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Lipid profile and high contents of cholesterol oxidation products (COPs) in different commercial brands of canned tuna

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ABSTRACT

Canned fish is submitted to processes that may degrade its lipids and form harmful compounds called cholesterol oxidation products (COPs). Samples of Brazilian commercial canned tuna were analyzed to evaluate the influence of different liquid mediums (oil and brine) on the fatty acid composition and formation of COPs. The exchange between fish lipids and the constituents of the covering liquid was highlighted by the high levels of linoleic acid found in tuna conserved in oil. High amounts of COPs were found. However, higher contents of COPs were found in tuna in brine (933.14 to 1914.23 μg/g) than in oil (698.24 to 1167.88 μg/g). This result was mainly promoted by the presence of pro-oxidant elements such as salt, as well as greater heat transfer in brine than in oil. This study showed that canned tuna is a potential source of exogenous COPs, indicating the role of liquid mediums in oxidative processes.

1. Introduction

Seafood products have been the subject of numerous studies due to the wide range of nutritional benefits related to their high contents of polyunsaturated fatty acids (PUFAs). These fatty acids play vital functions in structural and regulatory physiological activities and are associated with the prevention of cardiovascular diseases, inflammatory processes, neurocognitive disorders, and cancer (Innes & Calder, 2020).

Canning is considered one of the most important techniques of fish preservation. The shelf-stable attribute of canned fish allows increased access to fish, supporting a crucial role in human nutrition without the cold chains required during storage (Barbosa, Trigo, Campos, & Aubourg, 2019). Moreover, it attends the consumer demand for ready to eat food, being convenient as fast food. Thus, the consumption of canned fish, such as canned tuna, has increased over the last decades (Barbosa et al., 2019; Mata, Chanmalee, Punyasuk, & Thitamadee, 2020).

Canned products are submitted to a variety of industrial steps: storage pretreatments such as chilling and freezing, cooking, sterilization, and posterior storage (Aubourg, 2001). Accordingly, fish is exposed to different conditions that can cause nutritional and sensorial losses in the final product, as well as the formation of compounds known for their deleterious effects on human health (Aubourg, 2001; Barbosa et al., 2019; Zunin, Boggia, & Evangelisti, 2001).

Fish is a cholesterol-containing food that, when processed, represents a potential source of harmful compounds called cholesterol oxidation products (COPs) or cholesterol oxides (Dantas et al., 2015). Several studies have reported that cholesterol oxides are involved in physiological changes due to their cytotoxic, atherogenic, neurodegenerative, inflammatory, and carcinogenic effects (Sottero, Leonarduzzi, Testa, Gargiulo, Poli, & Biasi, 2019).

The structures of cholesterol oxides are similar to that of cholesterol, with an additional hydroxyl, ketone or epoxide group in the central nucleus, or a hydroxyl in the side chain. The presence of a double bond between carbons 5 and 6 facilitates the allylic hydrogen abstraction at C7 by lowering the activation energy required, originating 7-ketocholesterol, 7α-hydroxycholesterol, and 7β-hydroxycholesterol. Epoxidation leads to the formation of 5,6α- and 5,6β-epoxycholesterol, which are characterized as products of the oxidation of cholesterol by air. Besides, cholesterol oxides originated from the side-chain oxidation may also occur, with the predominant oxidation of the tertiary carbons C20 and

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C25 forming 20-hydroxycholesterol and 25-hydroxycholesterol, respectively (Hur, Park, & Joo, 2007; Smith, 1987).

Due to the high level of unsaturated compounds, such as polyunsaturated fatty acids and cholesterol, marine lipids are extremely prone to oxidation. Therefore, the high temperatures applied during cooking and sterilization may lead to oxidative processes in canned fish. In canning, the liquid mediums transfer the heat from retort to the fish muscle and may also change the lipid profile of the final product (Barbosa et al., 2019; Dantas et al., 2015; Mesías et al., 2015; Zunin et al., 2001). Thus, the aim of this study was to evaluate the influence of different liquid mediums (oil and brine) on the fatty acid composition and cholesterol oxides contents of Brazilian commercial canned tuna.

2. Material and methods

2.1. Standards, reagents, and solvents

The standard, undecanoic methyl ester, was purchased from Sigma (St. Louis, MO, USA) and the standard fatty acid mixture was purchased from SupelcoTM 37 (FAME Mix 18919, Bellefonte, PA, USA). Cholesterol and other standards, including 20α-hydroxycholesterol, 22(S)-hydroxycholesterol, 22(R)-hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, 7β-hydroxycholesterol, 5,6α-epoxycholesterol, and 5,6β-epoxycholesterol, were acquired from Sigma Chemical Company (St. Louis, MO, USA), while 25(R)-hydroxycholesterol and 7α-hydroxycholesterol were obtained from Steraloids (Wilton, NH, USA). The purity of the standards was at least 95%. High-performance liquid chromatography (HPLC) grade *n*-hexane and 2-propanol were obtained from Mscience (Darmstadt, Germany) and all other analytical grade solvents were from Merck (Darmstadt, Germany).

2.2. Samples

Samples of grated canned tuna conserved in brine and in oil were bought in supermarkets in the city of Rio de Janeiro, Brazil, in February 2015. Samples of both types of canned tuna, in brine and in oil, from three different brands (brand A, B, and C) were acquired. Six cans of each brand were selected, resulting in a total of 36 samples. Skipjack Tuna (*Katsuwonus pelamis*) was the species canned for all brands.

The ingredients listed on the can of each brand of tuna canned in brine were: brand A - tuna, water, salt, powder vegetable extract (potato, carrot, soybean); brand B - tuna, water, salt, vegetable broth; brand C - tuna, water, salt. The ingredients listed on the can of each brand of tuna canned in oil were: brand A - tuna, soybean oil, water, salt, powder vegetable extract (potato, carrot, soybean); brand B - tuna, edible oil, salt; brand C - tuna, soybean oil, salt.

Before analyses, the liquid medium was separated from the solid sample. The tuna was ground using a domestic processor and dried in an oven (Splabor, SP, Brazil) at 40 °C for 1 day for subsequent analyses.

2.3. Moisture and total lipids

The moisture content was determined according to AOAC (2002). The lipids were extracted and determined according to Bligh and Dyer (1959).

2.4. Fatty acid composition

Fish oil (25 mg) was submitted to saponification and methylation using BF_3 in methanol (Joseph & Ackman, 1992). The fatty acids were determined using a gas chromatographer (Shimadzu GC 2010, Tokio, Japan), equipped with a split injector (1:100), a flame ionization detector and a workstation. The chromatographic separation was performed in a fused silica CP-SIL 88 capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness) (Chrompack, Middelburg, The Netherlands). The chromatographic conditions were established according to Ferreira et al. (2017). Hydrogen was used as carrier gas (1 mL/min) and nitrogen as the make-up gas (30 mL/min). The chromatographic peaks of fatty acid methyl esters of the samples were identified by comparison with the retention time of fatty acid methyl ester standards and quantification was performed using the undecanoic methyl ester as internal standard.

2.5. Cholesterol and cholesterol oxides

Cholesterol and cholesterol oxides were obtained by direct saponification at room temperature for 22 h in the dark, as described by Saldanha, Sawaya, Eberlin, and Bragagnolo (2006).

Chromatographic analyses were performed using a Waters liquid chromatographer (Waters, Milford, MA, USA), equipped with on-line PDA (Waters 2998), and refractive index (RID-Waters, 2414) detectors. The analytical column was a Nova Pack CN HP (300 mm × 3.9 mm × 4 mm) (Waters, Milford, MA, USA) and the mobile-phase was *n*-hexane: 2-propanol (97:3, v/v) at a flow rate of 1 mL/min. The chromatographic conditions were established as described by Ferreira et al., (2017). Quantification was done using external standard curves of cholesterol (from 0.1 to 1.8 mg/mL) and cholesterol oxides (from 5 to 150 μg/mL). Epimeric 5,6 epoxides were quantified using a refractive index detector, since they do not absorb UV wavelengths.

To confirm the cholesterol oxide structures, samples were analyzed on a UHPLC Acquity chromatographer coupled to a TQD Acquity mass spectrometer (Micromass-Waters Manchester, England), with an APCI source configuration. A Phenomenex CN (250 mm × 4.3 mm × 5.0 μm) was used. The conditions were: isocratic mobile phase containing hexane: isopropanol (97:3), flow 1 mL/min, oven temperature 32 °C, and 10 μL of the sample. Ionization was performed in the APCI positive ion mode. The parameters were: corona current 21 μA; probe temperature 350 °C; source temperature 150 °C; cone voltage 30 V; extractor voltage 5 V. The main ions were determined in the selective ion monitoring (SIM) mode, where the selected ions were *m/z* 367, 369, 385, 401, and 403 (Saldanha et al., 2006). The compounds were identified by comparison of retention times of peaks in samples with those of reference standards and by *m/z* (Supplementary material Table 1).

Calibration curves, which varied from 0.1 to 1.0 mg/mL for cholesterol and 5.0 to 150.0 μg/mL for cholesterol oxides, were constructed for each analyte by plotting the chromatographic areas versus analyte concentration. All analytes demonstrated a good linearity response in the investigated range of concentrations, with correlation coefficients higher than 0.99. The LODs and LOQs were calculated as three and ten times the standard deviation, respectively, via the slope of the calibration curve. The LOD and LOQ values were in the ranges 6 to 70 ng/mL and 18 to 210 ng/mL for cholesterol and cholesterol oxides, respectively.

2.6. Statistical analysis

The experimental and analytical data were performed in triplicate. The repetitions were accepted when the coefficient of variation presented a medium level (<20%) for each sample (Vaz, et al., 2017). All quantitative results were submitted to mean variance sample comparison using ANOVA test (to compare more than two samples) and the differences were detected using a multiple mean pairwise-comparison by Tukey's test, at a significance level of 5%. An unpaired T-test, at 5% of significance, was carried out to compare the possible differences among the results obtained for tuna and liquid medium of each brand and type of liquid medium.

