International Journal of Food Science and Technology 2010, 45, 2227-2233

# Original article

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

Maria do Socorro M. Rufino,<sup>1,2a</sup> Jara Pérez-Jiménez,<sup>2b</sup> María Tabernero,<sup>2c</sup> Ricardo E. Alves,<sup>3\*</sup> Edy S. de Brito<sup>3</sup> & Fulgencio Saura-Calixto<sup>2</sup>

1 Federal Rural University of the Semi-Arid, Mossoró-RN, Brazil

2 Department of Metabolism and Nutrition, Institute for Food Science and Technology and Nutrition (ICTAN-CSIC), Madrid, Spain

3 Postharvest Physiology and Technology Laboratory, Embrapa Tropical Agroindustry, Fortaleza-CE, Brazil

(Received 6 April 2010; Accepted in revised form 22 July 2010)

**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<sup>-1</sup> g d.m.). 'CCP 76' cashew apples contained 28 g kg<sup>-1</sup> d.m. of extractable polyphenols and 13 g kg<sup>-1</sup> d.m. of ascorbic acid as well as a high amount of non-extractable condensed tannins (52 g kg<sup>-1</sup> d.m.). DF content was of 260 g kg<sup>-1</sup> d.m. in acerola fruit and of 209 g kg<sup>-1</sup> 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.

<sup>c</sup>Present address: Experimental Surgery Department, University Hospital La Paz, Paseo de la Castellana, 261, 28046 Madrid, Spain.

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

doi:10.1111/j.1365-2621.2010.02394.x

© 2010 The Authors. International Journal of Food Science and Technology © 2010 Institute of Food Science and Technology

<sup>\*</sup>Correspondent: Fax: (55) 85 3391 7222;

e-mail: elesbao@cnpat.embrapa.br

<sup>&</sup>lt;sup>a</sup>Present address: Food Technology Department, Federal University of Ceara, Av. Mister Hall, 2977, Pici, 60356-000 Fortaleza, CE, Brazil. <sup>b</sup>Present address: Institute of Advanced Chemistry of Catalonia-CSIC, c/Jordi Girona, 18-26, 08034 Barcelona, Spain.

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

## Chemicals and samples

Pepsin, glucose, tiourea and anthrone were obtained from Merck (Darmstadt, Germany). Amyloglucosidase, pancreatin, lipase,  $\alpha$ -amylase, 2,2'-Azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) [ABTS], 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-Azobis(2-methylpropionamidine) dihydrochloride [AAPH], 2,2 Diphenyl-1-picrylhydrazyl [DPPH<sup>•</sup>] gallic acid and galacturonic acid were obtained from Sigma-Aldrich Química, S.A. (Madrid, Spain). 2,4,6-Tri(2pyridyl)-5-triazine [TPTZ] was from Fluka Chemicals (Madrid, Spain). 3,6'-dihydroxy-spiro-[isobenzofuran-1-[3H],9'[9H]-xanthen]-3-one (Fluorescein) and iron IIIclorure-6-hydrate were from Panreac, Castellar del Vallés (Barcelona, Spain). All reagents used were of analytical grade.

Samples analysed were 'BRS 236' acerola and 'CCP 76' cashew apple. 'BRS 236' acerola was harvested in

Limoeiro do Norte, CE, Brazil and 'CCP 76' cashew apple in the Experimental Station of Embrapa Tropical Agroindustry, Pacajus, CE, Brazil. Two kilograms of each fruit was harvested in the second semester of 2007 at the commercial maturity stage (completely ripe), according to several parameters previously measured in them (Rufino et al., 2010b). Samples were taken from ten different trees and from different regions of them, to achieve a homogeneous sample nevertheless, as these fruits were clones, there were no high differences between them. After harvesting, the fruits were transported to the laboratory and processed in a domestic blender (Walita, Varginha, Brazil) to obtain a pulp and the seeds were discarded. Each fruit was divided into three subsamples and the pulp was freeze-dried (LH 4500; Terroni Fauvel, São Carlos, São Paulo, Brazil) and milled to a particle size of < 0.5 mm in a centrifugal milling.

