Impact of Air Frying on Cholesterol and Fatty Acids Oxidation in Sardines: Protective Effects of Aromatic Herbs

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Abstract: The high temperatures used to fry fish may induce thermo-oxidation of cholesterol, forming cholesterol oxidation products (COPs). COPs have been associated to coronary heart diseases, atherosclerosis, and other chronic diseases. Air fryers are an alternative thermal process technology to fry foods without oil, and are considered a healthier cooking method. This study is the 1st to evaluate the formation of COPs and the degradation of polyunsaturated fatty acids (PUFAs) in air-fried sardine fillets. Furthermore, we evaluated the effect of fresh herbs added as natural antioxidants to sardines subjected to air frying. Parsley (*Petroselinum crispum*), chives (*Allium schoenoprasum* L.), and a mixture of both herbs (*cheiro-verde*) were added in quantities of 0%, 2%, and 4%. Air frying significantly decreased the content of essential PUFAs, and increased the levels of COPs from 61.2 (raw) to 283 μg/g ($P < 0.05$) in the control samples. However, the use of herbs as natural antioxidants proved to be effective reducing such levels of COPs in most samples. The addition of 4% of *cheiro-verde* in air-fried sardines presented the best protective effect against lipid oxidation.

Keywords: air frying, cholesterol oxides, fatty acids, natural antioxidants, sardines

Practical Application: Fish is an important source of essential lipids. However, oxidized cholesterol products, which are formed during thermal processing, are potential hazards to human health. Air fryers present an alternative thermal process for frying food without oil, and this method of cooking is considered to be more convenient and healthier This study shows that the air frying increased the formation of cholesterol oxidation products and decreased the essential polyunsaturated fatty acids in sardine fillets. However, the lipid oxidation is significantly reduced by adding fresh herbs, such as parsley (*Petroselinum crispum*), chives (*Allium schoenoprasum* L.), or a mixture of both herbs (*cheiro-verde*) that are natural antioxidants.

Introduction

Frying, which is extensively employed in homes, restaurants, and industry, consists of dehydrating food immersed in hot oil. However, during frying, numerous reactions with oxygen, due to the high temperatures and the release of water, take place and consequently a succession of physical and chemical changes occur in the food products (Teruel and others 2015).

Air frying circulates hot air uniformly around the food instead of immersing it in hot oil and also reduces the fat contents in the fried food. Thus, the product is progressively dehydrated at a fixed frying temperature of 180 °C, while the crisp characteristic appears on the fried products with minimum variations in food quality (Andrés and others 2013; Heredia and others 2014; Sansano and others 2015). Air fryers are available on the market as an alternative to fry foods without oil, and are considered a convenient method of cooking; however, there are no studies regarding degradation of lipids by this thermal process.

Brazilian sardines (*Sardinella brasiliensis*) are an important source of fish commercialized in Brazil and are largely consumed due to their excellent nutritional quality and low cost. They contain proteins of high biological value, in addition to their lipid composition, especially *n*-3 polyunsaturated fatty acids (PUFAs), mainly eicosapentaenoic (EPA 20:5*n*-3) and docosahexaenoic acids (DHA 22:6*n*-3). The regular consumption of *n*-3 PUFAs is associated with health benefits (Casas and others 2014; FAO 2016; Zock and others 2016).

However, some authors have demonstrated that sardines also contain high levels of cholesterol (Saldanha and others 2008; Scherr and others 2015). These 2 types of compounds, *n*-3 PUFAs and cholesterol, are not chemically stable and this instability is influenced by their chemical structure, the presence of oxygen, light, metal ions, and the processing techniques (heat) used, among other factors (Dantas and others 2015; Barriuso and others 2017).

The temperature used and the time required to prepare fish and fish products are perhaps the main factors that contribute to the degradation of cholesterol, forming cholesterol oxidation products (COPs) (Dantas and others 2015; Leal-Castañeda and others 2017). These substances are structurally similar to cholesterol and are normally present in small quantities formed by endogenous metabolic processes. However, the exogenous cholesterol oxides

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doi: 10.1111/1750-3841.13967

A Publication of the Institute of Food Technologists
Journal of Food Science

formed during the processing of food can be potentially harmful to health (Dantas and others 2015) and these COPs are associated with the development of cardiovascular and neurodegenerative diseases, besides inflammation, atherosclerosis, mutagenesis, carcinogenesis, and cell death (Doria and others 2016; Barriuso and others 2017).

In recent years, polyphenolic compounds present in herbs and spices have been used as an alternative to replace synthetic antioxidants (Carocho and Ferreira 2013; Figueirêdo and others 2015). They have also attracted attention as they can prevent lipid oxidation in fish and fish products (Sancho and others 2011; Maqsood and others 2013; Frank and others 2014; Maqsood and others 2014; Figueirêdo and others 2015; Meleiro and others 2016; Tarvainen and others 2016; Vaisali and others 2016). Aromatic herbs are used worldwide by various culinary cultures and the antioxidant effects of parsley (*Petroselinum crispum*) and chives (*Allium schoenoprasum* L.) have been evaluated and proven (Zhang and others 2006; Jia and others 2012; Sęczyk and others 2015; Tang and others 2015; Sęczyk and others 2016). The aromatic herbs, parsley, and chives are good sources of essential nutrients such as vitamins and minerals as well as antioxidant compounds. The phenolic compounds in these herbs are the principal bioactive phytochemicals that have been identified in several studies, particularly the flavonoids (apigenin, apennine, malonyl-apennine, luteolin, crisoeriol, cosmosiin, quercetin, kaempferol, p-coumaric acid, myricetin, and isorhamnetin (Parvu and others 2010; Stan and others 2012; Farzaei and others 2013; Vlase and others 2013); gallic acid, coumaric acid, ferulic acid, and rutin in *A. schoenoprasum* (Kucekova and others 2011)). The combination of parsley and chives, also known as *cheiro-verde*, is an important fresh condiment commonly used in the Brazilian cuisine. It is usually finely chopped and then added to dishes such as soups, meats, fish, and sauces for salads and others. However, there are no data in the literature about the use of this herbal mixture as a natural antioxidant.

Considering the high degradation of cholesterol and PUFAs when heating fish, with the consequent formation of COPs, it is necessary to reevaluate the processing techniques for these foods, which are mainly affected by thermo-oxidation. The aim of the present study was to evaluate the impact of air frying on lipid degradation in sardine samples and the effects of adding aromatic herbs such as parsley, chives, and the mixture of both herbs (*cheiro-verde*) as natural antioxidants to protect the cholesterol and PUFAs from thermal degradation.

