Original article

**Acerola and cashew apple as sources of antioxidants and dietary fibre**

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**Summary**

Several tropical fruits have been described as natural sources of dietary fibre (DF) and phenolic compounds, associated with different health effects. The aim of this work was to ascertain the DF, phenolic compounds content (including non-extractable polyphenols, mostly associated with DF) and antioxidant capacity in acerola fruits and cashew apples from selected clones. ‘BRS 236’ acerola fruits presented a high antioxidant capacity because of the combination of both extractable polyphenols and l-ascorbic acid (providing together a Folin value of 170 kg d.m. g⁻¹). ‘CCP 76’ cashew apples contained 28 g kg d.m. of extractable polyphenols and 13 g kg d.m. of ascorbic acid as well as a high amount of non-extractable condensed tannins (52 g kg d.m.). DF content was of 260 g kg d.m. in acerola fruit and of 209 g kg d.m. in cashew apple. Acerola fruits and cashew apple should therefore be considered as new natural sources of DF and phenolic compounds.

**Keywords** Anacardium occidentale, antioxidant capacity, dietary fibre, Malpighia emarginata, polyphenols.

**Introduction**

Significant amount of bioactive constituents, such as dietary fibre (DF) or natural antioxidants, i.e. polyphenols, have been found during last years in many tropical fruits and related by-products (Jiménez-Escrig et al., 2001; Vasco et al., 2008; Rufino et al., 2010a). In many studies, all these constituents have been related to the prevention of several chronic diseases, such as cardiovascular disease or certain cancers (Bingham et al., 2003; Arts & Hollman, 2005). However, as tropical fruits include a wide variety of products, there still remain many fruits commonly consumed in tropical countries, but whose content in bioactive compounds is still totally or partially unknown.

Acerola (Malpighia emarginata), originally from the Antilles, can be found from South Texas, through Mexico and Central America to northern South America and throughout the Caribbean, being Brazil now the world’s largest producer, consumer and exporter (Assis et al., 2008). Acerola is characterised by a high vitamin C content, which is many times higher than that of other fruits that are considered good sources of this vitamin, such as guava, cashew apple, orange or lemon (Alves et al., 1995). Besides its consumption as a fruit, acerola presents a wide potential to be used in different juices and beverages rich in bioactive compounds, as it has been described in several works (Freitas et al., 2006; Mezadri et al., 2008; Lima et al., 2009; Sampaio et al., 2009).

The cashew (Anacardium occidentale) is native to Tropical America. Originating in Brazil, it has become naturalised in many tropical countries such as Vietnam, India, Nigeria, Tanzania, Ivory Coast, Mozambique and Benin. Cashew is formed by the developed peduncle (apple) attached to the nut (actual fruit composed of shell + kernel). The peduncle, which is also called pseudo-fruit, false fruit, cashew apple or simply cashew, represents the edible portion, in natura and also as
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Juices, pulp and preserves (Filgueiras et al., 1999). Apart from the usual presence of sugars and organic acids, one important characteristic of cashew apple is its high vitamin C content, four times higher than sweet orange (Akinwale, 2000).

For both acerola and cashew, several genetic improvement programmes have been developed to obtain clones allowing higher productivity. In particular, the clone ‘BRS 236’ acerola (Cereja) was obtained from the programme developed in Embrapa Tropical Agroindustry (Paiva et al., 1999) and the clone ‘CCP 76’ cashew was obtained at the Ceará Agricultural Research Corporation (Paiva et al., 2005). These particular clones were compared to others of the same fruits in previous works (Abreu et al., 2009; Sampaio et al., 2009). The study of nutritional characteristic of clones allows more reproducible results when compared to native fruits, besides the production advantages of clones.

Antioxidant capacity of acerola and cashew apple was recently determined by various techniques (Alves et al., 2008; Mezadri et al., 2008; Abreu et al., 2009; Sampaio et al., 2009; Rufino et al., 2010a). However, determinations were performed only in the supernatants of aqueous-organic extractions, without considering the antioxidant capacity associated with the residues of these extractions, which may have specific health properties (Serrano et al., 2009).

The aim of this work was to ascertain the DF and total phenolic contents of ‘BRS 236’ acerola fruits and ‘CCP 76’ cashew apples, and their antioxidant capacity. Based on these results further it would be possible to evaluate the application of these tropical fruits as antioxidants and sources of DF in functional foods or as naturally antioxidant dietary supplements.

