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Eugenia Klotzschiana O. Berg Fruits as New Sources of Nutrients: Determination of their Bioactive Compounds, Antioxidant Activity and Chemical Composition

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HIGHLIGHTS

- The *Eugenia klotzschiana* fruits have high fibre content.
- The iron content in the Cerrado pear is 55-times higher than common pear.
- The Cerrado pear presented high pulp yield.

Abstract: The Cerrado is one of the world's biodiversity hotspots and Brazil's second largest biome. Many native species of the Brazilian Cerrado provide fruits that have unique sensory characteristics and high nutritional value. This study aimed at characterizing the pulp of Eugenia klotzschiana O. Berg, concerning its proximal composition, bioactive compound content and antioxidant activity. The pulp under study had high moisture (89.47 g kg^{-1}) and caloric (96.07 kcal kg^{-1}) values whereas its contents of protein (0.59 g kg^{-1}) and lipids (2.35 g kg^{-1}) were low. The cerrado pear pulp also had high iron content (16.5 mg kg^{-1}) and dietary fiber (6.45 g kg^{-1}), besides 0.034-0,055 mg kg^{-1} carotenoids, 8.66 mg kg^{-1} ascorbic acid and 0.66 mg kg^{-1} total chlorophyll. Total phenolic compounds (333.41-566.33 mg EAG kg^{-1}) and flavonoids (225-50 mg EQ kg^{-1}) were found by extraction methods named Method 1 (water) and Method 2 (acetone+methanol), respectively. Thus, the cerrado pear can be an alternative to improving nutrient intake and to providing sustainable use of the native flora in the Cerrado.

Keywords: Cerrado pear; Nutrients; Flavonoids; Antioxidant capacity.

INTRODUCTION

The Cerrado is one of the world's biodiversity hotspots and Brazil's second largest biome, since it stretches over 2 million km^2 in Latin America and covers 22% of the Brazilian territory. With regard to its biological diversity, this region has been recognized as the richest savanna in the world because it has about 10,000 cataloged native plant species [1]. Expansion of agriculture and the use of modern technology in the Cerrado have generated undeniable socioeconomic benefits. However, areas that result from poor soil management with considerable erosion or invasion of exotic species can be found in the Cerrado. Around 55% of the original area was deforested or transformed by human action. In addition, the Cerrado has great biodiversity and endangered species of fauna and flora.

Many native species of the Brazilian Cerrado provide fruits that have unique sensory characteristics (color, aroma and flavor) and high nutritional value, regarding their levels of sugars, proteins, vitamins, minerals and compounds with high antioxidant activity. Among compounds that have functional properties in food, substances with antioxidant activities have received significant attention because they protect the human body against oxidative stress and prevent a number of chronic degenerative disorders [2]. Although these native fruits have high nutritional value, their economic aspect is undervalued; they have been consumed by the local population but still need to play more important roles in the industry and marketing [3-4]. Thus, studies have been conducted in order to characterize the native fruits of the Cerrado to promote appreciation of the importance of these products and encourage sustainable development [3, 5-6].

The cerrado pear (*Eugenia klotzschiana* O. Berg.), also known as *pera-do-campo* and *cabacinha-do-campo*, which belongs to the Myrtaceae family, is a native fruit of the Brazilian Cerrado and unknown to the food industry. The plant is a small 1-2-meter high shrub, with variable-sized fruits, which are yellow – when they are ripe – and have soft, astringent and sour pulp (due to its high acidity) [7-8]. The cerrado pear has been consumed fresh and used for producing jelly. Nevertheless, there is little knowledge about the cerrado pear due to its

restricted geographical distribution and the great difficulty in using its seeds directly for planting [9].

In the literature, few studies that describe the complete characterization of the pulp of *E. klotzschiana* O. Berg. fruits were found. This study aimed at characterizing the pulp of *E. klotzschiana* O. Berg., a native fruit of the Cerrado biome, concerning its chemical composition (proximal and mineral compositions), physical characteristics, bioactive compound content and antioxidant activity.