Materials and Methods

Samples and sample preparation

Sardines. Ten kilograms of fresh Brazilian sardines (*S. brasiliensis*) were obtained from Angra dos Reis, Rio de Janeiro, Brazil (Latitude: 23° 00′ 24″ S and Longitude: 44° 19′ 05″ W), in March 2016, and transported in a refrigerated truck to UFRRJ at Seropédica, Rio de Janeiro, Brazil. After evisceration, the sardines were immediately washed, homogenized, and separated into 8 lots. The sardines had an average weight of 88.34 ± 5.04 g and measured between 19 and 21 cm in length. One of them was analyzed on the same day the fish were acquired, corresponding to fresh (raw) sardines. The other samples were packed in polyethylene film and stored at 5 °C in a domestic refrigerator, until preparation the following day.

Herbs. Fresh parsley (*P. crispum*) and chives (*A. schoenoprasum* L.) were obtained from the Experimental Agricultural Station of EMBRAPA (Seropédica, Rio de Janeiro-Brazil). The herbs were selected, washed, and chopped (3 × 3 mm approximately). The moisture content of the fresh herbs was (94.70% ± 0.45; 87.50% ± 0.80 and 89.00% ± 0.30) for chives, parsley, and *cheiro-verde*, respectively. *Cheiro-verde* samples consisted of the combination of the mixture of the 2 herbs: 50% (w/w) of parsley and 50% (w/w) of chives. Two levels of parsley, chives, and *cheiro-verde* were employed, selected according to the concentration used in the local cuisine (2% and 4% [w/w]). The total phenolic was determined with *Folin-Ciocalteu* reagent, according to Singleton and Rossi (1965). The obtained results of these herbs were 8.34 ± 0.30, 5.71 ± 0.1, and 6.09 ± 0.42 mg GAE/g in parsley, chives, and *cheiro-verde*, respectively.

Preparation of sardine samples. Each treatment consisted of 12 fillets of raw sardines; the average weight of the sardine fillets was 61.66 ± 3.53 g and the dimensions 12 × 7 ± 0.5 cm. The control treatment was samples air-fried without the addition of herbs; the other treatments were sardines air-fried with the addition of 2% and 4% of parsley, chives, and *cheiro-verde,* respectively. The percentage of chopped herbs (g/100 g) was calculated according to the weight of the lot. After this, the herbs were added homogeneously on both sides of the sardine fillets. An electric air fryer was used for the thermal processing, (Air Fryer-Mondial, model: 4470-04, China); time and temperature were used according to the manufacturer's recommendations, simulating domestic use, with a nominal power of 1500 W. The samples were air-fried for 10 min; no oil was added to the air fryer chamber, and a fixed heating temperature of 180 °C was used according to the specifications of the equipment. The internal temperature (75 ± 0.5 °C) was monitored using a digital calibrated thermometer (Traceable Long Stem, VWR, Friendswood, Tex., U.S.A.) and the surface temperature of the processed sardines was approximately 78 ± 0.7 °C monitored by a laser thermometer (LASERGRIP 1080 infrared thermometer, ETEKCITY - Anaheim, Calif., U.S.A). After air frying, the samples were ground and homogenized in a multiprocessor (Walita, Brazil) to obtain a homogeneous mass. Convenient aliquots were taken for the analyses, which were carried out in triplicate.

Standards, reagents, and solvents. 20α-hydroxycholesterol (20α-OH), 22S-hydroxycholestrol (22S-OH), 22R-hydroxycholesterol (22R-OH), 25-hydroxycholesterol (25-OH), 7-ketocholesterol (7-keto), 7β-hydroxycholesterol (7β-OH), 5,6α-epoxycholesterol (5,6α-Ep), and 5,6β-epoxycholesterol (5,6β-Ep) were acquired from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Cholesterol (Chol), 25R-hydroxycholesterol (25R-OH), and 7α-hydroxycholesterol (7α-OH) were obtained 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 purities of the standards ranged from 95% to 99%. High-performance liquid chromatography (HPLC) grade *n*-hexane and 2-propanol were obtained from Vetec (Sigma, São Paulo, Brazil).

Analytical procedure

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

Fatty acid composition. For the analysis of fatty acids was used an aliquot of 25 mg of lipids, that was submitted to saponification and methylation using BF3 in methanol (Joseph and Ackman 1992). A GC instrument (GC 2010) from Shimadzu

Table 1–Moisture (g/100 g), total lipids (g/100 g dry basis), and cholesterol (mg/100 g dry basis) levels in raw, air-fried sardines (control), and air-fried sardines with chives, parsley, and *cheiro-verde* at 2 levels (2% and 4%).

	Raw	Control	Chives (2%)	Chives (4%)	Parsley (2%)	Parsley (4%)	*Cheiro-verde* (2%)	*Cheiro-verde* (4%)
Moisture	75.2^{A} (0.3)	55.2^{D} (0.3)	$58.0^{C;b}$ (0.5)	$59.5^{B;a}$ (0.4)	$53.7^{E;c}$ (0.3)	$57.2^{C;b}$ (0.1)	$54.8^{DE;c}$ (0.1)	$57.7^{C;b}$ (0.2)
Total lipid	16.5^{A} (0.2)	13.4^{C} (0.7)	$11.6^{D;c}$ (0.6)	$14.6^{B;a}$ (0.6)	$13.1^{C;b}$ (0.2)	$11.4^{D;c}$ (0.4)	$9.42^{E;d}$ (0.4)	$8.4^{E;d}$ (0.2)
Cholesterol	237.2^{A} (3.7)	136.4^{E} (0.4)	$150.6^{D;cd}$ (3.3)	$208.2^{B;a}$ (4.0)	$188.9^{C;b}$ (6.2)	$158.3^{D;c}$ (4.3)	$138.0^{E;d}$ (4.8)	$203.6^{B;a}$ (5.6)

Values represent means ± standard deviation ($n = 3$).
Capital letters in the same row indicate significant differences by the *Tukey* test ($P < 0.05$) between raw, control, and sardines fried with chives, parsley, and *cheiro-verde*, respectively.
Values followed by different lowercase letters in the same row differ from each other, according to the *Tukey* test ($P < 0.05$) in a factorial design (herbs and concentrations factors).

