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Antioxidant capacity and composition of pitanga seeds

Capacidade antioxidante e composição de sementes de pitanga

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ABSTRACT

Food industry generates a significant amount of seed wastes from the juice production, frozen pulps and jams. Considering that the characterization of wastes is the first step to determine their potential use, the aim of the present study was to determine the composition and the antioxidant capacity of seeds from pitanga fruits with different flesh colors (purple, red and orange). Chemical composition results revealed that pitanga seeds are a good source of insoluble dietary fiber, with low protein and fat levels, and no relevant differences were found among pitanga seeds from different flesh colors. Pitanga seed extracts had powerful antioxidant capacity that was partially correlated to their high phenolic content and showed some variation according to the pitanga flesh colors. Accordingly, it's suggested that this low value waste of pitanga processing, could be used as a source of natural antioxidants and dietary fiber, for animal and/or human nutrition.

Key words: *antioxidant capacity, DPPH, FRAP,* ***Eugenia uniflora*** *L.*

RESUMO

A indústria de alimentos gera quantidades significativas de resíduos de sementes a partir da produção de sucos, polpas congeladas e geleias de frutas. Considerando que a caracterização dos resíduos é o primeiro passo para determinar o seu uso potencial, o objetivo do presente estudo foi determinar a composição e a capacidade antioxidante de sementes de pitanga com diferentes colorações de polpa (roxa, vermelha e laranja). Os resultados da composição química revelaram que as sementes de pitanga são boas fontes de fibra alimentar insolúvel, com níveis baixos de proteína e gordura, e sem diferenças relevantes entre as sementes de pitangas de diferentes colorações. Os extratos das sementes apresentaram uma excelente capacidade antioxidante, que foi parcialmente correlacionada com o alto teor de fenólicos e apresentou alguma variação de acordo com a coloração da polpa das pitangas. Assim, sugere-se que esse resíduo de baixo valor, resultante do processamento da pitanga, poderia ser utilizado como fonte de antioxidantes naturais e de fibra alimentar, para a nutrição humana e/ou animal.

Palavras-chave: *capacidade antioxidante, DPPH, FRAP,* ***Eugenia uniflora*** *L.*

INTRODUCTION

The Brazilian cherry or pitanga (***Eugenia uniflora***) is a member of the Mirtaceae family. Mirtaceae is a pan-tropical family that occurs in South America, Southeast Asia, and Australia. Many species of Myrtaceae, including pitanga, are cultivated in home gardens for their edible fruit, and their leaves have been used in traditional medicine to treat several inflammatory conditions (REYNERSTON et al., 2008). In the Brazilian food industry, pitanga fruits have mostly been used to produce juice and frozen pulp.

Fruits are known as important sources of bioactive compounds. The pitanga frozen pulp has high content of total phenolics and carotenoids, which are known as antioxidant compounds (SPADA et al., 2008). Concerning to the phenolic compounds, the aqueous

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fraction of pitanga fruits contains antioxidant anthocyanins (cyanidin-3-*O*-β-glucopyranoside and delphinidin-3-*O*-β-glucopyranoside; EINBOND et al., 2004). Besides, the essential oil from pitanga fruits contains β-ocimene (7.4%), α-selinene (7.2%), β-selinene (5.2%), germacrene B (7.2%) and hexadecanoic acid (11.7%) and was demonstrated to have an interesting antioxidant activity (MARIN et al., 2008). However, few studies were found on bioactive compounds from pitanga seeds (OLIVEIRA et al., 2008).

Worldwide several million tons of agri-food solid wastes are produced annually and are likely to be discarded or used as low-value by-products (TALCOTT et al., 2003; ISCI & DEMIRER, 2007). Food industry generates a significant amount of seed wastes from the production of juice nectars, concentrates, jams, jelly powders and fruit bars. These wastes could be used as a source of ingredients for the food industry, since many fruit seeds have high content of antioxidant phenolic compounds (PURAVANKARA et al., 2000), dietary fiber (AL-FARSI & LI, 2008), oils (KOBORI & JORGE, 2005) and other components. This practice would reduce the impact on the environment, besides yielding value added ingredients (EMBRAPA INFORMAÇÃO TECNOLÓGICA, 2003).

