





Fatty acid profile and physicochemical, optical and thermal characteristics of *Campomanesia adamantium* (Cambess.) O. Berg seed oil

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Abstract

The aim of this study was to characterize the oil obtained from seeds of *Campomanesia adamantium* by physicochemical quality parameters, oxidative stability, antioxidant activity, quality indexes, optical and thermal stability and its fatty acid profile. These seeds were a relevant source of oil (83 mg g⁻¹) with high potential antioxidant activity (IC₅₀ = 25.32 µg mL⁻¹) evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) with induction period above of 50 hours. In addition, palmitic (53%) and oleic (34%) are the primary saturated and monounsaturated fatty acids. This oil showed excellent quality for edible vegetable oil and bioactive compounds. The thermal stability of this oil by thermogravimetric analysis/differential thermogravimetry (TGA/DTG) started at 154 and 231 °C under synthetic air and nitrogen atmospheres, respectively, and by differential scanning calorimetry (DSC) crystallization was onset at 4.94 °C. This study revealed as a novelty that the *C. adamantium* seeds are an excellent source of oil that presents best qualities, which makes it a great candidate for edible vegetable oil, as well as for production of soap, lotions and biofuel.

Keywords: antioxidant activity; Myrtaceae; nutritional quality; oxidative stability; thermal stability; vegetable oil.

Practical Application: Possibility of using of seeds of *C. adamantium* in human utilization for cooking purpose, as well as for production of soap, lotions and biofuel.

1 Introduction

The consumption of fruit species, including seeds or their by-products is widely recommended for human health promotion and disease prevention due to their nutritional properties (Liu, 2013; Ros & Hu, 2013; Pem & Jeewon, 2015). Among potential edible plant species is included *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae), found in savannas of South America (Global Biodiversity Information Facility, 2017). Fruits (pulp and including peel) of *Campomanesia* are widely used for human consumption *in natura* or utilizing their by-products (Viscardi et al., 2017); however, their seeds are discarded. Nowadays, the search for natural products of plant origin and their whole utilizing in food has increased. That can include seeds of *C. adamantium*, which oil we obtained.

Oils are composed of several classes of fatty acids classified according to the presence or absence of double bonds in their structure as saturated fatty acids (SFAs—without double bonds), monounsaturated fatty acids (MUFAs—with one double bond) and polyunsaturated fatty acids (PUFAs—with two or up to six double bonds). Besides, as *cis* or *trans* based on the configuration of the double bond and as *n*-3 or *n*-6 PUFAs depending on the

position of the first double bond of the fatty acid methyl-end (Orsavova et al., 2015; Briggs et al., 2017). Furthermore, MUFAs and PUFAs are highly susceptible to oxidative processes in the presence of heat, light, ionizing radiation, metal ions and metalloprotein, producing free radicals, which lead to rancidity and decrease oil quality, such as off-flavors, loss of color, altered nutrition value, can produce toxic substances, which can impair the health of consumers (Ahmed et al., 2016). Oil quality can be affected during its processing and storage, mainly PUFAs due to their likely degradation by heat, light and atmospheric oxygen actions, including heavy metals (Zhu et al., 2011; Vaskova & Buckova, 2015). On the other hand, vegetable oils present antioxidants, which slow up or reduce the speed of the oxidation effects in oils, quenching singlet oxygen and reacting or eliminate the free radicals or pro-oxidants, the most frequent in foodstuffs (Kaur et al., 2015).

The current study aimed to determine the fatty acid profile of the oil and its behavior characterization by physicochemical, potential antioxidant activity, nutritional quality, optical evaluation, oxidative and thermal stability analyses.