(Tokyo, Japan) equipped with a split injector (1:50), fused silica CP-SIL 88 capillary column 100 m x 0.25 mm i.d., 0.2 μm film thickness (Chrompack, Middelburg, the Netherlands), flame ionization detector, and workstation was used to identify the different compounds. The chromatographic conditions were: initial temperature, 100 °C (5 min) followed by an increase of 5 °C/min up to 160 °C (0 min), then 8 °C /min up to 230 °C (12 min); injector and detector temperatures were 250 and 280 °C, respectively. The equipment used hydrogen as the carrier gas at a flow rate of 1 mL/min and nitrogen as the make-up gas at 30 mL/min. Retention times of FAME standards were used to identify the chromatographic peaks of the samples, and the quantification was done by internal standardization, using undecanoic methyl ester as the internal standard. Factors for the conversion of fatty acid methyl esters to their corresponding triglycerides were used (Carpenter and others 1993).

Cholesterol and cholesterol oxides.

HPLC-PDA- Refrative index (RI). Cholesterol and cholesterol oxides were obtained by direct saponification performed with continuous agitation (2 g of the samples, 4 mL of a 50% aqueous solution of KOH, and 6 mL of ethanol) at 24°C for 22 h in the dark and the nonsaponifiable matter was extracted 4 times (10 mL of hexane for each extraction, totaling 40 mL) (Saldanha and others 2006).

HPLC analysis was performed with a Waters device (Milford, Mass., U.S.A.), equipped with Photodiode Array (PDA)/Refractive Index Detector (RID) detectors, rheodyne injector with a 20 μL loop, a tertiary solvent delivery system (Waters 600), oven heated column at 32 °C and software (Empower 2). The column used was a CN Hyperchrome 250 mm × 4.3 mm × 5.0 μm (Phenomenex, Colo., U.S.A.). The mobile-phase was *n*-hexane: 2-propanol (97:3, v/v) at a flow rate of 1 mL/min and an analysis time of 30 min. Quantification was done by external standardization, with concentrations ranging from 5.0 to 150.0 μg/mL for the oxides and from 0.1 to 2.0 mg/mL for the cholesterol, respectively. The epimeric 5,6-epoxides were quantified using a refractive index detector, because they do not absorb at Ultraviolet (UV) wavelengths. The cholesterol and other oxides were quantified using PDA detector.

Ultra Performance Liquid Chromatography (UPLC)-Atmospheric pressure chemical ionization (APCI)-Mass spectra (MS). In order to confirm the cholesterol oxide structures, chromatographic analyses were performed on a UPLC Acquity chromatographer coupled to a TQD Acquity Mass Spectrometer (Micromass-Waters Manchester, England), with an APCI source configuration, with a triple quadrupole mass spectrometer. A CN Hyperchrome 250 mm × 4.3 mm × 5.0 μm column (Phenomenex) was used. Isocratic mobile phase containing hexane:*n*-propanol (97:3), at a flow of flow 1 mL/min, oven temperature 32 °C, and 10 μL of the samples were injected into the UPLC. Ionization was performed in the APCI positive ion mode and the optimization conditions were adapted from (Ishida 2014). The ionization parameters were: full scan m/z 100–500, capillary voltage 1500 V, corona current 20 μA, drying gas temperature 350 °C, cone voltage 20 V, and vaporizer temperature 150 °C.

Statistical analysis

The analysis of variance (ANOVA) was employed on dependent variables and when significant differences were observed, the *Tukey* multiple range tests were applied. Multivariate analysis was used to describe the data from the quantification of cholesterol, fatty acids and COPs. Principal component analysis (PCA) was used to group similar samples and was performed on the standardized data to make sure all the elements had the same influence over the results. Hierarchical clustering on principle components (HCPC) was done to group samples with similar characteristics using the function HCPC in the FactoMineR. All analyses were performed using the software R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria) and the FactoMineR package version 1.32.

Results and Discussion

Effects of air frying on moisture, total lipid, and cholesterol

The moisture, lipid, and cholesterol contents in raw, hot air-fried control, and sardines with addition of parsley, chives, and *cheiro-verde* are shown in Table 1. The total lipids (g/100 g) and cholesterol (mg/100 g) were calculated on the dry basis.

The moisture content in raw sardines was 75.2 ± 0.3 g/100 g. In the air-fried sardines, the moisture ranged from 55.2 ± 0.3 to 59.5 ± 0.4 g/100 g. Air frying caused a significant loss of water in the control sardine samples, decreasing 20% in comparison to the initial levels. The lipid levels in raw sardines were 16.5 ± 0.2 g/100 g; while the control sardine samples presented 13.4 ± 0.7 g/100 g of lipids and the air-fried samples with the addition of herbs ranged from 8.4 to 14.6 g/100 g. The lipid contents decreased significantly after the air-fried processing, and the losses varied from 15.6% to 49%. Heating sardine fillets implies peripheral dehydration and decrease of lipids by dripping (García-Arias and others 2003; Saldanha and others 2008).

The cholesterol levels ranged from 237.2 ± 3.7 to 136.4 ± 0.4 mg/100 g in the raw and control samples, lower values than those obtained by Saldanha and others (2008) with 342 ± 2.7 to 219.0 ± 1.6 mg/100 g in raw and grilled Brazilian sardines, respectively. Air frying affected the cholesterol contents, producing a significant loss of this compound, approximately 34.9% in

Table 2–Fatty acid composition (g/100 g of oil) of raw, hot air-fried (control), and hot air-fried sardines with the addition of chives, parsley, and *cheiro-verde* at 2 levels (2% and 4%).