Fruit seeds have not generally received much attention as antioxidant sources, however SOONG & BARLOW (2004) demonstrated a significantly higher phenolic content and total antioxidant capacity in the seeds of fruits than in their edible portions. Antioxidants from residual sources could be used as natural food additives to increase the stability of foods by preventing lipid oxidation (MOURE et al., 2001). Besides its antioxidant capacity, phenolic compounds, mainly phenolic acids and flavonoids, possess other interesting health beneficial properties like anti-carcinogenic (BAILEY & WILIAMS, 1993), antimicrobial (TAKECHI et al., 1985), anti-mutagenic (LIVERIO et al., 1994) and anti-inflammatory activities (LANDOLFI et al., 1984), which could be exploited by the pharmaceutical industry.

Besides phenolic compounds, some fruit seeds contain other nutritionally important compounds that are usually underutilized. Blackcurrant or groselha (***Ribes nigrum***) seeds contain an exceptionally high level of the desirable polyunsaturated γ-linolenic acid (TRAITLER et al., 1984), while seeds of date have a high content of dietary fiber (AL-FARSI & LEE, 2008).

In the literature no data was found concerning to the composition or antioxidant capacity of pitanga seeds. Considering that the characterization of wastes is the first step to establish their potential use, the aim of the present study was to determine the composition and the antioxidant capacity of seeds from pitanga fruits with different flesh colors (purple, red and orange). These fruits were from trees cultivated at Embrapa Clima Temperado (RS, Brazil) and are being studied to yield cultivars adapted to the Brazil southern region l.

MATERIAL AND METHODS

Samples

The samples of purple, red and orange fleshed pitanga fruits (***Eugenia uniflora*** L.), were harvested at Embrapa Clima Temperado (Rio Grande do Sul, Brazil) in 2007. Each sample was a mixture of completely ripe fruits from various plant selections with the same flesh color. Three independent lots were separated, frozen at -18ºC and transported to the Federal University of Santa Maria. Fruits were thawed and the flesh (edible portions) was manually separated from the seeds. Seeds were dried in a conventional air-oven during 4 hours at 60ºC and then ground using a Wiley grinder with a 2mm sieve and stored at -18ºC until analysis.

Composition

Moisture weight loss was determined at 105ºC (method 925.10; AOAC, 1995). Ash content was determined at 550ºC (method 923.03) according to AOAC (1995). Protein content (N x 6.25) was determined by the microkjeldahl procedure (method 960.2) of the AOAC (1995). Total and insoluble dietary fiber was determined by the enzymatic-gravimetric methods (985.29 and 991.42) from AOAC (1995). Fat was extracted using chloroform and methanol as described by BLIGH & DYER (1959) and used for determination of the fat content and fatty acid profile. To prevent lipid oxidation during and after extraction, 0.02% butyl hydroxyl toluene was added to the chloroform used. The nitrogen free extract fraction (Nifext) was calculated by difference.

Fatty acid methyl esters (FAMES)

Aliquots (2-3mL) of the chloroform-lipid extract from BLIGH & DYER (1959) were evaporated at 50ºC using a vacuum pump. Fat was saponified in methanolic sulfuric acid solution as described by HARTMAN & LAGO (1973). Methylated samples were analyzed using an Agilent Technologies gas chromatograph (HP 6880) fitted with a capillary column DB-23 (60m x 0.25mm x 0.25μm, Agilent) and a flame ionization detector. The temperature of the injector port and the detector were set at 250ºC, and the carrier gas was nitrogen (0.6mL min^{-1}). After injection (1μL, split ratio 1:50), the oven temperature was hold at 140ºC for 5min, increased to 240ºC at a rate of 4ºC min^{-1} and hold at this temperature for 5min.

Phenolic compounds

The extraction of phenolic compounds was performed using the method of ESCARPA & GONZÁLEZ (2001) modified as follows. The homogenized samples (2g) were extracted in an ultrasonic bath at room temperature in the absence of light with an aqueous solution consisted of 800mL methanol and 50mL formic acid per liter. The samples were sequentially extracted with 6mL of the solution for 1h, 6mL for 30min and 3mL for 30min. After each extraction, the extracts were filtered in qualitative filter paper under vacuum. The combined filtrate was brought to a final volume of 25mL with the solution and stored at -18ºC until required for analysis.