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2 Materials and methods

2.1 Oil extraction

We collected the ripe fruits in Campo Grande, MS, Brazil (20°31'30"S, 54°36'2"W), on November 2017. Manually, seeds were removed from the fruits, cleaned and immediately dried in an oven under air circulation at 40 °C during 24 hours. The dried seeds were milled and refined using mortar and pestle. The oil extraction was carried out in ambient temperature by fixed maceration using hexane as a solvent. The supernatant was dried by rotary evaporator and the oil obtained was placed into amber and hermetic glass bottle and stored in a freezer at -18 °C for further analysis.

2.2 Physicochemical characterization

The acid value was determined by the method Ca 5a-40, refractive index (method Cc 7-25), relative density (method Cc 10a-25), iodine value (method Cd 1-25), saponification value (method Cd 3-25) and peroxide value (method 8-53) according to American Oil Chemists' Society (1990).

2.3 Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity of the antioxidant compound of the oil was investigated in vitro (Wei & Shibamoto, 2007). The assay tubes were stored in darkness for 60 min. We measured the absorbance in a spectrophotometer (Biochrom Libra S60PC Double Beam, Cambridge, UK) at 517 nm. Ethanol was used as a blank and ethanolic Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) solution as positive. We did the determinations in triplicate. The antioxidant activity was calculated as the inhibition percentage of the DPPH radical (%AA) as follows (Equation 1):

$$\%AA = \frac{Ad - (As - Ab)}{Ad} \times 100 \quad (1)$$

where: Ad = absorbance of the ethanolic DPPH solution; As = absorbance of the mixture of DPPH and sample; and Ab = absorbance of the blank.

2.4 Optical analysis: UV-visible and FTIR spectra acquisition

We diluted the oil in hexane (spectroscopic grade 99.9%). UV-visible absorption measurements were made utilizing spectrophotometer (Lambda 265 UV/Vis, Perkin Elmer, Waltham, MA, USA) and a quartz cuvette with 10 mm light path. The UV-visible absorption spectra were collected in the 200-800 nm range. Also, we analyzed the oil by spectrometer FTIR (Thermo Scientific NicolettiS5), obtaining the spectra in the infrared region between 500-4000 cm⁻¹ wavenumber range.

2.5 Oxidative stability by Rancimat method

The oxidative stability of the oil was determined according to the European Committee for Standardization (2003) by the Rancimat method utilizing the equipment Rancimat (873 Hersau,

Switzerland). The analyses were performed by adding 3.0 g of the oil into sealed glass reaction vessel at 110 °C and analyzing under a constant airflow rate of 10 L h⁻¹, which passed through the samples and then into measuring vessel containing 50 mL ultrapure water Mill-q in which the conductivity generated by volatile products during the oil degradation was measured as a function of time.

2.6 Methylation and fatty acid profile

We prepared the fatty acid methyl esters (FAMES) in ambient temperature (Dodds et al., 2005). The FAMES were analyzed by (GC 2010, Shimadzu) to obtain their peaks. The equipment used a gas chromatography-Flame ionization detector (GC-FID) and capillary column (BPX-70, 0.25 mm internal diameter, 30 m long, and 0.25 mm thick film). The injector temperature and the detector was 250 °C. The initial column temperature was kept at 80 °C for 3 min and then increased at 10 °C min⁻¹ and until reaching 140 °C, followed by an increase to 240 °C at 5 °C min⁻¹ for 5 min. We identified individual peaks of FAME by comparing their relative retention time with the standard of 37 FAMES (Supelco C22, 99% pure).

2.7 Nutritional quality index

The nutritional quality of the oil was determined based on its FA composition. The atherogenic index (AI) Equation 2 and the thrombogenic index (TI) Equation 3 considered the MUFA levels (Ulbricht & Southgate, 1991). The hypocholesterolemic: hypercholesterolemic ratio (HH) followed the Equation 4. The nutritional quality indexes were calculated (Santos-Silva et al., 2002).

