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Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil

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ABSTRACT

Four *Citrus* species (*C. sinensis*, cvs. Pera and Lima; *C. latifolia* Tanaka cv. Tahiti; *C. limettioides* Tanaka cv. Sweet lime and *C. reticulata*, cv. Ponkan) grown in Brazil were characterised in relation to contents of minerals, ascorbic acid, total polyphenols and antioxidant capacity of pulps and peels. In general, the peels demonstrated significantly higher contents of all compounds than the pulps ($p < 0.05$), with the exception of the Pera orange pulp that presented the highest acid ascorbic content (68 mg/100 ml), while the Tahiti lime peel presented the lowest (8 mg/100 g). Citrus showed high levels of potassium, calcium and magnesium, and the peels were considered sources of these minerals. The Ponkan mandarin peel presented the highest antioxidant capacity. The antioxidant capacity of citrus was correlated both to vitamin C and phenolics. Aside from citrus pulps, the peels are also good sources of bioactive compounds and minerals, and can be explored for their health promoting values in food products.

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1. Introduction

In recent years, clinical trials and epidemiological studies have established an inverse correlation between the intake of fruits and vegetables and the occurrence of chronic diseases, the most prevalent causes of death in the world. This protective effect has been attributed to the antioxidant properties, which coordinate and balance the body system to protect tissues and fluids from damage by reactive species or free radicals (Huang, Ou, & Prior, 2005; Patil, Jayaprakasha, Chidambara Murthy, & Vikram, 2009).

Citrus fruits (Rutaceae family) are an important source of antioxidants such as ascorbic acid, carotenoids, flavonoids, and other phenolic compounds (Abeyasinghe et al., 2007; Ghasemi, Ghasemi, & Ebrahimzadeh, 2009; Rapisarda, Bianco, Pannuzzo, & Timpanaro,

Abbreviations: AA, ascorbic acid; Ca, calcium; Cu, copper; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DRI, dietary reference intake; Fe, iron; FRAP, ferric reducing power; FW, fresh weight; K, potassium; Mg, magnesium; Mn, manganese; MT, million tons; SST, total soluble solids; Na, sodium; TA, titratable acidity; TPTZ, 2,4,6-tripyridyl-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; Zn, zinc.

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2008), and also some essential minerals for human nutrition (Gorinstein, Belloso, et al., 2001; Topuz, Topakci, Canakci, Akinci, & Ozdemir, 2005). Among the phenolic compounds, flavanones are the major group found in citrus. Studies have shown that intake of flavanones is associated with reduced risk of developing coronary heart disease, degenerative diseases and have received increasing attention as anti-carcinogenic compounds because of their anti-lipid peroxidation (Benavente-Garcia & Castillo, 2008).

Brazil is one of the major citrus producers, responsible for 20.5 million tons (MT) of citrus annually. Oranges (18.5 MT), mandarins (1 MT) and acid limes (1MT) are the major production (FAO, 2008). Approximately 2 MT are destined to the fresh fruits market, and according to data from IBGE – Brazilian Institute of Statistics Family Budget Surveys (IBGE, 2010), the citrus acquisition accounted for 25% of total fruits acquired by households in the country. The remaining production, about 90%, is destined to the juice industry (Ladaniya, 2008), generating a large amount of byproducts that account for 50% of the original whole fruit weight (Anagnostopoulou, Kefalas, Papageorgiou, Assimopoulou, & Boskou, 2006; Marín, Soler-Rivas, Benavente-Garcia, & Pérez-Alvarez, 2007).

During citrus fruit processing to make juice, peels are the primary byproducts. If unused, they become waste and a possible source of environmental pollution. These byproducts have been traditionally used as molasses for animal feed, fibre (pectin) and for fuel production (Li, Smith, & Hossain, 2006). However, studies

conducted on several fruits (citrus, apples, grapes and berries) have shown that peels are the major source of natural antioxidants. Therefore, phenolic compounds in peels and fruit byproducts can be used in food products as active ingredients or as substitutes for synthetic preservatives (Gorinstein et al., 2004; Ignat, Volf, & Popa, 2011), and have been associated with health problems.

Citrus species of various origins have been studied due to their phenolic compounds and antioxidant capacity (Abeyasinghe et al., 2007; Ghasemi, Ghasemi, & Ebrahimzadeh, 2009; Gorinstein, Beloso, et al., 2001; Rapisarda, Bianco, Pannuzzo, & Timpanaro, 2008). However, it is known that the chemical composition of fruits suffer variations according to climate, fertilisation applied, soil type, cultivar, fruit maturity, and even between parts of the same fruit. There are no studies comparing the potential health properties of pulp and peel of citrus from Brazil. Therefore, the aim of this study was to determine the antioxidant capacity, phenolic compounds, vitamin C and minerals of five commercial varieties of citrus from a Brazilian central region.