MATERIAL AND METHODS

Standards

Reagents (Folin & Ciocalteu's phenol reagent, ABTS 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) and BHT (3,5-Di-tert-4-butylhydroxytoluene) and standards (trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), quercetin (98% HPLC grade), gallic acid and glucose) were purchased from Sigma (Steinheim, Germany). Other reagents, i. e., sodium carbonate, chloroform, hexane, copper sulphate, sodium selenite, sodium sulfate, boric acid, 3,5 dinitrosalicylic acid, methyl red and potassium persulfate (Neon, São Paulo, Brazil); aluminum chloride hexahydrate, hydrochloric acid, sulfuric acid, acetone and glacial acetic acid (Vetec, Rio de Janeiro, Brazil); ethyl alcohol (Fmaia, São Paulo, Brazil); methyl alcohol, oxalic acid, sodium phosphate, ascorbic acid and 2,6-dichlorophenolindophenol (Dinâmica, São Paulo, Brazil); ferric chloride and ferrous sulphate (Alphatec SA, Rio de Janeiro, Brazil); and sodium nitrite (Synth, São Paulo, Brazil) were analytical grade. Enzymes were amylase, protease and amyloglucosidase.

Raw Material

Cerrado pears (*E. klotzschiana* O. Berg) were collected in areas of native vegetation, typical of the Cerrado, in Portelândia (17° 23′ S and 52° 38′ W), Goiás, Brazil, in the harvest season (from December 2014 to January 2015). Morphologically perfect and completely mature fruits were washed – to have all dirt removed – and dried at room temperature. The cerrado pear was considered ripe when its skin was yellow. This color was related to the luminosity (L*), ranging from 40.34 to 40.68, to the ratio of red to green (a*), ranging from 12.64 to 13.96 and to the ratio of yellow to blue (b*), ranging from 25.72 to 26.84, in agreement with the CIE system (*Commission Internationale de l'Eclairage*). Cerrado pears were frozen and the pulp and skin were separated manually from the seeds and homogenized immediately before analysis. The color of the cerrado pear pulp was related to the luminosity (L*), ranging from 56.70 to 65.20, to the ratio of red to green (a*), ranging from 3.18 to 4.74 and to the ratio of yellow to blue (b*), ranging from 33.72 to 37.48, in agreement with the CIE system.

Physical and chemical characterization

Individual measurements of mass, transverse and longitudinal diameters were carried out in 20 fruits by a digital caliper rule (Mitutoyo, São Paulo, Brazil). Fruit mass (FM), pulp mass (pulp, PM) and residue mass (RM) were obtained by direct individual weighing on a semi-analytical balance (Shimadzu, São Paulo, Brazil). Pulp yield was calculated by the formula (PM/FM)×100. Chemical analyses were performed in 4 repetitions. Values of titratable acidity, soluble solids and pH [10]; moisture, ash, protein, lipids and total dietary fiber [11] were determined. Carbohydrates were calculated by subtraction, by the following formula: 100 − (% moisture + % lipids + % protein + % total dietary fiber + % ash). The total energy value was estimated by considering the conversion factors of 4 kcal g^{-1}protein or carbohydrate and 9kcal g^{-1} lipid [12]. Total sugar contents (reducing and non-reducing ones) were determined in agreement with Miller [13]. Results were expressed as g glicose kg^{-1} fresh matter (FM). Mineral contents were evaluated in crushed and homogenized samples, which were dried in an oven at 105ºC and digested in HNO_3 and $HClO_4$, as described by

Malavolta [14]. Quantification of minerals found in the samples, i. e., calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), iron (Fe) and zinc (Zn), was carried out by dual beam atomic absorption spectroscopy GBC-XPLORAA-2 (GBC, Lakeside, Australia). Potassium (K) quantification was determined by a photoelectric flame photometer B-462. The phosphorus (P) was determined by colorimetry (molecular absorption spectrophotometry).