For Pearson's correlation test, Principal component analysis (PCA) and Hierarchical Clustering of Principal Components (HCPC) were applied using the following response variables: SFA, MUFA, PUFA, n3, n6, trans, cholesterol, 20-OH, 5,6α, 5,6β, 7-keto, 7α-OH, 7β-OH, and Total COPs. The Pearson's correlation test was applied to evaluate variable-variable correlations (r) and the correlation strength was determined using the empirical rule proposed by Teles et al. (2019). PCA

was used to condense the information of a number of correlated variables into a small number of uncorrelated variables called principal components (PCs) with minor loss of information. PCA was carried out using standardized variables to avoid bias due to variables of different magnitudes. HCPC was conducted to confirm the groups of samples suggested by PCA. All statistical analyses were performed using software R language and environment for statistical computing.

3. Results and discussion

3.1. Moisture and total lipid contents of canned tuna samples

The moisture level ranged from 24.60 ± 0.12 to 27.13 ± 0.03 g/100 g in tuna conserved in brine. Higher values were determined for tuna in oil, with contents varying from 31.93 ± 0.02 to 51.33 ± 0.02 g/100 g (Table 1). Significant differences were observed among the brands for both types of samples ($p < 0.05$). Other authors found varied moisture levels, from 52 to 64 g/100 g, in canned tuna in different vegetable oils (Bahurmiz, Al-Sa'ady, & Adzitey, 2018; Mohan, Remya, Murthy, Ravishankar, & Kumar, 2015; Stephen, Shakila, Jeyasekaran, & Sukumar, 2010). According to these studies, the results are affected by factors such as time and temperature of processing, the liquid medium used, as well as intrinsic characteristics of fish. These findings are close to the result assessed for samples from brand C in oil (51.33 g/100 g).

The total lipid amount varied from 4.21 ± 0.18 to 5.59 ± 0.18 g/100 g (dry basis) in tuna in brine and from 23.70 ± 0.04 to 43.99 ± 0.62 g/100 g in tuna in oil, with no significant differences between brand A and C ($p = 0.425$), respectively. Aberouman and Fazeli (2019) reported a lipid level of 36.27 g/100 g in tuna canned in oil, which was similar to the values observed for brand A (35.63 ± 0.03 g/100 g), while lower content was found by Marković, Mladenović, Cvijović, and Miljković (2015) (19.77 g/100 g).

The higher concentration of lipids determined for tuna in oil may be explained by the absorption of the filling medium (oil) by the fish muscle. A similar trend was described by Stephen et al. (2010) that reported an increment in the lipid level of tuna after canning with vegetable oil (from 9.5 to 19.5 g/100 g). Mohan et al. (2015) found an increase of approximately 450% in tuna canned with different vegetable oils (sunflower, coconut, and groundnut oil).

Most of the global tuna catch goes to the canning industry, and different species of tuna can be canned (Mata et al., 2020). Therefore, since fish composition varies with the species, the canning process may affect the fish in different ways and variable results can be obtained due to the use of different species.

Even within the same species, other factors that influence the composition of the raw fish (feeding and environmental conditions, age, catching season, fishing location) may affect the moisture and lipid levels of the final product (Bahurmiz et al., 2018). In addition, the parameters applied during the canning process may also be considered, making it difficult to compare the results obtained with previous studies

Table 1
Moisture and total lipid amount of canned tuna samples in brine and oil.

	Sample	Moisture(g/100 g)	Total lipids(g/100 g, db)
Tuna in brine	**Brand A**	24.60 ± 0.12^{a}	4.21 ± 0.18^{a}
	Brand B	27.13 ± 0.03^{c}	5.59 ± 0.18^{b}
	Brand C	26.92 ± 0.02^{b}	4.73 ± 0.42^{ab}
Tuna in oil	**Brand A**	42.30 ± 0.05^{B}	35.63 ± 0.03^{B}
	Brand B	31.93 ± 0.02^{A}	23.70 ± 0.04^{A}
	Brand C	51.33 ± 0.29^{C}	43.99 ± 0.62^{C}

Values represent the mean ± SD ($n = 3$). Different lowercase and capital letters in the same column indicate significant differences among brands for tuna in brine and oil, respectively. Statistical differences were obtained by applying multicomparison Tukey test for the means ($p < 0.05$).

3.2. Fatty acids composition of canned tuna and liquid medium

The fatty acid composition of tuna and liquid medium of samples conserved in brine and in oil is presented in Tables 2 and 3, respectively. The main fatty acids found in tuna canned in brine were palmitic (C16:0, from 32.81 ± 0.23 to 36.00 ± 0.26 g/100 g), oleic (C18:1*n*9c, from 15.37 ± 0.11 to 17.15 ± 0.12 g/100 g), stearic (C18:0, from 10.67 ± 0.08 to 12.84 ± 0.94 g/100 g) and docosahexaenoic (C22:6 ω3, from 4.44 ± 0.03 to 8.89 ± 0.06 g/100 g). In brine, tuna of brands B and C presented no significant differences for palmitic and oleic acids ($p = 0.717$ and $p = 0.083$, respectively). In oil, tuna and liquid medium of brands A and C presented no significant differences for oleic acid ($p = 0.334$ and 0.224 for tuna and liquid medium, respectively). Additionally, palmitic and docosahexaenoic acids in the liquid medium were similar for brands A and C. The fatty acid profile determined in this study is in agreement with those previously reported for tuna (Mesías et al., 2015; Truzzi et al., 2018).

Regarding the groups of fatty acids, the sum fatty acid levels decreased in the order of saturated (SFA) > monounsaturated (MUFA) > polyunsaturated (PUFA), for most cases showing significantly different results among the brands ($p < 0.05$) for most groups (Table 2). The sum of relevant PUFAs of the *n*3-series such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) varied from 4.73 ± 0.03 to 10.05 ± 0.07 in tuna conserved in brine, where DHA was the most prominent contributor.

A similar fatty acid profile was assessed in brine (liquid medium). Fatty acids like palmitic (from 27.57 ± 0.13 to 38.78 ± 0.28 g/100 g), oleic (from 15.48 ± 0.11 to 20.43 ± 0.33 g/100 g), stearic (from 8.29 ± 0.05 to 12.04 ± 0.09 g/100 g) and DHA (from 5.11 ± 0.04 to 14.98 ± 0.51 g/100 g) were found in higher levels. Although these were the main compounds observed in both fish and liquid medium of tuna conserved in brine, generally their contents varied significantly among the different brands ($p < 0.05$). Palmitic acid levels, for example, were higher in samples of fish than in samples of brine from brands A and B. However, a higher content of palmitic acid was determined in brine than in fish for brand C.

The presence of these fatty acids in brine indicates the migration of lipid components from the tuna to the liquid medium. The salt present in brine may disrupt the cell membranes and degrade proteins (Rhee, 1999), facilitating this migration. As a consequence, essential fatty acids may be liberated from the fish muscle to the covering liquid. In brine, the sum of EPA and DHA ranged from 5.45 ± 0.04 to 15.20 ± 0.51 g/100 g. In addition, these values were significantly higher in the liquid medium than in the fish in samples of brand A and B ($p < 0.05$).

Fish is recognized as an important source of EPA and DHA, which are known for their anti-inflammatory properties and potential to mitigate various metabolic and neurologic disorders (Innes & Calder, 2020). However, industrial processes like canning may compromise the quality of fish products from the nutritional point of view.

Regarding tuna conserved in oil, significant differences ($p < 0.05$) among brands were observed for most fatty acids (Table 3). The main fatty acid in tuna fish was linoleic acid (C18:2*n*6c, from 39.03 ± 0.38 to 49.61 ± 0.97 g/100 g), followed by oleic acid (from 18.94 ± 0.99 to 25.96 ± 0.08 g/100 g) and palmitic acid (10.48 ± 0.12 to 12.58 ± 0.70 g/100 g). PUFAs presented the highest contents, followed by MUFAs and SFAs, respectively. The contents determined for these different groups of fatty acids were significantly different among the brands ($p < 0.05$). Considering the *n*3-serie, the results determined for EPA + DHA varied from 0.96 ± 0.05 to 5.57 ± 0.32 g/100 g.

Concerning the oil; high levels of linoleic acid (from 48.58 ± 0.39 to 49.61 ± 0.97 g/100 g) and PUFAs (from 49.65 ± 0.39 to 51.05 ± 0.98 g/100 g) were assessed. The higher content of linoleic acid indicates a fatty acid profile different from that determined in tuna in brine. Since linoleic acid is commonly found in vegetable oils, such as soybean oil, its high level in tuna canned in oil suggests the absorption of fatty acids from the vegetable oils to the fish muscle, promoting changes in the lipid

Table 2
Fatty acid composition (g/100 g of lipids, dry basis) of the tuna and the liquid medium of canned tuna in brine.