2. Methods

2.1. Plant materials

Four species of citrus: two cultivars of sweet oranges (*Citrus sinensis* cv. Pera and cv. Lima); two species of limes (*C. latifolia* Tanaka cv. Tahiti and *C. limettioides* Tanaka cv. Sweet lime) and one cultivar of mandarin (*Citrus reticulata* Blanco cv. Ponkan) were harvested at a local farm in the state of Goiás, Midwest of Brazil. For each variety, 100 fruits were picked from trees in the four quadrants (North, South, East and West), using a randomized design on June 2010. For the analyses described in 2.3, 2.4 and 2.5 the fruits were separated into three 10-fruit sub-lots per cultivar. For the analyses described in 2.7, 2.8 and 2.9, 30 fruits of each cultivar were pooled, homogenised by powdering in liquid nitrogen and extracted in triplicate.

2.2. Reagents

The 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), catechin, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and the Folin-Ciocalteu reagents were obtained from Sigma Chemical Co. (St. Louis, MO). The mineral standards were obtained from Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical grade.

2.3. Titratable acidity, pH and total soluble solids

To characterise the maturity and quality of the fruit, the mean weight, the juice yield, the peel/whole fruit ratio, pH, total soluble solids (TSS), titratable acidity (TA) and (TSS/TA) ratio were determined. The pH values for the sample juice were measured with a digital pH metre. TSS, expressed as Brix, was measured with a portable refractometer (Instrutherm, model RT-30 ATC). TA was expressed as % of citric acid. These chemical measurements were determined according to AOAC (1995).

2.4. Moisture, ascorbic acid and mineral contents

The fruits were washed in tap water, separated into parts, pulp (juice vesicles and segments) and peel (flavedo and albedo), and homogenised. The moisture content of the samples was determined by drying 5 g samples at 105 °C until the weight was constant (AOAC, 1995). Ascorbic acid (AA) was determined by titration with an iodate potassium solution, after the samples were homogenised and acidified with sulphuric acid (20%), using starch

as an indicator. The end point of the reaction was the appearance of a blue colour (IAL, 2008). The AA content was expressed as mg of ascorbic acid per 100 g sample fresh weight (FW).

The mineral content was determined in dry ashed samples at 550 °C and dissolved in HCl according to AOAC (1995). Calcium (Ca), copper (Cu), magnesium (Mg), zinc (Zn), iron (Fe), and manganese (Mn) contents in the extract were measured using an atomic absorption spectrophotometer (Perkin-Elmer, model AAnalyst 400, Waltham, MA, USA). For the determination of Ca and Mg, a LaCl₃ solution was added. Potassium and sodium contents were determined by using a flame photometer (Corning, 410, NY, USA) with an air-propane flame. Minerals and trace elements were determined using the instrumental conditions recommended for each mineral and were calculated based on the respective standard curve.

2.5. Sample extraction for determination of total phenolics, DPPH scavenging capacity assay and FRAP assay

The fruits were washed with tap water, separated into pulp and peel, cut into small pieces, immediately homogenised by powdering in liquid nitrogen, and finally stored at – 20 °C until analysis. Powdered citrus tissues were extracted with 70% aqueous methanol (1 g of sample for each 20 ml of solvent) by stirring with a magnetic bar for 2 h, at 4 °C in triplicate. The extracts obtained were filtered through filter paper and used for the following determinations.

2.6. Folin-Ciocalteu reducing capacity and total phenolics

The determination was performed according to the Singleton and Rossi (1965) method. A 0.25 ml aliquot of extract was mixed with 0.25 ml of the Folin-Ciocalteu reagent and 2 ml of distilled water. After 3 min at room temperature, 0.25 ml of a saturated sodium carbonate (Na₂CO₃) solution was added, and the mixture placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a Ultrospec 2000 UV-Visible model spectrophotometer (Amersham Biosciences, Cambridge, UK). The control consisted of a methanol solution of catechin at different concentrations. The total phenolics were calculated subtracting the value of Folin-Ciocalteu reducing capacity due to ascorbic acid, using a standard curve according to Abe, Lajolo, and Genovese (2012). The results were expressed as mg of catechin equivalents/100 g of FW sample.

2.7. DPPH free radical scavenging activity

The extracts obtained above were used to assess the antioxidant capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to Brand-Williams, Cuvelier, and Berset (1995), with some modifications (Duarte-Almeida, Santos, Genovese, & Lajolo, 2006). A 50 µl aliquot of the previously diluted extract and 250 µl of DPPH (0.5 mM) were mixed, and after 25 min, the absorbance was measured at 517 nm using a Microplate spectrophotometer (Benchmark Plus, Biorad, Hercules, CA). The control consisted of a methanol solution of Trolox at different concentrations. The antioxidant capacity was expressed as µmoles Trolox equivalents/100 g of FW sample.

2.8. Ferric reducing power (FRAP) assay

The FRAP assay (Benzie & Strain, 1996) is based on the ability of phenolics to reduce Fe³⁺ to Fe²⁺. To prepare the FRAP reagent, 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride (10:01:01, v/v/v) were mixed. Twenty microlitre of previously diluted extract were added to 150 µl of reagent. The absorbance

was measured at 593 nm using a Microplate spectrophotometer (Benchmark Plus, Biorad, Hercules, CA). The analysis was performed in triplicate, using an aqueous Trolox solution as standard and the results were expressed as $\mu\text{moles Trolox equivalents}/100\text{ g}$ of FW sample.

