Investigation of bioactive compounds from various avocado varieties
(*Persea americana* Miller)

Laura Paulino MARDIGAN¹, Vanessa Jorge dos SANTOS², Patricia Tiemi da SILVA²,
Jesuí Vergílio VISENTAINER³, Sandra Terezinha Marques GOMES¹, Makoto MATSUSHITA³*

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**1 Introduction**

Avocado (*Persea Americana* Miller), is a fruit with significant nutritional quality, such as high levels of fatty acids, fibers, proteins, antioxidant compounds, vitamin E, β-carotene and minerals, particularly iron (Daiuto et al., 2014).

Antioxidants are compounds that are able to inhibit and/or reduce the effects of free radicals. They can be described as the compounds that protect cells against harmful effects of oxygenated and nitrogenous free radicals from oxidative processes (Soares et al., 2005). Antioxidant compounds, such as vitamin E and C, carotenoids, and phenolic compounds, can be found in avocado pulp (Ali et al., 2008). Avocado pulp can also provide an oil with great characteristics, rich in oleic acid (omega-9) and high levels of sterols. These substances together are able to positively influence the metabolic control of cholesterol, and prevent cardiovascular diseases (La Rosa et al., 2010). In 2016, Brazil obtained production of 195,492 tons (Food and Agriculture Organization of the United Nations, 2018).

There are a great number of avocado varieties. The main varieties include: Fortuna, Dourado, Hass, Ouro Verde, Fuerte, Quintal, Geada, Choquette, Prince, Margarida, Collinson, Breda, Manteiga, Beatriz and Booth (Empresa de Assistência Técnica e Extensão Rural de Minas Gerais, 2016). The aim of this study was to characterize five varieties of avocado (Breda, Choquette, Margarida, Ouro Verde, and Quintal) for their proximal composition, physical properties, fatty acid composition, antioxidant capacity, carotenoids and minerals.

**2 Materials and methods**

2.1 Sample acquisition and preparation

This study was performed with 10 fruits of each variety (Breda, Choquette, Margarida, Ouro Verde and Quintal). They were harvested on a fall morning, at the Experimental Farm of the State University of Maringá, with latitude 22°39’23”S, longitude 52°51’35”W and 378 m altitude.

All the fruits were washed with neutral soap and fresh water, sanitized with 1% chlorine solution, and selected according to the absence of injuries. Subsequently, they were cut in half and separated into the pulp, peel and core. The pulps were separated for the experimental analyses. They were then homogenized and vacuum packed in polyethylene bags with an aluminum layer, finally, they were stored frozen (-18 °C), until analyses.

2.2 Proximal composition and physical-chemical parameters

The following analyses were performed, according to the Association of Official Analytical Chemists (Latimer, 2016): moisture (method 968.11), ash (method 945.38) crude fiber (method 962.09), and crude protein (method 971.09), using 5.75 as the conversion factor (Brasil, 2003).

The total lipids content was determined by the method of Bligh & Dyer (1959). A Pocket Pal-1 portable digital refractometer (Atago, USA), with a scale from 0 to 35 °Brix, was used to determine the total soluble solids of the samples. The readings were taken directly from the equipment and the results were expressed in °Brix (method 968.11), ash (method 945.38) crude fiber (method 962.09), and crude protein (method 971.09), using 5.75 as the conversion factor (Brasil, 2003).

The total lipids content was determined by the method of Bligh & Dyer (1959). A Pocket Pal-1 portable digital refractometer (Atago, USA), with a scale from 0 to 35 °Brix, was used to determine the total soluble solids of the samples. The readings were taken directly from the equipment and the results were expressed in °Brix (method AOAC 925.09) (Latimer, 2016). The color parameters L*, a*, b*, c* and H* (°Hue) of avocado fruits was
determined using a Minolta CR-10 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) (Mardigan et al., 2014). The fruits weight was determined by analytical balance and the width and length were measured using a digital calipers (Mardigan et al., 2014). The pH value was determined by potentiometric pH measurement (Hanna Instruments, pH 300 model, Italy) (method AOAC 945.27) (Latimer, 2016).

2.3 Fatty acids composition

For fatty acids composition analysis, the total lipids were converted into methyl esters (FAME), according to Hartman & Lago (1973), modified by Maia & Rodriguez-Amaya (1993). The FAME were separated using a gas chromatograph, (Thermo, Trace GC Ultra, Italy), equipped with a flame ionization detector and CP-7420 fused silica capillary column (100 m × 0.25 mm × 0.25 µm, cyanopropyl). The gas flow rates were 1.2 mL/min for the carrier gas (H₂), 30 mL/min for the make-up gas (N₂) and 35 and 350 mL/min for H₂ and synthetic air, respectively, for the detector flame.

