CENTESIMAL AND MINERAL COMPOSITION, PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT CAPACITY FROM PITANGA (Eugenia uniflora L.) PULP

F. G. Zola¹, B. D. Oliveira², A.C. Rodrigues³, J. G. Taylor⁴, L.R. Cunha⁵, U. M. Pinto⁶, M. C. Bertoldi⁷

1- Department of Food, School of Nutrition – Federal University of Ouro Preto, Ouro Preto, MG – 37145-000 – Ouro Preto – MG – Brasil, Phone/Fax: (+55-31) 3559-1828 – e-mail: (flaguizola@gmail.com)
2- Department of Food, School of Nutrition – Federal University of Ouro Preto, Ouro Preto, MG – 37145-000 – Ouro Preto – MG – Brasil, Phone/Fax: (+55-31) 3559-1828 – e-mail: (brigida90@hotmail.com)
3- Department of Food, School of Nutrition – Federal University of Ouro Preto, Ouro Preto, MG – 37145-000 – Ouro Preto – MG – Brasil, Phone/Fax: (+55-31) 3559-1828 – e-mail: (adelinerodriguescoelho@yahoo.com.br)
4- Department of Chemistry, Institute of Physical and Biological Sciences – Federal University of Ouro Preto, Ouro Preto, MG – 37145-000 – Ouro Preto – MG – Brazil, Phone/Fax: (+55-31) 3559-1828 – e-mail: (jason@iceb.ufop.br)
5- Department of Food, School of Nutrition – Federal University of Ouro Preto, Ouro Preto, MG – 37145-000 – Ouro Preto – MG – Brasil, Phone/Fax: (+55-31) 3559-1828 – e-mail: (lrcunhaufv@yahoo.com.br)
6- Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences – University of São Paulo, São Paulo, SP – 35010-177 – São Paulo – SP – Brazil, Phone: (+55-11) 2648-0677; Fax: (+55-11) 2648-0677 – e-mail: (uelintonpinto@usp.br)
7- Department of Pharmacy, Faculty of Pharmacy – Federal University of Juiz de Fora, Governador Valadares, MG – 35010-177 – Governador Valadares – MG – Brazil, Phone/Fax: (+55-33) 3301-1000 – e-mail: (michele.bertoldi@ufjf.edu.br)

ABSTRACT – This study aims to assess the chemical composition and the antioxidant capacity from pitanga (Eugenia uniflora L.) pulp. The phenolic compounds were extracted from pulp by solid phase extraction and quantified by Folin-Ciocalteu assay. Antioxidant activity (AA) was measured by DPPH, ABTS and FRAP methods. Mineral and centesimal compositions were also determined. Pitanga is a good source of total polyphenols (104.6 mg AGE/100g pulp) and presented AA: (DPPH) 71.2 µmol Trolox/g pulp, (FRAP) 16.74 µmol sulfato ferroso/g pulp and (ABTS) 0.17 µmol Trolox/g of pulp. Pulp presented (g/100g of pulp): 3.68 ± 0.21proteins, 0.02 ± 0.03 lipids, 0.21 ± 0.04 ash, 10.31 ± 0.00 moisture, 2.06 ± 0.03 carbohydrates and 1.25 ± 0.05 fibers. Calcium was the trace element presented in highest amount (3.36 mg / g pulp). The study highlighted the contribution of phenolic and non-phenolic compounds on antioxidant potential of pitanga.

KEYWORDS: antioxidant activity; centesimal composition; mineral composition; phenolic compounds; solid phase extraction.

1. INTRODUCTION

Fruits and vegetables are foods that contribute to a healthy diet and decrease the risk of chronic non-communicable diseases (CNDs) due to in part their antioxidant activity, which is partially associated to the presence of phenolic compounds (Sreeramulu and Raghunath, 2010).
Phenolic compounds are secondary metabolites from plants characterized by a wide range of chemical structures and biological activities. Biological properties of these compounds has been attributed to their ability to scavenge free radicals (alkoxyl, superoxide, hydroxyl radical, nitric oxide, peroxynitrite oxidant); interact with enzymes (e.g. purines), transcription factors (NF-kB), receptors (e.g. estrogen receptors) which contribute to the reduction of oxidative stress common to cardiovascular diseases, neurodegenerative diseases, cancer, among other diseases (Fraga et al., 2010).