2.9. Statistical analysis

The data were expressed as means \pm standard deviations (SD) of three replicate determinations and then analysed by SPSS 17.0 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL). One way analysis of variance (ANOVA) and the Tukey's test were used to determine the differences among means. P values < 0.05 were regarded to be significant. Pearson's correlation analysis was performed between the antioxidant activity and the total phenolic or ascorbic acid contents.

3. Results and discussion

3.1. Physical characteristics

The quality parameters, including mean weight, juice yield, the peel/whole fruit ratio, pH, TSS, TA and TSS/TA ratio of citrus juices are shown in Table 1. The mean weight of fruits ranged from 109 ± 3 to 218 ± 13 g, with Pera and Lima varieties being the largest ones and Tahiti the smallest. The Lima orange had the highest juice yield (47.3%), and the Tahiti had the lowest (35.5%). The Ponkan mandarin and Pera orange had the highest peel/whole fruit ratio (29.1 ± 2.0 and 27.8 ± 0.8 , respectively) and the highest TSS value (11.0 ± 0.2 and 10.5 ± 0.3 , respectively), whereas the Lima orange had the lowest TSS value (7.7 ± 0.1). The consumption of oranges and tangerines depends on the reduction of acidity to a point where the juice becomes pleasing to the palate, with citric acid being the main organic acid present (Albertini et al., 2006). The TA value of Tahiti lime presented the highest value (4.37%), while the Sweet lime had the lowest (0.10%), followed by Lima orange (0.23%). The TSS/TA ratio is also an important parameter related with quality characteristics of citrus fruits, and among the citrus varieties evaluated, the Sweet lime had the highest value (85.9), and the Tahiti lime had the lowest (2.2).

As in most fruits, the maturity of citrus involves the accumulation of sugars and the loss of acidity (Ladaniya, 2008). Sweet oranges, mandarins, grapefruits, and pummelos are considered mature when their juice content and TSS/TA ratio have attained certain minimum limits for palatability (Albertini et al., 2006). In almost all citrus varieties that are used for table purposes (such as fresh fruits) and that are processed into juices, maturity is determined mainly on the basis of the ratio of total soluble solids to titratable acidity. For mandarins and oranges, the TSS/TA ratio of 8–14, according to the variety and local production, was considered necessary for good eating quality (Ladaniya, 2008). However,

the TSS/TA ratio is not considered a suitable index for determining the maturity of acid limes and lemons, whereas the acidity increases early in fruit development in these varieties, mainly by the increase in citric acid contents. In addition, varieties with low acidity, like Sweet lime and Lima orange, are characterised by a lack of citric acid, regardless of the ripening stage (Albertini et al., 2006). A juice volume of 30% (v/v) is the sole internal quality standard for North American lemons and limes, and lemons for processing should have higher juice volume (Ladaniya, 2008). In this study, significant differences between the citrus grown in the Midwest of Brazil were observed and were consistent with the characteristics of each variety as described. Therefore, the fruits studied were at a maturity state.

3.2. Moisture, ascorbic acid and mineral contents

The moisture contents of citrus varieties ranged from 87.3 ± 0.0 to $91.7 \pm 0.4\%$ for pulps and from 66.6 ± 0.2 to $79.3 \pm 0.1\%$ for peels (Table 2). Since citrus fruits are characterised by a good content of ascorbic acid, the content of this vitamin is considered a fundamental marker of the quality and value of these fruits, and their derivative products (Nagy, 1980). Among pulps, the Pera oranges showed the highest ascorbic acid content (68 mg/100 ml). The Tahiti lime and Ponkan mandarin pulps showed the lowest levels (41.4 ± 0.9 and 41.1 ± 2.5 mg/100 ml, respectively), with no significant difference between them. All ascorbic acid values are in agreement with a previous survey (Nagy, 1980) on the vitamin C contents for the principal citrus fruit from several countries (14–88 mg/100 ml of juice for sweet oranges, 15–45 mg/100 ml of juice for limes and about 15–55 mg/100 ml of juice for mandarins, respectively), except for Sweet lime, which showed a higher content (60.2 ± 2.2 mg/100 ml). The lower contents of ascorbic acid in mandarins compared to orange contents were previously reported by Nagy (1980) and Dhuique-Mayer, Caris-Veyrat, Ollitrault, Curk, and Amiot (2005). Due to many horticultural and climatic variables involved in citrus growing, it is not surprising that most investigators report wide ranges in vitamin C levels of different citrus fruit (Nagy, 1980).

Although citrus juices and pulp are recognised as providing an important source of vitamin C for human nutrition, there are other parts of the fruit which also contain this vitamin. These other parts of the fruit are not recognised in nutrition because they are generally the non-edible components, like peels. A wide variation in the levels of ascorbic acid was observed in peel analysis. The lowest level was observed in the Tahiti lime (6.84 ± 0.3 mg/100 g) and the highest in the Ponkan mandarin (47.6 ± 0.9 mg/100 g). The Ponkan mandarin and the Lima orange acid ascorbic contents were near to those presented by their respective pulps (Table 2).