Fatty acids	Canned tuna in brine					
	Brand A		Brand B		Brand C	
	Tuna	Liquid medium	Tuna	Liquid medium	Tuna	Liquid medium
C6:0	–	–	–	0.02 ± 0.01^{A}	–	0.04 ± 0.01^{A}
C8:0	$0.01 \pm 0.00^{a;\alpha}$	$0.02 \pm 0.00^{B;\beta}$	0.02 ± 0.00^{b}	0.01 ± 0.00^{A}	$0.03 \pm 0.00^{c;\beta}$	$0.02 \pm 0.00^{B;\alpha}$
C10:0	0.01 ± 0.00^{a}	–	$0.01 \pm 0.00^{a;\alpha}$	$0.01 \pm 0.00^{A;\alpha}$	$0.01 \pm 0.00^{a;\beta}$	$0.02 \pm 0.00^{B;\beta}$
C12:0	$0.07 \pm 0.00^{a;\alpha}$	$0.05 \pm 0.00^{A;\alpha}$	$0.16 \pm 0.01^{c;\beta}$	$0.07 \pm 0.00^{B;\alpha}$	$0.10 \pm 0.00^{b;\alpha}$	$0.14 \pm 0.00^{C;\beta}$
C14:0	$4.46 \pm 0.03^{a;\beta}$	$3.70 \pm 0.06^{A;\alpha}$	$6.60 \pm 0.05^{b;\beta}$	$4.85 \pm 0.01^{B;\alpha}$	$4.43 \pm 0.07^{a;\alpha}$	$5.35 \pm 0.04^{C;\beta}$
C15:0	$1.28 \pm 0.01^{a;\beta}$	$1.05 \pm 0.01^{A;\alpha}$	$1.56 \pm 0.01^{c;\beta}$	$1.14 \pm 0.00^{B;\alpha}$	$1.43 \pm 0.02^{b;\alpha}$	$1.43 \pm 0.01^{C;\alpha}$
C16:0	$32.81 \pm 0.23^{a;\beta}$	$27.57 \pm 0.13^{A;\alpha}$	$36.00 \pm 0.26^{b;\beta}$	$29.66 \pm 0.23^{B;\alpha}$	$35.76 \pm 0.90^{b;\alpha}$	$38.78 \pm 0.28^{C;\beta}$
C17:0	$1.72 \pm 0.01^{a;\beta}$	$1.29 \pm 0.02^{A;\alpha}$	$1.96 \pm 0.00^{b;\beta}$	$1.36 \pm 0.02^{A;\alpha}$	$1.99 \pm 0.00^{b;\alpha}$	$1.99 \pm 0.01^{B;\alpha}$
C18:0	$12.34 \pm 0.09^{b;\beta}$	$8.62 \pm 0.05^{A;\alpha}$	$10.67 \pm 0.08^{a;\beta}$	$8.29 \pm 0.05^{A;\alpha}$	$12.84 \pm 0.94^{b;\alpha}$	$12.04 \pm 0.09^{B;\alpha}$
C20:0	$0.52 \pm 0.00^{ab;\alpha}$	$0.55 \pm 0.01^{A;\beta}$	$0.58 \pm 0.00^{b;\beta}$	$0.53 \pm 0.01^{A;\alpha}$	$0.49 \pm 0.01^{a;\alpha}$	$0.66 \pm 0.00^{B;\beta}$
C21:0	$0.32 \pm 0.00^{a;\alpha}$	$0.35 \pm 0.00^{B;\beta}$	0.33 ± 0.01^{a}	0.35 ± 0.00^{B}	$0.37 \pm 0.01^{b;\beta}$	$0.14 \pm 0.00^{A;\alpha}$
C22:0	$0.08 \pm 0.00^{b;\alpha}$	$0.09 \pm 0.00^{B;\alpha}$	–	0.11 ± 0.00^{C}	$0.07 \pm 0.00^{a;\beta}$	$0.05 \pm 0.00^{A;\alpha}$
C23:0	$0.02 \pm 0.00^{a;\alpha}$	$0.26 \pm 0.02^{A;\beta}$	–	0.30 ± 0.03^{A}	0.02 ± 0.00^{a}	–
C24:0	$1.02 \pm 0.01^{a;\alpha}$	$4.02 \pm 0.11^{C;\beta}$	$1.92 \pm 0.01^{b;\alpha}$	$3.27 \pm 0.13^{B;\beta}$	$1.91 \pm 0.15^{b;\beta}$	$1.65 \pm 0.01^{A;\alpha}$
C14:1 cis	$0.06 \pm 0.00^{a;\alpha}$	$0.07 \pm 0.00^{A;\beta}$	–	0.08 ± 0.00^{B}	–	0.09 ± 0.00^{C}
C15:1	0.02 ± 0.00^{a}	–	–	0.01 ± 0.00^{A}	$0.02 \pm 0.00^{a;\alpha}$	$0.026 \pm 0.00^{B;\beta}$
C16:1 cis	$4.17 \pm 0.03^{b;\alpha}$	$4.15 \pm 0.07^{A;\alpha}$	$5.34 \pm 0.04^{c;\beta}$	$4.67 \pm 0.01^{B;\alpha}$	$3.60 \pm 0.11^{a;\alpha}$	$4.04 \pm 0.03^{A;\beta}$
C17:1	$0.59 \pm 0.00^{a;\alpha}$	$0.70 \pm 0.01^{B;\beta}$	$0.90 \pm 0.01^{c;\beta}$	$0.68 \pm 0.01^{B;\alpha}$	$0.73 \pm 0.07^{b;\beta}$	$0.17 \pm 0.00^{A;\alpha}$
C18:1 ω6 t	$0.17 \pm 0.00^{b;\alpha}$	$0.21 \pm 0.01^{B;\beta}$	$0.02 \pm 0.00^{c;\alpha}$	$0.21 \pm 0.01^{B;\beta}$	$0.12 \pm 0.01^{a;\beta}$	$0.02 \pm 0.01^{A;\alpha}$
C18:1 ω9 cis	$15.37 \pm 0.11^{a;\alpha}$	$20.43 \pm 0.33^{B;\beta}$	$17.15 \pm 0.12^{ab;\alpha}$	$20.27 \pm 0.15^{B;\beta}$	$17.01 \pm 0.42^{ab;\beta}$	$15.48 \pm 0.11^{A;\alpha}$
C20:1 ω9	$2.32 \pm 0.02^{b;\beta}$	$2.17 \pm 0.02^{B;\alpha}$	$1.55 \pm 0.01^{a;\alpha}$	$2.71 \pm 0.03^{C;\beta}$	$1.64 \pm 0.03^{a;\beta}$	$1.44 \pm 0.01^{A;\alpha}$
C22:1 ω9	$0.34 \pm 0.00^{b;\alpha}$	$0.37 \pm 0.01^{A;\beta}$	$0.26 \pm 0.01^{a;\alpha}$	$0.44 \pm 0.00^{B;\beta}$	$0.33 \pm 0.02^{b;\alpha}$	$0.72 \pm 0.00^{C;\beta}$
C24:1 ω9	$1.21 \pm 0.01^{ab;\beta}$	$0.90 \pm 0.01^{A;\alpha}$	$0.88 \pm 0.01^{a;\alpha}$	$1.10 \pm 0.01^{A;\beta}$	$1.39 \pm 0.03^{b;\beta}$	$0.86 \pm 0.01^{A;\alpha}$
C18:2 ω6 t	$0.56 \pm 0.00^{b;\beta}$	$0.38 \pm 0.01^{C;\alpha}$	$0.47 \pm 0.01^{a;\beta}$	$0.34 \pm 0.00^{B;\alpha}$	$0.51 \pm 0.01^{b;\beta}$	$0.02 \pm 0.00^{A;\alpha}$
C18:2 ω6 cis	$1.72 \pm 0.01^{a;\alpha}$	$1.87 \pm 0.00^{A;\beta}$	$1.72 \pm 0.01^{a;\beta}$	$1.68 \pm 0.22^{A;\alpha}$	$1.62 \pm 0.33^{a;\alpha}$	$3.47 \pm 0.02^{B;\beta}$
C18:3 ω6	$0.05 \pm 0.00^{a;\alpha}$	$0.06 \pm 0.01^{A;\alpha}$	$0.06 \pm 0.01^{a;\alpha}$	$0.05 \pm 0.01^{A;\alpha}$	$0.05 \pm 0.01^{a;\alpha}$	$0.04 \pm 0.01^{A;\alpha}$
C18:3 ω3	$0.16 \pm 0.01^{a;\beta}$	$0.11 \pm 0.00^{A;\alpha}$	$0.18 \pm 0.01^{a;\beta}$	$0.14 \pm 0.00^{A;\alpha}$	$0.11 \pm 0.01^{a;\alpha}$	$0.14 \pm 0.00^{A;\beta}$
C20:2 ω6	$0.29 \pm 0.00^{a;\beta}$	$0.23 \pm 0.00^{A;\alpha}$	$0.32 \pm 0.00^{b;\beta}$	$0.28 \pm 0.01^{A;\alpha}$	$0.28 \pm 0.03^{a;\alpha}$	$0.40 \pm 0.01^{B;\beta}$
C20:3 ω6	$1.85 \pm 0.01^{c;\alpha}$	$0.26 \pm 0.01^{A;\beta}$	$0.46 \pm 0.00^{b;\alpha}$	$1.75 \pm 0.02^{B;\beta}$	$0.13 \pm 0.01^{a;\alpha}$	$0.27 \pm 0.00^{A;\beta}$
C20:3 ω3	$1.37 \pm 0.01^{b;\beta}$	$1.26 \pm 0.02^{B;\alpha}$	$1.06 \pm 0.01^{a;\alpha}$	$1.06 \pm 0.03^{AB;\alpha}$	$0.92 \pm 0.17^{a;\beta}$	$0.15 \pm 0.00^{A;\alpha}$
C20:4 ω6	$0.13 \pm 0.00^{a;\beta}$	$0.09 \pm 0.00^{A;\alpha}$	$0.13 \pm 0.00^{a;\beta}$	$0.09 \pm 0.01^{A;\alpha}$	0.15 ± 0.01^{b}	–
C20:5 ω3 (EPA)	$1.17 \pm 0.01^{b;\beta}$	$0.22 \pm 0.00^{A;\alpha}$	$0.29 \pm 0.00^{a;\beta}$	$0.19 \pm 0.01^{A;\alpha}$	$0.34 \pm 0.00^{a;\alpha}$	$0.35 \pm 0.00^{B;\alpha}$
C22:6 ω3 (DHA)	$8.89 \pm 0.06^{c;\alpha}$	$14.98 \pm 0.51^{C;\beta}$	$4.44 \pm 0.03^{a;\alpha}$	$10.48 \pm 0.37^{B;\beta}$	$7.43 \pm 0.60^{b;\beta}$	$5.11 \pm 0.04^{A;\alpha}$
EPA + DHA	$\mathbf{10.05 \pm 0.07^{c;\alpha}}$	$\mathbf{15.20 \pm 0.51^{C;\beta}}$	$\mathbf{4.73 \pm 0.03^{a;\alpha}}$	$\mathbf{10.67 \pm 0.36^{B;\beta}}$	$\mathbf{7.77 \pm 0.60^{b;\beta}}$	$\mathbf{5.45 \pm 0.04^{A;\alpha}}$
∑SFA	$\mathbf{54.67 \pm 0.39^{a;\beta}}$	$\mathbf{47.58 \pm 0.02^{A;\alpha}}$	$\mathbf{59.84 \pm 0.42^{b;\beta}}$	$\mathbf{49.96 \pm 0.09^{B;\alpha}}$	$\mathbf{59.48 \pm 1.59^{b;\alpha}}$	$\mathbf{62.32 \pm 0.44^{C;\beta}}$
∑MUFA	$\mathbf{24.25 \pm 0.16^{ab;\alpha}}$	$\mathbf{29.00 \pm 0.46^{B;\beta}}$	$\mathbf{26.12 \pm 0.17^{b;\alpha}}$	$\mathbf{30.16 \pm 0.20^{B;\beta}}$	$\mathbf{24.82 \pm 0.64^{ab;\beta}}$	$\mathbf{22.84 \pm 0.16^{A;\alpha}}$
∑PUFA	$\mathbf{16.20 \pm 0.10^{b;\alpha}}$	$\mathbf{19.48 \pm 0.54^{C;\beta}}$	$\mathbf{9.13 \pm 0.06^{a;\alpha}}$	$\mathbf{16.07 \pm 0.60^{B;\beta}}$	$\mathbf{11.56 \pm 0.46^{a;\beta}}$	$\mathbf{9.95 \pm 0.07^{A;\alpha}}$
∑ω3	$\mathbf{11.59 \pm 0.07^{c;\alpha}}$	$\mathbf{16.58 \pm 0.54^{C;\beta}}$	$\mathbf{5.97 \pm 0.05^{a;\alpha}}$	$\mathbf{11.88 \pm 0.39^{B;\beta}}$	$\mathbf{8.80 \pm 0.78^{b;\beta}}$	$\mathbf{5.75 \pm 0.04^{A;\alpha}}$
∑ω6	$\mathbf{4.72 \pm 0.03^{a\beta}}$	$\mathbf{3.10 \pm 0.02^{A;\alpha}}$	$\mathbf{3.18 \pm 0.01^{a;\alpha}}$	$\mathbf{4.40 \pm 0.21^{A;\beta}}$	$\mathbf{2.86 \pm 0.33^{a;\alpha}}$	$\mathbf{4.22 \pm 0.03^{A;\beta}}$