Bioactive compounds

Total carotenoid contents were determined according to Nagata and Yamashita [15]. Two g pulp was extracted in 20 mL acetone:hexane (4:6) in the dark and then centrifuged for 3 minutes at 20°C at 15.000 rpm (ITR model 8BT, Med. Instruments, Warsaw, Mazowieckie, Poland). Afterwards, the extract was filtered through Whatman filter paper No. 4 and absorbance was measured at 453 ηm, 505 ηm, 645 ηm and 663 ηm. Results were expressed as mg β-carotene per kg^{-1} FM and mg lycopene per kg^{-1} FM. Total chlorophyll contents were determined according to Arnon [16]. One g pulp was homogenized in 30 mL acetone:water (80:20, v/v) and filtered through Whatman No. 4. The volume of the mixture was adjusted to 50 mL and absorbance was measured at 645 and 663 ηm. Total chlorophyll was calculated by the following equation: $20.2 \cdot A_{645} + 8.02 \cdot A_{663}$, where A_{645} is the absorbance at 645 ηm and A_{663} is the absorbance at 663 ηm. Results were expressed as mg per kg^{-1} FM. The ascorbic acid analysis was performed by the official method modified by Benassi and Antunes [17]. Five g fresh pulp was homogenized in 50 mL acid oxalic 2% and filtered through Whatman No. 4. Ten-mL aliquots were titrated with 0.2% dichlorophenol–indophenol. Results were expressed as milligrams of reduced AA per kg^{-1} fresh weight (FW). In order to determine total phenolic compounds, total flavonoids and antioxidant activity (DPPH, ABTS and FRAP methods), two extraction methods were compared. In Method 1, 20 g fresh pulp was homogenized in 100 mL water and filtered through Whatman No. 4 [18]. In Method 2, the procedure developed by Larrauri, Rupérez, and Saura-Calixto [19], with modifications, was employed as follows: 20 g fresh pulp was extracted sequentially with 40 mL methanol:water (50:50, v/v) at room temperature for 1 h and filtered through Whatman No. 4. The supernatant was recovered and extracted with 40 mL acetone:water (70:30, v/v) at room temperature for 60 min and filtered. Methanol and acetone extracts were combined and distilled water was added to make up the volume to 100 mL. Total flavonoid contents were determined by colorimetry, according to Subhasree *et al*. [20]. In the process, 250 µl extract was added to 1.5 mL distilled water and 150 µl 5% (m/v) $NaNO_2$. After 5 min, 300 µl 10% (m/v) $AlCl_3.6H_2O$ was added to the mixture, homogenized and left for 6 minutes at room temperature (25 ºC). Afterwards, 1.0 mL 1M NaOH was added to the mixture and the volume was adjusted to 5 mL. Absorbance of the mixture was measured at 510 nm. Quercetin was used as standard and results were expressed as g quercetin per kg^{-1} fresh fruit.

Total phenolic compounds and antioxidant activity

Total phenolic compounds were determined by the Folin–Ciocalteu assay, based on Waterhouse [21]. Results were expressed as mg gallic acid equivalent kg^{-1} FM (EAG kg^{-1}). An aliquot of crude extracts (100 µL) was mixed with 500 µL Folin–Ciocalteau reagent. Then, 7.4 mL water was added to it. After 1 min, a sodium carbonate solution (15%) was also added. After a 120-min reaction in the dark at room temperature, absorbance of the mixture was measured at 720 nm. The antioxidant activity was determined in crude extracts (Methods 1 and 2) by the ABTS, DPPH and FRAP methods. In the DPPH assay [22], 100 µL crude extract was added to 3.9 mL 60 µM 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution. After a 30-min incubation period at room temperature, in the dark, absorbance was measured at 515 nm. The antioxidant capacity was expressed as the concentration of antioxidant required to reduce the original amount of free radicals by 50% (EC_{50}). In the ABTS assay, ABTS radical cations ($ABTS^{\bullet+}$) were generated by reacting 5 mL aqueous ABTS solution (7 mM) and 88 µL potassium persulfate solution (140 mM) to make up the final concentration to 2.45 mM). The mixture, which was left in the dark for 14 h at room

temperature, was then diluted with ethanol to obtain absorbance of 0.7 ± 0.02 at 734 nm. Crude extracts (30 µL) were added to 3 mL ABTS radical solution in the dark and absorbance was measured at 734 nm after 6 min [23]. Antioxidant capacity of each sample was estimated by the FRAP assay, in agreement with the procedure described in the literature [24] with modifications. In the assay, 2.7 mL of freshly prepared FRAP reagent (TPTZ, $FeCl_3$ and acetate buffer) at 37 ºC was mixed with 90 µL fruit extract and 270 µL distilled water. A blank containing the FRAP reagent was used as reference and absorbance was determined at 595 nm after 30 min. Aqueous solutions of Fe (II) concentrations ranging from 100 to 1500 µM (Fe_2SO_4) were used for calibration.

Statistical analysis

Assays were performed in triplicate for each sample. Results were expressed as mean values ± standard deviation (SD). To prove significant differences between both extraction methods, the statistical analysis of the data was carried out by one-way analysis of variance, followed by the T test at 95% probability. To determine whether the bioactive compounds contributed to the antioxidant capacity, Pearson's correlation coefficients were calculated at 1% and 5% probability by the Student's t test for all variables.

RESULTS

The cerrado pear has a round base which decreases in longitudinal direction (piriformis format). Its skin is dark yellow and velvety. Inside, a yellow juicy pulp and cream-colored seeds can be found (Fig 1).

Figure 1. Photographic representation of cerrado pears (*E. klotzschiana* O.Berg*)*

The Table 1 showed physical characteristics of cerrado pear. Total titratable acidity (TTA) of the cerrado pear was 2.07 ± 0.01 g citric acid kg^{-1}, its pH was 3.00 ± 0.00 and total soluble solid (TSS) content was 6.00 ± 0.10 °Brix.