The identification of new sources of vitamin C is of great interest for public health. Ascorbic acid is known for a number of vital biological activities including synthesis of collagen, neurotransmitters,

Table 1
Standard quality parameters of different varieties of citrus from Brazil.^a

	Variety				
	Lima Orange	Pera Orange	Tahiti lime	Sweet lime	Ponkan mandarin
Average weight (g)	218 \pm 13 a	200 \pm 11 ab	109 \pm 3 d	152 \pm 13 c	178 \pm 7 bc
Juice yield (%)	47.3 \pm 0.8 a	40.2 \pm 3.4 bc	35.5 \pm 0.9 c	44.5 \pm 1.7 ab	41.4 \pm 0.7 b
Ratio peel/whole fruit (%)	16.7 \pm 1.2 b	27.8 \pm 0.8 a	19.3 \pm 1.0 b	18.2 \pm 2.7 b	29.1 \pm 2.0 a
pH	4.78 \pm 0.01 b	3.54 \pm 0.01 d	2.32 \pm 0.02 e	5.52 \pm 0.02 a	3.75 \pm 0.01 c
TSS (Brix)	7.7 \pm 0.1 d	10.5 \pm 0.3 a	9.8 \pm 0.2 b	8.7 \pm 0.2 c	11.0 \pm 0.2 a
TA (%)	0.23 \pm 0.02 d	1.25 \pm 0.01 b	4.37 \pm 0.10 a	0.10 \pm 0.01 e	0.84 \pm 0.01 c
TSS/TA ratio	34	9	2	86	13

^a Results are expressed as means \pm standard deviations (analyses from three replicates of 10 fruits per cultivar). Means in the same line with different letters are significantly different ($p < 0.05$).

Table 2
Moisture, ascorbic acid (AA), main minerals and trace elements in citrus pulp and peel.^a

	Pulps					Peels				
	Lima orange	Pera orange	Tahiti lime	Sweet lime	Ponkan mandarin	Lima orange	Pera orange	Tahiti lime	Sweet lime	Ponkan mandarin
Moisture	91.7 ± 0.4 a	88.3 ± 0.3b	88.5 ± 0.1b	91.5 ± 0.7 a	87.3 ± 0.0 c	70.3 ± 0.2 g	66.6 ± 0.2 h	72.6 ± 0.1 e	79.3 ± 0.1 d	77.1 ± 0.3 e
AA	46.1 ± 1.3 cd	68.1 ± 1.0 a	41.4 ± 0.9 e	60.2 ± 2.2b	41.1 ± 2.5 e	43.2 ± 0.4 de	24.3 ± 0.7 f	6.84 ± 0.3 g	22.6 ± 0.8 f	47.6 ± 0.9 c
Ca	18.7 ± 0.1 f	20.3 ± 0.2 f	tr	tr	16.0 ± 0.6 f	145.2 ± 6.5 c	165.4 ± 7.1b	214.2 ± 1.8 a	121.2 ± 5.6 d	85.5 ± 3.9 e
K	140.1 ± 2.2 d	139.0 ± 2.9 d	168.8 ± 6.7 bc	101.2 ± 4.9 e	140.5 ± 6.9 d	258.7 ± 11.0 a	266.0 ± 12.2 a	187.1 ± 5.8 b	142.1 ± 6.4 d	151.0 ± 1.3 cd
Na	2.0 ± 0.1 f	2.6 ± 0.1 f	2.8 ± 0.1 f	1.6 ± 0.1 f	2.9 ± 0.1 f	85.1 ± 4.2 a	60.1 ± 3.0 b	54.0 ± 0.4 c	37.7 ± 1.7 d	32.4 ± 1.5 e
Mg	10.3 ± 0.1 f	10.3 ± 0.2 f	8.5 ± 0.3 f	4.8 ± 0.1 g	9.4 ± 0.40 f	23.8 ± 1.2 c	27.8 ± 1.1b	41.2 ± 1.2 a	19.7 ± 0.8 d	13.4 ± 0.5 e
Fe	171.0 ± 6.6 d	256.4 ± 6.2 c	169.1 ± 7.8 d	106.8 ± 4.0 d	156.5 ± 4.1d	1008.6 ± 48.4 a	731.0 ± 31.0 b	768.7 ± 18.1b	943.4 ± 41.2 a	321.5 ± 8.5c
Cu	46.8 ± 0.6 e	35.6 ± 0.7 f	69.0 ± 0.9 b	25.3 ± 1.3g	48.9 ± 2.5 de	58.6 ± 2.9 c	88.3 ± 4.0 a	57.6 ± 2.8 c	72.7 ± 3.5 b	55.0 ± 1.2 cd
Mn	45.4 ± 0.4 ef	53.8 ± 2.2 e	36.7 ± 1.2 ef	33.8 ± 1.4 f	46.3 ± 1.5 ef	339.5 ± 14.0 a	212.1 ± 7.4 b	183.7 ± 8.7 c	200.3 ± 9.9 bc	95.3 ± 3.7 d
Zn	91.3 ± 2.9 e	67.2 ± 1.7 f	93.6 ± 1.7 de	61.7 ± 0.7 f	69.5 ± 3.4 f	352.0 ± 13.4 a	214.1 ± 2.7 b	200.3 ± 9.8 b	151.4 ± 7.2 c	111.2 ± 5.4 d