### 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<sup>-1</sup> solution in 0.08 M HCl–KCl buffer, pH 1.5, 40 °C, 1 h), pancreatin (1 mL of a 5 mg mL<sup>-1</sup> solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h) and  $\alpha$ -amylase (1 mL of a 120 mg mL<sup>-1</sup> 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



**Figure 1** Scheme of the determination of dietary fibre and associated antioxidants in acerola fruit and cashew apple.

AC: antioxidant capacity; EPP: extractable polyphenols; HT: hydrolysable tannins; CT: condensed tannins.

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 acetonic 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 H<sub>2</sub>SO<sub>4</sub> in methanol to release hydrolysable tannins (Figs 1 and S1) or to treatment with butanol/HCl/FeCl<sub>3</sub> 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/FeCl<sub>3</sub> (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<sup>-</sup> (Singleton et al., 1999).

#### Antioxidant capacity methods

DPPH<sup>•</sup> (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<sup>•</sup> methanolic solution (60  $\mu$ M). 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 DDPH<sup>•</sup>. The parameter EC<sub>50</sub>, which reflects 50% depletion of DPPH<sup>•</sup> free-radical, was expressed in terms of grams of fruit equivalent per gram of DPPH<sup>•</sup> in the reaction medium. The time taken to reach the steady state at EC<sub>50</sub> (t<sub>EC50</sub>) and the antiradical efficiency [AE] (AE =  $1/(EC_{50}*t_{EC50})$  were also determined.

 $ABTS^{\bullet+}$  assay (Re *et al.*, 1999):  $ABTS^{\bullet+}$  radical cation (ABTS<sup>•+</sup>) was produced by reacting 7 mM ABTS<sup>•+</sup> 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<sup>•+</sup> solution was diluted with methanol to an absorbance of 0.70  $\pm$  0.02 at 658 nm. After the addition of 100  $\mu$ L of sample or Trolox standard to 3.9 mL of diluted  $ABTS^{\bullet+}$  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  $\mu$ L), freshly prepared and warmed at 37 °C, was mixed with 90  $\mu$ L of distilled water and either 30  $\mu$ L 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.

Oxygen radical absorbance capacity (ORAC) assay (Ou *et al.*, 2001): Sample/blank was mixed with PBS buffer, AAPH and fluorescein. Fluorescence was recorded until it reached zero (excitation wavelength 493 nm, emission wavelength 515 nm) in a fluorescence spectrophotometer Perkin–Elmer LS 55 (Waltham, MA, USA) at 37 °C. Results were calculated using the differences of areas under the fluorescein decay curve between the blank and the sample and were expressed as Trolox equivalents.

#### Other determinations

Protein was determined using an automated nitrogen analyser FP-2000<sup>®</sup>; 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 furnance for 16 h at 550 °C quantified gravimetrically and ascorbic acid spectrophotometrically by using 2,6-diclorophenol (Strohecker & Henning, 1967).

#### Statistical analysis

All analyses were performed in triplicate and results were expressed as mean values  $\pm$  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<sup>-1</sup> 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<sup>-1</sup> d.m. (Saura-Calixto *et al.*, 2000).

Klason lignin content in acerola was  $135.4 \text{ g kg}^{-1}$  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–polysac-charides.

Total neutral sugars and uronic acids represent the major carbohydrate fraction of DF, reaching values of 76 and 57.5 g kg<sup>-1</sup> d.m. for acerola fruits and cashew apple, respectively. This portion of non-disgestible 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<sup>-1</sup> 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 aqueousorganic extracts from acerola and cashew apple can be seen in Table 2. Cashew apple exhibited high polyphenol content (28 g kg<sup>-1</sup> d.m.). In the case of acerola, the extraordinarily high value obtained (170 g kg<sup>-1</sup> d.m.) may be an overestimation as ascorbic acid (present in acerola at a concentration of 150 g kg<sup>-1</sup> 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<sup>-1</sup> d.m.).