Total phenolic content was determined using the method of SINGLETON & ROSSI (1965). An aliquot of 0.1mL of extract was mixed with 2.5mL of 0.25mol per liter Folin-Ciocalteu reagent. After 5min, 2mL of 1mol per liter Na_2CO_3 was added. The absorbance was determined at 740nm after 1h in the dark. Gallic acid was used as a standard for the calibration curve. The amount of total phenolic compounds was calculated and expressed as mg gallic acid per 100g sample.

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

A DPPH stable solution was used for determination of the total antioxidant activity of extracts according to BRAND-WILLIAMS et al. (1995). DPPH solution was previously diluted until its absorption at 515nm reached 1.10±0.02. The extract (0.05mL) was mixed with 1.9mL diluted methanolic DPPH solution. The antiradical power of the different extracts was determined by measuring the decrease of DPPH absorbance after 24 hours in the dark against a blank. Trolox was used as standard for the calibration curve and the results were expressed as mmol trolox equivalents per 100g sample.

Ferric-reducing antioxidant power (FRAP) assay

The method of BENZIE & STRAIN (1996) was used for FRAP assays. Ferric-2,4,6-trypyridyl-s-triazine (TPTZ) solution was prepared by mixing 2.5mL of 10mmol per liter TPTZ solution (prepared in 40mmol per liter HCl) with 2.5mL of 20mmol per liter $FeCl_3.6H_2O$ and 25mL of 0.3mol per liter acetate buffer at pH 3.6. The sample (40µL) was mixed with 1.2mL of ferric-TPTZ reagent and 120µL of Milli-Q® water and incubated at 37ºC during 15min. The absorbance of the colored complex formed with Fe^{+2} and TPTZ was taken at 593nm. Trolox was used as standard for the calibration curve and the results were expressed as mol trolox equivalents per 100g sample.

Statistical analysis

The experiment was conducted at a completely randomized design with three groups (colors of pitanga flesh) and three repetitions per group. All measurements were made in triplicate. Results were analyzed by one-way analysis of variance (ANOVA). Post hoc comparisons were made using Tukey´s test ($P<0.05$). The relationship between phenolic content and antioxidant capacity was evaluated by Pearson´s correlation. Statistical analyses were carried out using Statistica 6.0 (Copyright Sta Soft, Inc 1984-2001).

RESULTS AND DISCUSSION

Seeds chemical composition from purple, red and orange fleshed pitanga was similar (Table 1). No significant difference was observed in the moisture, ash, protein, total carbohydrate, and total and insoluble dietary fiber or Nifext fraction among samples. However, the fat content of seeds from purple fleshed pitanga were lower than that of the other samples ($P<0.05$). Seeds moisture content are similar to orange and guava seed wastes (55.4 and 43.3%, respectively), but much lower than passion fruit (80.9%; KOBORI & JORGE, 2005). Carbohydrate was the major nutrient found in pitanga seeds. This fraction was found to be composed mainly by insoluble dietary fiber, with a lower amount of digestible carbohydrates (Nifext fraction). Dietary fiber has important therapeutic implications for certain conditions such as diabetes, cardiovascular diseases and intestinal disorders in humans (SUTER, 2005). The insoluble fraction facilitates gastrointestinal transit and reduces constipation (SUTER, 2005). Besides, complex carbohydrates, like those found in

Table 1 - Chemical composition (g per 100g fresh weight) of seeds from purple, red and orange pitanga (***Eugenia uniflora*** L.).