^a Different letters in the same line represent significant differences between samples. Moisture expressed in (%); Ca, K, Na and Mg in mg/100 g of fresh weight; AA in mg/100 ml of pulps and in mg/100 g of fresh weight for peels; Fe, Cu, Mn and Zn in µg/100 g of fresh weight. Tr = traces.

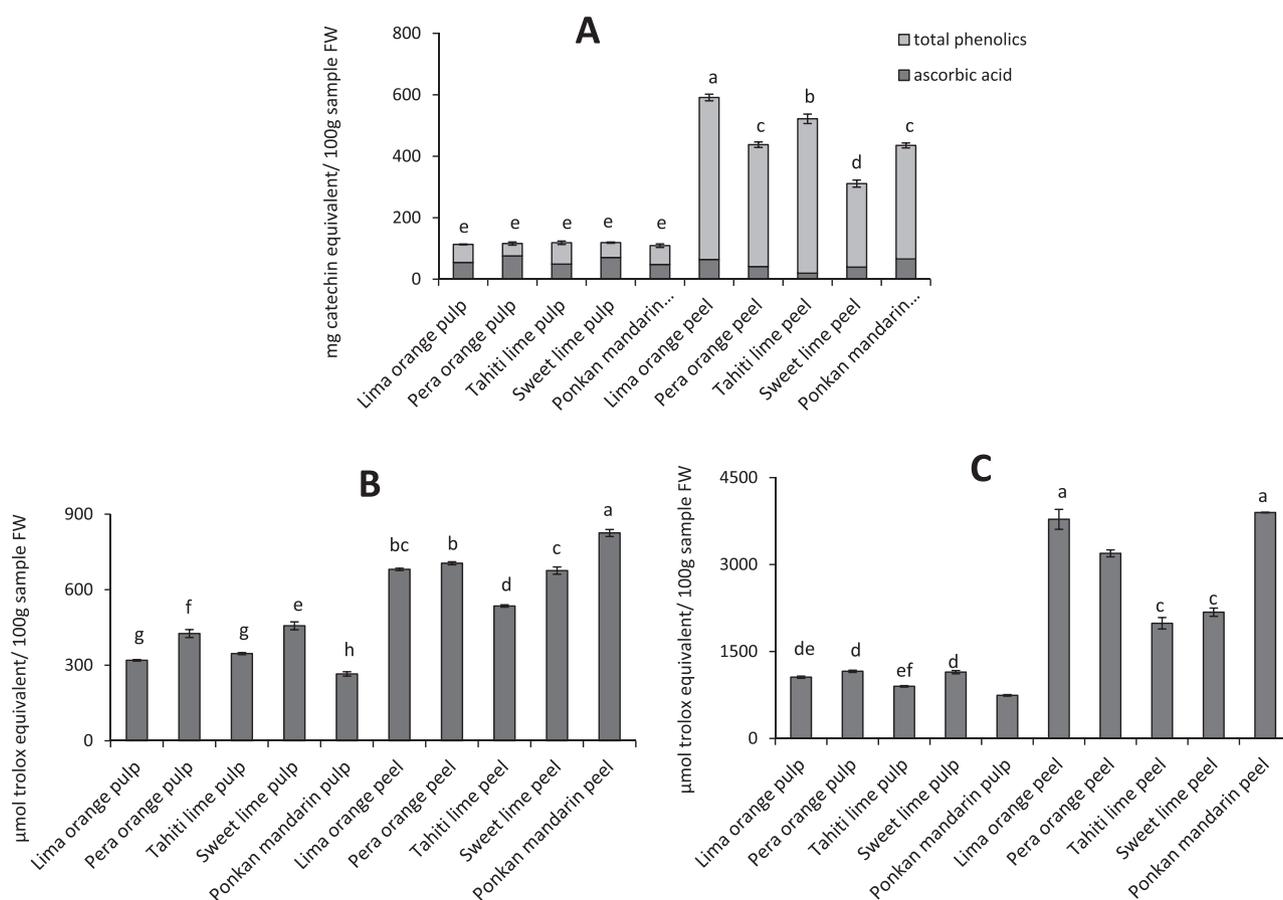


Fig. 1. Folin–Ciocalteu reducing capacity (A) and antioxidant capacity determined by DPPH (B) and FRAP (C) of citrus pulps and peels. Bars with different letters are significantly different ($p < 0.05$).

steroid hormones and carnitine, responsible for the conversion of cholesterol into bile acid. In terms of antioxidant activity, its reactivity with oxygen and nitrogen reactive species, and concentrations in human fluids and tissues, make it a likely scavenger of these species. It is also thought to be involved in the regeneration of tocopherol and prevent lipid peroxidation. Its intake has been demonstrated to reverse vascular endothelial vasomotor dysfunction in bronchial circulation of patients with coronary heart disease and seems to protect against gastric cancer (Evans & Halliwell, 2001; Patil et al., 2009).