$$AI = \frac{C12:0 + 4 \times C14:0 + C16:0}{\sum MUFA + \sum \omega - 6 + \sum \omega - 3} \quad (2)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5 \times \sum MUFA + 0.5 \times \sum \omega - 6 + 3 \times \sum \omega - 3} \quad (3)$$

$$HH = \frac{C18:1Cis-9 + C18:2\omega-6 + C20:4\omega-6 + C18:3\omega-3 + C20:5\omega-3 + C22:5\omega-3 + C22:6\omega-3}{C14:0 + C16:0} \quad (4)$$

2.8 Thermogravimetric analysis/ differential thermogravimetry (TGA/DTG)

A thermobalance TGA Q50, TA Instrument was used. The TGA was calibrated with nickel for temperature settings and with 100 and 1000 mg standards for weight accuracy. Samples (~ 3 mg) were heated in a platinum pan from 10 to 550 °C at a heating rate of 2 °C/min under nitrogen (N₂) and synthetic air atmosphere gases (60 mL min⁻¹). The data processed by Universal Analyses 2000 software version 3.7A (TA Instruments).

2.9 Differential Scanning Calorimetry (DSC)

We obtained the DSC curves in a calorimeter model DSC Q20 with the RCS90 coupled to a cooling system, both TA Instruments. Samples (~ 5 mg) using aluminium crucibles (Tzero standard) as support and reference, at a heating rate of 10 °C min⁻¹, cycle heating followed by cooling to temperatures between -80 °C and 25 °C, under inert nitrogen (N₂) atmosphere

with a flow of 60 mL min⁻¹. The data were processed with the help of Universal Analyses 2000 software version 3.7A (TA Instruments).

2.10 Data analysis

All experiments were carried out in triplicates. Data were analysed by ANOVA using the R Core Team (R Foundation for Statistical Computing, 2019). The significance of the differences between means for individual acids level were considered at $p < 0.001$, and no significant differences observed between classes of acids in this oil ($P = 0.383$).

3 Results and discussion

3.1 Extraction yield and physicochemical characterization

The yield oil extraction was 83%, is excellent and is close to *Brassica napus* L. (Citeau et al., 2018). The physicochemical parameters used demonstrated that the *C. adamantium* seeds are a source of excellent oil and compared to established conventional parameters and other edible oils (Table 1).

3.2 Antioxidant activity

The antioxidant activity of this oil was $IC_{50} = 25.32 \mu\text{g mL}^{-1}$, shown to be higher and more effective than Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), a positive control, in a ratio of 1:2. This analysis demonstrated that *C. adamantium* seed oil is a rich source of natural antioxidant.

3.3 UV-visible and FTIR

The UV-Visible absorption spectra found two leading absorbance bands at 236 and 293 nm (Figure 1), which are related to π electronic transitions observed on unsaturations, conjugated nonbonding electron system and aromatic compounds (Spatari et al., 2017). The absorption at 236 nm may be attributed to different compounds present in the vegetable oil, such as phytocholesterols (phytosterols and phytosterols), tocopherol and others (Gonçalves et al., 2018; Jolayemi et al., 2018). The absorption band at 293 can be attributable to tocopherols and FAs (palmitic, oleic, linoleic and stearic acids) (Lapčikova et al., 2018). Intake of oils rich in phytocholesterols and tocopherols are related with prevention of the cardiovascular diseases, diabetes, obesity, and others (Ogbe et al., 2015; Shahidi & Camargo, 2016).

The characteristic weak band around at 3006 cm⁻¹ is associated with the higher composition of acyl group of

MUFAs, featured to oleic acid and SFAs (palmitic acid) (Guillén & Cabo, 1997).

3.4 Oxidative stability of the seed oil of *C. adamantium*: Rancimat

The induction period of the oil was above 50 hours. Higher oxidative stability is attributed to the abundance of the long-chain FAs (99.38%), higher quantities of palmitic and oleic acids and a smaller amount of PUFAs (5.66%) (Damanik & Murkovic, 2018). Beyond to a lower percentage of α -linolenic (0.10%) compared to linoleic acids (5.56%) (Damanik & Murkovic, 2018). Furthermore, this behavior can be attributable to the combination and synergistic interaction of natural several antioxidant compounds, such as tocopherols, tocotrienols, phytosterols and phytosterols (Hassanien et al., 2014; Damanik & Murkovic, 2018).