The sample injection volume was 2 µL, using a sample split ratio of 1:80. The injector and detector temperatures were 240 °C. The column temperature was initially set at 165 °C for 7 min, followed by a heating rate of 4 °C/min, until reaching 185 °C, where it remained for 3 min, then heated at 6 °C/min until the column reached 235 °C, where it was maintained for 1.67 min. The FAME were identified by their retention time using Sigma standards 189-19 (USA). The fatty acids were quantified according to Joseph & Ackman (1992), using methyl tricosanoate (23:0, Sigma, EUA) as the internal standard.

2.4 Antioxidant capacity assays

Extract preparation

The extraction process involved the dissolution of 5 g of avocado pulp in 15 mL of methanol, under stirring, for 5 min. The solution was filtered using a Buckner funnel and then stored in an amber flask (Mardigan et al., 2014).

DPPH assay (2,2 Diphenyl-1-picrylhydrazyl)

For the DPPH assay, 25 µL of the extract was added to a test tube with 2 mL of DPPH solution. After 30 min of reaction time, the absorbance was measured using a UV-vis spectrophotometer (Thermo Scientific, Genesys 10 UV, USA), at 517 nm (Ma et al., 2011). The calibration curve was established using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), as standard where the concentration was between 200-300 mmol Trolox/L. The results were expressed as µmol Trolox equivalents (TE)/g of fresh mass.

ABTS assay (2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))

The ABTS assay was performed by combining 30 µL of each sample, respectively, to a test tube with 3 mL of ABTS solution. After 6 min, the absorbance was read at 734 nm (Thermo Scientific, Genesys 10 UV, USA) (Rufino et al., 2007). The calibration curve was constructed using Trolox, curve concentrations were between 600 - 3000 mmol Trolox/L. The results were expressed as µmol TE/g of fresh mass.

FRAP assay Ferric reducing antioxidant power

The FRAP assay involved adding 100 µL of the sample in a test tube with 300 µL of distilled water and 3 mL of FRAP reagent (2,4,6-tri(2-pyridyl)-1,3,5-triazine). After 30 min of reaction time, the solution absorbance was read at 593 nm (Thermo Scientific, Genesys 10 UV, USA) (Rufino et al., 2006). Trolox was used to construct the calibration curve, where the concentration was between 80 - 550 mmol Trolox/L. The results were expressed as µmol TE/g of fresh mass.

ORAC assay (Oxygen Radical Absorbance Capacity)

Antioxidant capacity analysis by the ORAC assay, was performed according to Zulueta et al. (2009). A stock solution of fluorescein (1.03 mmol/L) prepared in phosphate buffer solution (K₃HPO₄ and KH₂PO₄, 75 mmol/L, pH 7.00), was used to obtain the working solution. The sample extracts were respectively diluted in a phosphate buffer solution with pH 7.0 and a concentration of 0.075 mol L⁻¹ and then 25 µL aliquots were transferred to microplate wells. Subsequently, 150 µL of fluorescein were added and after 5 min of reaction time, at 37 °C, 25 µL of 2,2’-azobisis(2-methylpropionamidine) dihydrochloride (AAPH) was added. The absorbance readings were then started.

The blank was prepared in a similar manner, however, 25 µL of phosphate buffer solution was added instead of the sample extract. Fluorescence was monitored using an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Trolox solutions were used to construct the calibration curve, in a concentration that was between 200 - 1000 µmol Trolox/L. The results were expressed as µmol TE/g of fresh mass.

ORAC assay Lipophilic (L)

For this assay, 25 mg of each oil sample was solubilized in 1.5 mL of acetone and 4.5 mL of 7% randomly methylated β-cyclodextrin (RMCD) solution (50% acetone: 50% water, v/v). The extracts were diluted with 7% RMCD in acetone/water (50:50, v/v), until they reached a concentration appropriate to be within the concentration range of the standard curve. The 7% RMCD solution was used as a blank and to dissolve the Trolox. The L-ORAC assay was performed at 37 °C on a Victor™X4 spectrofluorimeter (PerkinElmer, USA), using 96-well black microplates, and the excitation/emission measurement was made from the top of the plate. The final L-ORAC value was calculated using a linear regression model between Trolox concentration (µmol/L) and the area under the decreasing curve of fluorescein, according to Prior et al. (2003).

