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Physicochemical and antioxidant properties of the pequi (*Caryocar brasiliense* Camb.) almond oil obtained by handmade and cold-pressed processes

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Abstract

The aim of this study is to characterize physical and chemically and determine the antioxidant capacity of pequi almond oils (PAO) extracted by handmade and by cold-pressing. Both oils showed good quality by acid, peroxide and thiobarbituric acid values. The fatty acid (FA) profile showed a significant presence of monounsaturated FA, mainly oleic acid (53.48 to 55.41 %); saturated FA, such as palmitic acid (33.30 to 35.89 %); and polyunsaturated FA (PUFA), such as linoleic acid (5.85 to 7.23 %). The total phenolic (TP) and carotenoid content ranged in concentration from 87.56 to 392.00 mg GAE/100 g and 36.03 to 262.40 mg/100 g, respectively. The tocopherol and phytosterol results indicated the predominant presence of α-tocopherol (52 to 67 %) and stigmasterol (63 to 68 %). The antioxidant capacity of PAO as measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) method oscillated from 58.48 mg/mL to 76.46 mg/mL (IC_{50}), from 10.61 to 40.46 µmol TE/g by the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS$^{\bullet+}$) method, and from 113.93 to 280.85 µmol TE/100 g and 164.49 to 277.86 µmol TE/100 g, by the lipophilic and hydrophilic oxygen radical absorbance capacity (ORAC) methods, respectively. The oils presented a good oxidative and thermal stability by Rancimat method (IP of 7.33 a 15.91 h) and curves thermogravimetric and differential scanning calorimetry (To 337-363 °C and 159-184 °C, respectively). The results confirmed the presence of compounds that conferred antioxidant capacity and oxidative and thermal resistance for PAO made by handmade or cold-pressing, indicating that these oils can potentially be used for food and non-food applications.

Introduction

In Brazil, there is an abundant supply of oilseed that is used to produce vegetable oils; however, it is still necessary to harness more oil from alternative seeds in each region. Therefore, pequi along with other new alternatives, such as jatropha *(Jatropha curcas)*, turnip (*Raphanus sativus* L.), buriti *(Mauritia flexuosa)*, macaw palm *(Acrocomia aculeata)*, and a wide variety of oilseed appear as species to be exploited (PNA, 2006). Composition data physicochemical properties and antioxidant capacity for many of these alternative vegetable oils have not been reported (Ramadan *et al*., 2012).

Pequi is found in Brazilian Cerrado and belongs to the Caryocaceae family and *Caryocar* genus (Vera *et al*., 2007; Bernardes *et al.*, 2008). The fruit has a layer of yellowish pulp that surrounds a thin layer of thorns, and the innermost portion contains a white almond that is rich in oil (Sousa *et al*., 2011). The almond is considered an unexplored commercial by-product of the pulp processing; however, it can be roasted for the production of oil and used as a culinary ingredient in a tamale-like cake, cake, condiments, or fresh consumption (Segall *et al*., 2006; Rabêlo *et al.*, 2008).

Pequi almond oil (PAO) is composed mainly of fatty acids (FA) such as oleic (50.2%), palmitic (42.3%), and stearic acids (1.5%) and minor amounts of myristic, palmitoleic, linoleic, and linolenic acids (Mata *et al*., 2009). This FA composition gives the PAO nutritional value because oleic acid consumption is related to a decrease in LDL-c (low-density lipoprotein) and a reduction of coronary disease risk (Ramadan *et al.*, 2012). Despite the poor characterization of the minor components, because of its FA composition, PAO is considered a good raw

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material source for the cosmetic and food industry. In folk medicine, PAO is used as an anti-inflammatory, to heal wounds, and for the treatment of rheumatic and muscle pains (Oliveira *et al*., 2010; Passos *et al.*, 2003). Recent research points to the role of oil in inflammation and tissue repair in experimentally induced lesions (Leite *et al.*, 2009; Da Silva Quirino *et al*., 2009).

Oil extraction from the pequi almond is currently performed by a rustic technique on a small scale using water at high temperatures, which is characterized as a handmade process (Aquino *et al.*, 2009). However, cold-pressed PAO, which is a well-known technological process that preserves the level of bioactive compounds that have antioxidant properties (Hoed, 2010), is not found in local markets as it is for other vegetable oils.

Since little attention has been paid to the importance of the knowledge about the antioxidants and physico-chemical properties of PAO extracted by handmade and cold-pressed processes. The present study aims to investigate aspects related to the quality, FA composition, content of bioactive compounds, antioxidant capacity and thermal profile of these oils.

Materials and Methods

Pequi almond oil

PAO, extracted from the pequi almond (*Caryocar brasiliense* Camb.) by both handmade and cold-pressed processes, was provided by the Brazilian Agricultural Research Corporation, EMBRAPA (Brasilia, Distrito Federal). Ripe pequi fruits were randomly collected for oil processing from Community Água Boa 2, located in the Rio Pardo de Minas city (latitude 15° 33' 40.12" S and longitude 42° 23' 35.16" W, Minas Gerais, Brazil), where the almonds were characterized as a by-product. Handmade oils were obtained from a cooperative in the Vereda Funda community located in Rio Pardo de Minas city. The oil was obtained by thoroughly cooking and then crushing a sun-dried almond for approximately 45 min in water that was pre-heated to 60-70°C. The supernatant oil resulting from the cooking process was separated and warmed to loosen the remaining water and then filtered (Ferreira *et al.*, 2011). The cold-pressed oil was extracted at an EMBRAPA facility using a hydraulic press (Marconi, MA-098, Brazil) at a pressure of 9 tons. Handmade oil (PAA) and cold-pressed oil (PAP) were classified according to the crop as PAA10 and PAP11 (2009/2010 crop), PAA20 and PAP21 (2010/2011 crop), and PAA30 and PAP31 (2011/2012 crop). All chemicals used were obtained from Sigma-Aldrich and Merck and were of analytical grade.