3.5 Fatty acids profile

We identified a total of nine FAs and their percentages ranging from 0.1% to 53.02%. Palmitic and oleic are the most abundant fatty acids, as well as has been found in other studies (Renes et al., 2018; Zoidis et al., 2018). Beyond, these two FAs are found in high amount in manufactured edible products using non-conventional processing (Monteiro et al., 2018; Coutinho et al., 2019; Ferreira et al., 2019; Guimarães et al., 2019; Silveira et al., 2019). The FAs profile and the percentage of triglycerides are summarized in Table 2.

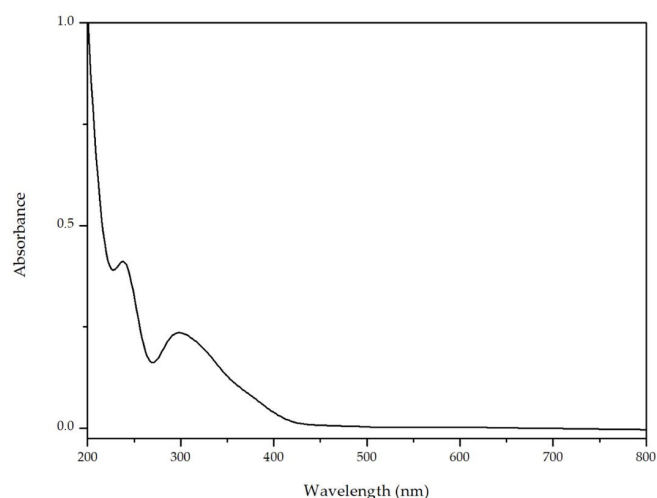


Figure 1. UV-Vis spectra of the *Campomanesia adamantium* seed oil diluted with hexane in 0.1.

Table 1. Physicochemical characterization and quality of the *Campomanesia adamantium* seed oil in comparison with parameters of conventional edible oils.

Parameter	<i>C. adamantium</i>	Conventional edible oils
Iodine value ($\text{gI}_2/100 \text{g}^{-1}$)	24.95 ± 18.61	Coconut oil (15 ± 19) (Louheranta et al., 1998)
Acid value (mg KOHg^{-1})	7.22 ± 0.26	Palm oil (10) (Food and Agriculture Organization of the United Nations, 2001)
Saponification value (mg KOHg^{-1})	196	Olive oil (184-196) (Food and Agriculture Organization of the United Nations, 2015)
Peroxide value ($\text{meq. O}_2/\text{kg}^{-1} \text{ oil}$)	12.93	≤ 20 meq. (Food and Agriculture Organization of the United Nations, 2015)
Refraction value at 40 °C	1.47	Olive oil (1.47) (Food and Agriculture Organization of the United Nations, 2015)
Relative density at 25 °C	0.91	Olive oil (0.91-0.92) (Food and Agriculture Organization of the United Nations, 2015)

Table 2. The fatty acids profile revealed in the *Campomanesia adamantium* seed oil and their mean and standard deviation (SD) in percentage (%).

Fatty acid	Mean \pm SD (%)
Caproic (C6:0)	0.42 \pm 0.21
Myristic (C14:0)	0.20 \pm 0.03
Palmitic (C16:0)	53.02 \pm 0.34
Palmitoleic (C16:1)	3.97 \pm 0.58
Stearic (C18:0)	2.45 \pm 0.84
Oleic (C18:1 n9c)	34.13 \pm 0.84
Linoleic (C18:2 n6c)	5.56 \pm 0.29
α -Linolenic (C18:3 n3c)	0.10 \pm 0.0
Lignoceric (C24:0)	0.14 \pm 0.05
Total SFA	56.23
Total MUFA	38.10
Total PUFA	5.66
Total FAs	100

SFA = Saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; FA = fatty acid.