Materials and methods

Methods

Dietary fibre determination

Dietary fibre was measured as indigestible fraction, based on a procedure previously described (Saura-Calixto et al., 2000). This method combines enzymatic treatments and separation of digestible compounds by dialysis using physiological conditions (temperature and pHs), obtaining the fraction of food that is not digested in the small intestine and reach the colon, composed of two fractions: a soluble fraction (supernatant) and an insoluble fraction (residue of enzymatic digestion) (Fig. 1). Total DF was calculated as the sum of insoluble DF components [resistant starch, non-starch polysaccharides (NSP), Klason lignin, resistant protein, ash, extractable polyphenols, proanthocyanidins, and hydrolysable phenols] plus soluble DF components (NSP and extractable polyphenols).

Samples (0.3 g) were incubated with pepsin (0.2 mL of a 300 mg mL\(^{-1}\) solution in 0.08 m HCl–KCl buffer, pH 1.5, 40 °C, 1 h), pancreatin (1 mL of 5 mg mL\(^{-1}\) solution in 0.1 m phosphate buffer, pH 7.5, 37 °C, 6 h) and \(\alpha\)-amylase (1 mL of a 120 mg mL\(^{-1}\) solution in 0.1 m Tris–maleate buffer, pH 6.9, 37 °C, 16 h). Samples were centrifuged (15 min, 3000 g) and supernatants removed. Residues were washed twice with 5 mL of distilled water, and all supernatants were combined. Each supernatant was incubated with 100 µL of amyloglucosidase for 45 min at 60 °C before being transferred to dialysis membranes (12 000–14 000 molecular weight cutoff, Visking dialysis tubing; Medicell International Ltd., London, UK) and dialysed against water for 48 h at 25 °C to eliminate digestible compounds.

NSP were hydrolysed with 1 m sulphuric acid at 100 °C for 90 min and spectrophotometrically measured by anthrone assay (Loewus, 1952). The residue was weighed to determine insoluble DF, and resistant
protein and ash were determined in it (see 'Other Determinations'). In this residue, after treatment with sulphuric acid (12 m, 20 °C for 3 h; dilution to 1 m and incubation for 2 h, 100 °C), NSP were determined spectrophotometrically as neutral sugars (by anthrone assay) and uronic acids and klason lignin was determined gravimetrically (Loewus, 1952; Scott, 1979).

**Extraction of antioxidants**

Figure S1 shows a scheme of the treatment applied to the samples to determine antioxidant capacity. In a capped centrifuge tube, 0.5 g of sample was placed; 20 mL of acidic methanol/water/HCl (50:50, v/v; pH 2) was added and the tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged at 2500 g in a Thermo Heraeus MegaFuge 11 (Thermo Fisher, Waltham, MA, USA) for 10 min and the supernatant was recovered. Twenty millilitres of acetone/water (70:30, v/v) was added to the residue, and shaking and centrifugation are repeated. Methanolic and acetic extracts were combined and used to determine the antioxidant capacity associated with extractable antioxidants (Figs 1 and S1). The residues of these extractions were subjected either to hydrolysis with H2SO4 in methanol to release hydrolysable tannins (Figs 1 and S1) or to treatment with butanol/HCl/FeCl3 to determine proanthocyanidins or condensed tannins (Figs 1 and S1).

**Determination of antioxidant compounds**

Total polyphenols in extracts were determined according to the Folin-Ciocalteu method in supernatants (Figs 1 and S1) (Singleton et al., 1999). Test sample (0.5 mL) was mixed with 1 mL of Folin-Ciocalteu reagent and swirled. After 3 min, 10 mL of sodium carbonate solution (75 g L⁻¹) was added and mixed. Additional distilled water was mixed thoroughly by inverting the tubes several times. After 1 h, the absorbance at 750 nm was recorded. The results were expressed as g gallic acid equivalents (GAE) g kg⁻¹.