Physicochemical analyses and fatty acid composition

American Oil Chemists' Society (AOCS, 2004) standards were used to determine acidity (mg KOH/g oil), thiobarbituric acid test (TBA) (ng/g) and peroxide value (PV) (mEq O_2/kg oil) of almond oil (methods Ca 5a-40, 19-90 Cd and Cd 8b-90, respectively).

Fatty acids methyl esters (FAME) were obtained after boron trifluoride (BF3) 14 % esterification according to the Ce 2-66 method (AOCS, 2004). The FA were analyzed using gas chromatograph (GC) (Shimadzu, GC-2010 Plus, Kyoto, Japan) equipped with a split injector system and an autosampler. FA separation was carried out on an SP-2560 column with dimensions of 100 m×0.25 mm. Helium was used as the carrier gas (1 mL/min). FAME separation was carried out using a temperature gradient between 140 and 240°C, and detection was performed with a flame ionization detector (FID) at 260°C. The identification of the chromatographic peaks was made by comparing the retention time of the sample peaks with a standard mixture of 37 FAME (Supelco®, 37 component FAME Mix, United States). The results are expressed as percentage area for each identified peak.

Polar fraction extraction from oils

The polar fraction of the oil was obtained according to the methodology described by Montedoro *et al.* (1992), which was then used to quantify the total phenolic (TP) and antioxidant capacity using the hydrophilic oxygen radical absorbance capacity (ORAC) method and the $ABTS^{\bullet+}$ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) assay.

Total phenolics and carotenoids

TP quantification was based on the colorimetric method by Swain and Hills (1959) using the Folin-Ciocalteu reagent adapted to a 96-well microplate reading (Biotek®, Synergy HT, United States). A standard curve with gallic acid was used for quantification, and the results are expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of oil (mg GAE/100 g). Total carotenoids were determined following a method by Minguez-Mosquera *et al.* (1991), which is based on the dilution of the sample in cyclohexane and then reading at 472 nm using spectrophotometric equipment (Thermo Spectronic® 20, Genesystm, United States). The result is expressed as micrograms of carotenoid per 100 grams of oil (μg/100 g).

Tocopherols and phytosterol profile

The tocopherol composition of PAO was determined according to the Ce 8-89 method (AOCS, 2004), with minor modifications. The oil samples were diluted with hexane and then filtered through a 0.22 µm polytetrafluoroethylene (PTFE) membrane filter. The samples were analyzed using high performance liquide chromatography (HPLC) (Shimadzu, CBM-20A, Kyoto, Japan) consisting of an RF-10AXL fluorescence detector (excitation = 296 nm and emission = 330 nm). A normal silica phase column (Sim-pack CLC-SIL. 250×4.6 mm internal diameter with 0.5 µm particle size) was used with hexane/isopropanol (99: 1 v/v) as a mobile phase. The system was operated isocratically at a flow rate of 1 mL/min. The identification of the tocopherols (α-, β-, γ-, and δ-tocopherol) was conducted by comparing the HPLC retention times with those of standard compounds (Sigma-Aldrich, United States) under the same operating conditions, and the quantification was based on an external standard method. The results are expressed in mg/kg of oil.

The phytosterol composition was determined as described by Almeida (2009) with minor modifications. The standard 5-α-cholestane (1 mg/mL hexane) was added to each sample and evaporated under a nitrogen (N_2) steam before the saponification with 3% methanolic KOH (potassium hydroxide) at 50 ± 2°C for 3 hours. The organic phase was collected and transferred to a test tube, and the extraction process was repeated three times. The three extracts were mixed with a vortex for 30 seconds, evaporated, and resuspended in 150 µL of hexane before injection into a GC system. The samples were injected into GC (Shimadzu, GC-2010 Plus, Kyoto, Japan) equipped with an FID detector and a fused silica capillary column LM 5 (5% fenil 95% metilpolisiloxane, 60 m×0.25 mm internal diameter with 0.25 µm particle size). The GC program settings were as follows: column temperature at 150°C increasing 10°C/min until 300°C; detector and injector temperatures at 300°C; helium at 1 mL/min and 1/50 of the split ratio. The sterols were identified by comparing the relative retention times to campesterol, stigmasterol, and β-sitosterol standards from Sigma-Aldrich. The results are expressed in mg/kg of oil.

Antioxidant capacity of PAO

DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The analysis of DPPH$^{\bullet}$ radical scanning was performed according to Blois (1985) and Brand-Williams, Cuvelier and Berset (1995) with modifications. Aliquots of oil were diluted with butanol at different concentrations (12.5 to 250 mg/mL) and were added to the DPPH$^{\bullet}$ 6.10-5 mol/L solution (butanolic medium). The reduction of the DPPH$^{\bullet}$ radical was measured at 517 nm in a spectrophotometer after 30 min in the dark. The decrease in the value of the optical density of the samples was correlated with the control (without sample); therefore, the percentage of antioxidant protection was established according to the equation: % protection = 100 - (sample Abs x 100)/control Abs. The values of half-maximal inhibitory concentration (IC_{50}) were calculated using the equation obtained from a curve prepared with different concentrations of the sample.