The fruits from Eugenia uniflora L. known as pitanga in Brazil, with common names Brazilian cherry, Surinam cherry or south cherry, are native from the Atlantic forest, belonging to the family Myrtaceae (Bagetti et al., 2011). Parts of the plant have been used in the medicinal folk, particularly for making teas from its leaves, which are rich in tannins, glicosides and flavonoids (Pio et al., 2005). Despite its beneficial effects, as far as we know, no study has been determined trace elements in fruit of Eugenia uniflora L. as well as the content of phenolic compounds, which has been quantified together non-phenolic reducing compounds as interferents.

This work aimed to characterize the centesimal and mineral composition, the total phenolic content as well as to determine the in vitro antioxidant activity from pitanga pulp.

2. MATERIALS AND METHODS

2.1. Extraction and Quantification of Phenolic Compounds

Orange pitanga fruits (Eugenia uniflora L.), collected in Ouro Preto, MG, Brazil, were washed and sanitized with sodium hypochloride at 50 mg L⁻¹ for 15 min. Seeds were manually removed and the pulp was homogenized and kept frozen at -20°C until use. Phenolic compounds from the pulp were extracted using solid phase extraction (SPE) according to the procedure described by Bertoldi (2009). Briefly, the pitanga pulp was thawed and mixed with 1:1:1 (v/v/v) ethanol:methanol:acetone solution and filtered (crude extract). Solvents were evaporated at 40°C using a rotary evaporator and aqueous extract was onto C18 Waters Sep-Pak Vac 35cc 10g 20 cm³ minicolumns (Waters Corporation, Milford, MA) previously conditioned with acidified methanol (0.01%). Phenolic compounds were adsorbed onto the cartridge and eliminated by methanol, while sugars, organic acids (ascorbic acid) and other water-soluble compounds were removed by washing the column. The extract enriched in phenolic compounds was named phenolic extract. The total phenolic content of the extracts was determined by the Folin-Ciocalteu assay (Shahidi and Naczk, 1996) and expressed as mg of galic acid equivalent per L (mg AGE L⁻¹).

2.2. Centesimal and Mineral Composition

The contents of moisture, ash, total lipids, proteins, carbohydrates, total soluble solutes and fibers was determined according to the Association of Official Analytical Chemists (AOAC, 2003) and Instituto Adolfo Lutz (IAL 2005). Results were expressed as g/100 g of pulp. The carbohydrate content was calculated by subtracting the sum of protein, total lipids, moisture and ash from 100%. The experiment was performed in triplicate and results were expressed as average and standard deviation.

Trace elements were determined by Total Reflection X-Ray Fluorescence (TXRF). The analysis was performed on a S2-PICOFOX instrument (Bruker AXS, Berlin, Germany) with a molybdenum anode operating at 50 kV and 750 mA. Results were obtained by integration of the signal on a Bruker
Spectra software (version 6.1.5.0) with quantification being performed by means of comparing with internal standards of known concentration. The minerals were expressed as mg g⁻¹ of fruit.

2.3. Antioxidant Activity

The antioxidant activities (AA) of the crude and phenolic extracts from pitanga pulp was determined by the ABTS+ radical method, as described by Cai et al. (2004) and by DPPH free radical method, according to the procedure described by Brand-Williams et al. (1995). Trolox (0-2000 μM) was used as an external standard for both assays and the results were expressed as μM Trolox equivalent per gram of fresh pulp. Additionally, antioxidant activity was expressed as half maximal inhibitory concentration (IC₅₀) which indicates the concentration of phenolic extract that provides 50% of inhibition of DPPH absorbance. Additionally, the ferric-reducing antioxidant power (FRAP) was estimating according to the procedure described by Benzie and Strain (1996). Standard aqueous solutions of iron (II) sulfate heptahydrate (FeSO₄·7H₂O) ranging in concentration from 500 - 2000 μM were used for calibration. Results were expressed as μmol Fe²⁺ per gram of fresh fruit.