Fatty acids	Raw	Control	Chives (2%)	Chives (4%)	Parsley (2%)	Parsley (4%)	*Cheiro-verde* (2%)	*Cheiro-verde* (4%)
C12:0	0.22^{BC}	0.15^{CD}	$0.23^{ABC;a}$	$0.24^{AB;a}$	$0.32^{A;a}$	$0.11^{D;b}$	$0.07^{D;b}$	$0.11^{D;b}$
C14:0	16.05^{A}	15.14^{A}	$14.44^{A;a}$	$14.37^{A;a}$	$15.83^{A;a}$	$10.30^{B;b}$	$7.84^{C;c}$	$10.35^{B;b}$
C15:0	2.23^{BC}	2.58^{ABC}	$2.94^{A;a}$	$2.71^{AB;ab}$	$2.64^{AB;abc}$	$2.21^{BC;bc}$	$2.00^{C;c}$	$2.25^{BC;c}$
C16:0	37.49^{D}	42.53^{BC}	$44.63^{AB;ab}$	$45.92^{A;a}$	$44.55^{AB;ab}$	$39.78^{CD;c}$	$42.31^{BC;bc}$	$40.82^{C;c}$
C17:0	1.35^{B}	1.95^{A}	$1.94^{A;a}$	$1.79^{AB;a}$	$1.88^{A;a}$	$1.97^{A;a}$	$1.74^{AB;a}$	$1.96^{A;a}$
C18:0	5.49^{C}	10.60^{A}	$7.20^{BC;b}$	$7.04^{BC;b}$	$7.35^{BC;b}$	$8.75^{AB;b}$	$8.81^{AB;a}$	$9.21^{AB;a}$
C20:0	0.41^{AB}	–	$0.48^{A;a}$	$0.44^{A;a}$	$0.44^{AB;ab}$	$0.37^{B;b}$	–	$0.09^{C;c}$
C21:0	1.11^{A}	0.18^{C}	$0.50^{B;a}$	$0.43^{B;a}$	$0.51^{B;a}$	$0.17^{C;b}$	$0.16^{C;b}$	$0.19^{C;b}$
C22:0	0.17^{B}	–	$0.23^{A;a}$	$0.02^{B;b}$	$0.20^{AB;ab}$	$0.20^{AB;ab}$	–	–
C23:0	–	0.01^{B}	–	$0.02^{B;b}$	0.02^{b}	$0.18^{B;b}$	$0.95^{A;a}$	$0.20^{B;b}$
C14:1	0.66^{A}	0.03^{D}	$0.26^{B;a}$	$0.26^{B;ab}$	$0.26^{B;ab}$	$0.15^{C;b}$	$0.03^{D;c}$	$0.15^{C;b}$
C15:1	0.25^{A}	0.23^{A}	$0.25^{A;a}$	$0.27^{A;a}$	$0.25^{A;a}$	$0.18^{AB;ab}$	$0.12^{B;b}$	$0.19^{AB;ab}$
C16:1	7.03^{AB}	7.43^{A}	$6.14^{CD;b}$	$6.57^{BC;a}$	$5.76^{DE;bc}$	$5.25^{EF;d}$	$4.96^{F;d}$	$5.40^{EF;d}$
C17:1	0.48^{A}	0.37^{BC}	$0.42^{AB;a}$	$0.40^{ABC;ab}$	$0.37^{BC;ab}$	$0.35^{BC;ab}$	$0.32^{C;b}$	$0.39^{BC;ab}$
C18:1*n*9*t*	–	0.22^{B}	–	$0.28^{A;a}$	$0.17^{B;b}$	$0.17^{B;b}$	$0.09^{C;c}$	$0.19^{B;b}$
C18:1*n*9*c*	6.35^{C}	9.90^{A}	$7.09^{C;c}$	$8.74^{B;b}$	$7.01^{C;c}$	$8.46^{B;b}$	$9.15^{AB;a}$	$8.59^{B;b}$
C20:1*n*9	0.19^{A}	0.06^{B}	$0.07^{B;b}$	$0.06^{B;b}$	$0.07^{B;b}$	$0.12^{AB;a}$	$0.13^{AB;a}$	$0.11^{AB;a}$
C22:1*n*9	–	0.04^{BC}	$0.05^{ABC;abc}$	$0.03^{CD;c}$	$0.04^{C;bc}$	$0.09^{A;a}$	$0.08^{A;a}$	$0.08^{AB;ab}$
C24:1*n*9	0.57^{B}	1.28^{A}	$0.41^{BC;b}$	$0.19^{C;c}$	$0.43^{BC;b}$	$1.32^{A;a}$	$1.25^{A;a}$	$1.41^{A;a}$
C18:2*n*6*t*	0.34^{C}	0.59^{A}	$0.33^{C;b}$	$0.32^{C;b}$	$0.29^{C;b}$	$0.46^{B;a}$	$0.42^{B;a}$	$0.45^{B;a}$
C18:2*n*6*c*	2.22^{AB}	1.48^{C}	$2.36^{AB;a}$	$1.45^{C;b}$	$1.90^{BC;ab}$	$2.62^{A;a}$	$2.40^{AB;a}$	$2.56^{AB;a}$
C18:3*n*6	–	–	–	–	–	$0.58^{B;b}$	$0.84^{A;a}$	$0.81^{A;a}$
C18:3*n*3	2.19^{A}	0.79^{C}	$1.51^{B;b}$	$1.28^{BC;b}$	$1.45^{B;b}$	$2.56^{A;a}$	$2.29^{A;a}$	$2.45^{A;a}$
C20:3*n*6	–	–	–	–	–	$0.36^{B;b}$	$0.46^{AB;ab}$	$0.48^{A;a}$
C20:3*n*3	0.94^{A}	0.53^{B}	$0.34^{C;a}$	$0.27^{C;a}$	$0.40^{BC;a}$	$0.37^{BC;a}$	$0.35^{C;a}$	$0.36^{BC;a}$
C20:4*n*6	0.26^{A}	0.24^{AB}	$0.23^{AB;a}$	$0.12^{ABC;ab}$	$0.14^{ABC;ab}$	$0.06^{C;b}$	$0.14^{ABC;ab}$	$0.10^{BC;ab}$
C20:5*n*3	3.11^{A}	0.76^{E}	$1.14^{DE;d}$	$1.55^{CD;cd}$	$1.18^{DE;d}$	$3.04^{A;a}$	$1.92^{C;c}$	$2.49^{B;b}$
C22:6*n*3	8.11^{A}	1.14^{E}	$1.77^{DE;d}$	$3.98^{C;c}$	$2.67^{D;d}$	$7.55^{A;a}$	$5.36^{B;b}$	$5.90^{B;b}$
ΣSFA	64.52^{B}	73.15^{A}	$72.59^{A;a}$	$72.98^{A;a}$	$73.84^{A;a}$	$64.05^{B;b}$	$63.89^{B;b}$	$65.19^{B;b}$
ΣMUFA	15.53^{CD}	19.56^{A}	$14.69^{DE;b}$	$16.80^{B;a}$	$14.36^{E;b}$	$16.09^{BC;a}$	$16.13^{BC;a}$	$16.51^{B;a}$
ΣPUFA	17.17^{A}	5.53^{E}	$7.68^{D;d}$	$8.97^{D;c}$	$8.03^{D;cd}$	$17.60^{A;a}$	$14.18^{C;b}$	$15.60^{B;b}$
Σ*n*3	14.35^{A}	3.22^{F}	$4.76^{E;f}$	$7.08^{D;d}$	$5.70^{E;e}$	$13.52^{A;a}$	$9.92^{C;b}$	$11.20^{B;b}$
Σ*n*6	2.82^{AB}	2.31^{C}	$2.92^{BC;b}$	$1.89^{C;b}$	$2.33^{C;b}$	$4.08^{A;a}$	$4.26^{A;a}$	$4.40^{A;a}$
ΣTrans	0.34^{D}	0.81^{A}	0.33^{E}	$0.60^{BC;ab}$	$0.46^{D;c}$	$0.63^{B;a}$	$0.51^{CD;bc}$	$0.64^{A;a}$