	Soluble DF		Insoluble DF		Total DF		
	Acerola	Cashew	Acerola	Cashew	Acerola	Cashew	
Total neutral sugars	16.3 ± 0.5	9.3 ± 0.6	27.0 ± 1.2	28.6 ± 1.0	43.3 ± 1.3	37.9 ± 1.2	
Uronic acids	28.5 ± 1.6	$15.5 \pm 0.8$	$4.2 \pm 0.6$	4.1 ± 0.2	32.7 ± 1.7	19.6 ± 0.8	
Klason lignin	-	-	135.4 ± 0.0	99.0 ± 12.8	135.4 ± 0.0	99.0 ± 12.8	
Resistant protein	-	-	$26.5 \pm 0.0$	41.2 ± 0.04	$26.5 \pm 0.0$	41.2 ± 0.04	
Ash	-	-	$9.2 \pm 0.0$	8.3 ± 0.0	$9.2 \pm 0.0$	8.3 ± 0.0	
Polyphenols	9.6 ± 0.8	n.d.	$3.6 \pm 0.2$	$3.9 \pm 0.3$	13.2 ± 0.8	3.9 ± 0.3	
DF	54.4 ± 1.8	24.8 ± 1.0	205.9 ± 1.3	185.1 ± 12.8	260.3 ± 2.3	209.9 ± 12.9	

**Table 1** Content and composition of dietary fibre (g kg<sup>-1</sup> d.m.) of acerola fruit and cashew apple<sup>a</sup>

n.d., non-detected.

<sup>a</sup>Mean value  $\pm$  standard deviation, n = 3.

Both acerola and cashew apple contained significant amount of hydrolysable tannins in the residues of aqueous-organic extractions (3.9 g kg<sup>-1</sup> d.m. in acerola and 12.1 g kg<sup>-1</sup> d.m. in cashew apple). Acerola also contained 52 g kg<sup>-1</sup> 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; Cerdá *et al.*, 2005).

Antioxidant capacity associated with phenolic compounds in the different supernatants obtained (Fig. S1) was determined by FRAP,  $ABTS^{\bullet+}$ ,  $DPPH^{\bullet}$  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 mix of fruits consumed in 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<sup>•+</sup> 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.

# Polyphenols and antioxidant capacity associated with dietary fibre

Antioxidant capacity and phenolic compounds associated with DF (soluble and insoluble) are shown in Table 3. Extractable polyphenols associated with soluble DF and extractable polyphenols and hydrolysable tannins associated with insoluble DF were all observed in acerola fruits, whereas in cashew apples all the polyphenols associated with DF were associated with its insoluble fraction. Although values in Table 3 cannot be directly compared with those in Table 2, as the extraction methods are different (aqueous-organic vs enzymatic extraction), a significant proportion of the polyphenols is clearly associated with DF.

Table 2 Polyphenols and antioxidant capacity of acerola fruit and cashew apple in aqueous-organic extracts and their residues<sup>a</sup>