3. RESULTS AND DISCUSSION

3.1. Phenolic Compounds

The pulp yield of pitanga was 72 g pulp/100 g of fresh fruit. The total phenolic contents (TPC) of crude and phenolic extracts were 126.1 mg AGE/100 g fresh fruit and 104.6 mg AGE/100 g fresh fruit, respectively. A large variation (from 95.0 to 799.8 mg GAE 100g⁻¹ of fresh pulp) in values of total phenolic content for pitanga has been found (Bagetti et al., 2011; Abe et al., 2012; Denardin et al., 2015). In general, variations in the composition of phenolic compounds in fruits are associated to intrinsic (specie, cultivar and maturity) and extrinsic (cultivation conditions, seasonality, transport, storage) characteristics as well as the extraction method or solvents used for extraction of these compounds (Cardello and Cardello 1998; Moura et al., 2011). Additionally, according to Vissotto et al. (2013), differences in phenolic content of fruits may be likely related to the lack of specificity of Folin-Ciocalteu method for predicting phenolic compounds content since non-phenolic reducing compounds including ascorbic acid are detected by Folin-Ciocalteu reagent. For pitanga, levels of ascorbic acid varying from 0.086 mg 100g⁻¹ to 42.9 mg 100g⁻¹ fresh fruit have been reported for different varieties from pitanga (Denardin et al., 2015) that might interfere in the results of phenolic content of the fruit. In the present study, ascorbic acid was eliminated by solid phase extraction (SPE) in order to improve the specificity of the method, as suggested by Sánchez-Rangel et al. (2013), which avoided an overestimation of 20.5% in the total phenolic content from pitanga pulp.

3.2. Centesimal and Mineral Composition

The centesimal and mineral compositions of pitanga pulp are presented in Table 1 and Table 2, respectively. Protein content was higher in our study (3.68 ± 0.21 g 100g⁻¹ of pulp) than those normally reported for this fruit in previous reports (0.68 to 1.62 g 100g⁻¹ of pulp), while lower values of lipids were found in our study as compared to those from the literature (0.26 to 0.5 g 100g⁻¹ of pulp) (Bagetti et al., 2011; TACO, 2011). Calcium was the most abundant mineral present in pitanga pulp, followed by potassium, iron, chlorine and zinc. To our knowledge, this is the first report using TXRF.
to determine the mineral composition of pitanga. Variations in centesimal and mineral composition are expected due to reasons already described in the present study.

Table 1 - Centesimal composition of pitanga pulp (g 100g⁻¹ pulp).

<table>
<thead>
<tr>
<th>Component</th>
<th>Value (± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>3.68 ± 0.21</td>
</tr>
<tr>
<td>Lipids</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>Ash</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>Moisture</td>
<td>85.78 ± 0.20</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>10.31 ± 0.51</td>
</tr>
<tr>
<td>Fibers</td>
<td>2.06 ± 0.51</td>
</tr>
<tr>
<td>Total soluble solutes (°Brix)</td>
<td>5.2 ± 0.60</td>
</tr>
</tbody>
</table>

Values are the mean values of triplicates ± standard deviation.

Table 2 - Spectroscopy results determined by Total Reflection X-ray Fluorescence TXRF of trace elements found in pitanga (mg g⁻¹ pulp).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Content of trace elements in pitanga pulp (mg g⁻¹ fresh pulp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (K)</td>
<td>0.90 ± 0.04</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>3.36 ± 0.04</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>0.60 ± 0.01</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.17 ± 0.00</td>
</tr>
<tr>
<td>Chlorine (Cl)</td>
<td>0.56 ± 0.09</td>
</tr>
<tr>
<td>Chrome (Cr)</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.07 ± 0.00</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>NF±</td>
</tr>
</tbody>
</table>

*NF – not found

3.3. Antioxidant Activity

*In vitro* antioxidant activities of crude and phenolic extracts from pitanga, determined by three different antioxidant assays (DPPH, ABTS and FRAP), are presented in Table 3.