$ABTS^{\bullet+}$ radical scavenging activity

The antioxidant capacity of PAO was measured using the Trolox equivalent antioxidant capacity (TEAC) assay according to Re *et al*. (1999) and adapted for microplates. The $ABTS^{\bullet+}$ radical cation was prepared by adding potassium persulfate. Measurement of the TEAC was determined by comparing the decrease in absorption after using 20 µL of the PAO extract, a reagent blank, or a Trolox standard with 200 µL of 7 mM $ABTS^{\bullet+}$. Absorbance at 734 nm was monitored 6 min after the addition of the reactant at a temperature of 25°C. The TEAC value is expressed as Trolox equivalents (TE) in micromoles per gram of sample (µmol TE/g).

ORAC

Both lipophilic and hydrophilic antioxidant capacities were determined using the ORAC assay as described by Prior *et al.* (2003). For the lipophilic method, oil samples were diluted in acetone:water 1:1 (v/v) with β-cyclodextrin to 7%, and for the hydrophilic method, the extracts were diluted in a phosphate buffer with a pH of 7.4. Analyses were conducted on a plate reader (Biotek®, Synergy HT, USA). Para aBiotek® automated plate reader with 96-well plates. AAPH was used as a peroxyl radical generator, trolox as a standard, and fluorescein as a fluorescent probe. All samples and reagents were dissolved in 75 mM phosphate buffer, pH 7.4. A 25 µL aliquot of the diluted sample, the blank, or the trolox calibration solution (6.25-100 µM) was mixed with 150 µL of 40 nM fluorescein and incubated for 15 min at 37°C before an injection of the 25 µL AAPH solution (173 mM). The fluorescence was recorded at 37°C every 1 min for 100 min at respective excitation and emission wavelengths of 485 (filter 485/20) and 535 nm (filter 528/20). The final ORAC values were calculated using the net area under the decay curves

and expressed as µmol TE/100 g oil.

Oxidative stability test and thermal analyses

The oxidative stability was determined in Rancimat® apparatus (Metrohm, 743 Rancimat, Switzerland) with the PC 743 Rancimat® 1.0 software, heating 3.0 g of oil at a temperature of 120°C, under a constant air flow (20 L/h), according to method Cd 12b-92 (AOCS, 2004). The result were expressed as the induction period (IP) and determined automatically.

Thermal properties were analysed through a thermogravimetric analyzer (Shimadzu, DTG-60/60H, Kyoto, Japan), in the following conditions: temperature range at 25-700°C, with heating rate 10°C/min, in air atmosphere (100 mL/min) in open platinum crucibles containing 8.6 mg of sample. A Shimadzu software was used to analyse the data of three independent measurements: the thermogravimetric (TG), first derivate thermogravimetric (DTG) and differential thermal analysis (DTA) curves.

The thermal behavior of tested oils was also determined using differential scanning calorimetry (DSC) (Shimadzu, DSC-60, Kyoto, Japan). The equipment was calibrated using high-purity metallic indium as a standard (melting temperature of 156.6°C). Oil samples (2 mg) were weighed in an aluminium pan and then it was partially closed (central hole made with 0.8 mm needle) and placed under an air atmosphere (100 mL/min), the calometric running at the 25-600 °C temperature range and heating rate 10°C/min.

Statistical analysis

The results were subjected to Hartley's test to evaluate the homogeneity of variances. The non-homogeneous samples (PHartley < 0.05) were subjected to the Box-Cox test for transformation. The results were then analyzed by univariate ANOVA followed by the Tukey Honest Significant Difference (HSD) test at the 5% significance level for the homogeneous samples to compare the means. However, the Kruskal-Wallis test was applied to the remaining non-homogeneous results. To compare the handmade and cold-pressed samples, normality and homogeneity of variances were determined by the Shapiro-Wilk test. The non-homogeneous samples (PShapiro-Wilk < 0.05) were transformed using the Box-Cox test. The homogeneous samples were subjected to Student's t test at 5% significance, and the Mann-Whitney test was applied to the non-homogeneous samples.

Results and Discussion

Physicochemical analyses and fatty acid composition

Table 1 summarises the physicochemical properties and the FA composition of PAO extracted by handmade and by cold-pressing. For the acid value, the mean values ranged between 0.42 to 1.89 mg KOH/g of oil and were within the established value determined by Codex Alimentarius (1999) for virgin and pressed oils (4.0 mg KOH/g oil). These results demonstrate the absence of marked hydrolysis of the FA composing the oils. Mata *et al.* (2009) and Mariano (2014) found higher acid value for PAO (3.86 and 3.56 mg KOH / g, respectively) then the ones obtained in this study.