2.5 Carotenoids

The carotenoids were extracted from 0.20 g of lyophilized sample with 10 mL of a hexane-acetone mixture (6:4 v/v), under stirring for 1 min. The tubes were incubated for 9 min, centrifuged and then, immediately, the absorbance was measured at 450 nm.
(Thermo Scientific, Genesys 10 UV, USA). A calibration curve was obtained using β-carotene as the standard. The results were expressed as mg of β-carotene/100 g of sample (Neves et al., 2015).

2.6 Mineral contents

The samples were digested by carbonization in a muffle at 550 °C for 6 h. Then, 10 mL of 5% nitric acid was added to the sample, and the mixture was heated until complete dissolution of the ashes, followed by filtration. After the sample had reached room temperature, the solution was transferred to a 25-mL volumetric flask and the volume was quenched with deionized water (method AOAC 941.12) (Latimer, 2016).

The minerals iron (Fe), calcium (Ca) and manganese (Mn), were determined in an AA 240 FS flame atomic absorption spectrophotometer (Varian, Australia). Sodium (Na) was determined by atomic emission spectrometry. Hollow cathode lamps were used. The current was set, according to the manufacturer’s recommendations.

For the calibration curves, individual reference solutions containing 1000 mg/L of each element were used (Specsol, Brazil). For the different points of the calibration curve, the solutions were prepared by successive dilutions, from a mixed solution of 100 mg/L prepared from the original solutions.

The Schinkel solution, composed of 10 g/L CsCl and 100 g/L LaCl (Fluka, Germany), was added to all reference solutions and samples at a final concentration of 0.5% (v/v). The analytical procedures followed the protocols established by the American Public Health Association (2005).

2.7 Statistical analyses

All analyses were performed in quadruplicate. The results obtained in the experiment were submitted to analysis of variance (ANOVA) and the means were compared using Tukey’s test (p<0.05). All the statistic procedures were performed in the SAS program, version 9.0 (SAS Institute, Inc. Cary, NC).

3 Results and discussion

3.1 Proximal composition and physical-chemical parameters

The proximal composition (Table 1) of the pulps revealed a high moisture content, followed by a high total lipids content, particularly highlighting Quintal variety. Tango et al. (2004) studied Quintal variety and found a similar total lipid content for the Manteiga variety. For the total soluble solids of Hass variety, in agreement with Vinha et al. (2013) reported 6.68 °Brix similar values of pH and soluble solids for the Manteiga variety (pH 6.19 and 9.32 °Brix). According to Vieites et al. (2012), a* indicates the red/green intensity (+ red/- green). Among the five varieties, only the Breda variety had a greater presence of green pigment in the pulp. The b* parameter indicates the blue/yellow intensity (- blue/+ yellow). A significant difference in the b* value was observed among the varieties and Choquette had the greatest concentration of yellow pigment in the pulp.

Ouro Verde and Quintal varieties presented the greatest longitudinal diameters (Table 2). Similar results were found by Oliveira et al. (2013) of 18.5 and 15.4 cm, for Ouro Verde and Quintal varieties, respectively.

The values for pH were within the values quoted in the literature. Concerning soluble solids, Quintal variety had a relatively lower value than Choquette. Santos et al. (2015) found similar values of pH and soluble solids for the Manteiga variety (pH 6.19 and 9.32 °Brix). Vinha et al. (2013) reported 6.68 °Brix for the total soluble solids of Hass variety, in agreement with the current study.

3.2 Fatty acids composition

Table 3 shows that avocado oil mainly contains monounsaturated fatty acids (60%), followed by saturated fatty acids (29%) and a significant amount of polyunsaturated fatty acids (11%).