	Purple	Red	Orange
Moisture	57.5±0.4	58.6±0.7	57.0±0.0
Ash	0.8±0.1	0.8±0.0	0.6±0.0
Protein	3.7±0.2	3.6±0.2	3.3±0.2
Fat	0.5±0.0	0.7±0.0 *	0.7±0.0 *
Total carbohydrate[&]	37.5±0.5	36.4±0.9	38.4±0.2
Total fiber	24.7±0.1	23.4±0.1	23.0±0.1
Insoluble fiber	23.7±0.2	23.3±0.0	23.0±0.0
Nifext fraction[&]	12.8±0.5	13.0±0.9	14.4±0.2

Results are mean ± standard deviation (n=3). *Significantly different from purple samples ($P<0.05$). [&]Calculated by difference.

the dietary fiber fraction, are also very important in the nutrition of ruminants (FOX et al., 1992). Therefore, the insoluble dietary fiber of pitanga seeds could be evaluated for use in human and/or animal nutrition. However, it is important to evaluate if the lectins recently described in pitanga seeds (OLIVEIRA et al., 2008) could be a limiting factor for this application.

Table 2 presents the fatty acid composition of pitanga seeds. Pitanga seeds had a high proportion of unsaturated fatty acids (60-70%) being 13-16% monounsaturated fatty acids (MUFA) and 45-47% polyunsaturated fatty acids (PUFAS). PUFAS, especially the n-3 fatty acids, are considered desirable compounds in the human diet because of their effect in reducing the incidence of cardiovascular disease (LEAF & WEBER, 1988). Pitanga seeds had MUFA proportion similar to that of black currant seed residue (HELBIG et al., 2008).

In seeds from purple and red pitanga the predominant unsaturated fatty acid was linoleic acid (C18:2n6c), followed by oleic acid (C18:1n9c), while seeds from orange pitanga had á-linolenic acid (C18:3n3) as the second most abundant unsaturated fatty acid. YI et al. (2008) also found linoleic acid as the predominant fatty acid in berry seeds press residues. Palmitoleic acid was found only in seeds from purple and red pitanga. Seeds from purple and red pitanga had higher linoleic acid and lower á-linolenic acid content than seeds from orange pitanga ($P<0.05$). Seeds from red pitanga had higher oleic acid (C18:1n9c) followed by seeds from orange and purple pitanga ($P<0.05$).

The only saturated fatty acid found was palmitic acid, which was found in higher proportion in seeds from orange pitanga when compared to the other samples ($P<0.05$). Tomato seeds also had high content of palmitic acid, but followed by stearic acid (CANTARELI et al., 1993), which was not found in pitanga seeds.

Table 2 - Fatty acid composition (% of total fatty acids) of seeds from purple, red and orange pitanga.

Fatty acids	Purple	Red	Orange
C16:0	29.8±0.6 [b]	30.3±1.7 [b]	34.2±0.0 [a]
C16:1n7c	1.4± 0.0 [a]	1.5±0.1 [a]	ND
C18:1n9c	12.5±0.5 [c]	15.0±0.3 [a]	13.7±0.1 [b]
C18:2n6c	38.3± 0.9 [a]	36.0±2.0 [a]	28.6±0.0 [b]
C18:3n3	8.7±0.4 [b]	9.2±0.7 [b]	18.3±0.0 [a]
NI	9.3±0.3	8.0±2.2	5.3±0.0

Results are mean ± standard deviation (n=3). Means that have no common letters within the same row are statistically different ($P<0.05$). C12:0, C14:0, C14:1n5, C18:0, C18:1n9t, C18:2n6t C20:1n9, C20:4n6, C20:5n3, C22:0, C22:5n3 and C22:6n3 were not detected. NI: Non-identified compounds.

Seeds from orange pitanga had higher phenolic content than seeds from purple and red pitanga (Table 3, $P<0.05$). Total phenolic content of pitanga seeds is very similar to that found for jackfruit seed (***Artocarpus heterophyyllus*** Lam.) (SOONG & BARLOW, 2004) and it is about 5 to 10-fold higher than that found on grape pomace powder from winemaking industry (YI et al., 2008) or on the edible portion of various fruits (VINSON et al., 2001). Hence, pitanga seeds can be considered good sources of phenolics.