Values represent the mean ± SD ($n = 3$). Different lowercase and capital letters in the same row indicate significant differences (Tukey test) among brands for tuna and liquid medium, respectively. Greek letters in the same row inside each brand point out significant differences (unpaired T test) between tuna and liquid medium. All statistical analysis was performed at 5% of significance.

profile of fish (Li, Liang, Zhang, & Gao, 2016).

In addition, fatty acids such as palmitic acid, which is typical of fish metabolism, were determined in high levels in the oil. These results confirm the findings of other authors concerning the exchange of fatty acids between the fish and the filling media (Caponio, Gomes, & Summo, 2003; Mesías et al., 2015; Mohan et al., 2015; Stephen et al., 2010). The sum of EPA and DHA was lower in the oil than in tuna, suggesting minor losses of these constituents.

According to Gonçalves (2011), most of the fish moisture is involved in the myofibrillar structure and connective tissue, which can easily break down due to their constitution, into actin / myosin (myofibrillar proteins) in detriment of collagen and elastin (stroma proteins). Thus, the muscle fibers are easily broken during the processing of grated tuna. Through this process, lipids, especially those that contain unsaturated fatty acids, which have a lower melting point, liquefy and migrate to the liquid medium. However, since brine and oil present different viscosities, polyunsaturated fatty acids (like EPA and DHA) and even saturated fatty acids, which have a higher melting point, may migrate and disperse in the liquid. This may explain why the levels of fatty acids were higher in samples in brine, indicating the interaction between the components of the muscle and the liquid medium, than in oil.

Caponio et al. (2003) investigated oils (extra virgin olive oil, olive oil, soybean oil, sunflower oil, and corn oil) used as liquid mediums in canned tuna and pointed out the occurrence of fatty acids of high molecular weight and high degree of unsaturation that are characteristic of fish, in particular DHA.

Mesías et al. (2015) evaluated tuna canned in different liquid mediums (brine, sunflower oil, and olive oil) and found higher levels of linoleic acid in tuna canned with vegetable oils, presenting the following results: from 4.1 to 4.4 g/100 g for tuna in brine, from 0.9 to 33.8 g/100 g for tuna in sunflower oil, and from 4.8 to 5.6 g/100 g for tuna in olive oil. This study demonstrated the contribution of the liquid medium to tuna lipids, since the highest content of linoleic acid was found in samples conserved in sunflower oil, which commonly presents linoleic acid as its main fatty acid.

Oxidative processes are amply described in processed fish products. It is well known that the conditions to which fish lipids are exposed during canning may induce the oxidation of fatty acids (Aubourg, Gallardo, & Medina, 1997; Barbosa et al., 2019; Naseri & Rezaei, 2012). Aubourg et al. (1997) evaluated the effect of the canning process on tuna (*Thunnus alalunga*) canned with olive oil and salt. The results showed that cooking reduced the PUFAs content from 46.10 (raw fish) to 43.27 (cooked fish) g/100 g. A higher degradation occurred when the samples were sterilized, with levels varying from 31.80 to 37.10 g/100 g. The

Table 3
Fatty acid composition (g/100 g of lipids, dry basis) of the tuna and the liquid medium of canned tuna in oil.