Two FAs (palmitic and oleic) are the majority in *C. adamantium* oil, which can be used for food applications, soap, lotions and biofuel production (Mba et al., 2015).

This oil present low values of atherogenic (1.23) and thrombogenic indexes (2.52) and high value of hypocholesterolemic: hypercholesterolemic ratio (0.75), which is recommended for human consumption, because they are related to disease prevention and human health promotion (Wood et al., 2008; Fernandes et al., 2018).

3.6 TGA/DTG

In both N₂ (Figure 2) and synthetic air atmospheres (Figure 3).

The first mass decomposition event temperature can be attributed to the loss of moisture linked by hydrogen bonds of the sample. The second mass loss event is attributable to natural antioxidant decomposition (Juhász et al., 2012; Martins et al., 2012). The third mass loss event is related to FAs of the *C. adamantium* seed oil.

The presence of one high peak in this oil is explained by the higher percentage of the SFAs and MUFAs, represented by palmitic and oleic acids, respectively compared to PUFA (linoleic and linolenic acids) (Martin-Ramos et al., 2017). The events of this oil in N₂ and synthetic air atmospheres are shown in Table 3.

3.7 DSC

The DSC curves of the crystallization and melting behavior of the oil are illustrated in Figure 4.

The crystallization temperature of the oil was onset at 4.94 °C, offset (-21.15 °C) and peak enthalpy (39.24 J/g). DSC is a crucial technique used to recognize food product features (Farah et al., 2018). The onset crystallization temperature recorded in this study can be explained by the interaction of a higher amount of the SFAs (palmitic and stearic) effect against unsaturated

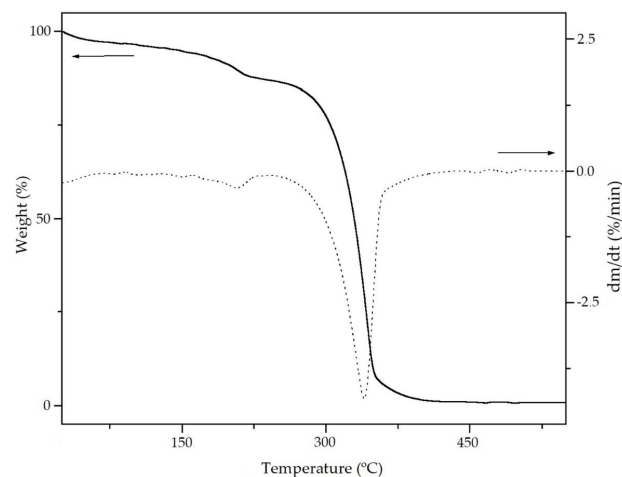


Figure 2. The TGA/DTG curves of the *Campomanesia adamantium* seed oil at 2 °C/min heating from 25 °C to 550 °C in nitrogen (N₂) air atmosphere flow at 60 mL min⁻¹.

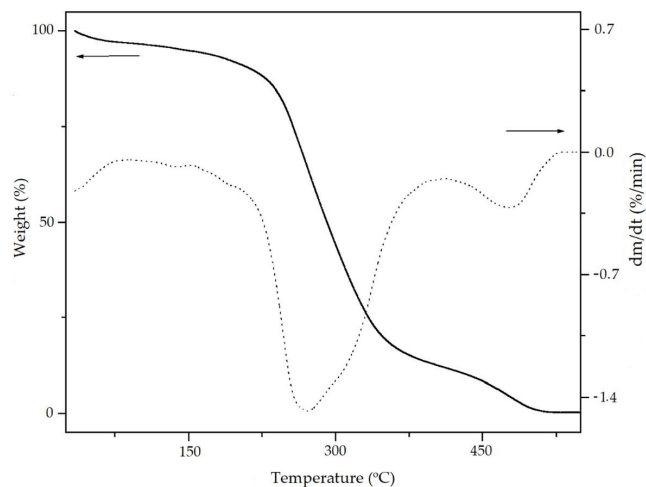


Figure 3. The TGA/DTG curves of the *Campomanesia adamantium* seed oil at 2 °C/min heating from 25 °C to 550 °C in synthetic air atmosphere flow at 60 mL min⁻¹. First derivative plot of mass per time unit (dm/dt).