Values are means ($n = 6$).
Different capital letters in the same row indicate significant differences among raw, control, and sardines with added chives, parsley, and cheiro-verde by the Tukey test ($P < 0.05$).
Different lowercase letters in the same row differ according to the Tukey test ($P < 0.05$) to a factorial design (herbs and concentrations factors).

control samples compared to the raw sardines. Other authors have found similar effects in microwave, roasted, fried, and grilled fish (Candela and others 1997; Echarte and others 2001; Ozogul and others 2015). The loss of cholesterol contents in heated sardines could be attributed to oxidative processes (Saldanha and others 2008). Among air-fried sardines with the herbs, the cholesterol levels varied from 138.0 ± 4.8 to 208.2 ± 4.0 mg/100 g. The addition of herbs showed a protective effect on the air-fried samples, however, sardines with 4% chives presented the highest protective effect (34.8%), followed by 4% *cheiro-verde* (31.9%). Thus, the use of herbs at the levels applied in the present study protected cholesterol from thermal degradation, and the phenolic compounds present in the plants are probably responsible for this effect.

Effects of air frying on fatty acids

The fatty acid compositions in sardines expressed as g/100 g of oil are presented in Table 2. The main fatty acids found in sardines were palmitic (C16:0), myristic (C14:0), stearic (C18:0), docosahexaenoic DHA (C22:6 *n*3), and palmitoleic (C16:1 *n*7). The air fryer caused an increase of the total saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) contents in the thermal processed samples, between 11.8% and 20.6 %, respectively. Similar results were reported by Leal-Castañeda and others (2017) in fish oil after microwave heating; Gladyshev and others (2006) in boiled and roasted humpback salmon; and García-Arias and others (2003) in boiled and grilled sardines.

Air frying in sardines caused a significant decrease of PUFAs, approximately 70.2% in control sardines, with losses of essential fatty acids; there was a higher degradation of the *n*-3 series (80.7%), mainly DHA 85%. These results are in agreement with those reported by other authors in sardines after heating (Candela and others 1998; García-Arias and others 2003; Saldanha and others 2008) and in other fish samples: hake (Saldanha and Bragagnolo 2007); in salmon, mackerel, sardine, and tuna, using different cooking methods (microwaving, boiling, and grilling) (Moussa and others 2014). Other authors also reported the influence of thermal processing (boiling, frying and microwave) on fatty acid contents in kutum roach (*Rutilus frisii kutum*) samples (Hosseini and others 2014). The amount of PUFA decreased significantly after the heating treatment and was probably due to oxidative reactions. PUFAs are very susceptible to oxidation and are easily incorporated into the chain mechanism of lipid peroxidation (Hsieh and Kinsella 1989; Saldanha and Bragagnolo 2008), and tend to decrease with elevated temperatures. During the air fryer processing, sardines are subjected to heating and oxygen present in the chamber, and these 2 factors can accelerate the oxidative deterioration of the PUFAs in sardines. Other studies have also

Table 3–Cholesterol oxides (μg/g dry basis) of raw, air-fried sardines without herbs (control) and with the addition of chives, parsley, and *cheiro-verde* at 2 levels (2% and 4%).

Cholesterol oxides	Raw	Control	Chives (2%)	Chives (4%)	Parsley (2%)	Parsley (4%)	"*Cheiro-verde*" (2%)	"*Cheiro-verde*" (4%)
5,6α	21.6^{D} (0.9)	68.8^{A} (0.6)	43.6$^{B;a}$ (0.1)	–	22.4$^{D;c}$ (0.7)	22.4$^{C;b}$ (1.0)	12.0$^{E;d}$ (2.0)	–
5,6β	–	63.4^{A} (1.2)	30.8$^{B;a}$ (3.4)	13.0$^{D;c}$ (0.0)	–	–	7.6$^{E;d}$ (0.9)	24.0$^{C;b}$ (3.1)
20α	–	3.8^{D} (0.2)	7.9$^{B;b}$ (0.9)	9.3$^{B;b}$ (1.0)	11.2$^{A;a}$ (0.5)	5.8$^{C;c}$ (0.0)	1.5$^{E;d}$ (0.0)	0.2$^{EF;d}$ (0.0)
22R	7.2^{C} (0.3)	1.1^{E} (0.0)	10.4$^{B;b}$ (1.5)	2.1$^{DE;d}$ (0.2)	12.5$^{A;a}$ (0.5)	6.1$^{C;c}$ (0.1)	6.4$^{C;c}$ (0.4)	3.3$^{D;d}$ (0.0)
22S	–	26.2^{B} (3.6)	50.3$^{A;a}$ (2.1)	1.8$^{F;e}$ (0.2)	6.3$^{E;d}$ (0.6)	12.9$^{C;b}$ (1.5)	10.8$^{D;c}$ (0.5)	20.0$^{C;b}$ (1.0)
25OH	8.3^{C} (0.5)	23.8^{A} (0.4)	5.8$^{D;c}$ (0.2)	0.8$^{E;d}$ (0.0)	8.0$^{C;b}$ (0.5)	8.4$^{C;b}$ (0.5)	0.7$^{E;d}$ (0.1)	13.9$^{B;a}$ (0.3)
25R	–	28.5^{B} (0.1)	28.4$^{B;b}$ (1.2)	21.6$^{C;c}$ (1.3)	36.1$^{A;a}$ (3.1)	11.6$^{D;d}$ (0.7)	34.0$^{A;ab}$ (0.9)	12.3$^{D;d}$ (0.7)
7-keto	7.1^{E} (1.0)	64.9^{A} (0.6)	56.2$^{B;a}$ (2.9)	20.7$^{D;c}$ (2.8)	39.3$^{C;b}$ (1.2)	3.3$^{EF;d}$ (0.4)	1.6$^{F;d}$ (0.1)	4.4$^{EF;d}$ (0.4)
7β-OH	8.3^{A} (0.0)	0.8^{EF} (0.0)	1.4$^{D;c}$ (0.2)	2.6$^{B;a}$ (0.3)	1.7$^{C;b}$ (0.0)	1.0$^{E;d}$ (0.1)	0.7$^{F;e}$ (0.0)	1.1$^{E;d}$ (0.0)
7α-OH	8.7^{A} (0.1)	1.6^{B} (0.1)	1.1$^{C;a}$ (0.1)	0.7$^{DE;bc}$ (0.2)	0.5$^{DE;cd}$ (0.0)	0.3$^{F;e}$ (0.0)	0.7$^{D;b}$ (0.0)	0.4$^{EF;d}$ (0.0)
Total	61.2^{E} (2.8)	282.9^{A} (6.8)	235.9$^{B;a}$ (12.6)	72.6$^{DE;c}$ (6.0)	138.0$^{C;b}$ (7.1)	71.8$^{D;c}$ (4.3)	76.0$^{D;c}$ (4.9)	79.6$^{D;c}$ (5.5)