The antioxidant capacity of pitanga seeds was expressed as equivalents of trolox, which is a hydrosoluble analog of vitamin E. Seeds from orange pitanga had slightly higher radical scavenging capacity in the DPPH assay than seeds from purple pitanga ($P<0.05$), but both had DPPH values similar to red pitanga (Table 3). In contrast, seeds from purple pitanga had higher ferric reducing antioxidant power than all the other samples ($P<0.05$; Table 3). These screening tests indicate that pitanga seeds appear to be promising sources of antioxidants, since their activity in the DPPH assay was about two-times higher than that presented by the pulp of various tropical fruits including grape, açaí (***Euterpe oleracea***) and baguaçu (***Eugenia umbelliflora*** *Berg*) (KUSKOSKI et al., 2006). However, further studies are required to completely characterize the potential of extracts from pitanga seeds as antioxidant additives in foods or in the treatment of free radical-associated disorders.

Generally, a positive relationship between total phenolics and antioxidant activity has been reported previously (ALONSO et al., 2002). In the present study the phenolic content had a positive correlation with antioxidant capacity measured by the DPPH assay ($r^2 = 0.72$, $P<0.05$). Although this result suggests a role for phenolic compounds in the scavenging of DPPH radical, further studies on the complete composition of the extracts are required to confirm this hypothesis. In contrast to the DPPH assay, the antioxidant capacity assessed by the FRAP method had no significant correlation with total phenolic content, suggesting that compounds that are not evaluated in the total phenolic assay may be more important to the FRAP antioxidant capacity. Carotenoids are known to be important antioxidant compounds in pitanga flesh (SPADA et al., 2008). However, it is unlikely that pitanga seeds have an appreciable amount of these antioxidant compounds, since they had a white to pale green color. The

Table 3 - Antioxidant capacity and phenolic content of extracts from purple, red and orange pitanga.

Samples	Phenolic content (g gallic acid per 100g)	Antioxidant capacity	
		DPPH (mmol trolox per 100g)	FRAP (mmol trolox per 100g)
Purple	2.5±0.2 [b]	14.6±0.6 [b]	26.2±2.8 [a]
Red	2.6±0.0 [b]	16.7±1.3 [a,b]	6.1±0.7 [b]
Orange	2.8±0.2 [a]	17.4±1.4 [a]	6.4±0.4 [b]

Results are mean ± standard deviation (n=3) and are expressed per 100g of dried seed used to prepare the extract. DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power. Means that have no common letter within the same column are different (P<0.05).

discrepancy between results of DPPH and FRAP assay may be related to the different mechanisms involved in each evaluation. In the FRAP assay the antioxidant capacity is measured as the ability to reduce 2,4,6-trypyridyl-s-triazine-Fe(III) complex to 2,4,6-trypyridyl-s-triazine-Fe(II) complex (BENZIE & STRAIN, 1996) and may be related to the presence of iron-chelating compounds in the samples. In contrast, the DPPH assay involves a fast electron transfer process from phenolic compounds to the DPPH radical (BRAND-WILLIAMS et al., 1995; MARTINS et al., 2009). In a recent study MARTINS et al. (2009) showed that results of DPPH were more correlated to the antioxidant capacity of compounds *in situ*, than the FRAP assay.

Flavonoids are considered the most powerful antioxidants among the polyphenol compounds (SHAHID et al., 1992; SOOBRATTEE et al., 2005). LU & FOO (2003) showed the presence of an array of polyphenols in blackcurrant seeds, such as anthocyanins, consisting of the rutinosides and glucosides of delphinidin, cyanidin, myricetin, quercetin, kaempferol, dihydroquercetin and aureusidin, as well as the phenolic acids 1-cinnamoyl- and 1-p-coumaroyl-b-D-glucosides. Thus, it is possible that flavonoid compounds could be the major responsible for the antioxidant capacity of pitanga seeds. However, more studies are needed to identify the phenolic profile of pitanga seeds.

CONCLUSIONS

Pitanga seeds had powerful antioxidant capacity that was partially correlated to their high phenolic content and showed some variation according to the pitanga flesh colors. Accordingly, we suggest that this low value waste of pitanga processing, could be used as a source of natural antioxidants. No relevant differences were found in the composition among seeds from pitanga of different colors. Results revealed that pitanga seeds are a good source of insoluble dietary fiber, which could be explored for use in animal and/or human nutrition. However, more studies are necessary to determine if some antinutritional factor like cyanogenic glycosides or lectins could be a limitant factor for this application.

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