When evaluated by TBA method (measuring the hydroperoxides decomposition products), the obtained values (1.50 to 6.75 ng/g) were lower than the recommended (99.0 ng/g) for refined canola oil (AOCS, 2004). This fact should be related to the majority presence of palmitic and oleic acids in the samples, once the test is more sensitive to unsaturated fatty acid (UFA) containing two or more double bonds (Silva, 2007). The PV was also lower (0.37 to 4.44 mEq O_2/kg) to the maximum allowed of 15 mEq O_2/kg (Alimentarius, 1999) for virgin oils and cold pressed, indicating a good quality of them in terms of degree of lipid oxidation. This result matches with the ones obtained by Mata *et al.* (2009) using oil of pequi almond.

No difference between handmade and cold pressed samples were observed for the acidity and TBA parameters, however, the PV of the pressed oils was lower by 70% of the one obtained to the handmade oil, which is indicative of a lower primary oxidative degradation (Table 1).

Among the SFA, two were present in high concentrations: palmitic acid (C16:0), which ranged from 33.30% (PAP11) to 35.89% (PAP21), and stearic acid (C18:0), which ranged from 2.20% (PAP21) to 2.63% (PAA10). Among the monounsaturated fatty acids (MUFA), oleic acid (C18:1) had the highest concentration, ranging from 53.48% (PAP21) to 55.41% (PAA20) (Table 1). This is a favorable composition to ensure high oxidation resistance. In almond, Lima *et al.* (2007) found similar saturated fatty acid (SFA) and UFA contents of 47.17 and 52.48%, respectively.

The polyunsaturated fatty acids (PUFA) content was predominantly linoleic acid (C18:2), ranging from 5.85% (PAA10) to 7.23% (PAP11). Although there is a slight difference between the content of PUFA in the analyzed oils, their average grade was higher in cold-pressed oils (6.73%) compared

Table 1. Physicochemical properties and fatty acids profile (percentage of the total FAs) from pequi almond oils obtained by handmade (PAA) and by cold-pressed (PAP) process

Parameters	PAA10	PAA20	PAA30	PAA (Mean)	PAP11	PAP21	PAP31	PAP (Mean)
Physicochemical								
Acid value (mg KOH/g)	0.52±0.00^{ab}	0.56±0.08^{ab}	0.42±0.00^{a}	0.51±0.07	1.89±0.00^{b}	0.84±0,03^{ab}	0.45±0,06^{ab}	1.09±0.68
TBA (ng/g)	2.00±0.00^{a}	4.50±0.66^{b}	6.75±0.95^{c}	4.72±2.07	1.50±0.00^{a}	4.91±0.63^{bc}	5.42±0.24^{bc}	4.51±1.55
Peroxide value (mEq O_2/kg)	1.88±0.17^{bc}	1.63±0.18^{b}	4.44±0.15^{d}	2.91±1.44*	2.25±0.35^{c}	0.38±0.08^{a}	0.37±0.08^{a}	0.85±0.88
Fatty acids (%)								
C14:0	0.37±0.00^{d}	0.34±0.00^{a}	0.35±0.00^{b}	0.35±0.00*	0.36±0.00^{c}	0.38±0.00^{f}	0.38±0.00^{e}	0.37±0.00
C16:0	34.88±0.09^{c}	34.56±0.05^{b}	35.51±0.03^{d}	34.92±0.11	33.30±0.02^{a}	35.89±0.04^{e}	35.14±0.03^{d}	34.78±0.39
C16:1	0.55±0.00^{a}	0.52±0.00^{a}	0.52±0.01^{a}	0.53±0.01*	0.60±0.00^{b}	0.85±0.00^{c}	0.52±0.00^{a}	0.66±0.05
C18:0	2.63±0.01^{c}	2.52±0.01^{c}	2.34±0.05^{b}	2.50±0.04	2.63±0.01^{c}	2.20±0.01^{a}	2.28±0.02^{ab}	2.37±0.07
C18:1	55.14±0.08^{c}	55.41±0.14^{c}	54.35±0.02^{b}	54.97±0.17	55.38±0.03^{c}	53.48±0.07^{a}	54.32±0.02^{b}	54.39±0.28
C18:2	5.85±0.01^{a}	5.94±0.02^{b}	6.54±0.02^{d}	6.11±0.11*	7.23±0.01^{e}	6.39±0.01^{c}	6.59±0.00^{d}	6.73±0.13
C23:0	0.60±0.05^{ab}	0.71±0.07^{ab}	0.58±0.02^{ab}	0.63±0.03	0.51±0.01^{a}	0.81±0.07^{b}	0.76±0.01^{b}	0.69±0.05
∑ SFA	38.46±0.15^{c}	38.13±0.21^{b}	38.59±0.08^{c}	38.39±0.08	36.80±0.05^{a}	39.28±0.06^{d}	38.56±0.03^{c}	38.21±0.37
∑ MUFA	55.68±0.14^{c}	55.93±0.24^{c}	54.87±0.04^{b}	55.49±0.17	55.98±0.06^{c}	54.33±0.12^{a}	54.85±0.04^{b}	55.05±0.25
∑ PUFA	5.85±0.02^{a}	5.94±0.03^{b}	6.54±0.04^{d}	6.11±0.11*	7.23±0.02^{e}	6.39±0.01^{c}	6.59±0.00^{d}	6.73±0.13

Values are means ± standard error (n = 3), different superscript letters in the same row differ significantly, according to the Tukey HSD or Kruskal-Wallis tests ($p < 0.05$)

*PAA differs statistically from the PAP, according Student's t test or the Mann-Whitney test ($p < 0.05$). TBA: thiobarbituric acid test. ∑ SFA: total saturated fatty acids; ∑ MUFA: total monounsaturated fatty acids; ∑ PUFA: total polyunsaturated fatty acids

to the handmade ones (6.11%). The comparisons demonstrate that the content of PUFA in the handmade PAO is approximately 9 % lower than in the cold-pressed samples, suggesting a relation between the additional heat treatment, different processing times and the oxidation of PUFA in the handmade oils and others factors.