The Dietary Reference Intake (DRI) is the daily intake level of a nutrient that is considered to be sufficient to meet the requirements of 97–98% of healthy individuals and prevent the development of diseases. The DRI (ANVISA, 1998) of vitamin C is 45 mg/day for a Brazilian adult. In Brazil, fruits are classified as “rich in” or a “source of” a vitamin or a mineral when they provide 30% or 15% of the element’s Dietary Reference Intake specific for age and gender, per 100 g of fruit (ANVISA, 2005). In this sense, citrus juices and pulps can be considered “rich in” vitamin C. The contents found in the present research confirm that 100 ml of juice of all citrus pulp

varieties studied provide 100% of DRI. Ponkan mandarin and Lima orange peels also provide 100%, while the varieties of other peels studied provide 50% or less of dietary intake per 100 g.

Eight minerals were determined, including 4 major elements (K, Ca, Na and Mg) and 4 trace elements (Cu, Fe, Mn and Zn), as shown in Table 2. All main minerals and trace elements were higher in peels than in pulps, except for Tahiti pulp which presented the second highest content of Cu and K. Potassium was the most abundant element presented in citrus peels and pulps, followed by Ca and Mg.

The citrus fruits showed high potassium content (140 mg in 100 g of orange pulp), while the sodium content is relatively low (2 mg/100 g orange pulp). The ratio of K and Na in oranges plays an important role in maintaining the electrolyte balance of cells in the human body (Ladaniya, 2008). However, peels showed 40 times more sodium than pulps. The lowest K content was observed in Sweet lime pulp (101.2 ± 4.9 mg/100 g FW) and the highest value in Pera and Lima orange peels (266.0 ± 12.2 and 258.7 ± 11.0 mg/100 g FW, respectively).

The highest Ca content was found in the Tahiti lime peel (214.2 ± 1.8 mg/100 g FW), which can be classified as a “source of” calcium for adults. On the other hand, calcium contents of Tahiti and Sweet lime pulps could not be detected. Therefore, the citric acid in citrus may act as a chelating agent and thus increase the Ca absorption by preventing the formation of insoluble salts (Ladaniya, 2008).

In addition, many antioxidant defenses depend on micronutrients. Some minerals are components of antioxidant enzymes: superoxide dismutase depends on Mn, Cu and Zn; catalase depends on Fe, and glutathione peroxidase on Se (Evans & Halliwell, 2001). Magnesium is also present in mitochondria and other enzymes important in energy transfer (Ladaniya, 2008). Analysis of Mg in the fruits revealed that Tahiti lime peel has the highest content (41.2 ± 1.2 mg/100 g FW) in relation to other peels and pulps. A portion of 100 g of this peel may provide more than 15% of the Mg dietary recommendation intake for people of any age or gender. Thus, it was classified as a “source of” Mg.

Of all microelements evaluated, Fe was detected in a higher concentration than Cu, Mn and Zn for both citrus pulps and peels. Peels showed 2 to 8 times more Fe content than pulps, but cannot be classified as “rich in” or a “source of” Fe. Apart from this, it is also known that the ascorbic acid present in citrus improves the bioavailability of Fe (Ladaniya, 2008). The Lima orange peel showed the highest Mn content (339.5 ± 14.0 µg/100 g FW), which is approximately seven times higher than those of pulps. According to the Mn dietary recommendations, it can be classified as “source of” Mn.

Besides Mn, Zn and Cu are also important for the body and supplied by citrus fruits (Gorinstein, Belloso, et al., 2001; Topuz et al., 2005). The DRI for Zn and Cu are 7 mg/day and 0.9 mg/day for an adult, respectively (ANVISA, 2005). Hence, pulps and peels (100 g) cannot be considered “rich” or “source of” these minerals. Secondly, according to Gorinstein, Zachwieja, et al. (2001), the contents of most minerals and especially trace elements in plants are known to be very low. However, in terms of biological activity they are strikingly strong. When they are incorporated into organomineral complexes, their ability is enhanced a thousand fold and sometimes a million fold over the activity of a simple ionic state.

3.3. Total phenolic contents and *in vitro* antioxidant capacity

The antioxidant capacity of fruits and vegetables is an important indicator of their *in vitro* potential as health promoters. Several methods have been developed to evaluate the antioxidant capacity of fruits or other plants and animal tissues; for instance, phenolics in fruits have been monitored spectrophotometrically

using the Folin–Ciocalteu reagent. The antioxidant capacity by DPPH and FRAP assays was selected to determine the total antioxidant capacities of citrus pulps and peels. The results are shown in Fig. 1.