Table 1. Proximal composition of avocado varieties (Persea americana Miller).

|                | Ouro verde | Breda | Quintal | Margarida | Choquette |
|----------------|------------|-------|---------|-----------|-----------|
| Moisture (%)   | 76.94 ± 0.14 | 78.06 ± 0.11 | 79.15 ± 0.89 | 83.06 ± 0.80 | 76.23 ± 0.13 |
| Ash (%)        | 0.19 ± 0.17 | 0.17 ± 0.11 | 0.17 ± 0.08 | 0.22 ± 0.11 | 0.31 ± 0.09 |
| Lipids (%)     | 13.1 ± 1.08 | 11.8 ± 0.97 | 14.18 ± 0.72 | 8.8 ± 0.39 | 12.01 ± 0.67 |
| Crude fiber (%)| 5.01 ± 0.90 | 5.13 ± 0.76 | 4.10 ± 0.47 | 5.02 ± 0.52 | 5.59 ± 0.28 |
| Crude protein (%) | 2.08 ± 0.78 | 1.61 ± 0.51 | 1.40 ± 0.68 | 1.01 ± 0.88 | 1.91 ± 0.17 |

Different letters signify significant differences between lines (p<0.05), according to the Tukey method.
The monounsaturated fatty acids level mainly reflects the oleic acid (18:1 n-9) content, which is the major acid in avocado oil of all varieties. The Choquette and Quintal varieties had significant 18:1n-9 contents. Tango et al. (2004) found similar amounts of oleic acid in the Fuerte (61%) and Hass (47%). varieties. Ferrari (2003) also studied this variety and found 22% linoleic acid. Salas et al. (2000) described avocado mesocarp oil with linoleic acid contents varying from 10 to 14%.

According to Salgado et al. (2008b), the fatty acid composition of avocado oil confirms the possibility of using it as a substitute for olive oil or as a raw material by the food industry, due to its physicochemical similarity and taste.

### Table 2. Physical chemical parameters of pulp from avocado varieties (*Persea americana* Miller).

|       | Quintal   | Margarida | Choquette | Ouro verde | Breda     |
|-------|-----------|-----------|-----------|------------|-----------|
| L     | 58.90± 1.61 | 55.60± 1.22 | 52.10± 0.36 | 52.10± 1.99 | 46.96± 1.28 |
| a     | -0.96± 0.68 | -1.40± 0.10 | -1.56± 0.32 | -1.86± 1.05 | -2.16± 1.36 |
| b     | +31.30± 1.55 | +35.63± 0.58 | +38.26± 0.77 | +32.50± 2.70 | +35.50± 1.55 |
| c     | 29.96± 2.04 | 37.06± 3.06 | 33.20± 1.73 | 32.56± 2.70 | 35.56± 1.56 |
| H (hue) | 92.03± 1.51 | 89.90± 2.30 | 85.56± 1.10 | 89.10± 4.17 | 88.56± 4.45 |
| pH value | 7.20± 1.33a | 6.55± 1.72b | 6.18± 2.08c | 6.50± 1.99bc | 6.70± 1.65b |
| Soluble solids (° Brix) | 7.67± 2.17 | 7.20± 2.11 | 6.50± 2.08 | 6.38± 2.18 | 8.97± 1.89 |
| Length (cm) | 16.33± 1.84 | 12.33± 0.76 | 17.33± 1.54 | 14.67± 0.96 | 14.67± 0.62 |
| Width (cm) | 6.00± 1.24 | 4.00± 1.36 | 3.33± 2.01 | 3.33± 1.75 | 3.33± 1.77 |
| Weight (g) | 845.00± 0.75 | 533.33± 0.61 | 582.67± 0.99 | 540.00± 0.16 | 540.00± 0.81 |

Different letters signify significant differences between lines (p < 0.05), according to the Tukey method.