Table 3 - Antioxidant activity of crude and phenolic extracts from the pulp of pitanga, determined by DPPH, ABTS and FRAP methods.

<table>
<thead>
<tr>
<th>Extract/ Antioxidant activity assay</th>
<th>DPPH (µM Trolox g⁻¹ pulp)</th>
<th>DPPH, IC50 (mg GAE L⁻¹ phenolic extract)</th>
<th>ABTS (µM Trolox g⁻¹ pulp)</th>
<th>FRAP (µmol Fe²⁺ g⁻¹ pulp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>71.2 ± 3.2</td>
<td>147.2 ± 10.9</td>
<td>0.17 ± 0.0</td>
<td>16.74 ± 0.7</td>
</tr>
<tr>
<td>Phenolic extract</td>
<td>12.2 ± 0.9</td>
<td>488.2 ± 15.0</td>
<td>8.83 ± 0.1</td>
<td>34.87 ± 2.3</td>
</tr>
</tbody>
</table>

The ability of phenolic compounds from pitanga to scavenge the DPPH radical was evaluated and the antioxidant activity was 12.2 µmol Trolox g⁻¹ pulp and 488.2 mg GAE L⁻¹ of phenolic extract (IC50). Several studies have reported values varying from 5.6 to 42.0 µM Trolox g⁻¹ fresh pulp (Prado, 2009; Bagetti *et al.*, 2011; Moura *et al.*, 2011; Abe *et al.*, 2012) or, in terms of IC₅₀, ranging from
36.78 to 110.91 mg GAE L\(^{-1}\) (Denardin et al., 2015). Higher antioxidant activities has been found for purple variety of pitanga as compared to orange and red varieties, which is probably associated with the higher content of anthocyanins in purple fruits (Bagetti et al., 2011; Denardin et al., 2015).

In general, these variations in the antioxidant activity from fruits are associated with the differences in the chemical composition of the extract obtained from fruit, particularly related the type and relative levels of phenolic compounds in the extract, which may vary according to several reasons previously described in the present study. A strong positive correlation between antioxidant activity and phenolic content of pitanga has been demonstrated in several studies, suggesting that phenolic compounds are primarily responsible for the antioxidant activity of the fruits (Bagetti et al., 2011; Denardin et al., 2015). However, data present in Table 3 for DPPH assay indicate that non-phenolic reducing compounds, including ascorbic acid, considerably contribute to antirradical activity of the fruit.

The ability of antioxidants from pitanga to reduce the radical cation ABTS\(^{+}\) to ABTS was 8.83 μM Trolox g\(^{-1}\) of fresh pulp. As far as we know, this is the first result of antioxidant activity from pitanga determined by ABTS method.

The ability of antioxidants from pitanga to reduce ferric iron (Fe\(^{3+}\)) to the ferrous ion (Fe\(^{2+}\)) was evaluated by FRAP method and shown to be 34.87 μmol Fe\(^{+2}\) g\(^{-1}\) fresh fruit. Similar result was found for orange variety of pitanga (33.17 μmol g\(^{-1}\) fresh fruit), an intermediate value between those determined for red (23.43 μmol g\(^{-1}\)) and purple (81.62 μmol g\(^{-1}\)) varieties from this fruit (Denardin et al., 2015).

4. CONCLUSIONS

Pitanga is a fruit rich in substances that contribute to its antioxidant capacity, indicating that both phenolic and non-phenolic compounds present antirradical properties, which suggests that its regular consumption might contribute to reduce the risk of CNDs.

5. REFERENCES


Campinas State University – UNICAMP. (2011). *Brazilian Table of Food Composition – TACO* (versão 4, 4. ed.). Campinas: UNICAMP/NEPA.


