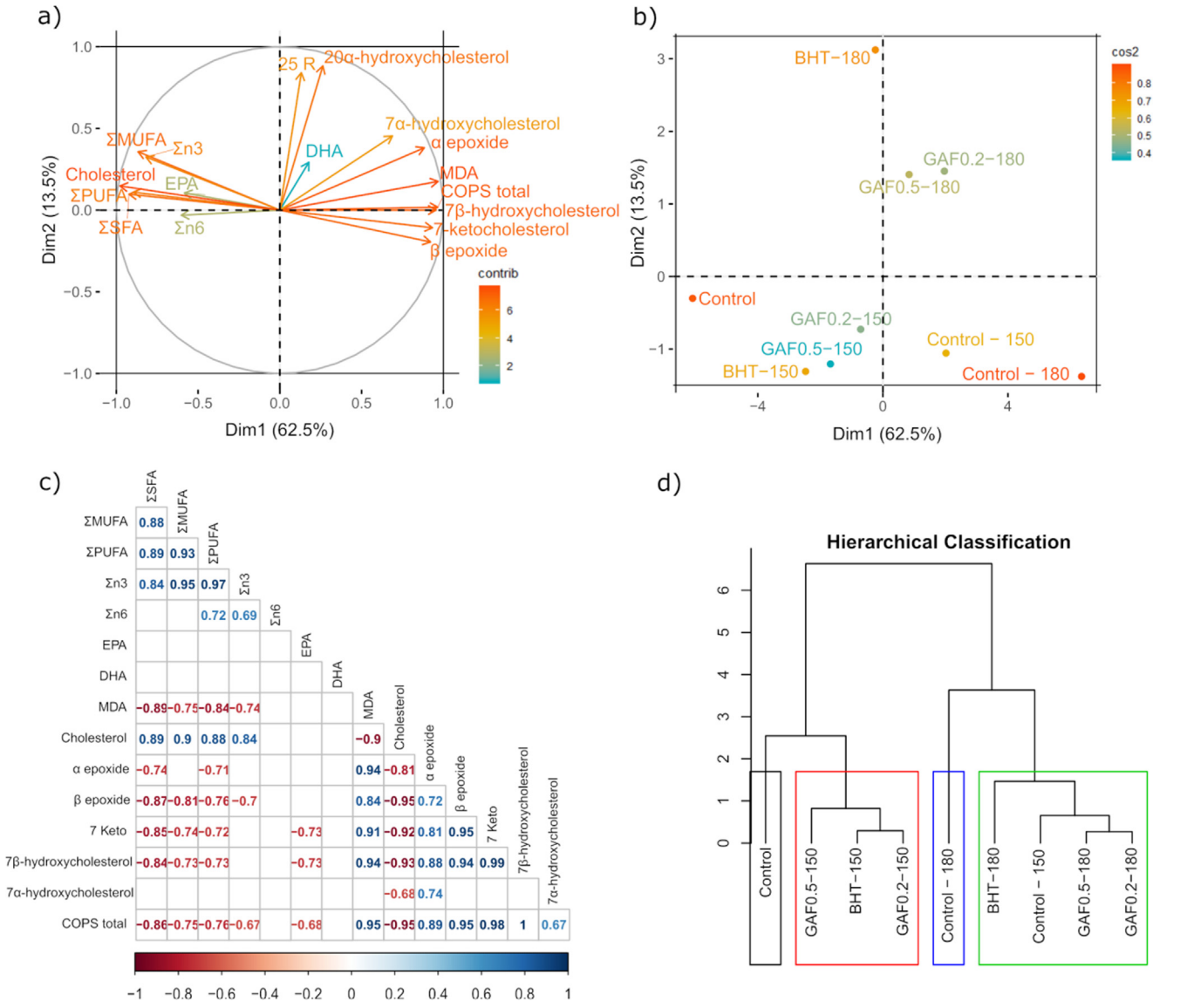

Fig. 5. Multivariate analyses of variables total fatty acids composition, cholesterol, and COPs in treatments with ground *aroeira* fruit, BHT, and controls. Principal component analyses for variables (a) and for treatments (b), only significant r values (numbers inside the correlogram) are presented in correlogram (c), negative or positive correlation are represented by red or blue numbers respectively, and hierarchical clustering from principal components (d).

manuscript with help from the other authors. Elizabeth A.F.S. Torres reviewed and edited the article. All authors revised and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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