Fatty acids	Tuna canned in oil					
	Brand A		Brand B		Brand C	
	Tuna	Liquid medium	Tuna	Liquid medium	Tuna	Liquid medium
C6:0	–	–	–	–	–	0.08 ± 0.00^{A}
C8:0	–	0.01 ± 0.00^{A}	–	0.05 ± 0.00^{C}	–	0.04 ± 0.00^{B}
C10:0	–	–	–	–	–	–
C12:0	–	–	–	–	–	–
C14:0	0.22 ± 0.00$^{a;\beta}$	0.10 ± 0.01$^{A;\alpha}$	0.59 ± 0.01$^{b;\beta}$	0.17 ± 0.01$^{A;\alpha}$	0.17 ± 0.01$^{a;\beta}$	0.11 ± 0.00$^{A;\alpha}$
C15:0	0.06 ± 0.00$^{a;\beta}$	0.02 ± 0.00$^{A;\alpha}$	0.18 ± 0.02$^{b;\beta}$	0.04 ± 0.00$^{A;\alpha}$	–	0.02 ± 0.00^{A}
C16:0	10.55 ± 0.17$^{a;\alpha}$	11.19 ± 0.13$^{A;\alpha}$	12.58 ± 0.70$^{b;\alpha}$	12.22 ± 0.74$^{A;\alpha}$	10.48 ± 0.12$^{a;\alpha}$	11.59 ± 0.21$^{A;\beta}$
C17:0	0.13 ± 0.02$^{a;\beta}$	0.01 ± 0.00$^{A;\alpha}$	0.24 ± 0.04$^{b;\beta}$	0.15 ± 0.04$^{B;\alpha}$	0.11 ± 0.02$^{a;\alpha}$	0.11 ± 0.02$^{B;\alpha}$
C18:0	3.36 ± 0.01$^{a;\alpha}$	3.36 ± 0.07$^{A;\alpha}$	5.06 ± 0.40$^{b;\beta}$	4.54 ± 0.03$^{B;\alpha}$	3.42 ± 0.06$^{a;\alpha}$	3.46 ± 0.02$^{AB;\beta}$
C20:0	0.36 ± 0.02$^{a;\alpha}$	0.32 ± 0.00$^{A;\alpha}$	0.35 ± 0.05$^{a;\alpha}$	0.39 ± 0.03$^{B;\beta}$	0.39 ± 0.01$^{a;\beta}$	0.32 ± 0.00$^{A;\alpha}$
C21:0	0.05 ± 0.00$^{a;\alpha}$	0.05 ± 0.01$^{A;\alpha}$	0.15 ± 0.01$^{b;\beta}$	0.06 ± 0.01$^{A;\alpha}$	–	0.06 ± 0.01^{A}
C22:0	–	–	–	–	–	0.01 ± 0.00^{A}
C23:0	–	–	–	–	–	–
C24:0	0.33 ± 0.00$^{a;\beta}$	0.04 ± 0.00$^{A;\alpha}$	1.05 ± 0.11$^{b;\beta}$	0.14 ± 0.01$^{B;\alpha}$	–	0.05 ± 0.00^{A}
C14:1 cis	–	–	–	–	–	–
C15:1	–	–	–	–	–	–
C16:1 cis	0.27 ± 0.01$^{a;\beta}$	0.11 ± 0.00$^{A;\alpha}$	0.76 ± 0.00$^{b;\beta}$	0.20 ± 0.00$^{B;\alpha}$	0.19 ± 0.00$^{a;\beta}$	0.11 ± 0.00$^{A;\alpha}$
C17:1	0.08 ± 0.00$^{a;\beta}$	0.06 ± 0.01$^{A;\alpha}$	0.14 ± 0.02$^{a;\beta}$	0.07 ± 0.01$^{A;\alpha}$	–	0.05 ± 0.01^{A}
C18:1 ω6 t	–	0.07 ± 0.01^{A}	–	0.17 ± 0.02^{B}	–	0.14 ± 0.01^{B}
C18:1 ω9 cis	22.83 ± 0.45$^{b;\alpha}$	25.52 ± 0.05$^{A;\beta}$	18.94 ± 0.99$^{a;\alpha}$	23.95 ± 1.48$^{A;\beta}$	22.66 ± 1.13$^{b;\alpha}$	25.96 ± 0.08$^{A;\beta}$
C20:1 ω9	0.25 ± 0.00$^{a;\alpha}$	5.22 ± 0.10$^{A;\beta}$	–	5.06 ± 0.14^{A}	–	5.29 ± 0.35^{A}
C22:1 ω9	–	–	–	–	–	–
C24:1 ω9	0.12 ± 0.01$^{a;\beta}$	0.01 ± 0.01$^{A;\alpha}$	0.39 ± 0.03$^{b;\beta}$	0.03 ± 0.01$^{A;\alpha}$	0.22 ± 0.29$^{a;\beta}$	0.01 ± 0.01$^{A;\alpha}$
C18:2 ω6 t	–	–	0.09 ± 0.01^{a}	–	–	–
C18:2 ω6 cis	45.00 ± 0.09$^{b;\alpha}$	48.58 ± 0.39$^{A;\beta}$	39.03 ± 0.38$^{a;\alpha}$	49.61 ± 0.97$^{A;\beta}$	46.49 ± 0.92$^{b;\alpha}$	49.17 ± 1.84$^{A;\beta}$
C18:3 ω6	0.22 ± 0.00$^{a;\beta}$	0.21 ± 0.01$^{B;\alpha}$	0.30 ± 0.02$^{b;\beta}$	0.04 ± 0.00$^{A;\alpha}$	0.24 ± 0.01$^{a;\beta}$	0.21 ± 0.01$^{B;\alpha}$
C18:3 ω3	5.18 ± 0.01$^{b;\beta}$	0.03 ± 0.00$^{A;\alpha}$	4.66 ± 0.11$^{a;\beta}$	0.03 ± 0.00$^{A;\alpha}$	5.25 ± 0.13$^{b;\alpha}$	0.03 ± 0.00$^{A;\alpha}$
C20:2 ω6	0.42 ± 0.01$^{c;\alpha}$	0.42 ± 0.01$^{A;\alpha}$	0.36 ± 0.02$^{b;\alpha}$	0.43 ± 0.03$^{A;\beta}$	0.06 ± 0.00$^{a;\alpha}$	0.43 ± 0.01$^{A;\beta}$
C20:3 ω6	–	–	0.47 ± 0.04$^{a;\beta}$	0.02 ± 0.01$^{A;\alpha}$	–	0.01 ± 0.01^{A}
C20:3 ω3	0.15 ± 0.01$^{b;\beta}$	0.02 ± 0.01$^{A;\alpha}$	0.07 ± 0.00$^{c;\alpha}$	0.06 ± 0.01$^{A;\alpha}$	–	0.02 ± 0.01^{A}
C20:4 ω6	–	0.05 ± 0.00^{A}	0.10 ± 0.00$^{b;\beta}$	0.05 ± 0.00$^{A;\alpha}$	0.07 ± 0.00$^{a;\beta}$	0.04 ± 0.00$^{A;\alpha}$
C20:5 ω3 (EPA)	0.18 ± 0.01$^{a;\beta}$	0.16 ± 0.01$^{A;\alpha}$	0.18 ± 0.01$^{a;\alpha}$	0.20 ± 0.01$^{A;\beta}$	0.21 ± 0.01$^{a;\beta}$	0.12 ± 0.07$^{A;\alpha}$
C22:6 ω3 (DHA)	1.67 ± 0.07$^{b;\beta}$	0.16 ± 0.00$^{A;\alpha}$	5.39 ± 0.32$^{c;\beta}$	0.61 ± 0.02$^{B;\alpha}$	0.74 ± 0.04$^{a;\beta}$	0.16 ± 0.02$^{A;\alpha}$
EPA + DHA	**1.85 ± 0.06$^{a;\beta}$**	**0.33 ± 0.01$^{A;\alpha}$**	**5.57 ± 0.32$^{b;\beta}$**	**0.81 ± 0.02$^{B;\alpha}$**	**0.96 ± 0.05$^{a;\beta}$**	**0.28 ± 0.09$^{A;\alpha}$**
∑SFA	**15.05 ± 0.23$^{a;\alpha}$**	**15.12 ± 0.22$^{A;\alpha}$**	**20.19 ± 0.88$^{b;\beta}$**	**17.77 ± 0.79$^{B;\alpha}$**	**14.56 ± 0.07$^{a;\alpha}$**	**15.84 ± 0.15$^{A;\beta}$**
∑MUFA	**23.56 ± 0.47$^{b;\alpha}$**	**30.99 ± 0.06$^{A;\beta}$**	**20.24 ± 1.05$^{a;\alpha}$**	**29.50 ± 1.36$^{A;\beta}$**	**23.07 ± 1.41$^{b;\alpha}$**	**31.52 ± 0.41$^{A;\beta}$**
∑PUFA	**52.83 ± 0.16$^{a;\beta}$**	**49.65 ± 0.39$^{A;\alpha}$**	**50.65 ± 0.16$^{a;\alpha}$**	**51.05 ± 0.98$^{A;\alpha}$**	**53.07 ± 1.08$^{a;\alpha}$**	**50.20 ± 1.95$^{A;\alpha}$**
∑ω3	**7.18 ± 0.08$^{b;\beta}$**	**0.38 ± 0.01$^{A;\alpha}$**	**10.30 ± 0.22$^{c;\beta}$**	**0.90 ± 0.01$^{B;\alpha}$**	**6.20 ± 0.15$^{a;\beta}$**	**0.33 ± 0.09$^{A;\alpha}$**
∑ω6	**45.64 ± 0.08$^{b;\alpha}$**	**49.27 ± 0.39$^{A;\beta}$**	**40.35 ± 0.37$^{a;\alpha}$**	**50.14 ± 0.99$^{A;\beta}$**	**46.87 ± 0.92$^{b;\alpha}$**	**49.87 ± 1.86$^{A;\alpha}$**

Values represent the mean ± SD ($n = 3$). Different lowercase and capital letters in the same row indicate significant differences (Tukey test) among brands for tuna and liquid medium, respectively. Greek letters in the same row inside each brand point out significant differences (unpaired T test) between tuna and liquid medium. All statistical analysis was performed at 5% of significance.

authors used variable combinations of time and temperature and found the highest PUFAs degradation when lower temperatures were used for a longer time (110 °C, 120 min).

Naseri and Rezaei (2012) showed that canning reduced the content of EPA and DHA in approximately 53 and 45%, respectively, in canned sprat. Nonetheless, since the samples evaluated in the current study are from the market, fish samples were not analyzed before canning. Thus, authenticated findings concerning the oxidation of fatty acids cannot be concluded.

Several authors have reported higher content of PUFAs or MUFAs in comparison with SFAs in fresh tuna (Stephen et al., 2010; Truzzi et al., 2018). Thus, considering the fatty acid profile determined in tuna in brine (SFAs > MUFAs > PUFAs), it is possible to suggest that oxidative reactions and consequent degradation of PUFAs during canning occurred (Aubourg et al., 1997).

Tuna conserved in oil showed a profile close to typical ones found in tuna and other fish species (PUFA > MUFA > SFA) (Stephen et al., 2010; Truzzi et al., 2018). However, the main contributor to the high level of PUFAs was the linoleic acid that migrated to the fish muscle, making it difficult to observe the possible oxidative reactions proposed for the tuna samples in brine.

The liquid medium applied in canning directly influences the lipid oxidation rates, since it creates a pro-oxidant or antioxidant environment depending on its composition. Medina et al. (1998) demonstrated that tuna canned in brine presented higher oxidation rates than tuna conserved in vegetable oils (virgin olive oil, refined olive oil, and refined soybean oil). The authors reported that the aqueous environment (brine) made fish lipids more prone to oxidation due to the accumulation of unsaturated fatty acids at the oil–water interface. Moreover, they measured the content of tocopherols and phenols in order to suggest their antioxidant activity. Thus, the antioxidant effect of the oils was attributed to the migration of hydrophilic phenols from the oils to the water-muscle interface, where lipid oxidation occurs.

Salt is recognized as a pro-oxidant element (Mariutti & Bragagnolo, 2017); however, its mechanism of action is not completely elucidated. It may facilitate the access of oxidizing agents to lipids, liberate iron from iron-containing molecules and inhibit the activity of antioxidant enzymes (Mariutti & Bragagnolo, 2017; Rhee, 1999). Moreover, a greater heat transfer is noticed in brine than in oil (Xavier et al., 2007), leading to a more pronounced lipid thermo-oxidation.

On the contrary, the antioxidant potential of vegetable oils can be

highlighted due to the presence of polyphenols in their composition. Refined oils are commonly submitted to chemical and physical processes that may compromise their polyphenol content, making them more susceptible to oxidative deterioration. However, they are enriched with synthetic or natural antioxidants (Redondo-Cuevas, Castellano, Torrens, & Raikos, 2018). These considerations indicate several ways in which the liquid medium may directly affect the quality of the canned product concerning their sensorial and nutritional quality.