### Table 3. Fatty acids composition of avocado varieties (*Persea americana* Miller).

| Fatty acids | Variety |
|------------|---------|
|             | Ouro verde | Choquette | Breda | Quintal | Margarida |
| 16:0        | 201.89± 8.94 | 217.60± 5.39 | 206.85± 3.30 | 219.17± 11.00 | 142.72± 18.2 |
| 16:1n-7     | 76.57± 5.93 | 67.39± 1.67 | 78.98± 6.35 | 48.09± 2.29 | 30.76± 7.85 |
| 18:0        | 5.23± 0.62  | 7.04± 0.26  | 4.92± 0.36  | 8.03± 0.45  | 4.93± 1.35  |
| 18:1n-9     | 250.96± 7.19 | 446.8± 2.66 | 227.56± 6.79 | 393.92± 1.61 | 315.56± 12.17 |
| 18:1n-7     | 32.11± 4.15 | 41.61± 1.44 | 37.33± 3.09 | 25.87± 1.13 | 25.63± 5.05 |
| 18:2n-6     | 108.74± 6.85 | 106.20± 2.54 | 93.65± 7.98 | 83.53± 4.10 | 90.57± 10.24 |
| 18:3n-3     | 7.11± 0.75  | 8.11± 0.02  | 7.11± 0.64  | 3.26± 0.22  | 7.01± 1.59  |
| SFA         | 207.11± 9.44 | 224.65± 5.65 | 211.78± 3.53 | 227.19± 11.36 | 147.65± 20.12 |
| MUFA        | 359.64± 15.80 | 555.83± 9.23 | 343.87± 14.96 | 467.88± 3.16 | 371.94± 24.27 |
| PUFA        | 115.85± 7.59 | 114.31± 5.25 | 100.76± 8.61 | 86.79± 4.29 | 97.59± 11.69 |
| n-6         | 108.74± 6.85 | 106.20± 2.54 | 93.65± 7.98 | 83.53± 4.10 | 90.57± 10.24 |
| n-3         | 7.11± 0.75  | 8.11± 0.02  | 7.11± 0.64  | 3.26± 0.22  | 7.01± 1.59  |
| n-6/n-3     | 15.29± 0.75 | 13.09± 0.30 | 13.17± 0.07 | 25.63± 0.76 | 12.91± 1.85 |
| PUFA/SFA    | 0.56± 0.02  | 0.51± 0.01  | 0.48± 0.04  | 0.38± 0.01  | 0.66± 0.02  |

Different letters signify significant differences between lines (p < 0.05), according to the Tukey method. SFA (Saturated Fatty Acids), MUFA (Monounsaturated Fatty Acid), PUFA (Polyunsaturated Fatty Acid).

### 3.3 Antioxidant capacity

The antioxidant capacity reflects the efficacy of the natural antioxidants to protect the vegetal product against oxidative damages and loss of commercial and nutritional value. In general, the cultivars analyzed, presented similar results for the antioxidant assays (Figure 1).

Regarding the DPPH antioxidant capacity, the Breda variety had the highest capacity (1.11 μmol TE/g). These results are similar to that found by Wang et al. (2010), for the Hass variety (1.3 μmol TE/g). Quintal variety had the highest ABTS’ radical scavenging capacity assay (41%). Villa-Rodríguez et al. (2011), studied the Hass variety produced in all four seasons of the year and found higher values compared to this study.

Breda variety possessed the highest FRAP antioxidant capacity (145%), whereas Ouro Verde variety exhibited the highest ORAC value (23%). Besides total ORAC, L-ORAC assay was also performed, to verify the amount of antioxidant...
in the lipophilic part of the avocado. The results showed that the Margarida variety had the highest content (200 μmol TE/g). Villa-Rodríguez et al. (2011) studied the Hass variety and found 500 μmol TE/g. The results indicate that the avocado varieties studied represent a valuable source of lipophilic antioxidants.

### 3.4 Carotenoids

The carotenoid content in fruits and vegetables depends on several factors, such as genetic variety, maturation stage, post-harvest storage and preparation (Capecka et al., 2005). Some studies have shown that avocado presents carotenoids and lipophilic compounds with benefits to health (Ding et al., 2007). In this study, the highest levels of carotenoids were found in the Ouro Verde variety. Vinha et al. (2013) studied some parts of Hass avocado cultivated in the region of Portugal and found similar results for the pulp (0.810 mg β-carotene/100 g of sample). Lu et al. (2005) found 0.600 mg β-carotene/100 g of the pulp of Hass variety. Wang et al. (2010) studied the peel, core and pulp of seven varieties of avocado cultivated in California, and the highest β-carotene content found was 0.770 mg/100 g of the pulp. The results found in this study (Table 4), corroborate the literature data.

### 3.5 Mineral contents

Table 5 shows the mineral composition of the five varieties, considering values of iron, calcium, manganese and sodium. Calcium was the major macronutrient in all the varieties, and the
Quintal variety presented the highest value. Salgado et al. (2008a) found lower results for this mineral (0.35 mg/100 g).

Regarding micronutrients, higher contents were found for iron. Salgado et al. (2008a) found similar results for this mineral in the Hass variety (19.7 mg/100 g).

4 Conclusion

All the avocado varieties analyzed in this study presented fibers, lipids, proteins, carotenoids and minerals, and the oleic acid was the major lipid component for all the samples. The fruits presented significant values for L-ORAC, DPPH, FRAP, ABTS and ORAC assays and can be considered sources of antioxidant compounds. Concerning these analyses, Margarida and Quintal varieties could be highlighted due to their greater values among the varieties studied.

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