Table 1. Physical characteristics of the cerrado pear (*E. klotzschiana* O. Berg*)* from the Cerrado (Portelândia, Goiás, Brazil)

Variables	Mean[a] ± SD[b]	Amplitude	
		Minimum	Maximum
Longitudinal diameter (cm)	8.96±0.87	7.8	10.3
Transverse diameter (cm)	5.95±0.59	4.9	6.7
Mass (g)			
Fruit	101.73±29.24	74.42	172.53
Residue	38.34±13.90	24.20	72.29
Pulp	67.75±15.84	49.52	100.24
Pulp yield (g kg^{-1})	64.35±3.16	58.10	67.17

[a] Mean of 20 fruits

[b]Standard deviation

Table 2 shows the chemical characteristics of the cerrado pear pulp. The pulp had high moisture content (89.47g kg^{-1}) which makes the fruit lie within the class of fleshy and succulent tropical fruits. The fragility of the skin makes the cerrado pear highly susceptible to enzymatic and microbial deterioration, a fact that makes its preservation difficult.

Table 2. Chemical characteristics (g kg^{-1}) and total energy value (kcal kg^{-1}) of the cerrado pear (*E. klotzschiana* O. Berg) from the Cerrado (Portelândia, Goiás, Brazil).

Variables (expressed as fresh matter - FM)	Means ± Standard deviation
Moisture	89.47 ± 0.29
Ash	0.04 ± 0.00
Protein	0.59 ± 0.01
Lipids	2.35 ± 0.14
Carbohydrates	7.50 ± 0.40
Dietary fiber	6.45
Soluble fiber	1.36
Insoluble fiber	5.09
Caloric value	96.07 ± 2.33
Total sugars	1.35±0.03
Reducing sugars	1.15±0.01

Values of total, reducing and non-reducing sugars were 1.35, 1.15 and 0.2 g kg^{-1}, respectively, similar to the one found for murici (1.83 g kg^{-1}). These attributes affect consumers' acceptability directly [6]

Mineral composition of the cerrado pear pulp is shown in Table 3. Among the minerals evaluated in this work were the quantity of iron, manganese, copper, potassium, phosphorus, magnesium and zinc.

Table 3. Mineral contents of the cerrado pear (*E. klotzschiana* O. Berg*)* (Portelândia, Goiás, Brazil) and its contribution to dietary reference intake (%DRI).

Minerals (mg $100g^{-1}$ FM)	Cerrado pear	%DRI
Potassium	625.00	13.29
Phosphorus	131.00	18.71
Calcium	140.00	1.16
Magnesium	54.00	12.85
Iron	16.50	206.25
Manganese	1.10	47.82
Copper	0.57	63.33
Zinc	1.61	14.36

Since there is no information available in the literature regarding bioactive compounds, phenolic compound contents and antioxidant capacity of the cerrado pear, reference data on other small fruits were used by this study. Table 4 shows ascorbic acid, total chlorophyll and total carotenoids in β-caroteno and licopeno.

Table 4. Bioactive compounds (mg kg^{-1} fresh matter) of the cerrado pear (*E. klotzschiana* O. Berg) from the Cerrado (Portelândia, Goiás, Brazil)

Variables	Means± Standard deviation
Total chorophyll	0.660 ± 0.00
Total carotenoids (β-caroteno)	0.034 ± 0.01
Total carotenoids (licopeno)	0.055 ± 0.01
Ascorbic acid	8.660 ± 0.07

Table 5 shows results of total phenolic compounds (TPC), total flavonoids and antioxidant activity of the cerrado pear pulp by both extraction methods under study.

In this study, TPC content extracted by Method 2 (566 mg EAG kg^{-1}) was significantly higher than the one found by Method 1 (333 mg EAG kg^{-1}). Concerning flavonoid contents, Method 2 (550 mg EQ kg^{-1}) was significantly more efficient than Method 1 because its extraction was 2-fold higher than the one of the latter (225 mg EQ kg^{-1}).

In the DPPH assay, low EC_{50} value represented high antioxidant activity. The cerrado pear pulp had low EC_{50} in both extractions (0.74 µg mL^{-1} and 0.78 µg mL^{-1} in Method 1 and Method 2, respectively). In this study, EC_{50} of the cerrado pear was higher than the ones reported for other Cerrado native fruits, such as cagaita (14.15 µg mL^{-1}) [5].