3.3. Cholesterol and cholesterol oxides of canned tuna and liquid medium

Cholesterol content of the fish samples varied from 190.95 ± 4.61 to 421.11 ± 37.83 mg/100 g in tuna in brine and from 135.90 ± 1.84 to 226.93 ± 34.50 mg/100 g in tuna in oil, in dry basis, respectively (Table 4). Significant differences ($p < 0.05$) were observed among the brands for both types of samples, but the liquid mediums from brands B and C presented similar values ($p = 0.588$). These levels were higher than those determined by Stephen et al. (2010), who reported levels from 183.61 to 185.80 mg/100 g in tuna canned with vegetable oil.

Results showed the migration of cholesterol from tuna muscle to its liquid medium, which was more evident when tuna was conserved in oil. The oil presented cholesterol levels varying from 113.09 ± 0.86 to 259.12 ± 0.64 mg/100 g, while contents from 17.27 ± 3.03 to 32.95 ± 7.23 mg/100 g were found in brine. Significant differences among some brands ($p < 0.05$) were detected in both types of samples. Due to its molecular structure, cholesterol presents a greater affinity with the nonpolar environment promoted by oils (Jandacek, Webb, & Mattson, 1977). Thus, its migration is favored in this type of liquid medium. No data was found in the literature reporting the quantification of cholesterol in liquid medium of canned tuna for comparison.

Considering both types of tuna samples and liquid mediums, the following COPs were identified and quantified by HPLC-RID-PDA, as well as confirmed by UHPLC-APCI-MS: 20α-hydroxycholesterol (20α-OH), 22(R)-hydroxycholesterol (22R-OH), 22(S)-hydroxycholesterol (22S-OH), 25-hydroxycholesterol (25-OH), 25R-hydroxycholesterol (25R-OH), 7-ketocholesterol (7-keto), 7α-hydroxycholesterol (7α-OH), 7β-hydroxycholesterol(7β-OH), 5,6α- epoxycholesterol (5,6α-EP), and 5,6β-epoxycholesterol (5,6β-EP). The chromatograms of cholesterol and cholesterol oxides obtained by HPLC-RID-PDA in the evaluated samples are shown in Fig. 1.

UHPLC-APCI-MS has been widely applied to identify cholesterol oxides in fish samples. These compounds were previously determined in *Sardinella brasiliensis* (Ferreira et al., 2017; Saldanha, Benassi, & Bragagnolo 2008), *Merluccius hubbsi* (Saldanha & Bragagnolo, 2008), and fish oil (de Oliveira et al., 2020). COPs are commonly found in fish muscle due to their production by the fish metabolism (Osada, Kodama, Cui, Yamada, & Sugano, 1993).

Nine COPs were found in tuna conserved in oil (20α-OH, 22R-OH, 22S-OH, 25-OH, 7-keto, 7α-OH, 7β-OH, 5,6α-EP, and 5,6β-EP), which presented some significant differences among brands ($p < 0.05$) for tuna and liquid medium The main cholesterol oxide determined was 5,6α-EP (from 452.68 ± 0.00 to 908.00 ± 0.68 μg/g), followed by 5,6β-EP (from 99.66 ± 11.62 to 148.91 ± 11.30 μg/g). The highest total COPs content was found in samples from brand C (1167.88 ± 11.07 μg/g), followed by brand A (819.95 ± 76.24 μg/g) and then B (698.24 ± 16.74 μg/g). As observed for cholesterol, COPs also migrated from tuna to the liquid medium ($p < 0.05$), where levels of total COPs ranging from 899.72 ± 6.41 to 1684.19 ± 0.61 μg/g were determined. Considering the individual cholesterol oxides, 22S-OH, 25-OH, 25R-OH, 7α-OH, and 7β-OH were not identified in the oil.

For the samples of tuna in brine, ten compounds were identified: 20α-OH, 22R-OH, 22S-OH, 25-OH, 25R-OH, 7-keto, 7α-OH, 7β-OH,

Table 4
Cholesterol (mg/100 g, dry basis) and cholesterol oxides (μg/g, dry basis) contents of the tuna and the liquid medium of canned tuna in brine and in oil.

Tuna in brine						
	Tuna		**Liquid medium**			
COPs						
	Brand A	**Brand B**	**Brand C**	**Brand A**	**Brand B**	**Brand C**
20α-OH	85.91 ± 0.13$^{a;\alpha}$	153.02 ± 0.25$^{b;\beta}$	165.17 ± 1.69$^{c;\beta}$	94.88 ± 0.66$^{C;\beta}$	30.58 ± 0.26$^{B;\alpha}$	11.98 ± 0.89$^{A;\alpha}$
22R-OH	31.11 ± 1.22$^{a;\alpha}$	–	68.54 ± 0.44$^{b;\beta}$	100.81 ± 10.95$^{C;\beta}$	37.80 ± 0.59^{B}	25.14 ± 1.14$^{A;\alpha}$
5,6α-EP	504.14 ± 8.59$^{a;\alpha}$	1246.65 ± 46.33$^{b;\alpha}$	1296.70 ± 28.08$^{b;\alpha}$	1338.76 ± 12.92$^{B;\beta}$	1245.35 ± 12.63$^{A;\alpha}$	1465.66 ± 44.96$^{C;\beta}$
5,6β-EP	110.99 ± 1.81$^{a;\alpha}$	244.87 ± 4.81$^{b;\alpha}$	222.94 ± 3.67$^{b;\beta}$	229.69 ± 23.92$^{B;\beta}$	256.80 ± 49.24$^{B;\alpha}$	116.59 ± 7.92$^{A;\alpha}$
22S-OH	21.11 ± 0.65^{a}	59.30 ± 13.45^{b}	–	–	–	–
25-OH	10.81 ± 1.05$^{b;\alpha}$	68.77 ± 1.18$^{c;\beta}$	4.99 ± 0.03$^{a;\alpha}$	52.39 ± 0.21$^{C;\beta}$	44.60 ± 0.15$^{B;\alpha}$	28.54 ± 0.03$^{A;\beta}$
25R-OH	25.50 ± 1.15$^{a;\alpha}$	104.04 ± 1.25$^{c;\beta}$	33.78 ± 0.60$^{b;\alpha}$	85.53 ± 0.48$^{A;\beta}$	93.01 ± 0.73$^{B;\alpha}$	121.36 ± 0.17$^{C;\beta}$
7-Keto	60.69 ± 2.35$^{b;\beta}$	33.23 ± 1.22$^{a;\alpha}$	32.94 ± 1.29$^{a;\alpha}$	51.68 ± 1.07$^{B;\alpha}$	108.22 ± 1.29$^{C;\beta}$	13.44 ± 3.97$^{A;\beta}$
7α-OH	47.99 ± 1.15$^{b;\alpha}$	–	8.87 ± 0.19$^{a;\alpha}$	85.53 ± 0.48$^{C;\beta}$	57.38 ± 0.27^{B}	13.82 ± 0.52$^{A;\beta}$
7β-OH	34.89 ± 0.72$^{c;\beta}$	4.35 ± 0.14$^{a;\alpha}$	14.84 ± 0.56$^{b;\alpha}$	12.61 ± 0.31$^{A;\alpha}$	29.66 ± 0.31$^{C;\beta}$	18.26 ± 0.29$^{B;\beta}$
Total COPs	**933.14 ± 6.97$^{a;\alpha}$**	**1914.23 ± 56.11$^{b;\alpha}$**	**1848.77 ± 29.79$^{b;\alpha}$**	**2051.88 ± 24.71$^{B;\beta}$**	**1903.40 ± 40.38$^{A;\alpha}$**	**1814.79 ± 54.78$^{A;\alpha}$**
Cholesterol	**190.95 ± 4.61$^{c;\beta}$**	**421.11 ± 37.83;β^{b}**	**271.38 ± 1.24$^{a;\beta}$**	**32.95 ± 7.23$^{B;\alpha}$**	**18.05 ± 1.99$^{A;\alpha}$**	**17.27 ± 3.03$^{A;\alpha}$**
Tuna in oil						
	Tuna		**Liquid medium**			
COPs						
	Brand A	**Brand B**	**Brand C**	**Brand A**	**Brand B**	**Brand C**
20α-OH	46.16 ± 6.38$^{b;\alpha}$	27.15 ± 0.24$^{a;\alpha}$	74.89 ± 0.538$^{c;\alpha}$	130.90 ± 0.48$^{B;\beta}$	26.42 ± 0.75$^{A;\alpha}$	135.18 ± 0.21$^{B;\beta}$
22R-OH	22.05 ± 0.11$^{b;\beta}$	6.87 ± 0.04$^{a;\alpha}$	15.21 ± 0.61$^{ab;\beta}$	10.62 ± 0.14$^{A;\alpha}$	12.38 ± 2.99$^{A;\beta}$	9.20 ± 0.43$^{A;\alpha}$
5,6α-EP	529.69 ± 67.18$^{a;\alpha}$	452.68 ± 0.00$^{a;\alpha}$	908.00 ± 0.68$^{b;\alpha}$	1278.35 ± 0.00$^{B;\beta}$	762.28 ± 2.02$^{A;\beta}$	1509.02 ± 0.00$^{C;\beta}$
5,6β-EP	99.66 ± 11.62$^{a;\beta}$	112.67 ± 16.90$^{a;\beta}$	148.91 ± 11.30$^{b;\beta}$	83.77 ± 2.71$^{B;\alpha}$	90.58 ± 3.18$^{B;\alpha}$	24.19 ± 0.00$^{A;\alpha}$
22S-OH	26.49 ± 0.27^{b}	–	9.22 ± 0.49^{a}	–	–	–
25-OH	–	1.85 ± 0.01 ^{g}	–	–	–	–
25R-OH	–	–	–	–	–	–
7-Keto	52.84 ± 1.16$^{c;\beta}$	32.55 ± 0.29$^{b;\beta}$	11.65 ± 0.14$^{a;\beta}$	7.98 ± 1.60$^{A;\alpha}$	8.06 ± 1.73$^{A;\alpha}$	6.60 ± 0.09$^{A;\alpha}$
7α-OH	16.33 ± 0.09^{b}	3.60 ± 0.02^{a}	–	–	–	–
7β-OH	26.73 ± 0.14^{a}	60.88 ± 0.21^{b}	–	–	–	–
Total COPs	**819.95 ± 76.24$^{b;\alpha}$**	**698.24 ± 16.74$^{a;\alpha}$**	**1167.88 ± 11.07$^{c;\alpha}$**	**1511.62 ± 3.4$^{B;\beta}$**	**899.72 ± 6.41$^{A;\beta}$**	**1684.19 ± 0.61$^{C;\beta}$**
Cholesterol	**226.93 ± 34.50$^{b;\alpha}$**	**212.11 ± 35.01$^{b;\beta}$**	**135.90 ± 1.84$^{a;\alpha}$**	**259.12 ± 0.64$^{B;\alpha}$**	**113.09 ± 0.86$^{A;\alpha}$**	**246.27 ± 9.69$^{B;\beta}$**

Values represent the mean ± SD ($n = 3$). Different lowercase and capital letters in the same row indicate significant differences (Tukey test) among brands for tuna and liquid medium, respectively. Greek letters in the same row for each brand point out significant differences (unpaired T test) between tuna and liquid medium. All statistical analysis was performed at 5% of significance.