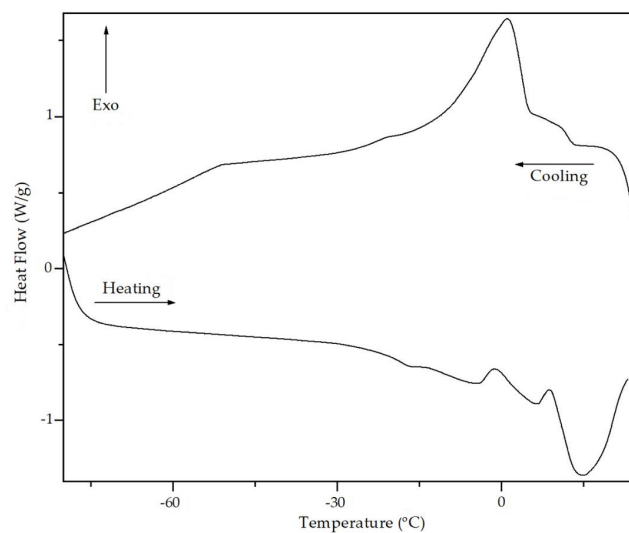


Figure 4. Differential scanning calorimetry (DSC) curves of the *Campomanesia adamantium* seed oil.

Table 3. Temperature of mass loss of the *Campomanesia adamantium* seed oil under nitrogen (N₂) and synthetic air atmospheres.

Atmosphere	Event	Temperature range (°C)		Event mass loss (%)	Residual mass (%)
		T _i	T _f		
N ₂	1 st	89.07	160.62	2.65	0.73
	2 nd	160.62	231.49	6.78	
	3 rd	231.49	418.46	86.26	
Synthetic air	1 st	78.72	112.58	0.81	0.26
	2 nd	112.58	154.31	1.51	
	3 rd	154.31	414.18	82.96	

T_i = Initial temperature; T_f = final temperature.

(oleic, linoleic and palmitoleic) acids, as well as the influence of unsaturation on the crystallization temperature of the oil (Rodrigues et al., 2006).

The oil heating behavior was observed at -13.12 °C (3.977 J/g), -0.37 °C (4.465 J/g) and 9.41 °C (29.95 J/g), which correspond to PUFAs, MUFAs and SFAs, respectively.

4 Future studies

We recommend to future sensory studies of *Campomanesia adamantium* seed oil as projective methods, sensory description using trained panel and hedonic test with consumer perception of food (Torres et al., 2017; Gambaro, 2018; Vital et al., 2018), as well the determination and characterization of metal and non-metal components in this oil according to the geographical origin (Rocha et al., 2019).

5 Conclusions

Campomanesia adamantium seed oil is an excellent source of bioactive compounds whose presence was proven by UV-Visible characterization. Besides, this oil demonstrates high oxidative and thermal stabilities and antioxidant activity, which are directly associated with its excellent quality. The shorter band that appeared at 3006 cm⁻¹ by FTIR analysis and the low iodine value demonstrates that the oil presents a high content of saturated and monounsaturated fatty acids. The lipidic profile confirmed that this oil has similar proportions of saturated and unsaturated fatty acids of which palmitic acid is the major saturated fatty acid while oleic is the monounsaturated fatty acid and presents good nutritional quality. Thus, these qualities found in the *C. adamantium* seed oil make it a great candidate for edible vegetable oil, which can be used for cooking and deep frying food, as well as for production of soap, lotions and biofuel.

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