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Food Chemistry 114 (2009) 693–695

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Short Communication

Free radical-scavenging behaviour of some north-east Brazilian fruits in a DPPH· system

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ARTICLE INFO

Article history:
Received 23 January 2008
Received in revised form 21 August 2008
Accepted 30 September 2008

Keywords:
Tropical fruits
Radical scavenger
DPPH·
Kinetics

ABSTRACT

The antiradical capacity (radical-scavenging capacity, RSC) of seven tropical fruit from the Brazilian north-east (açaí, acerola, cashew apple, mangaba, murici, umbu and uvaia) were studied using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) assay. To determine their RSC, the second-order rate constants (k_2) for the oxidation of these extracts by DPPH· were calculated. The values of k_2 were compared to natural and synthetic antioxidants. The k_2 values (l/mol g s), in methanol at 25 °C, were 38.0, 29.7, 21.3, 20.1, 10.1, 9.54 and 5.47 for acerola, cashew apple, mangaba, umbu, açaí, uvaia and murici, respectively.

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1. Introduction

Natural antioxidants present in foods have attracted interest because of their safety and potential nutritional and therapeutic effects. It is recognised that besides a role in endogenous defence of plants, human consumption of dietary antioxidants affords protection against some pathological events. Generation of active oxygen and free radicals is important both in food systems and in biological systems (Espin, Soler-Rivas, Wichers, & García-Viguera, 2000; Suja, Jayalekshmy, & Arumughan, 2004). In foods, the process of autoxidation and development of rancidity is caused by free radicals (Shahidi, Janitha, & Wanasundara, 1992). Lipid peroxidation leads to the development of off-flavours and undesirable chemical compounds (Angelo, 1996). In living systems, free radicals attack key biological molecules, leading to many degenerative disease conditions such as cancer, inflammation, atherosclerosis, and ageing (Labuza, 1971).

In the past years, much attention has been devoted to ascorbic acid, tocopherol, tocotrienols, β-carotene (Rice-Evans & Miller, 1995), and natural antioxidants of plant origin, because they have potential application in the food industry for increasing the stability and shelf life of food products. Moreover, they also find use as nutraceuticals and phytoceuticals, as they have significant impact on the status of human health and disease prevention (Noguchi & Nikki, 2000).

The antioxidant activity can be expressed in terms of radical-scavenging ability, during reaction with a specific radical such as [DPPH·] or [LOO·]. The ability to scavenge stable DPPH· is a widely used method to evaluate radical-scavenging capacity (RSC) in a relatively short time compared to other methods (Brand-Williams, Cuvelier, & Berset, 1995; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998). Free radical scavenging is the generally accepted mechanism for how antioxidants inhibit lipid oxidation.

The scavenging of DPPH· by radical scavengers can be summarised as

$$\mathrm{DPPH^{\cdot}} + \mathrm{FE} \rightarrow \mathrm{DPPH{-}H} + \mathrm{A^{\cdot}}, \quad (1)$$

$$\mathrm{DPPH} + \mathrm{A^{\cdot}} \rightarrow \mathrm{DPPH{-}A}, \quad (2)$$

$$\mathrm{A^{\cdot}} + \mathrm{A^{\cdot}} \rightarrow \mathrm{A{-}A}, \quad (3)$$

where FE is a scavenger of the fruit extract, A· is a radical.

The newly formed radical (A·) can mainly follow radical–radical interaction to render stable molecules, via radical disproportionation (collision of radicals with abstraction of an atom by one radical from another – Eqs. (2) and (3)), although these secondary reactions are greatly hindered (Aruoma, 1998; Chimi, Cillard, Cillard, & Rahmani, 1991). It is important to notice that when studying fruit extracts there is the existence of several possible radical scavenger species in the extracts.

There is no reported study on the radical-scavenging capacity of several tropical fruits using a kinetic model. The aim of this study was to characterise kinetically the free radical-scavenging capacity

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doi:10.1016/j.foodchem.2008.09.098

of acerola (*Malpighia emarginata*), açaí (*Euterpa oleracea*), cashew apple (*Anacardium occidentale*), mangaba (*Hancornia speciosa*), murici (*Byrsonima crassifolia*), umbu (*Spondias tuberosa*) and uvaia (*Eugenia pyriformis*) by using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·), in order to discover their potential as a source of antioxidants.

2. Material and methods

2.1. Samples

Acerola (*Malpighia emarginata*) was collected at Limoeiro do Norte, CE, Brazil. Açaí (*Euterpa oleracea*), cashew apple (*Anacardium occidentale*) and murici (*Byrsonima crassifolia* L.) were collected at Embrapa Experimental Station at Paraipaba, CE, Brazil. Uvaia (*Eugenia pyriformis*) was collected at Alagoinha, CE, Brazil. Mangaba (*Hancornia speciosa*) and umbu (*Spondias tuberosa*) were collected at Picos, PI, Brazil. 2,2-Diphenyl-1-picrylhydrazyl (DPPH·) was supplied by Sigma (St. Louis, MO). All other reagents (acetone and methanol) were of analytical grade and were supplied by Vetec Quimica Fina (Duque de Caxias, RJ, Brazil).