Calculation of the antioxidant activity was equivalent to the edible part of the fruit (0.08 and 0.09 g pulp in Method 1 and Method 2, respectively) to neutralize 1 g $DPPH^{\bullet}$. The cerrado pear had 220.80 and 319.20 µmol TE g^{-1} in the ABTS assay in Method 1 and Method 2, respectively.

Table 5. Total flavonoids, total phenolic compounds and antioxidant activity of the cerrado pear pulp (*E. klotzschiana* O. Berg*)* from the Cerrado (Portelândia, Goiás, Brazil)

Variables	Method 1	Method 2
Total phenolic compounds (mg EAG kg^{-1})	333.41 ± 0,80[b]	566.33 ± 0.98[a]
Total flavonoids mg. EQ kg^{-1} FM)	225.00 ± 0.10[b]	550.00 ± 0.74[a]
Antioxidant activity - DPPH (EC_{50} µg mL^{-1})	0.74[a]	0.78[a]
Antioxidant activity - DPPH (g g^{-1} DPPH)	0.08[a]	0.09[a]
Antioxidant activity - ABTS (µmol TE.g^{-1} FM)	220.80[a]	319.20[b]
Antioxidant activity - FRAP (µM Fe^{2+} g^{-1} FM)	0.47 ± 0.01[b]	0.80 ± 0.01[a]

Mean ± standard deviation; n=4; TE: Trolox Equivalent; FM: fresh matter. Averages on the same line followed by different letters have significant difference ($p<0.05$).

Therefore, Method 2 had higher values of phenolics, flavonoids and antioxidant activity, thus, corroborating results found by other authors [4]. However, this comparison is important because the aqueous extraction is one of the most common forms of consumption by the population and involves low-cost reagents.

DISCUSSION

The pulp mass is the most important physical characteristic of the fruit in terms of economic use [25] (Table 1). The cerrado pear from Goiás had higher pulp yield (64.35 g kg^{-1}) than the one found in araticum (45.9 g kg^{-1}) (*Annona crassiflora* Mart) [26]. Also, from a conservation point of view, the determination of acidity is an important factor in defining the durability of a product. The pH and titratable acidity that were found by this study corroborate what had already been reported by Donadio and Moro [7], who consider the cerrado pear an acid fruit that increases our durability. The TSS/TTA ratio provides better evaluation of the fruit flavor, since it is more representative than isolated measurements of sugar contents or acidity [27]. The TSS/TA ratio found by this study was 2.89 ± 0.02, similar to the one reported for *Genipa americana* L. fruits (2.04) [25].

The comparison of the proximal compositions of the cerrado pear and other fruits from the Cerrado biome shows that the moisture content of the cerrado pear is similar to the ones of cagaita (*E. dysenterica* DC.) and murici (*Byrsonima crassifolia* L. RICH) (80.87 g $kg^{-1}$and 93.12 g kg^{-1}, respectively) [3,6]. Its protein content is similar to the ones of cagaita (0.60 and 0.63 g kg^{-1}, respectively) [28,3] whereas its lipid content is similar to the ones of araticum and coquinho-azedo (*Butia Capitata* Mart.) (2.14 and 2.73 g kg^{-1}, respectively) [29].

The cerrado pear has high fiber content, i. e., a portion of 100 grams of the fruit represents 25% of the daily requirement of dietary fiber recommended for an adult [30] Similar results were found by Bramorsk *et al*. [31] for camarinha (*Gaylussacia brasiliensis*) (6.53 g kg^{-1}), a fact that shows the potential of Cerrado native fruits and their contribution to the nutritional value of the human diet. Fiber is important to human health because it has the ability to absorb water in the large intestine, increase intestinal motility and reduce the risk of colon problems, such as constipation and cancer. Besides, fiber reduces serum levels of triglycerides and glucose [32]. Besides, since 100 g of an edible portion had 96.07 kcal, the caloric value of the cerrado pear is considered high [33].

The pulp gave important contribution to the DRI of iron, manganese, copper, potassium, phosphorus, magnesium and zinc. The zinc content was found cerrado pear pulp showed to be close to what had been reported by Fidalski [34] for orange, a common fruit very consumed in the human diet. Its iron content (16.5 mg kg^{-1}) was 55-fold higher than the one reported for common pears (0.30 mg kg^{-1}) [35]. Iron is an important mineral because it is responsible for the oxygen storage and use in the body blood [35].