The values of FA are in agreement with those found by other authors who studied the lipid fraction of pequi almonds (Lima *et al.*, 2007; Mata *et al.*, 2009). The oleic acid levels are also similar to those present in olive oil (55 to 83%) (Firestone, 2005). From a health point of view, MUFA are related to a decrease in the level of LDL cholesterol (low-density lipoprotein) and the maintenance of HDL cholesterol (high-density lipoprotein) level in humans and animals. This is indeed one of the greatest benefits of oils rich in oleic (such as PAO) over other seed oils that are highly polyunsaturated, where PUFA reduce both serum LDL cholesterol and HDL levels in the body (Ramadan *et al.*, 2012). In general, the PAO showed good quality, which might be related to presence of the SFA and MUFA - less susceptible to degradation, despite the heterogeneity of the samples.

Total phenolics and carotenoids

The total carotenoids and phenolic compounds from PAO obtained by handmade and cold-pressing processes are shown in Table 2. This investigation reveals a high phenolic content in the oils that were studied. Statistical analysis revealed significant differences in the phenolic content that ranged from 87.56 (PAP11) to 392.00 mg GAE/100 g oil (PAA30), which are significant amounts when compared to what Lima *et al.* (2007) detected in almond: 122 mg GAE/100 g. Additionally, according to Hoed (2010), in general, the oils contain a TP content that ranges from 10 to 400 mg/100 g of oil.

The TP content observed in the handmade oil was significantly higher (approximately 55%) than the cold-pressed samples. This result is not in agreement with recent research that shows that oils from cold-pressed edible seeds can retain more beneficial compounds, including those that are natural phenolic antioxidants (Ramadan *et al.*, 2012). The results presented here indicate that the phenolic composition

Table 2. Bioactive compounds from pequi almond oils obtained by handmade (PAA) and by cold-pressed treatment (PAP)

Bioactive compounds	PAA10	PAA20	PAA30	PAA (Mean)	PAP11	PAP21	PAP31	PAP (Mean)
Total Phenolics (mg GAE/100 g)	200.89±7.59^{c}	163.11±7.70^{bc}	392.00±6.67^{d}	252.00±31.80*	87.56±6.29^{a}	155.03±9.05^{b}	96.44±13.08^{a}	113.01±10.40
Total Carotenoids (µg/100 g)	262.40±0.56^{a}	56.84±3.59^{ab}	36.03±0.55^{b}	118.42±36.13	159.83±8.72^{ab}	68.05±0.86^{ab}	41.58±0.37^{ab}	89.82±18.09
Tocopherols (mg/kg)								
α-tocopherol	86.21±4.96^{ab}	80.54±0.65^{a}	91.25±4.07^{ab}	86.00±2.57	79.74±0.29^{a}	94.11±0.45^{ab}	100.06±3.57^{b}	91.49±4.01
β-tocopherol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
γ-tocopherol	60.49±3.92^{a}	59.96±0.74^{a}	84.38±4.67^{b}	68.28±5.33	53.53±0.28^{a}	46.62±0.29^{a}	91.32±2.99^{b}	63.82±8.82
δ-tocopherol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total tocopherols	146.57±8.89^{ab}	140.50±1.39^{a}	175.63±8.74^{bc}	154.27±7.57	133.27±0.57^{a}	140.73±0.74^{a}	191.92±6.56^{c}	155.31±11.78
Fitosterols (mg/kg)								
Campesterol	41.47±1.56	45.40±7.16	44.00±3.34	43.58±2.56	48.09±1.50	40.76±1.30	38.01±2.22	42.82±1.77
Stigmasterol	537.00±16.76	484.04±13.33	555.03±39.99	521.65±15.31	488.43±38.94	482.09±14.05	653.42±106.83	527.30±36.74
β-sitosterol	217.63±12.02	204.72±11.17	278.84±25.09	228.09±13.62	232.92±11.56	220.98±10.50	273.16±51.66	238.50±12.90
∑ Fitosterols	796.10±30.01	734.16±28.56	877.86±61.74	793.32±27.63	769.44±49.74	743.83±25.61	964.59±160.71	808.62±49.33

Values are means ± standard error (n = 3), different superscript letters in the same row differ significantly, according to the Tukey HSD or Kruskal-Wallis tests ($p < 0.05$)

* PAA differs statistically from the PAP, according Student's t test ($p < 0.05$). n.d.: Result not detected. ∑ Fitosterols: sum of the phytosterols: campesterol, stigmasterol and β-sitosterol

of the handmade oils may have been affected by water presence in the extraction and/or by the thermal treatment to which these samples were subjected, increasing the phenolic concentration by its release from bound forms. Nederal *et al*. (2012) observed that the oils produced from roasted pumpkin seeds had twice the amount of TP when compared to cold-pressed ones. Terpinc and colleagues (2011) showed that a heat treatment at 160°C applied to *Camelina sativa* seeds increased the content of TP by the release of insoluble phenolics, while below this temperature, soluble phenolic conjugates were released. So, it is suggested to reconsider the assertion that only cold-pressed oils have the greatest health benefits, as is often advertised.