Values represent means ± standard deviation in triplicates.
Different capital letters in the same row indicate significant differences among all treatments, by the *Tukey* test ($P < 0.05$). Different lowercase letters in the same row indicate differences, according to the *Tukey* test ($P < 0.05$) to a factorial design (herbs and concentrations factors).

observed a remarkable decrease in the PUFA contents in sardine samples after heating (García-Arias and others 2003; Saldanha and others 2008).

On the other hand, the addition of herbs to the air-fried sardines showed a protective effect against fatty acid degradation. The sardines with 4% parsley obtained the highest protective effect of PUFAs in relation to the control samples (216%). The effectiveness of herbal protection decreased in the following order: 4% parsley > 4% *cheiro-verde* > 2% *cheiro-verde* > 4% chives > 2% parsley > 2% chives. This fact is probably due the presence of the phenolic compounds in these plants, acting as natural antioxidants.

The aromatic herbs, parsley and chives, are good sources of bioactive compounds (Parvu and others 2010; Farzaei and others 2013; Vlase and others 2013). The flavonoids are the most dominant compound present in parsley (Pápay and others 2012), and they are known to act as antioxidants. Diverse studies have shown a potential role in the extracts of phenolic compounds derived from these herbs as natural antioxidants (Wong and Kitts 2006; Zhang and others 2006; Farzaei and others 2013; Vlase and others 2013; Parvu and others 2014).

In addition, results from other studies indicate a potential role in the extracts of phenolic compounds derived from parsley as natural antioxidants suggesting that this culinary herb can be used as an alternative to synthetic antioxidants, especially in fat-based foods, whereas parsley can be used to suppress lipid oxidation (Guerra and Lajolo 2005; Jia and others 2012).

The antioxidant activity of chives is also related to the presence of a variety of sulfur-containing compounds and their precursors (Vlase and others 2013), in addition to other bioactive compounds such as polyphenols (Stajner and Varga 2003; Štajner and others 2006; Bezmaternykh and others 2014).

Thermal degradation in lipids is a complex process and it is well established that during and after high temperature processing, chemical and biochemical reactions can occur, resulting in the loss of quality and damage to PUFAs, which could lead to primary and secondary lipid oxidation products (Boran and others 2006; Selmi and Sadok 2007; Saldanha and others 2008). Thus, this important loss of PUFAs in the control samples showed the high impact of air frying on sardines. In contrast, the addition of herbs to most of the treatments protected these fatty acids from degradation.

Effects of air frying on COPs

In the present study, the main oxides determined in raw sardines were: 5,6α-EP, 25-OH, 7-keto, 7β-OH, and 7α-OH. The presence of oxidized cholesterol in the nonheated marine products suggests that this may be characteristic of the metabolism of the fish (Osada and others 1993). After thermal processing, the COPs found in the control samples and the sardines with herbs were: 5,6α-EP, 5,6β-EP, 20α-OH, 22R-OH, 22S-OH, 25-OH, 25R-OH, 7-keto, 7β-OH, and 7α-OH (Table 3).

Air frying resulted in an increase (78.3%) of the total COPs, from 61.2 to 282.9 μg/g in the raw and control samples, respectively. Despite the convenience of the heat processing using an air fryer without the use of oil, the data obtained in this study demonstrate the high impact on air-fried sardines (control treatment), forming high levels of COPs. As described in the literature the influence of cholesterol oxides on human health deserves some emphasis, because the exogenous cholesterol oxides formed during processing of foods can be potential harmful to health, affecting the cellular metabolism, changing the membrane composition and proprieties, and they are more cytotoxic than cholesterol and are associated with the development of cardiovascular and neurodegenerative diseases, among others, which can cause deleterious effects such as inflammation, atherosclerosis, mutagenic and carcinogenic, and cell death. (Thanan and others 2014; Laparra and