	Extractable polyph	enols	Hydrolysable tan	nins	Condensed tannins	
	Acerola	Cashew	Acerola	Cashew	Acerola	Cashew
Content (g kg <sup>-1</sup> d.m.)	170.4 ± 9.5	28.0 ± 0.2	3.9 ± 0.1	12.1 ± 0.7	N.d.	52.0 ± 0.8
FRAP ( $\mu$ mol Trolox g <sup>-1</sup> d.m.)	1640.29 ± 94.51	245.44 ± 14.65	14.87 ± 0.82	56.37 ± 4.15	n.d.	n.d.
ORAC ( $\mu$ mol Trolox g <sup>-1</sup> d.m.)	816.55 ± 57.59	287.31 ± 57.63	173.92 ± 20.74	344.65 ± 65.25	n.d.	n.d.
ABTS <sup>•+</sup> ( $\mu$ mol Trolox g <sup>-1</sup> d.m.)	1249.83 ± 19.72	188.16 ± 0.92	n.d.	n.d	n.d.	60.53 ± 0.02
DPPH•						
$EC_{50}$ (g d.m. g <sup>-1</sup> DPPH <sup>•</sup> )	0.56 ± 0.003	4.81 ± 0.04	28.65 ± 1.65	5.95 ± 0.23	n.d.	n.d.
t <sub>EC50</sub> (min)	9.32 ± 0.59	25.97 ± 1.22	16.53 ± 1.25	22.10 ± 2.83	n.d.	n.d.
AE <sup>b</sup>	0.193	0.008	0.002	0.008	n.d.	n.d.

d.m., dry matter; N.d., Non-detected; n.d., non-determined; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity. <sup>a</sup>Mean value ± standard deviation, *n* = 3.

<sup>b</sup>Antiradical Efficiency, AE =  $1/(EC_{50}*t_{EC50})$ .

© 2010 The Authors International Journal of Food Science and Technology © 2010 Institute of Food Science and Technology International Journal of Food Science and Technology 2010

	Soluble DF		Insoluble DF					
	Extractable polyphenols <sup>b</sup>		Extractable polyphenols <sup>c</sup>		Hydrolysable tannins <sup>d</sup>		Condensed tannins <sup>e</sup>	
	Acerola	Cashew	Acerola	Cashew	Acerola	Cashew	Acerola	Cashew
Content (g kg <sup>-1</sup> d.m.)	9.6 ± 0.8	N.d.	3.6 ± 0.2	3.9 ± 0.3	3.8 ± 0.3	16.9 ± 1.1	N.d.	88.0 ± 4.0
FRAP (µmol Trolox g <sup>-1</sup> d.m.)	51.22 ± 1.68	N.d.	14.92 ± 0.45	40.35 ± 1.41	8.37 ± 0.84	82.35 ± 4.15	n.d.	n.d.
ORAC (µmol Trolox g <sup>-1</sup> d.m.)	86.00 ± 2.86	N.d.	91.92 ± 9.50	99.50 ± 8.07	146.21 ± 15.14	469.27 ± 87.34	n.d.	n.d.
ABTS <sup>•+</sup> (µmol Trolox g <sup>-1</sup> d.m.) DPPH <sup>•</sup>	N.d.	N.d. N.d.	N.D.	N.D.	n.d.	n.d.	n.d.	111.23 ± 0.88
EC <sub>50</sub> (g d.m. g <sup>-1</sup> DPPH•)	34.64 ± 1.36	N.d.	41.91 ± 2.85	16.62 ± 0.11	262.28 ± 1.88	28.28 ± 0.15	n.d.	n.d.
<i>t</i> EC <sub>50</sub> (min)	13.00 ± 1.93	N.d.	38.88 ± 0.08	23.97 ± 3.13	5.24 ± 0.21	11.90 ± 0.43	n.d.	n.d.
AE <sup>f</sup>	0.002	N.d.	0.001	0.003	0.001	0.003	n.d.	n.d.

Table 3 Polyphenols and antioxidant capacity associated with dietary fibre of acerola fruit and cashew apple<sup>a</sup>

d.m., dry matter; N.d., Non-detected; n.d., non-determined; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity. <sup>a</sup>Mean value  $\pm$  standard deviation, n = 3.

<sup>b</sup>Supernatant 1 in Fig. 1.

<sup>c</sup>Supernatant 2 in Fig. 1.

<sup>d</sup>Supernatant 3 in Fig. 1

<sup>e</sup>Supernatant 4 in Fig. 1.

<sup>f</sup>Antiradical Efficiency, AE =  $1/(EC_{50}*t_{EC50})$ .

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  $\mu$ mol 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 reproductibility of results when compared to native fruits.