Proanthocyanidins (condensed tannins) not extracted by the previous aqueous-organic procedure were measured at 555 nm after hydrolysis with butanol/HCl/FeCl3 (3 h, 100 °C) in supernatants (Figs 1 and S1) (Reed et al., 1982). Results were compared with carob pod (*Ceratonia siliqua*) proanthocyanidin standard (Nestlé, Ltd., Vers-Chez-les Blanes, Switzerland), which has been reported to be a suitable standard for proanthocyanidins determination (Pérez-Jiménez et al., 2009). Hydrolysable tannins were measured by hydrolysis with methanol and sulphuric acid for 20 h at 85 °C (Figs 1 and S1) (Hartzfeld et al., 2002). Concentration was estimated by the Folin–Ciocalteu method and expressed as g GAE kg⁻¹ (Singleton et al., 1999).

**Antioxidant capacity methods**

DPPH* (Free-Radical Scavenging) Assay (Brand-Williams et al., 1995; Sánchez-Moreno et al., 1998): After adjusting the blank with methanol, 0.1 mL of the sample was mixed with 3.9 mL of a DPPH* methanolic solution (60 μM). The absorbance at 515 nm was measured until the reaction reached the plateau depending
on the sample, this time may be between 1 and 60 min. A calibration curve at that wavelength was made to calculate the remaining DPPH. The parameter EC50, which reflects 50% depletion of DPPH free-radical, was expressed in terms of grams of fruit equivalent per gram of DPPH in the reaction medium. The time taken to reach the steady state at EC50 (tEC50) and the antiradical efficiency [AE] (AE = 1/(EC50*tEC50)) were also determined.

**ABTS** assay (Re et al., 1999): ABTS** radical cation (ABTS**) was produced by reacting 7 mM ABTS** stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS** solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 658 nm. After the addition of 100 µL of sample or Trolox standard to 3.9 mL of diluted ABTS** solution, absorbance readings were taken every 20 s, using a Beckman DU-640 (Beckman Instruments Inc. Fullerton, CA, USA) spectrophotometer. The reaction was monitored during 6 min. The percentage inhibition of absorbance versus time was plotted, and the area below the curve (0–6 min) was calculated. Solutions of known Trolox concentrations were used for calibration.

Ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996; Pulido et al., 2000): FRAP reagent (900 µL), freshly prepared and warmed at 37 °C, was mixed with 90 µL of distilled water and either 30 µL of test sample or standard or appropriate reagent blank. Reading at the absorption maximum (595 nm) was taken every 15 s, using a spectrophotometer. The readings at 30 min were selected for calculation of FRAP values. Solutions of known Trolox concentrations were used for calibration.

**Other determinations**

Protein was determined using an automated nitrogen analyser FP-2000®; Dumas Leco Corp. (St. Joseph, MI, USA). Fat content was determined using a Soxhlet System HT (FOSS, Höganäs, Sweden) extractor with petroleum ether. Ash content was determined with an electric muffle furnace for 16 h at 550 °C quantified gravimetrically and ascorbic acid spectrophotometrically by using 2,6-dichlorophenol (Strohecker & Henning, 1967).

**Statistical analysis**

All analyses were performed in triplicate and results were expressed as mean values ± standard deviation on dry matter (d.m.) basis.

**Results and discussion**

**Dietary fibre**

The content and composition of DF in acerola and cashew apple, including neutral sugars, uronic acids, Klason lignin, resistant protein, ash and polyphenols, are presented in Table 1.

Acerola fruits and cashew apple had a total DF content of 260 and 209 g kg⁻¹ d.m., respectively, most of it insoluble DF (79% on acerola fruits and 88% in cashew apple). This content is in the same range as the values reported for some common fruits such as apples, oranges or bananas, in which it ranges from 170 to 360 g kg⁻¹ d.m. (Saura-Calixto et al., 2000).

Klason lignin content in acerola was 135.4 g kg⁻¹ d.m. Klason lignin is the gravimetric residue obtained from the sulphuric acid treatment typically performed to solubilize and hydrolyse polysaccharides in DF analysis; it is made up of lignin with an associated mixture of protein–polyphenols–polysaccharides.

Total neutral sugars and uronic acids represent the major carbohydrate fraction of DF, reaching values of 76 and 57.5 g kg⁻¹ d.m. for acerola fruits and cashew apple, respectively. This portion of non-digestible carbohydrates reaches the colon where it is potentially fermentable by colonic microbiota. Some of the metabolites generated during colonic fermentation of carbohydrates, as short chain fatty acids (especially butyrate), have been described as beneficial for intestinal health (Wong et al., 2006). Finally, cashew apple DF contained 41.2 g kg⁻¹ d.m. of resistant or indigestible protein.