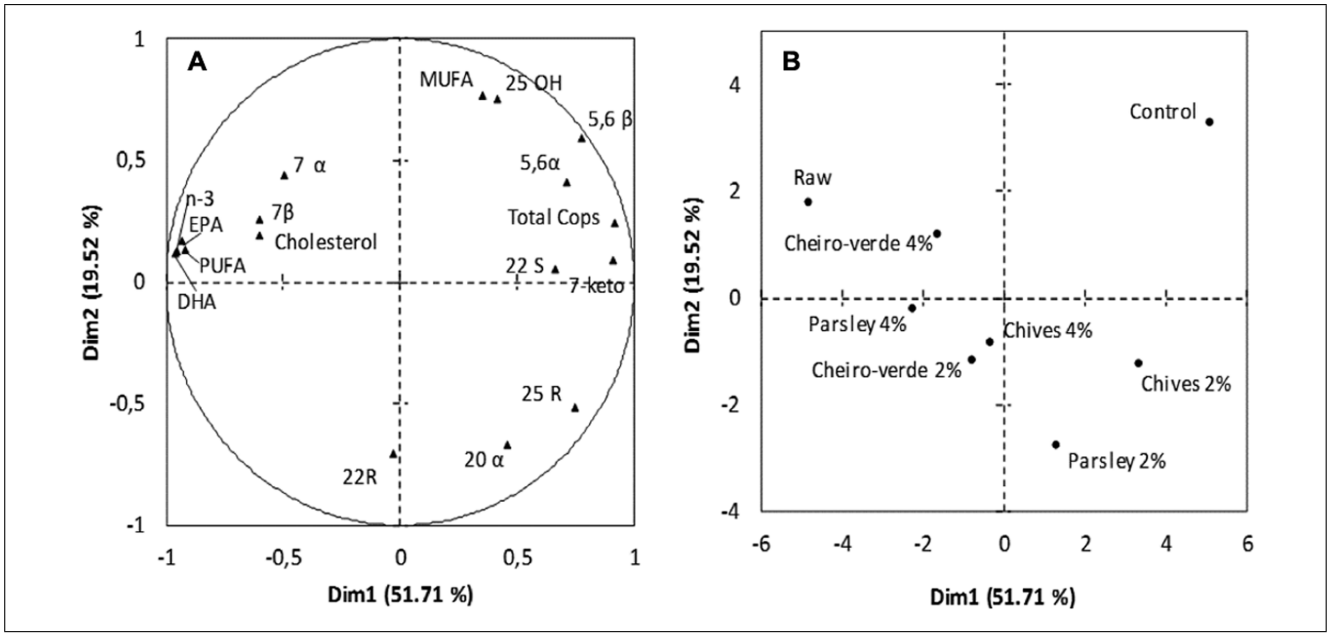

Figure 1–Principal components analysis (PCA) plots as a function of cholesterol oxides, cholesterol and fatty acids. (A) PCA loading plot for response variables (B) score plot for treatments of raw and air-fried sardines without herbs (control) and with addition of chives, parsley, and *cheiro-verde* at 2 levels (2% and 4%).

others 2015; Barriuso and others 2017; Doria and others 2016; Kulig and others 2016).

The amount of COPs formed in products depends on a number of parameters, such as the method of preparation and thermal treatment. There are no studies on the formation of cholesterol oxides in fish treated thermally using an air fryer. However, the increase of COPs after heating has been reported in other studies of fish and fish products in different types of thermal processes such as boiling, steaming, and baking (Hong and others 1996); pan-frying with oil (Echarte and others 2001); frying (Al-Saghir and others 2004); microwave, frying, grilling, and roasting (Astiasarán and others 2007); grilling (Saldanha and Bragagnolo 2007; Saldanha and Bragagnolo 2008); baking and frying (Dean and others 2009); and baking in electric or steam-convection ovens (Freitas and others 2015). Large levels of COPs are formed when the food is subjected to direct heat (Morgan and Armstrong 1992). Heating is a well-known lipid oxidation inducer, since high temperatures produce large amounts of free radicals due to the acceleration of propagation reactions and to the decomposition of lipid hydroperoxides (Otaegui-Arrazola and others 2010), accelerating the oxidative process.

In cholesterol thermo-oxidation process, the predominant compounds are those derived from the oxidation of C-7 carbon, although the epoxidation route includes the formation of 5,6-epoxides (Chien and others 1998). The 5,6α- and 5, 6β-epoxycholesterols were identified as products of cholesterol oxidation by air (Guardiola and others 1996). The processing parameters of sardines in an air fryer, with a high temperature (180 °C) and air flow into the chamber provided the ideal conditions for the formation of high levels of the 5,6-epoxides in processed sardine samples. Thus, the high levels of epoxides could be explained by the epoxidation of cholesterol molecules, due to the air frying of the sardine fillets. The increase of 7-Keto could possibly be due to dehydration of 7α- and 7β-hydroperoxides and/or dehydrogenation of 7α- and 7β-hydroxycholesterols.

Although thermal treatment by air frying significantly increased the levels of COPs in control samples, the addition of herbs minimized the cholesterol degradation process, inhibiting the formation of high levels of cholesterol oxides in most samples with herbs. The sardines with 4% parsley presented the highest protective effect (74.6%) compared to control samples. In these samples, a decrease of 5,6α-EP, 5,6β-EP, and 7-keto was also observed, probably due the bioactive compounds present in these aromatic herbs, acting as natural antioxidants, and inhibiting the free radical chain reaction and consequent epoxidation of cholesterol. The most effective antioxidants are those that act by disrupting the free radical chain reactions. The effects shown in the studied herbs could probably be attributed to the particular chemical structures present in the polyphenols; the aromatic ring feature and highly conjugated system with multiple hydroxyl groups make these compounds good electron or hydrogen atom donors, neutralizing free radicals and other reactive oxygen species (ROS) (Zhang and Tsao 2016). In contrast, sardines with 2% chives showed the highest level of COPs (235.9 μg/g), which was the lowest protective effect (16.6%) among the percentages and herbs tested in this work. The phenolic compounds present in parsley and chives have been shown to act as natural antioxidants in different research by different authors (Jia and others 2012; Parvu and others 2014; Pereira and Tavano 2014; Tang and others 2015; Sęczyk and others 2016).

As determined in the present study, Tarvainen and others (2016) reported similar findings using natural antioxidants to reduce cholesterol oxidation in baked salmon treated with rosemary and oregano extracts. Furthermore, Vaisali and others (2016) showed that phenolic compounds (quercetin, rutin, and caffeic acid) were effective in preventing the oxidation of sardine oil.