As for the bioactive compounds the cerrado pear pulp presented low chlorophyll content, and carotenoid contents and vitamin C content important for human health. Low chlorophyll content found in the cerrado pear pulp was due to the maturation process, in which chlorophyll is degraded, thus, causing changes in the color of the pericarp to gradual extinction and increasing the synthesis or expression of carotenoid pigments [36]. Carotenoid contents of the cerrado pear pulp were 0.034 mg β-carotene kg^{-1} and 0.055 mg lycopene kg^{-1} FM. In the human organism, β-carotene undergoes oxidation, resulting in two aldehydes called retinal, which are converted to Vitamin A by biochemical reactions [36]. Vitamin C content found by this study for the cerrado pear pulp was similar to the one reported by Park *et al*. [37] for pear (8 mg kg^{-1}) (*Pyrus communis*). The intake of food containing vitamin C is required because of the important roles it plays in the human organism. Ascorbic acid acts as substrate in hydroxylation, wound healing, immune responses, nourishing the cells, and, therefore, essential to human metabolism. Besides, vitamin C acts as an antioxidant and can prevent oxidative damage [38].

In both extraction methods studied, TPC content of the cerrado pear (333-566 mg EAG kg^{-1}) was higher than the value reported by Rocha *et al*. [28] for acetone solution 70% (217 mg EAG kg^{1}). Thus, the cerrado pear had higher TPC than native fruits from the Cerrado, such as cagaita (111 mg EAG kg^{-1}), gabiroba (*Campomanesia adamantium*) (259 mg EAG kg^{-1}), pitanga (*Eugenia punicifolia*) (327 mg EAG kg^{-1}) [28] and uvaia (*Eugenia pyriformis* Cambess) (127 mg EAG kg^{-1}) [39]. In the classification proposed by Vasco, Ruales and Kamal-Eldin [40], in both extraction methods, the cerrado pear is classified as medium TPC (100-500 mg EAG kg^{-1}). Phenolic compounds are secondary metabolic products that are associated with mechanisms of adaptation and plant defense against ultraviolet rays, microorganisms and insects. Regarding human health, fruit and vegetable intake has been associated with low risks of chronic diseases. This effect is associated with polyphenols and their effects on the human organism [6].

Independent of the extraction methods, flavonoid contents found by this study were higher (225-550 mg EQ kg^{-1}) than the ones reported by Nascimento *et al*. [41] for other species of *Eugenia sp.* (16 mg EQ kg^{-1}). The intake of flavonoid-rich fruits is recommended because they increase the antioxidant capacity of the body and protect it against lipid peroxidation. Regarding flavonoids found in Brazilian fruits, the most frequently reported in literature are myricetin, quercetin and kaempferol.

The antioxidant activity by DPPH assay expressed in EC_{50} of the cerrado pear was more efficient that BHT (854 µg mL^{-1}), ascorbic acid (4 µg mL^{-1}) and gallic acid (2 µg mL^{-1}), which have been commonly used as antioxidants by the food industry. The antioxidant activity can be attributed to the presence of ascorbic acid, phenolic compounds and carotenoids, substances which have been confirmed as potent antioxidants [39].

The values of antioxidant activity by ABTS assay for two methods (220.80-319.20 µmol TE g^{-1}) were similar to the ones of guava (*Psidium cattleyanum* Sabine) (242.30 µmol ET g^{-1}) and uvaia (336.29 µmol ET g^{-1}) reported by Pereira *et al*. [27]. In the FRAP method, the cerrado pear had 0.47 µM $Fe^{2+}g^{-1}$ FM and 0.80 µM Fe^{2+} g^{-1} FM in Method 1 and Method 2, respectively. Similar values were found for *Canarium odontophyllum* fruits (0.27 and 1.74 µM Fe^{2+} g^{-1} FM) [42].

CONCLUSION

This study provides information about *E. klotzschiana* O. Berg fruits, which are unknown and unexplored in the Brazilian Cerrado. It contributes to develop knowledge and stimulate sustainable use of these fruits by the food industry and the population.

The cerrado pear showed that it has high nutritional value, mainly high fiber and iron contents. The cerrado pear proved that it is a very interesting fruit regarding its carotenoids, flavonoids, phenolic compounds, ascorbic acid compositions and, consequently, its antioxidant activity. Thus, the cerrado pear can be considered a good source of these compounds and can be consumed as a result of the sustainable use of the Cerrado biome.