Table 1 also shows that polyphenols are present on DF in acerola and cashew apples, lending antioxidant capacity to it, as discussed in the next paragraph.

**Polyphenols and antioxidant capacity**

Extractable polyphenol content determined in aqueous-organic extracts from acerola and cashew apple can be seen in Table 2. Cashew apple exhibited high polyphenol content (28 g kg⁻¹ d.m.). In the case of acerola, the extraordinarily high value obtained (170 g kg⁻¹ d.m.) may be an overestimation as ascorbic acid (present in acerola at a concentration of 150 g kg⁻¹ d.m.), like many other compounds, can react with Folin-Ciocalteau reagent (Prior et al., 2005). Cashew apple also contained ascorbic acid, but at a much lower concentration (13 g kg⁻¹ d.m.).
Both acerola and cashew apple contained significant amounts of hydrolysable tannins in the residues of aqueous-organic extractions (3.9 g kg\(^{-1}\) d.m. in acerola and 12.1 g kg\(^{-1}\) d.m. in cashew apple). Acerola also contained 52 g kg\(^{-1}\) g non-extractable condensed tannins. With regard to cashew apple, these non-extractable polyphenols were even more abundant than extractable polyphenols. Although non-bioavailable in the small intestine, these non-extractable polyphenols linked to DF would reach the colon, where the microbiota may release them and modify their structure, giving place to some absorbable metabolites and to antioxidant metabolites that can improve the colonic antioxidant status (Gonthier et al., 2003; Cerdà et al., 2005).

Antioxidant capacity associated with phenolic compounds in the different supernatants obtained (Fig. S1) was determined by FRAP, ABTS\(^{•+}\), DPPH\(^{•}\) and ORAC (Table 2). It was high in both acerola fruits and cashew apples in comparison with other South America fruits known for their high antioxidant contents or with the Spanish Mediterranean diet (Serrano et al., 2007; Vasco et al., 2008; Rufino et al., 2010a). Hydrolysable and condensed tannins in cashew apples likewise presented high antioxidant capacity. Only ABTS\(^{•+}\) could be applied to condensed tannins, as butanol/HCl interferes in the other antioxidant capacity assays. Results were lower than the antioxidant capacity measured in the extractable polyphenol fraction.

<table>
<thead>
<tr>
<th>Table 1 Content and composition of dietary fibre (g kg(^{-1}) d.m.) of acerola fruit and cashew apple(^a)</th>
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<tbody>
<tr>
<td><strong>Soluble DF</strong></td>
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<tr>
<td>Acerola</td>
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<tr>
<td>Total neutral sugars</td>
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<tr>
<td>Uronic acids</td>
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<td>Klason lignin</td>
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<td>Resistant protein</td>
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<td>Ash</td>
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<td>Polyphenols</td>
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<td>DF</td>
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n.d., non-detected.

\(^a\)Mean value ± standard deviation, \(n = 3\).

<table>
<thead>
<tr>
<th>Table 2 Polyphenols and antioxidant capacity of acerola fruit and cashew apple in aqueous-organic extracts and their residues(^a)</th>
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<tbody>
<tr>
<td><strong>Extractable polyphenols</strong></td>
</tr>
<tr>
<td>Acerola</td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>Content (g kg(^{-1}) d.m.)</td>
</tr>
<tr>
<td>FRAP (μmol Trolox g(^{-1}) d.m.)</td>
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<tr>
<td>ORAC (μmol Trolox g(^{-1}) d.m.)</td>
</tr>
<tr>
<td>ABTS(^{•+}) (μmol Trolox g(^{-1}) d.m.)</td>
</tr>
<tr>
<td>DPPH(^{•})</td>
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<tr>
<td>(t_{50}) (min)</td>
</tr>
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<td>AE(^b)</td>
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\(^b\)Antiradical Efficiency, AE = 1/(EC\(_{50}\)\(^*\)\(t_{50}\)).
Table 3 Polyphenols and antioxidant capacity associated with dietary fibre of acerola fruit and cashew apple