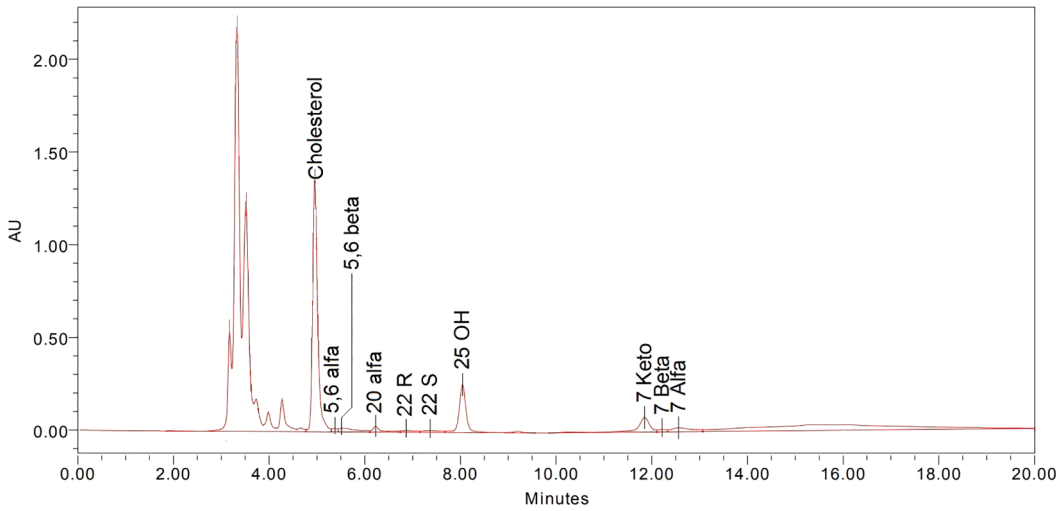

Fig. 1. Chromatogram by HPLC-RID-PDA of cholesterol and cholesterol oxides (tuna canned in brine sample).

5,6α-EP, and 5,6β-EP. As observed in tuna in brine, 5,6α-EP was the most representative COP (from 504.14 ± 8.59 to 1296.70 ± 28.08 μg/g), followed by 5,6β-EP (from 110.99 ± 1.81 to 244.87 ± 4.81 μg/g). High levels of 20α-OH were also measured (from 85.91 ± 0.13 to 165.17 ± 1.69 μg/g). Some statistical differences were detected among brands for both tuna and liquid medium.

The total COPs contents of tuna canned in brine were significantly higher than the ones determined in tuna in oil ($p < 0.05$), the values increased as follows: brand A (933.14 ± 6.97 μg/g) < brand C (1848.77 ± 29.79 μg/g) < brand B (1914.23 ± 56.11 μg/g). In addition, higher levels of COPs were also found in the liquid medium (brine) when compared to tuna ($p < 0.05$), where values varied from 1814.79 ± 54.78 to 2051.88 ± 24.71 μg/g.

COPs were also detected in canned tuna conserved in brine by Zunin et al. (2001). They evaluated different brands from Italy and reported contents varying from 37.7 to 328.9 μg/g of total COPs, with higher levels of 7-keto (from 10.1 to 139.2 μg/g), 7β-OH (from 7.0 to 50.1 μg/g) and 5,6β-EP (from 3.1 to 52.8 μg/g), respectively.

Cholesterol oxidation is a complex process that may be influenced by several factors and occur through different mechanisms, leading to the formation of diverse COPs at varied levels (Barriuso, Ansorena, & Astiasarán, 2017). Although COPs are mainly formed during food processing and storage, they are commonly found in unprocessed fish. Indeed, COPs are endogenously formed in animals by enzymatic or non-enzymatic reactions (Hur et al., 2007; Smith, 1987). Therefore, the initial cholesterol and COPs contents present in raw samples directly affect the formation of cholesterol oxides during canning.

Cholesterol oxidation in food occurs via an auto-oxidative process, similar to the oxidation of unsaturated fatty acids, that is based on the formation of free radicals. This mechanism requires the presence of oxygen and is induced by light, heat, photosensitizers, metals and oxygen and nitrogen reactive species (Dantas et al., 2015; Lehtonen, Lampi, Riuttamäki, & Piironen, 2012; Mariutti & Bragagnolo, 2017). Therefore, besides intrinsic factors of fish muscle (fish composition), extrinsic factors such the conditions of processing and storage may also induce or minimize the cholesterol oxidation (Domínguez, Pateiro, Gagaoua, Barba, Zhang, & Lorenzo, 2019).

Regarding intrinsic factors, the fatty acid profile and the amounts of pro-oxidants (heme-proteins, metals, enzymes) and antioxidants (vitamins, antioxidant enzymes or peptides) are determinant in the development of oxidative processes. Moreover, other aspects that can modify the meat composition, such as the diet, can be considered (Domínguez et al., 2019).

Among the extrinsic parameters involved in COPs formation, processing temperature, heating time, storage conditions and packing (presence of light and oxygen), as well as levels of pro-oxidants and antioxidants play an important role (Dantas et al., 2015; Hur et al., 2007). In the case of canned tuna, the liquid medium applied may also be highlighted as a crucial aspect.

In the current study, higher levels of COPs were found in samples conserved in brine than in oil, in both liquid medium and fish. The oxidative stability of canned tuna depends on the balance of anti- and pro-oxidant elements present in the liquid medium. Salt present in brine is commonly known as an oxidation inducer, while vegetable oils have high content of bioactive compounds with antioxidant properties.

The influence of salt on COPs formation has been reported. In a study performed by Kang et al. (2008), the addition of salt increased the COPs content of Gulbi (*Pseudosciaena manchurica*) during drying and storage. Cholesterol oxides were also detected in commercial samples of dried salted shrimp (Soto-Rodríguez, Campillo-Velázquez, Ortega-Martínez, Rodríguez-Estrada, Lercker, & Garcia, 2008). A study was conducted to monitor the formation of COPs in dried salted shrimp during cooking, sun drying, and storage. The authors identified COPs such as 7α-OH, 7β-OH, 5,6α-EP, 5,6α-EP, 25-OH, and 7-keto, as well as demonstrated the pronounced formation of COPs during storage, when total COPs content reached a value of 886.6 μg/g after 90 days of storage in polypropylene boxes at room temperature (Becerra, Flores, Valerio-Alfaro, Soto-Rodriguez, Rodríguez-Estrada, & García, 2014).

Other important factors are the fatty acid profile and the pH of the liquid medium. High concentrations of unsaturated fatty acids may also induce the formation of COPs, since radicals and oxygenated species formed during lipid oxidation can exert a pro-oxidant effect (Lehtonen et al., 2012). Therefore, the high content of PUFAs present in oil contributes to the content of COPs determined in tuna conserved in oil. On the other hand, the lower pH of brine created a pro-oxidant environment (Mozuraityte, Kristinova, Rustad, & Storrø, 2016). Low pH values can reduce the protein–protein interaction resulting in the liberation of water to the fish surface and consequent protein solubilization that causes a greater exposure of lipids to pro-oxidant agents (Liu et al., 2010). In addition, the viscosity of the liquid medium also influences lipid oxidation by facilitating or hindering the movement of molecules (Hur et al., 2007). Therefore, the presence of water, as seen in brine, may increase the formation of COPs.

Cooking and sterilization, which require the use of high temperatures, are the main process steps involved in the formation of COPs in canned products. High temperatures may reduce the activation energy necessary for the hydrogen abstraction originating radicals that initiate the oxidative reactions (Barriuso et al., 2017).

Besides the time and temperature applied during the heating process, heat transfer is affected by the dimensions, shapes and material of cans, the thermo-physical properties of the product, and the liquid medium used (Xavier et al., 2007). When considering the liquid medium, differences in heat penetration may be attributed to the viscosity and flow behavior when exposed to heat stress. The higher the viscosity the slower the heat transfer rate (Mohan, Remya, Ravishankar, Vijayan, & Srinivasa Gopal, 2014). Xavier et al. (2007) evaluated the heat transfer in tuna canned with different filling mediums in tin-free steel cans and demonstrated that oil was more resistant to heat penetration than sauce, brine and curry.

Oxidation may also be induced by heavy metals contained in tuna and in the cans (Eboh, Mepba, & Ekpo, 2006). The pressure applied in the retort system can increase the rate of lipid oxidation in fish muscle since it promotes the release of water and/or metal ions from hemoprotein (Mesías et al., 2015). In addition, in tuna samples which are finely minced or grated, the contact with oxygen and other pro-oxidant agents increases (Zunin et al., 2001).