According to Aquino *et al.* (2009), the analysis of carotenoids is important to determine the quality of the oil extracted, as indicated by the loss of these very unstable compounds during processing. The levels of total carotenoids detected in PAO ranged from 36.03 (PAA30) to 262.40 ug/100 g (PAA10) (Table 2), which are significant amounts when compared to what Lima *et al.* (2007), detected in almond: 295 ug/100 g. The almonds used to obtain oils for the present study were subjected to the extraction process in the presence of oxygen and the use of high temperatures (especially the handmade samples), which may have been detrimental by causing a reduction in the amount of carotenoids in the PAO. When compared, there was no significant difference between handmade samples and samples that were pressed.

Tocopherols and phytosterol composition

Table 2 shows the tocopherols and phytosterol composition of the PAO. The total tocopherols content obtained from the PAO analyzed ranged from 133.27 mg/kg (PAP11) to 191.92 mg/kg (PAP31). However, the handmade and cold-pressed oils had similar contents (154.27 and 155.31 mg/kg, respectively).

The main isomer of tocopherol in the *C. brasiliense* almond oil was α-tocopherol with a content between 79.74 (in PAP11) and 100.06 mg/kg (in PAP31), representing 52 to 67% of the total. This was followed by γ-tocopherol, whose content varied between 46.62 (PAP21) and 91.32 mg/kg (PAP31). Cardoso *et al.* (2013) identified α-tocopherol, α-tocotrienol, γ- tocopherol, and γ-tocotrienol in cooked pequi pulp. These are the first reported values

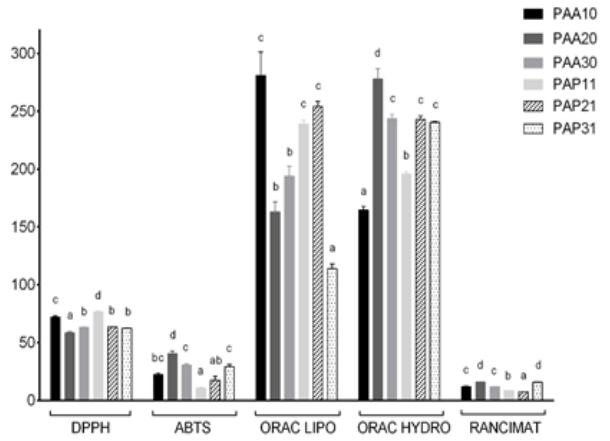

Figure 1. Antioxidant capacity and oxidative stability of the pequi almond oils obtained by the handmade (PAA) and cold-pressed treatments (PAP).

Values are means ± standard error (n = 3), different superscript letters in the same method differ significantly, according to the Tukey HSD or Kruskal-Wallis tests ($p < 0.05$). Units: DPPH: IC_{50} (mg/mL). ABTS: µmol TE/g. ORAC: µmol TE/100 g. Rancimat: induction period in hours

that offer a profile of the tocopherol isomers in PAO, and their presence is attractive from a nutritional standpoint.

The α- and γ-tocopherol isomers proved to be the major tocopherols in vegetable oils and fats, contributing to their stability against oxidation. The α-tocopherol isomer is useful to human nutrition and is the most effective antioxidant among the isomers of tocopherol, while γ-tocopherol has 10-35 % (Ramadan *et al*., 2012). The level of α-tocopherol found in the present study of PAO approximates that detected in cold-pressed blueberry, red raspberry, blackberry, and cranberry seed oils (21-151 mg/kg) (Parry Jr., 2006), which gives good stability and antioxidant capacity to PAO.

The phytosterol content of the analyzed oils showed values between 734.16 and 964.59 mg/kg (stigmasterol, β-sitosterol and campesterol) which are in agreement with values reported in the literature for most oils (1000 to 5000 mg/kg) (Gunstone and Padley, 1997). Of the phytosterols found in PAO, stigmasterol had the highest content, with values ranging between 482.09 and 653.42 mg/kg of the phytosterol content, followed by β-sitosterol, with 204.72-278.84 mg/kg and campesterol (48.09-38.01 mg/kg). Matos (2007) reported that the PAO obtained by cold-pressing contained β-sitosterol (1200 mg/kg), campesterol (800 mg/kg), and squalene (640 mg/kg).

According to Almeida (2009), phytosterols promote the reduction of total cholesterol because these sterols decreases the absorption of exogenous cholesterol from the diet, thereby reducing the risk of heart disease. There is no significant difference between the handmade and the cold-pressed oils, and there were few effects from external factors on the concentration of the phytosterols, such as crop, environmental factors, or extraction type, according to what is reported in the literature (Ramadan *et al*., 2012).

Antioxidant capacity and oxidative stability of the PAO

To characterize the antioxidant potential of the PAO obtained by the two treatments (handmade and cold-pressed), the DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and ORAC methods were used, and the results are shown in Figure 1. According to the antioxidant capacity that was evaluated using the DPPH• method, the PAA20 sample had a low IC_{50} value (58.48 mg/mL) and therefore an increased activity of DPPH$^{\bullet}$ radical scavenging. In contrast, the PAP11 sample had a higher IC_{50} value (76.46 mg/mL). These data demonstrate that bioactive compounds from PAO were able to react directly with the radical and can be beneficial when ingested. Chen *et al.* (2014) and Jiao *et al*. (2014) found IC_{50} values of 8.53 and 152.84 mg/mL in cumin and pumpkin seed oils, respectively, using the DPPH$^{\bullet}$ assay.