2.2. Extracts preparation and chemical analysis

The extracts were obtained in the year of harvest, and stored at 4 °C until analysed. The fruits were crushed, weighed, and extracted sequentially with 40 ml methanol:water (50:50, v/v) and 40 ml acetone:water (70:30, v/v) at room temperature for 60 min. The supernatants from these extractions were combined and made up to 100 ml with distilled water (Saura-Calixto, Goñi, Mañas, & Abia, 1991) and assayed spectrophotometrically at 700 nm by the Folin–Ciocalteau method, using gallic acid as standard (Obanda & Owuor, 1997). The results were expressed as mg of gallic acid/100 g of fruit. The fruits were also analysed for vitamin C (Strohecker & Henning, 1967).

2.3. Free radical scavenging by DPPH·

DPPH was dissolved in methanol. Experiments were performed on freshly prepared solutions of radical, which in the absence of radical scavengers were stable for more than 1 day. The presence of methanol was required to enhance the solubility of the extract to reach pseudo-first order assay conditions, where the initial fruit extract concentration was much lower than the initial radical concentration ($[DPPH^{\cdot}]_0 \gg [FE]_0$). Specific antiradical activity assay conditions were as follows: DPPH· 60 μM; acerola 20–50 g/l, açaí 0.1–10 g/l, cashew apple 50–200 g/l, mangaba 10–100 g/l, murici 2–8 g/l, umbu 50–200 g/l and uvaia 60–100 g/l.

The free radical-scavenging activity of fruits was measured using the method described by Brand-Williams et al. (1995), with modifications. A 0.06 mM solution of DPPH in methanol was prepared and an aliquot of 100 μl of the antioxidant/fruit extract solution was added to 3.9 ml of the DPPH solution. The decrease in absorbance at 515 nm was measured at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 min, and then every 5 min until the reaction reached a plateau. The spectrophotometric assays were recorded using a UV–Vis Genesis spectrophotometer (Spectronic Instruments, Rochester, NY). Temperature was controlled at 25 °C with a circulating water bath. As a reference the solvent mixture instead of the extract was used. The results were obtained in triplicate and expressed as mean ± SD.

2.4. Kinetic analysis

Fitting of the experimental data was carried out by using the Levenberg–Marquardt method (Marquardt, 1963) implemented in Origin v. 6.0 programme for Windows. Recording of spectrophotometric data was taken until the disappearance of DPPH· in the presence of fruit extracts occurred.

Second-order rate constants (k_2) were calculated, to determine the RSC of the tropical fruits. The antioxidant of the fruit extract was depleted from the medium under pseudo first-order conditions ($[DPPH^{\cdot}]_0 \gg [FE]_0$), following the equation:

$$\frac{d[FE]}{dt} = -k[FE], \tag{4}$$

$$[FE] = [FE]_0 \times \exp(-kt), \tag{5}$$

where [FE] is the fruit extract concentration, $[FE]_0$ is the initial fruit extract concentration, k is the pseudo-first order kinetic rate constant, and t is the time.

The concentration of DPPH· was calculated by mass balance using the following equation:

$$[DPPH^{\cdot}] = [DPPH^{\cdot}]_0([FE]_0 \times \exp(-kt)), \tag{6}$$

where $[DPPH^{\cdot}]$ is the radical concentration, $[DPPH^{\cdot}]_0$ is the initial radical concentration, k is the pseudo-first order kinetic rate constant, and t is the time. $[DPPH^{\cdot}]$ concentration in the reaction medium was calculated according to the method of Brand-Williams et al. (1995) obtained from the calibration curve with the equation as determined by linear regression:

$$Abs_{515\ nm} = 0.0137[DPPH^{\cdot}] - 0.029, \tag{7}$$

where $[DPPH^{\cdot}]$ is expressed in μM.

The pseudo-first order kinetic rate constant (k) was linearly dependent on the concentration of the fruit extract. The second-order rate constant (k_2) was determined by the equation (Mukai, Morimoto, Kikuchi, & Nagaoka, 1993; Shi & Niki, 1998):

$$\frac{d[DPPH^{\cdot}]}{dt} = -k_2 \cdot [FE] \cdot [DPPH^{\cdot}]. \tag{8}$$

This method to calculate the second-order kinetic rate constant (k_2) differs from the method presented by Brand-Williams et al. (1995), which is based on the assumption that the DPPH· concentration is much lower than the antioxidant concentration ($[DPPH^{\cdot}]_0 \ll [AH]_0$).

3. Results and discussion

The results showed that the absorbance decreased as a result of a colour change from purple to yellow, as the radical was scavenged by antiradicals, through donation of hydrogen, to give the reduced form DPPH–H (Eq. (3)). The oxidation reaction is truly a second-order reaction since two species react to form one or more oxidation products. The reaction may be assumed as a pseudo-first order reaction if one of the reactants is in excess, as assumed by Brand-Williams et al. (1995). Some authors do not make this simplification (Mukai et al., 1993; Shi & Niki, 1998), as in this work. Since a simplification is not made, the obtained kinetic parameters may be more accurate.