Folin–Ciocalteu reducing capacity (Fig. 1A) was similar for the five citrus pulps, ranging from 109.16 ± 4.21 to 118.94 ± 2.03 mg catechin equivalent/100 g of FW, and those with lower and higher Folin–Ciocalteu reducing capacity were Ponkan mandarin and Sweet lime, respectively, but no significant difference between the varieties was observed. All citrus peels evaluated presented very high Folin–Ciocalteu reducing capacity, ranging from 310.18 ± 10.91 (Sweet lime) to 575.06 ± 8.51 (Lima orange) mg catechin equivalent/100 g of FW, with significant differences between almost all varieties. These values were 2.5 to 4 times higher than for pulps. However, the Folin–Ciocalteu reducing capacity is related to the content of both phenolics and ascorbic acid. For pulps, due to the higher content of vitamin C, the Folin–Ciocalteu reducing capacity results only 35% (Pera orange) to 59% (Tahiti lime) from the presence of phenolics (Fig. 1A). The contribution of ascorbic acid for the Folin–Ciocalteu reducing capacity of peels is lower than that presented by pulps, of about 4 (Tahiti lime) to 15% (Ponkan mandarin).

Previous studies had already shown higher levels of phenolic compounds in citrus peel compared to their segments (Abeyasinghe et al., 2007; Gorinstein, Belloso, et al. 2001; Guimarães et al., 2010). A study with 21 citrus varieties (Ramful, Baborun, Bourdon, Tarnus, & Aruoma, 2010) found that the amount of total phenolics in peels ranged from 188.2 ± 6.5 to 766.7 ± 5.7 mg of gallic acid equivalent/100 g of FW. According to Ignat et al. (2011), phenolic compounds may act as protective agents against UV lights, pathogens and predators in fruits and vegetables. Peels may contain higher concentrations of these compounds because they are in the outer part of the fruit, so they are more predisposed to the synthesis of phenolic compounds.

The *in vitro* antioxidant capacity of all peels was higher than those of pulps, both in terms of the DPPH radical scavenging capacity (Fig. 1B) and the FRAP assay (Fig. 1C). For pulps, the highest value of antioxidant capacity measured using the DPPH assay was presented by Sweet lime (456.7 ± 15.7 µmol of trolox equivalent/100 g of FW), and presented by Pera orange (1158.8 ± 19.22 µmol of trolox equivalent/100 g of FW) when using the FRAP assay, but with no significant differences between the Pera and Lima oranges. Ponkan mandarin showed the lowest values of antioxidant capacity in both assays (265.6 ± 8.7 µmol of trolox equivalent/100 g of FW for DPPH and 744.0 ± 12.7 µmol of trolox equivalent/100 g of FW for FRAP). However, in relation to peels, the Ponkan mandarin showed the highest antioxidant capacity (825.4 µmol of trolox equivalent/100 g of FW for DPPH and 3897.9 µmol of trolox equivalent/100 g of FW for FRAP) in both assays. In the FRAP assay, no significant difference was found with the Lima orange peel value (3780.8 ± 171.5 µmol of trolox equivalent/100 g of FW). The Tahiti lime peel showed the lowest antioxidant capacity in both assays (535.3 µmol of trolox equivalent/100 g of FW for DPPH and 1987.1 µmol of trolox equivalent/100 g of FW for FRAP); in the FRAP assay, no significant difference was found with the Sweet lime peel value (2178.8 ± 71.5 µmol of trolox equivalent/100 g of FW). These results are probably associated with phenolics and AA contents.

Similar to the results in the present study, Ghasemi et al. (2009) evaluated the DPPH radical scavenging capacity of the 13 most commonly available citrus in Iran, and the Ponkan mandarin peel showed the highest activity compared with other peels and tissues. This result was associated with the high phenolic and flavonoid contents of this plant. While flavonoids are abundant elsewhere in the plant kingdom, there are several compounds (e.g., flavanones, flavanone glycosides and polymethoxylated flavones) unique to

Table 3

Correlation coefficients between DPPH, FRAP, Folin Ciocalteu reducing activity, total phenolic contents and AA for pulps and peels from five Brazilian citrus.

Correlations	Pulps	Peels	Pulps and peels
Folin × total phenolics	−0.177	0.983 ^c	0.997 ^c
Folin × AA	0.475	0.168	−0.342
Folin × DPPH	0.801	0.108	0.818 ^b
Folin × FRAP	0.615	0.362	0.874 ^b
Total phenolics × DPPH	−0.719	−0.443	0.773 ^b
Total phenolics × FRAP	−0.662	0.196	0.845 ^b
AA × DPPH	0.863	0.915 ^a	−0.205
AA × FRAP	0.845	0.926 ^a	−0.257
DPPH × FRAP	0.864	0.863	0.946 ^b

^a Correlation is significant at $p < 0.05$.

^b Correlation is significant at $p < 0.01$.

^c Correlation is significant at $p < 0.001$.

citrus, which are relatively rare in other plants (Li et al., 2006). Flavanones in orange were previously used as markers to differentiate citrus varieties. The main flavanone glycosides in oranges and mandarin species are hesperidin and narirutin (Albertini et al., 2006).