By the ABTS$^{\bullet+}$ method the antioxidant capacity ranged from 10.61 µmol TE/g (PAP11) to 40.46 µmol TE/g (PAA20). Kesen *et al*. (2014) found values for ABTS$^{\bullet+}$ in olive oil between 1.3 and 1.90 µmol TE/g, which are lower than the values found in this study. Handmade oil samples were characterized by a statistically higher level of antioxidant capacity (31.15 µmol TE/g) compared to the cold-pressed samples (19.14 µmol TE/g) when assessed by this method, which may be related to processing.

A variance of 113.93 µmol TE/100 g (PAP31) to 280.85 µmol TE/100 g (PAA10) was observed in the almond pequi oils evaluated by the lipophilic ORAC method. These values are higher than those found by Eller *et al.* (2010) in tomato seed oils (0.96 to 1.47 µmol TE/100 g). For the hydrophilic extract, the variation between samples ranged from 164.49 (PAA10) to 277.86 µmol TE/100 g (PAA20). According to the United States Department of Agriculture (USDA, 2010), hydrophilic ORAC values of extra virgin olive oil are in the range of 146 to 1150 µmol TE/100 g.

The evaluated oils differed in oxidative-induction time at Rancimat® method (7.33 to 15.91h), but, in general, possess a good oxidative stability may be due to the majority presence of SFA and MUFA and others minority constituents. According to Ramadan *et al.* (2012), the oxidative stability of vegetable

Table 3. Thermal analysis summary of pequi almond oils obtained by handmade (PAA) and by cold-pressed treatment (PAP) in the first event, air atmosphere and heating rate 10 °C/min.

Samples	TG/DTG				DSC			
	T_o (°C)	Ti (°C)	Tf (°C)	Mass Loss (%)	T_o (°C)	Ti (°C)	Tf (°C)	T_p (°C)
PAA10	342.90±4.10	212.74±1.90^a	379.96±5.28	51.88±2.79	165.13±10.39	160.26±3.00^{ab}	298.82±33.89	164.27±0.60^a
PAA20	351.61±17.46	236.32±1.25^b	380.40±7.57	53.26±4.39	160.28±13.79	165.98±4.11^{bc}	334.04±35.04	173.19±8.35^a
PAA30	348.96±6.53	231.44±2.43^b	382.28±7.78	54.79±4.94	175.41±3.26	155.60±2.76^{ab}	302.50±19.19	301.16±17.84^b
PAA (Mean)	347.82±5.66	228.59±3.68	380.88±3.50	53.31±2.11	166.68±5.54	161.24±2.33	312.95±17.50	212.87±28.43*
PAP11	338.20±4.91	208.10±0.74^a	381.42±4.22	54.02±0.49	159.58±13.92	151.92±1.67^a	306.81±16.30	304.19±13.76^b
PAP21	337.66±4.43	233.59±0.92^b	377.82±7.77	52.36±2.21	184.72±2.94	154.75±1.36^{ab}	339.58±2.65	319.19±3.57^b
PAP31	363.10±13.58	238.71±2.45^b	396.80±4.28	63.44±4.93	167.44±9.07	176.24±2.58^c	343.59±29.18	321.26±18.00^b
PAP (Mean)	346.32±6.05	226.80±4.80	385.35±4.06	56.61±2.33	169.00±7.09	161.75±4.39	328.80±12.72	312.03±7.15

Values are means ± standard error (n = 3), different superscript letters in the same column differ significantly, according to the Tukey HSD ($p < 0.05$). *PAA differs statistically from the PAP according to Student's t test or the Mann-Whitney test ($p < 0.05$)

TG: Thermogravimetric. DTG: Derivative Thermogravimetric. DTA: Differential Thermal Analysis. DSC: Dynamic Scanning Calorimetry. T_o: early extrapolated of the thermal event; Ti: initial temperature; Tf: final temperature; T_p: peak temperature

oils and fats depends on the composition of FA, the presence of small bioactive compounds soluble in fat and the initial amount of hydroperoxides. Refined soy oil (rich in PUFA) presented IP of 3.87h, in the same study conditions (data not shown).

The oils showed a strong antioxidant capacity and oxidative stability with the overall methods in this study and demonstrated what it takes to obtain a prominent position in relation to other seed oils from fruits, bringing addition compounds with possible health benefits and potential applicability in processes that use high temperature.

Thermal analyses

The thermal data (TGA / DTG and DSC) of all samples are similar due to their similar chemical composition, and are shown in Table 3. Figure 2 showns the curves of oil samples PAA30 and PAP31 at air atmosphere. The TG / DTG curves showed at least three weight loss steps (Figure 2a; Table 3). The To is the early extrapolated of the thermal event and is used for comparison of the initial material decomposition (Matos and Machado, 2004). The To of the analyzed oils varied between 337 and 363 °C, with no statistical differences in oxidative stability. According Saad *et al.* (2008), oils` To typically varies only a few degrees Celsius, being higher when the oil has a greater amount of antioxidant compounds.