Even though the consumption of raw fish has grown in recent years (Kawai and others 2012), thermal processing is still one of the most commonly used forms of preparing fish. Moreover, despite the convenience of the heat processing using an air fryer without

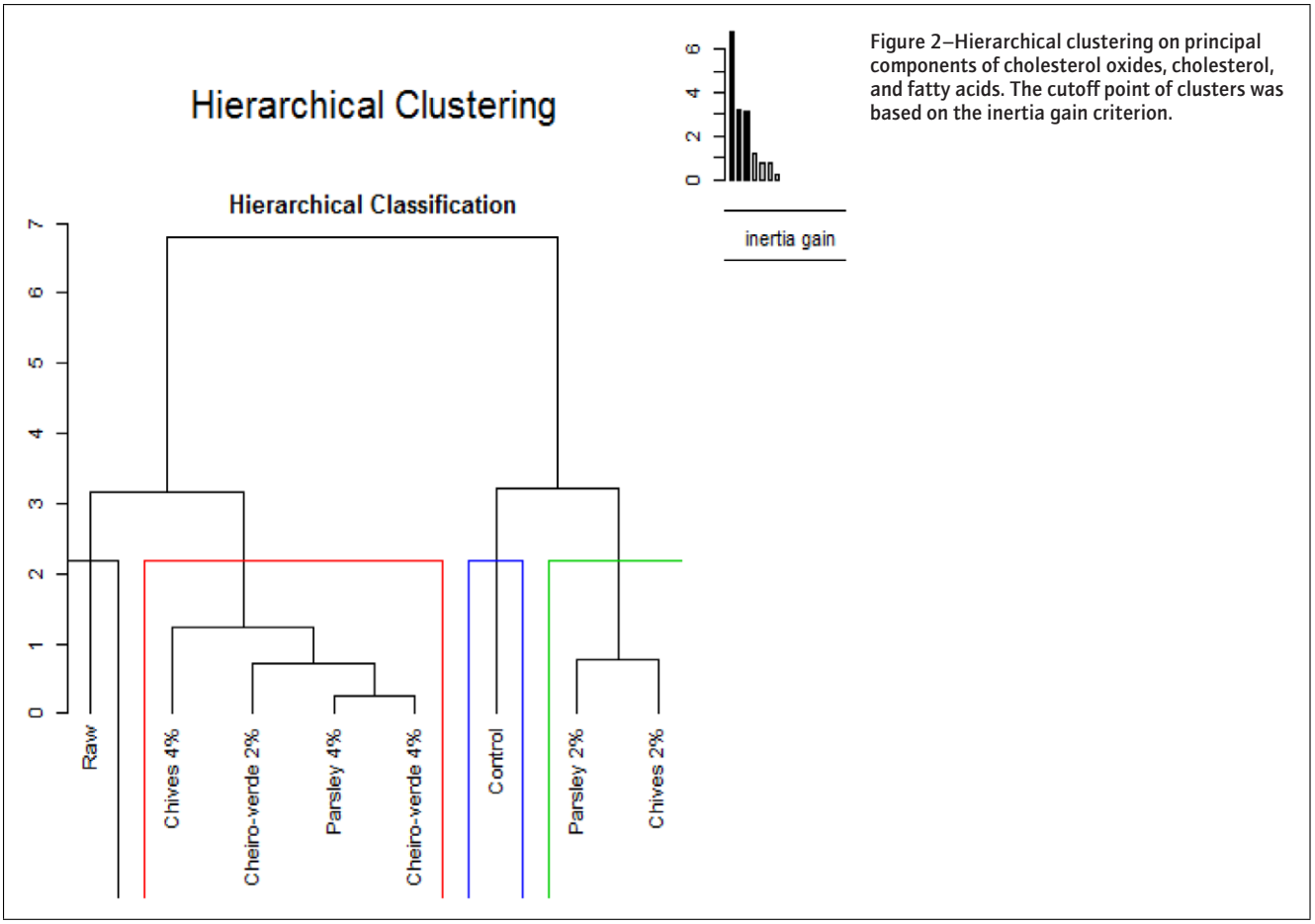

Figure 2–Hierarchical clustering on principal components of cholesterol oxides, cholesterol, and fatty acids. The cutoff point of clusters was based on the inertia gain criterion.

the use of oil, the data obtained in this study, demonstrates the high impact on air-fried sardines (control treatment), forming high levels of COPs, which contrasts with the use of aromatic herbs that proved to be effective in most samples to inhibit such high levels of COPs formation. Due to the health hazards of synthetic additives, natural antioxidants are under intensive investigation to be used as safe alternatives to synthetic compounds. Thus, the use of plant bioactive compounds is being evaluated as potentially effective additives to prevent lipid oxidation in fish and fish products.

Correlation and PCA

The PCA plot clearly showed the high cholesterol, EPA, and DHA in sardines while these values decrease after air frying with concomitant increase of the COPs content in control samples (Figure 1B). The results showed that 7-Keto presented a strong correlation with total COPs and showed a negative correlation with EPA, DHA, *n*-3, and PUFA adequately represented by PC1 and PC2 (Figure 1A). The cutoff point of the HPCP suggested 4 groups (Figure 2). The raw and control samples formed 2 groups (Cluster 1 and 4, respectively) with 1 member in each group, as both treatments presented different results. On the other hand, the treatment of sardines with 4% parsley, 4% chives, 2% *cheiro-verde*, and 4% *cheiro-verde* formed Cluster 2; while 2% parsley and 2% chives formed the Cluster 3 (Figure 2).

Overall, the PCA analysis indicates that the addition of herbs protected the sardines during air frying, especially, the sample with 4% *cheiro-verde*. Thus, suggesting a probable synergistic effect between the antioxidant compounds present in both herbs.

Conclusion

The data obtained in this study showed that air frying presented a great impact on the lipid quality of sardines. In spite of the convenience of the air fryer, the results showed a significant decrease in PUFAs contents and high levels of COPs formation in the control treatment. On the other hand, the use of the aromatic herbs, parsley (*P. crispum*), chives (*A. schoenoprasum* L.), and their mixture (*cheiro-verde*) proved to be effective for most samples. The addition of 4% *cheiro-verde* to the sardine samples presented the best protective effect on the lipid oxidation for air frying.

Acknowledgments

The authors thank the Natl. Brazilian Research Foundations (CAPES) for financial support. Alexandra C.H.F. Sawaya thanks P. Mazzafera (FAPESP grant 2008/58035-6) for use of the UHPLC-MS equipment.

Author Contributions

Fernanda S. Ferreira undertook most of the experimental work presented in this paper. Tatiana Saldanha designed, supervised, and organized the study. Laura M. Keller compiled the data. Davy W.H. Chávez did the statistical analysis. Alexandra C.H.F. Sawaya was responsible for the mass spectrometer analyses. Geni R. Sampaio and Elizabeth A.F.S. Torres supervised and organized the study. Fernanda S. Ferreira and Tatiana Saldanha predominantly interpreted the results and drafted the manuscript with help from the other authors.

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