#### Acknowledgments

The authors acknowledge the financial support from the CAPES, CNPq, ICTAN-CSIC, EMBRAPA, UFERSA and European Union (FP6 Contract no.: 0015279).

#### References

- Abreu, C.R.A., Maia, G.A., Figueiredo, R.W. et al. (2009). Quality, bioactive compounds, and antioxidant activity of cashew apple (*Anacardium occidentale* L.) from commercial early dwarf clones. *Acta Horticulturae*, 841, 451–454.
- Akinwale, T.O. (2000). Cashew apple juice: its use in fortifying the nutritional quality of some tropical fruits. *European Food Research* and Technology, 211, 205–207.
- Alves, R.E., Chitarra, A.B. & Chitarra, M.I.F. (1995). Postharvest physiology of Acerola (*Malpighia emarginata* D.C.) fruits: maturation changes, respiratory activity and refrigerated storage at ambient and modified atmospheres. *Acta Horticulturae*, **370**, 223–230.
- Alves, R.E., Brito, E.A., Rufino, M.S.M. & Sampaio, C.G. (2008). Antioxidant activity measurement in Tropical fruits: a case study with acerola. *Acta Horticulturae*, **773**, 299–305.
- Arts, I.C. & Hollman, P.C. (2005). Polyphenols and disease risk in epidemiological studies. *American Journal of Clinical Nutrition*, 81, 317–325.
- Assis, S.A., Fernandes, F.P., Martins, A.B.G. & Faria Oliveira, O.M.M. (2008). Acerola: importance, culture conditions, production and biochemical aspects. *Fruits*, 63, 93–101.
- Benzie, I.F.F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry*, 239, 70–76.
- Bingham, S., Day, N.E., Luben, R. et al. (2003). Dietary fibre in food and protection against colorectal cancer in the European Prospec-

© 2010 The Authors

tive Investigation into Cancer and Nutrition (EPIC): an observational study. *Lancet*, **361**, 1496–1501.

- Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wis*senchaft und Technologie, 28, 25–30.
- Cerdá, B., Periago, P., Espín, J.C. & Tomás-Barberán, F.A. (2005). Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability. *Journal of Agricultural and Food Chemistry*, **53**, 227–235.
- Filgueiras, H.A.C., Alves, R.E., Mosca, J.L. & Menezes, J.B. (1999). Cashew apple for fresh consumption: research on harvest and postharvest technology in Brazil. *Acta Horticulturae*, **485**, 155– 160.
- Freitas, C.A.S., Maia, G.A., Sousa, P.H.M., Brasil, I.M. & Pinheiro, A.M. (2006). Storage stability of acerola tropical fruit juice obtained by hot fill method. *International Journal of Food Science and Technology*, **41**, 1216–1221.
- Gonthier, M.P., Donovan, J.L., Texier, O., Felgines, C., Remesy, C. & Scalbert, A. (2003). Metabolism of dietary procyanidins in rats. *Free Radicals in Biology and Medicine*, **35**, 837–844.
- Hartzfeld, P.W., Forkner, R., Hunter, M.D. & Hagerman, A.E. (2002). Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. *Journal of Agricultural and Food Chemistry*, **50**, 1785–1790.
- Jiménez-Escrig, A., Rincón, M., Pulido, R. & Saura-Calixto, F. (2001). Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *Journal of Agricultural and Food Chemistry*, 49, 5489– 5493.
- Lima, A.S., Maia, G.A., Sousa, P.H.M., Prado, G.M. & Rodrigues, S. (2009). Storage stability of a stimulant coconut water–acerola fruit juice beverage. *International Journal of Food Science and Technology*, 44, 1445–1451.
- Loewus, F.A. (1952). Improvement in the anthrone method for determination of carbohydrates. *Analytical Chemistry*, **24**, 219.
- Mezadri, T., Villaño, D., Fernández-Pachón, M.S., García-Parrilla, M.C. & Troncoso, A.M. (2008). Antioxidant compounds and antioxidant activity in acerola (*Malpighia emarginata* DC.) fruits and derivatives. *Journal of Food Composition and Analysis*, 21, 282– 290.
- Ou, B., Hampsch-Woodill, M. & Prior, R.L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the flourescent probe. *Journal of Agricultural and Food Chemistry*, **49**, 4619–4626.
- Paiva, J.R., Correia, M.P.F., Freire, F.C.O., Braga Sobrinho, R. & Jucá, W. (1999). Seleção massal de acerola em plantio comercial. *Pesquisa Agropecuária Brasileira*, 34, 505–511.
- Paiva, J.R., Barros, L.M., Cavalcanti, J.J.V. et al. (2005). Selection of dwarf cashew clones for commercial plantation in Aracati County, Ceará State, Brazil. Revista Ciência Agronômica, 36, 338–343.
- Pérez-Jiménez, J., Arranz, S. & Saura-Calixto, F. (2009). Proanthocyanidin content in foods is largely underestimated in the literature data: an approach to quantification of the missing proanthocyanidins. *Food Research International*, **42**, 1381–1388.
- Prior, R.L., Wu, X. & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, **53**, 4290–4302.
- Pulido, R., Bravo, L. & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing antioxidant power assay. *Journal of Agricultural and Food Chemistry*, **48**, 3396–3402.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS

radical cation decolorization assay. *Free Radicals in Biology and Medicine*, **26**, 1231–1237.

- Reed, J.D., McDowell, R.T.E., Van Soest, P.J. & Horvath, P.R.J. (1982). Condensed tannins: a factor limiting the use of cassava forage. *Journal of the Science of Food and Agriculture*, **33**, 213–220.
- Rufino, M.S.M., Alves, R.E., Brito, E.S., Silveira, M.R.R.S. & Moura, C.F.H. (2010b). Quality for fresh consumption and processing of some non-traditional tropical fruits from Brazil. *Fruits*, 64, 361–370.
- Rufino, M.S.M., Alves, R.E., Brito, E.S., Pérez-Jiménez, J., Saura-Calixto, F. & Mancini Filho, J. (2010a). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, **121**, 996–1002.
- Sampaio, C.G., Morais, S.M., Rufino, M.S.M., Alves, R.E. & Brito, E.S. (2009). Quality, bioactive compounds, and antioxidant activity of Brazilian acerola clones. *Acta Horticulturae*, 841, 463–466.
- Sánchez-Moreno, C., Larrauri, J.A. & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, **76**, 270– 276.
- Saura-Calixto, F., Garcia-Alonso, A., Goñi, I. & Bravo, L. (2000). In vitro determination of the indigestible fraction in foods: an alternative to dietary fiber analysis. Journal of Agricultural and Food Chemistry, 48, 3342–3347.
- Scott, R.W. (1979). Colorimetric determination of hexauronic acids in plant materials. *Analytical Chemistry*, **51**, 936–941.
- Serrano, J., Goñi, I. & Saura-Calixto, F. (2007). Food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity. *Food Research International*, 40, 15–21.
- Serrano, J., Puuponen-Pimiä, R., Dauer, A., Aura, A.M. & Saura-Calixto, F. (2009). Tannins: current knowledge of food sources, intake, bioavailability and biological effects. *Molecular Nutrition and Food Research*, 53, s310–s329.
- Singleton, V.L., Orthofer, R. & Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, **299**, 152–178.
- Strohecker, R. & Henning, H.M. (1967). Analisis de vitaminas: metodos comprobados. Madrid: Paz Montalvo.
- Vasco, C., Ruales, J. & Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry*, **111**, 816–823.
- Wong, J.M., De Souza, R., Kendall, C.W., Emam, A. & Jenkins, D.J. (2006). Colonic health: fermentation and short-chain fatty acids. *Journal of Clinical Gastroenterology*, 40, 235–243.

#### **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.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.