3.4. Correlation coefficients of Folin–Ciocalteu reducing capacity, AA, total phenolic content, DPPH and FRAP

Recent studies with citrus have demonstrated that the antioxidant efficiency may be attributed, in a significant part, to their phenolic content (Gorinstein, Zachwieja, et al., 2001; Rapisarda, Bianco, Pannuzzo, & Timpanaro, 2008). Other studies (Abeyasinghe et al., 2007; Xu et al., 2008) have shown that ascorbic acid has played a major role in the antioxidant capacity of citrus fruits. As the results are divergent, a direct correlation between antioxidant capacity and total phenolic or ascorbic acid contents of samples was demonstrated by the Pearson's coefficient $p < 0.01$, $p < 0.05$ or $p < 0.001$ (Table 3).

For pulps, good correlation coefficients of Folin–Ciocalteu reducing capacity and DPPH or FRAP assay were found, supporting similar results reported by Rapisarda et al. (2008) and Xu et al. (2008), but a significant correlation could not be found among them. However, considering the results for peels, the correlations between the Folin–Ciocalteu reducing capacity and the antioxidant capacity by both assays were weak. These results are in agreement with other studies with citrus peel (Anagnostopoulou et al., 2006; Ghasemi et al., 2009). For pulps and peels together, the correlations between Folin–Ciocalteu reducing capacity and antioxidant capacity by DPPH and FRAP were significantly higher ($r = 0.818$ and 0.874 , respectively, with $p < 0.01$). In general, extracts with a high Folin–Ciocalteu reducing capacity showed a high antioxidant capacity.

Reporting to Huang, Ou, and Prior (2005), the Folin–Ciocalteu reagent is nonspecific for phenolic compounds since it measures sample reducing capacity through electronic transfer-based antioxidant capacity, and thus it can be reduced by many non-phenolic compounds such as vitamin C. The low antioxidant activity of Ponkan mandarin (DPPH assay) and Tahiti lime (FRAP assay) pulps may be linked to lower levels of ascorbic acid when compared with other pulps evaluated, since the varieties showed similar Folin–Ciocalteu reducing capacity. The same was observed with peels, with Ponkan mandarin having highest AA contents and antioxidant capacity, while Tahiti lime having the lowest.

In this sense, the ascorbic acid absorbance was subtracted of the value of Folin–Ciocalteu reducing capacity (Fig. 1A). Thus, the correlations between total phenolics and ascorbic acid with the Folin–Ciocalteu reducing capacity and antioxidant capacity assays were done. The total phenolics content of peels correlated highly with the Folin–Ciocalteu reducing capacity for peels and for pulps

and pulps together, but no correlation was found for pulps separately. These results confirm the results shown in Fig. 1A, which demonstrate that the Folin–Ciocalteu reducing capacity for citrus peels is 85–96%, and results from phenolic compounds, whereas for pulps it is only 35–59%. Yet, total phenolics correlated significantly with both antioxidant capacities assay (DPPH and FRAP) for pulps and peels together.

The AA contents correlated highly with two *in vitro* antioxidant capacities assays, for pulps and peels separately. Studies with citrus (Rapisarda et al., 2008; Xu et al., 2008) also showed good correlations between ascorbic acid and DPPH or FRAP assays. According to Abeyasinghe et al. (2007), based on the FRAP assay, the antioxidant capacity caused by vitamin C was higher than the one caused by flavonoids (naringin and hesperidin) in four varieties of citrus from China. However, negative correlations were observed between the AA contents and both antioxidant assays for peels and pulps together, showing that peels showed lower acid ascorbic contents than pulps, but higher antioxidant capacity than pulps.

A direct correlation between the two methods of antioxidant activity demonstrated high correlation coefficients at $p < 0.01$ (0.946) for pulps and peels together). Some studies suggest this may occur by chemistry similarity between the two assays; both are based on the electron transfer reaction. Nonetheless, there are divergences in the literature on DPPH (Huang, Ou, & Prior, 2005). Other studies suggest that the interaction of antioxidant compounds with the DPPH radical depends on its structural conformation (Rapisarda et al., 2008), and so, the DPPH assay is less sensitive as compared to FRAP. In fact, the relationship between the antioxidant activity by both assays and the phenolic compounds or vitamin C depends on several factors, such as chemical structure of individual components, the synergistic interaction among them, and the specific conditions applied in different assays (Huang et al., 2005).

In conclusion, the antioxidant capacity of citrus does not seem to be a property of a single phytochemical compound, but is correlated both to vitamin C and phenolic constituents. Citrus fruits grown in Brazil provide a variety of bioactive compounds that are vital in health promotion and disease prevention. In addition to citrus pulp and juices, peels are also promising sources of compounds which can be used for their health properties in food products. They can be applied as a source of functional compounds, or as natural preservatives, improving the lipid oxidation of meals and fat products. Therefore, the present study provided very important information to guide the practice of agriculture and of the food industry in the development of new products, which are expected to be safe and health-promoting.

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