Time and conditions of storage are also pivotal in oxidative processes in cholesterol-containing foods. Moreover, thermal processing, even for short periods as occurs in the sterilization step, leads to continued oxidative processes of the cholesterol molecule (Vicente, Sampaio, Ferrari, & Torres 2012). Moreover, important polyunsaturated fatty acids may be degraded by canning, as well as migrate to the liquid medium, which is commonly not consumed. Thus, it is important to better evaluate the fatty acid composition of fish products in order to review processing practices from the perspective of health benefits.

Furthermore, the current study highlights the potential of canned fish products as sources of exogenous COPs, which are subjects of remarkable importance in the public health area. COPs are associated with the development of inflammatory processes, cell death, atherosclerosis, carcinogenesis, and neurodegenerative diseases (Sottero et al., 2019).

Regarding the consequences of canning on fish lipids, it is critical to limit the extent of the oxidation process that leads to the degradation of fatty acids and the formation of COPs. Antioxidants are considered one of the most suitable and practical strategies to control or minimize oxidation in food. Thus, the addition of natural sources of antioxidants such as herbs, spices and fruit may present promising alternatives (de Oliveira et al., 2018).

Canned tuna is a product that presents advantages related to preservation and convenience. In addition, aspects such as its price, taste and

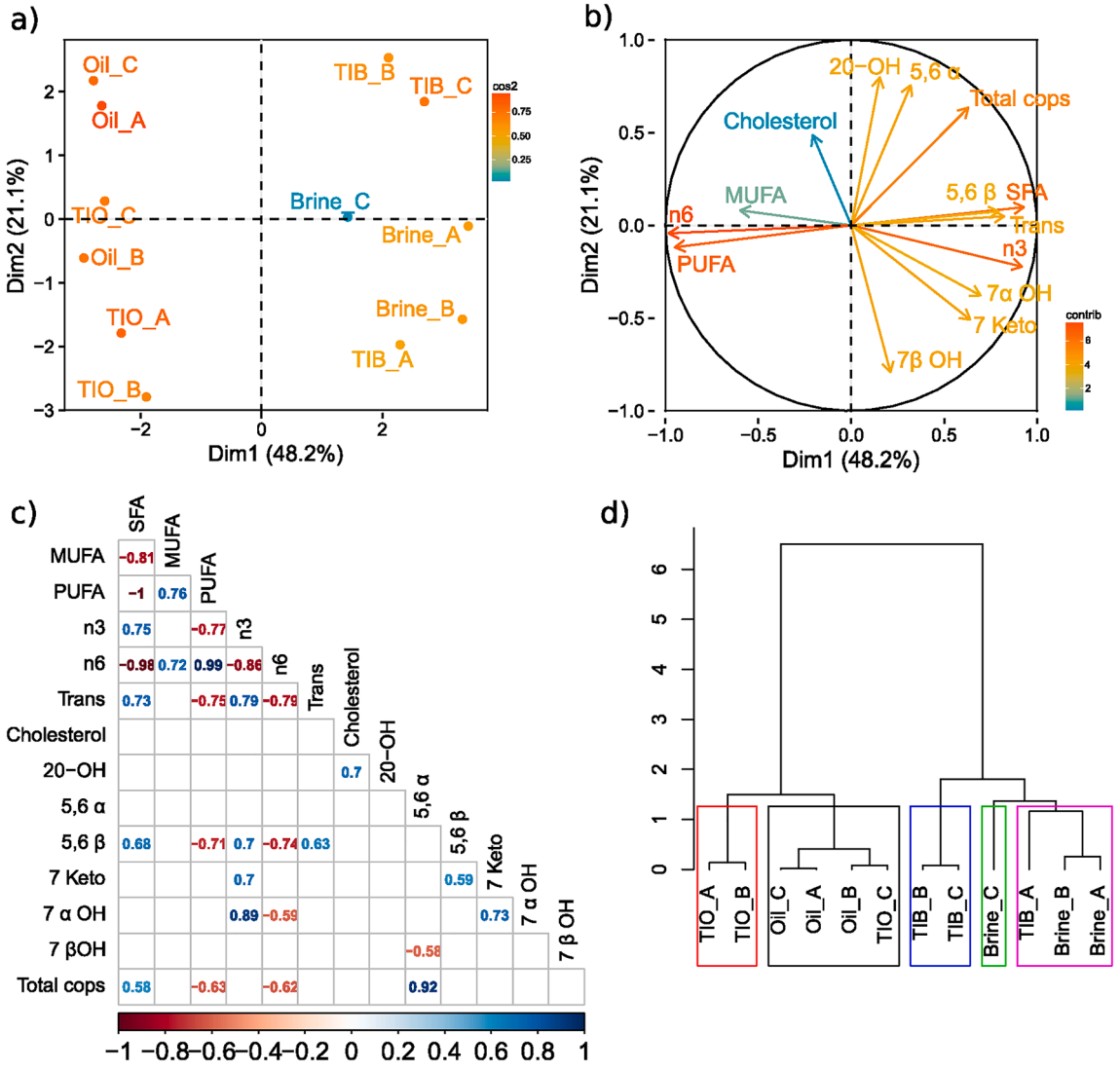

Fig. 2. Score plots (PC1 *vs* PC2) of PCA performed for variables (MUFA, PUFA, *n*3, *n*6, cholesterol, and cholesterol oxides) (a), and for samples (b). The correlogram was carried out on the same variables used in PCA, where blue and red values mean significant positive and negative correlation, respectively (c). Hierarchical clustering from principal components HCPC (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nutritional value also contribute to the consumer demand. Since canned tuna is one of the most consumed fishery products worldwide, studies of its characteristics (including price, taste, packaging, processing, safety, and nutrition) must be conducted (Kumar, 2018; Kumar & Kocour, 2015). The recent literature has discussed diverse issues: the presence of heavy metals, histamine and other contaminants in canned tuna, the lack of information in its labels, methods to better identify each species, optimization of parameters during the different steps of processing, among others (Barbosa et al., 2019; Kumar, 2018; Mata et al., 2020; Mesías et al., 2015; Mohan et al., 2015; Peivasteh-Roudsari et al., 2020). However, few studies have investigated the effects of canning on the lipid quality of tuna and the formation of COPs, as well as the influence of the liquid medium applied.

3.4. Principal component analysis

The first two PCs explain 48.7% and 22%, respectively, which is ~ 70% of the original data variability. Sample TIB_B and TIB_C (Fig. 2a) had greater amounts of 22α-OH, 5,6α-EP, and total COPs (Fig. 2b) than TIB_A. Besides theses oxides, TIB_A presented relevant quantities of 7β-OH, 7α-OH, and 7-keto. Although these tuna samples were all conserved in brine, they are from different brands and were probably submitted to different processing conditions. Tuna samples conserved in oil and their liquid mediums (TIO_A, TIO_B, TIO_C, Oil_B, and Oil_C) showed the highest amounts of PUFAs and fatty acids from the *n*6-series (Fig. 2a and 2b). This confirms the exchange between fatty acids from the fish and the oil, mainly when considering linoleic acid.

Regarding correlations; PUFAs presented strong positive correlation with Σ*n*6 (0.98) (Fig. 2c). Cholesterol presented moderate positive correlation with 22α-OH (0.7). The total COPs presented significant moderate negative correlation with PUFAs (-0.66), suggesting that products, such as radicals derived from lipid oxidation of PUFAs can exert a pro-oxidant effect on cholesterol. As expected, total COPs showed a strong positive correlation with 5,6α-EP, the main cholesterol oxide present in samples.

Fig. 2d shows four groups of samples that were formed according to their similarities by applying HCPC. The groups were divided as follows: group 1 (samples of tuna in oil from brands A and B), group 2 (samples of oil from brands A, B, and C, and tuna conserved in oil from brand C), group 3 (samples of brine from brands A and B, and tuna in brine from brand A), and group 4 (samples of tuna in brine from brands B and C and brine from brand C).

Samples of tuna conserved in oil are placed in two different groups, demonstrating the variation among the brands. In addition, samples of fish and liquid medium from the same brand are also placed in different groups (samples conserved in oil from brands A and B, for example), highlighting the migration of lipids. Similar trends were noticed in samples conserved in brine as well.

4. Conclusion

The results showed the strong influence of the liquid medium on the fatty acid profile of tuna, which was demonstrated by an exchange of fatty acids between the fish muscle and the covering liquid. The high levels of linoleic acid found in tuna conserved in oil highlight this effect. In samples of tuna in brine, this exchange caused the migration of important fatty acids from the fish to the brine. The liquid medium played an important role in COPs formation, which was more pronounced in samples of tuna in brine. This result was mainly promoted by the presence of pro-oxidant elements such as salt, as well as greater heat transfer in brine than in oil. Thus, data from this study suggested that canned tuna samples are potential sources of exogenous COPs and revealed that the liquid medium is a key factor in oxidative processes. These considerations demonstrate that the canning process should be carefully studied to better elucidate mechanisms that cause losses in the nutritional value of these products and the formation of COPs. Moreover, strategies to minimize oxidation in canned fish products should be investigated.

Author contributions

Natalie Marinho Dantas undertook almost most of the experimental work presented in this paper. Vanessa S. de Oliveira interpreted the results and drafted the manuscript with help from the other authors. Geni R. Sampaio interpreted the results and drafted the manuscript with help from the other authors. Yane Sane Koppe Chrysostomo contributed to the experimental analysis. Davy W. H. Chávez did the statistical analysis. Ormindo D. Gamallo helped with experimental works. Alexandra C. H. F. Sawaya was responsible for the mass spectrometry analyses. Elizabeth A. F. S. Torres supervised and organized the study. Tatiana Saldanha designed, supervised, organized the study, interpreted the results and drafted the manuscript with help from the other authors. 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129334.

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