In the presence of the tropical fruit extracts, a decrease in the absorbance at 515 nm was measured until the fruit antioxidant was depleted under pseudo-first order assay conditions. The pseudo-first order rate constant, k was linearly dependent on the initial radical scavenger concentration ($[FE]_0$).

The second-order rate constants, k_2, which represents the rate at which DPPH can be oxidised by 1 g of fruit, were calculated from Eq. (8) and are presented in Table 1. This rate constant is related to the RSC present in the fruit extracts and a higher k_2 value corresponds to a better RSC. Five fruits have exhibited more than one kinetic period: açaí, mangaba, murici, umbu and uvaia. These fruits may possess several natural antioxidants in their composition with

Table 1
Vitamin C and extractable polyphenols in fruit pulps and second-order kinetic rate constants (k_2) for the reaction with DPPH·.

	k_2 (Phase 1) [l/mol g s]	k_2 (Phase 2) [l/mol g s]	k_2 (Phase 3) [l/mol g s]	Vitamin C [mg/100 g]	Extractable polyphenols [mg/100 g]
Fruit extract					
Açaí (*Euterpa oleracea*)	10.1 ± 0.75	1.17 ± 0.10	0.16 ± 0.01	84.0 ± 10.0	518 ± 115.5
Acerola (*Malpighia emarginata*)	38.0 ± 2.57			1360 ± 9.5	1060 ± 53.0
Cashew apple (*Anacardium occidentale*)	29.7 ± 1.12			190 ± 5.7	118 ± 3.7
Mangaba (*Hancornia speciosa*)	21.3 ± 0.64	1.51 ± 0.11		431 ± 6.7	172 ± 31.1
Murici (*Byrsonima crassifolia*)	5.47 ± 0.34	0.31 ± 0.02		148 ± 4.0	937 ± 38.9
Umbu (*Spondias tuberosa*)	20.1 ± 2.71	2.70 ± 0.31	0.11 ± 0.02	18.4 ± 1.8	90.4 ± 2.2
Uvaia (*Eugenia pyriformis*)	9.54 ± 0.44	0.75 ± 0.3		39.3 ± 5.2	127 ± 3.3
Antioxidant (natural and synthetic)					
α-Tocopherol[a]	37.1 ± 0.90				
BHT[a]	3.70 ± 0.90				

Each value is the mean ± standard deviation of three replicate experiments.
[a] Data from Suja et al. (2004).

different scavenging capacity and in this case the scavenging of DPPH· will be carried out by all the antioxidants present in the fruit. Antioxidants with higher RSC will scavenge the DPPH· radicals at a higher rate whilst antioxidants with lower RSC will take more time to reduce the amount of DPPH· present in the assay.

The RSC obtained for the tropical fruits studied herein was compared to that of other synthetic (BHA and BHT) and natural (α-tocopherol) antioxidants. α-Tocopherol (vitamin E) is a natural antioxidant abundant in oils and other foodstuffs with high fat content, such as butter and margarine (Shahidi et al., 1992). Vitamin E was the most powerful antioxidant assayed with a k_2 value of 31.7 l/mol g s (Suja et al. 2004).

The order of RSC, according to k_2 values (the higher k_2 value, the better the RSC) was acerola > α-tocopherol > cashew apple > mangaba > umbu > açaí > uvaia > murici > BHT (Table 1). The tropical fruits studied herein possessed k_2 values ranging from 5.47 to 38.0 l/mol g s (Table 1). The results showed significantly higher activity for acerola, cashew apple, mangaba, umbu and açaí, compared to BHT.

The results for vitamin C and total extractable phenolics are presented on Table 1. The order for vitamin C was acerola > mangaba > cashew apple > murici > açaí > uvaia > umbu; with values ranging from 18.4 to 1360 mg/100 g. The values for total extractable phenolics were between 90.4 and 1060 mg/100 g, and again acerola presented the higher value. The activity of acerola, which has a high ascorbic acid content, was also significantly higher than that of α-tocopherol. A study has demonstrated that ascorbic acid scavenges radicals faster than (+)-catechin, ethyl gallate and α-tocopherol (Sawai & Moon, 2000).

The activity of the Brazilian tropical fruits was also high, when compared to other antioxidants reported in the literature. Suja et al. (2004) have studied the RSC of antioxidant compounds of sesame (*Sesamum indicum* L.) and found that the purified sesamol showed an RSC of 40.0 l/mol g s and others purified compounds showed an RSC below 5.0 l/mol g s. Acerola showed a significant activity, only 5.1% lower than purified sesamol. The results also showed significant higher activity for cashew apple, mangaba, umbu and açaí.

Acknowledgments

To CAPES, CNPq, EMBRAPA, UFERSA and European Union (FP6 Contract no.: 0015279) for financial support.

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