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REFERENCES

1. Myers, N.; Mittermeier, R.A; Mittermeier, C.G; Fonseca, G.A.B.; Kent, J. Biodiversity hotspots for conservation priorities. *Nature* 2000, *403*, 853-858.
2. Kaur, C.; Kapoor, H.C. Antioxidants in fruits and vegetables – the millennium's health. *Int J Food Sci Tech* 2001, *36*, 703-725.
3. Cardoso, L.M.; Martino, H.S.D.; Moreira, A.V.B.; Ribeiro, S.M.R.; Sant'Ana, H.M.P. Cagaita (*Eugenia dysenterica* DC.) of the Cerrado of Minas Gerais, Brazil: Physical and chemical characterization, carotenoids and vitamins. *Food Res Int* 2011, *44*, 2151–2154.
4. Almeida, M.M.B.; Sousa, P.H.M.; Arriaga, A.M.C.; Prado, G.M.; Magalhães, C.E.C.; Maia, G.A.; Lemos, T.L.G. Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Res Int* 2011, *44*, 2155-2159.
5. Roesler, R.; Malta, L.G.; Carrasco, L.C.; Pastore, G. Evaluation of the antioxidant properties of the Brazilian Cerrado fruit *Annona crassiflora* (araticum). *J Food Sci* 2006, *71*, 2.
6. Souza, V.R.; Pereira, P.A.P.; Queiroz, F.; Borges, S.V. Carneiro, J.D.S. Determination of bioactive compounds, antioxidant activity and chemical composition of Cerrado Brazilian fruits. *Food Chem* 2012, *134*, 381-386.
7. Donadio, L.C.; Moro, F.V. Potential of brazilian *Eugenia myrtaceae* - as ornamental and as a fruit crop. *Acta Hortic* 2004, *632*, 65-68.
8. Faria Junior, J.E.Q. *O gênero Eugenia (Myrtaceae) nos estados de Goiás e Tocantins, Brasil.* Master's thesis in Botanics. Universidade de Brasília, Brasília, 2010.
9. Ribeiro, J.F.; Silva, J.C.; Batmanian, G.J. Fitossociologia de tipos fisionômicos do Cerrado em Planaltina-DF. *Rev Bras Botân* 1985, *8*, 131-142.
10. Zenebon, O.; Pascuet, N.S.; Tiglea, P. *Métodos físico-químicos para análise de alimentos*. Instituto Adolfo Lutz: São Paulo, Brazil, 2008; pp. 85-158.
11. *Association of Official Analytical Chemists Official methods of analysis of the Association of Official Analytical Chemists* - AOAC, 16rd ed; AOAC: Washington, Brazil, 2008.
12. Merril, A.L.; Watt, B.K. *Energy value of foods: Basis and derivation*, United States Department of Agriculture: Washington, US, 1973; pp. 105
13. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chim* 1959, *31*, 426- 428.
14. Malavolta, E.; Vitti, G.C.; Oliveira, S.A. *Avaliação do estado nutricional das plantas: princípios e aplicações*, 2sd ed.; Potafos: Piracicaba, 1997, pp. 319.
15. Nagata, M. Yamashita, I. Simple method for simultaneous determination of chlorophyll an carotenoids in tomato fruit. *J Japan Soc Food Sci Technol* 1992, *39*, 925-928.
16. Arnon, D.I. Copper enzymes in isolated chloroplasts polyphenoloxidase in beta vulgaris. *Plant Physiol* 1949, *24*, 1–15.
17. Benassi, M.T.; Antunes, A.J. A comparison of meta-phosphoric and oxalic acids as extractant solutions for the determination of vitamin C in selected vegetables. *Arq Biol Tech* 1988, *31*, 507-513.
18. Lima, C.A.; Faleiro, F.G.; Junqueira, N.T.V.; Cohen, K.O.; Guimarães, T.G. Características físico-químicas, polifenois e flavonoides amarelos em frutos de espécies de pitaias comerciais e nativas do cerrado. *Rev Bras Frutic* 2013, *35*, 565-570.
19. Larrauri JA, Rupérez P and Saura-calixto F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *J Agric Food Chem* 1997, *45*, 1390-1393.
20. Subhasree, B.; Baskar, R.; Laxmi keerthana, R.; Lijina Susan, R.; Rajasekaran, P. Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chem* 2009, *115*, 1213–1220.
21. Waterhouse, A.L. Polyphenolics: Determination of total phenolics. In *Current Protocols in Food Analytical Chemistry*; Wrolstad R.E., Ed., John Wiley & Sons: New York, US, 2002; pp. I1.1.1–I1.1.8.
22. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol* 1995, *28*, 25–30.
23. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay free radical. *Biol Med* **1999**, *26*, 1231–1237.

24. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Bioch* 1996, *239*, 70–76.
25. Hamacek, F.; Moereira, A.V.B.; Martino, H.S.D.; Ribeiro, S.M.R.; Pinheiro-Santana, H.M. Valor nutricional, caracterização física e físico-química de jenipapo (Genipa americana L.) do cerrado de Minas Gerais. *Alim Nutr* 2013, *24*, 73-77.
26. Pimenta, A.C.; Rosa, P.S.; Zuffellato-Ribas, K.C.; Koehler, H.S. Caracterização de plantas e de frutos de araticunzeiro (*Annnona crasssiflora* Mart.) nativos no cerrado matogrossense. *Rev Bras Frutic* 2014, *36*, 892-899.
27. Pereira, M.C.; Steffens, R.S.; Jablonski, A.; Hertz, P.F.; Rios, A.O.; Vizzotto, M.; Flores, S.H. Caracterization an antioxidant potential of Brazilian fruits from the Myrtaceae family. *J Agric Food Chem* 2012, *60*, 3061−3067.
28. Rocha, W.S.; Lopes, R.M.; Silva, D.B.; Vieira, R.F.; Silva, J.P.; Agostini-Costa, T.S. Compostos fenólicos totais e taninos condensados em frutas nativas do cerrado. *Rev Bras Frutic* 2011, *33*, 1215-1221.
29. Lopes, R.M.; Silva, J.P.; Vieira, R.F.; Silva, D.B.; Gomes, I.S. Composição de ácidos graxos em polpa de frutas nativas do cerrado. *Rev Bras Frutic* 2012, *34*, 635-640.
30. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes. National Academic Press, Washington D.C. (1999–2011)
31. Bramorski, A.; Cherem, A.R.; Mezadri, T.; Melo, S.S.; Deschamps, F.C.; Gonzaga, L.V.; Rochenbach II and Fett R. Chemical composition and antioxidante activity of *Gaylussacia brasiliensis* (camarinha) grown in Brazil. *Food Res Int* 2011, *44*, 2134–2138.
32. Lai, T.N.H.; Andre, C.; Rogez, H.; Mignolet, E.; Nguyen, T.B.T.; Larondelle, Y. Nutritional composition an antioxidant property of the sim fruit (*Rhodomyrtus tomentosa*). *Food Chem* 2015, *168*, 410–416.
33. Brasil. Resolução - RDC nº 54, de 12 de novembro de 2012. Dispõe sobre o Regulamento Técnico sobre Informação Nutricional Complementar. Diário Oficial da União 19 nov. 2012.
34. Fidalski, J.; Auler, P.A.M.; Tormem, V. Relations among Valencia orange yields with soil and leaf nutrients in northwestern Paraná, Brazil. *Braz Arch Biol Technol* 2000, *43*, doi: 10.1590/S1516-89132000000400006.
35. USDA-ARS (US Department of Agriculture, Agricultural Research Service) USDA nutrient database for standard reference, Release 25, Software 1.2.2, from the Nutrient Data Laboratory Page on the World Wide Web. 2015. http://www.nal.usda.gov/fnic/foodcomp [7 October 2016].
36. Schoefs, B. Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis. *Trends Food Sci Technol* 2002, *13*, 361–371.
37. Park, Y.I.M.H.; Ham, K.; Kang, S.; Park, Y.N.J.; Leontowicz, H.; Leontowicz, M.; Trakhtenberg, S.; Gorinstein, S. Quantitative assessment of the main antioxidant compouns, antioxidant activities and FTIR spectra from commonly consumed fruits, compared to standard kiwi fruit. *Food Sci Technol* 2015, *63*, 346-352.
38. Oliveira, M.C.; Schoffen, J.P.F. Oxidative Stress Action in Cellular Aging. *Braz Arch Biol Technol* 2010, *55*, 1333-1342.
39. Rufino, M.S.M.; Alves, R.E.; Brito, E.S.; Pérez-Jiménez, J.; Saura-Calixto, F.; Mancini-Filho, J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chem* 2010, *121*, 996-1002.
40. Vasco, C.; Ruales, J.; Kamal-Eldin, A. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chem* 2008, *111*, 816–823.
41. Nascimento, V.T.; Moura, N.P.; Vasconcelos, M.A.S.; Maciel, M.I.S.; Albuquerque, U.P. Chemical characterization of native wild plants of dry seasonal forests of the semi-arid region of northeastern Brazil. *Food Res Int* 2011, *44*, 2112-2119.
42. Chew, L.Y.; Nagendra, P.; Amin, I.; Azrina, A.; Lau, C.Y. Nutritional composition and antioxidant properties of *Canarium odontophyllum* Miq (dabai) fruits. *J Food Comp Anal* 2011, *24*, 670–677.