<table>
<thead>
<tr>
<th></th>
<th>Soluble DF</th>
<th>Insoluble DF</th>
<th>Hydrolysable tannins</th>
<th>Condensed tannins</th>
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<tr>
<td></td>
<td>Extractable polyphenols&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Extractable polyphenols&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Hydrolysable tannins&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Condensed tannins&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Acerola</td>
<td>Cashew</td>
<td>Acerola</td>
<td>Cashew</td>
</tr>
<tr>
<td>Content (g kg&lt;sup&gt;−1&lt;/sup&gt; d.m.)</td>
<td>9.6 ± 0.8</td>
<td>N.d.</td>
<td>3.6 ± 0.2</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>FRAP (μmol Trolox g&lt;sup&gt;−1&lt;/sup&gt; d.m.)</td>
<td>51.22 ± 1.66</td>
<td>1.88</td>
<td>14.92 ± 0.45</td>
<td>40.35 ± 1.41</td>
</tr>
<tr>
<td>ORAC (μmol Trolox g&lt;sup&gt;−1&lt;/sup&gt; d.m.)</td>
<td>86.00 ± 2.86</td>
<td>N.d.</td>
<td>91.92 ± 9.50</td>
<td>99.50 ± 8.07</td>
</tr>
<tr>
<td>ABTS&lt;sup&gt;+&lt;/sup&gt; (μmol Trolox g&lt;sup&gt;−1&lt;/sup&gt; d.m.)</td>
<td>N.d.</td>
<td>N.d.</td>
<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td>DPPH&lt;sup&gt;+&lt;/sup&gt;</td>
<td>N.d.</td>
<td>N.d.</td>
<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td>eC&lt;sub&gt;50&lt;/sub&gt; (g d.m. g&lt;sup&gt;−1&lt;/sup&gt; DPPH&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>34.64 ± 1.36</td>
<td>N.d.</td>
<td>41.91 ± 2.85</td>
<td>16.62 ± 0.11</td>
</tr>
<tr>
<td>eT&lt;sub&gt;50&lt;/sub&gt; (min)</td>
<td>13.00 ± 1.93</td>
<td>N.d.</td>
<td>38.88 ± 0.08</td>
<td>23.97 ± 3.13</td>
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<tr>
<td>AE&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.002</td>
<td>N.d.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

<sup>d.m., dry matter; N.d., Non-detected; n.d., non-determined; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity.</sup>

Also, all these polyphenol fractions associated with DF exhibited high antioxidant capacity according to the different methodologies employed. For comparative purposes, antioxidant capacity associated with DF of a mixture of the fruits consumed in the Spanish diet was 17.7 μmol Trolox g<sup>−1</sup> d.m. by FRAP assay (Serrano et al., 2007), much lower than antioxidant capacity associated with DF either of acerola or of cashew apple. Moreover, the antioxidant capacity associated with DF in ‘BRS 236’ acerola fruits and ‘CCP 76’ cashew apples may be of nutritional significance as these antioxidant compounds would reach the colon intact and they could produce various beneficial effects as discussed earlier.

Conclusions

In summary, this study provides new nutritional data on the composition of ‘BRS 236’ acerola fruits and ‘CCP 76’ cashew apples. Acerola fruits presented a high antioxidant capacity as determined by various assays because of the combination of high concentrations of extractable polyphenols (170 g kg<sup>−1</sup> d.m.) together with an exceptional amount of ascorbic acid. Cashew apples contained 28 g kg<sup>−1</sup> d.m. of extractable polyphenols and a high amount of non-extractable condensed tannins (52 g kg<sup>−1</sup> d.m.), both conferring it as well a high antioxidant capacity. A significant part of these polyphenols, especially in the case of cashew apples, were associated with DF, showing specific health properties. Acerola fruits and cashew apples also presented a high DF content (about 200 g kg<sup>−1</sup> d.m.). Owing to their content in bioactive compounds, acerola fruits and cashew apples have considerable nutritional and health potential. Moreover, these two clones present production advantages and a higher reproducibility of results when compared to native fruits.

Acknowledgments

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Supporting Information
Additional Supporting Information may be found in the online version of this article:
Figure S1. Scheme of the determination of antioxidant capacity of the dry matter in acerola fruit and cashew apple.

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