The first decomposition event initiated between the temperatures of 208-238°C and ended at 377-396°C with a weight loss of 51-63% that could be attributed to the decomposition of MUFA and PUFA. Marques *et al*. (2014) stated that the first stage is the most important to assess the thermal stability of the oil, once it starts the thermal decomposition of the UFA. However, according Vecchio *et al*. (2009) there is no consensus on this assignment.

The PAO showed higher values than the observed for olive oil, as reported by Vecchio *et al.* (2008) (160 and 370 °C for the first two overlapping peaks), demonstrating improved thermal stability. According to Smith *et al.* (2007) the oxidative stability of oils is influenced by the amount and type of metal, natural antioxidants, phospholipids, free FA, mono- and diglycerides, polymers and the number of double bonds in the oil.

The second stage of decomposition started at a temperature of 375-399°C and completed between 402-440°C, representing from 12 to 22% of mass loss. The third event, with a weight loss of 2-29%, occurred in the temperature range of 404-441°C to 477-481°C. The second and the third event is related to the thermal decomposition of the SFA and the complete decomposition of lipid molecules initiated on the first stage (Vecchio *et al.*, 2009). The fourth event (493-499°C to 601-614°C), generated a weight loss of 7 to 8% and is related to the decomposition of the polymerized material formed during heating. The residue that is obtained at the end of the oil decomposition is composed of heat-resistant carbonaceous polymeric compounds (Fonseca and Yoshida, 2009; Vecchio *et al.*, 2009).

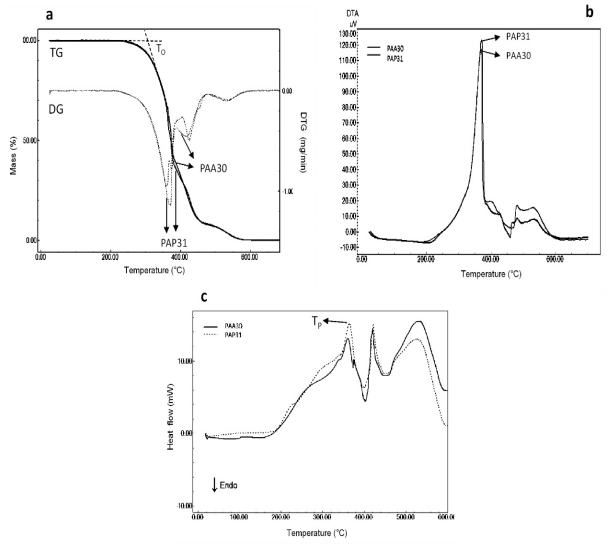

Figure 2. Thermal analysis of pequi almond oils obtained by handmade (PAA30) and by cold-pressed treatment (PAP31) in heating rate 10°C/min, air atmosphere (100 ml/min). (a) TG and DTG curves (8,6 mg). (b) DTA (8,6 mg). (c) DSC (2 mg)

The events observed in TG / DTG curves are exothermic transitions, as evidenced by the peaks of DTA curve (Figure 2b). These events are characteristic of decomposition/combustion resulting from the oxidation of oils. In the first exothermic event occurred a greater release of energy (represented by the most pronounced peak) starting from 190-210°C and maximum temperature (T_p) of 344-368°C. More stages of thermal decomposition were observed resulting from the exothermic oxidation and loss of mass of the oil.

DSC curves of all the samples showed similar and complex profiles with at least three high peaks (Figure 2c; Table 3), indicating that the thermal oxidation may be characterized by at least three exothermic steps attributed to volatilization and/or combustion of triacylglycerols.

The oxidation of PAO began to 159-184°C (To) within the temperature range reported for edible oils (130-180°C) (Aguerrebere *et al.*, 2011). The first thermal event began between 151 and 176°C and the samples PAP31 and PAA20 showed the highest oxidative resistance when compared to the others. Furthermore, the first DSC peak occurred in the temperature range of 164.27 and 321.26°C and the samples PAP31, PAP21, PAP11 and PAA30 had the higher temperatures.

The 2 °, 3 ° and 4 ° exothermic peaks held between 298-339 °C to 393-404°C, 393-404 to 448-455 and 451-457°C to 588-594°C, respectively, and were statistically similar between samples. These findings are relevant since it is the first report of the experimental thermo-oxidative characteristics of PAO obtained by handmade and cold pressed process, which is the basis for more in-depth characterization studies.

Conclusions

This study revealed that PAO that are extracted by handmade or cold-pressed possess a good quality and interesting FA profiles. They have a predominant presence of oleic acid and compounds with antioxidant properties, such as phenolics, carotenoids, tocopherols, and phytosterols, which may be of interest for food and non-food applications. Futhermore, the PAO has good oxidative and thermal resistance. The phytochemical compounds with beneficial health effects bring attention to the almond that is a common byproduct and generally not valued by pequi producers, which may increase the number of new products that can be developed from the fruit.

The oils had similar compositions, however, the handmade pequi almond oil showed a lower content of PUFA, a higher peroxide value, total phenolics content, and an antioxidant capacity verified by the $ABTS^{\bullet+}$ method in relation to cold-pressed oils. This demonstrated a possible influence of thermal processing on the degradation of PUFA, formation of peroxides and release of phenolic compounds with antioxidant capacity. More studies are needed to determine the variables that influence the processing of PAO.

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