

## Brazilian Cerrado fruit araticum (*Annona crassiflora* Mart.) as a potential source of natural antioxidant compounds

\*Arruda, H. S., Pereira, G. A. and Pastore, G. M.

*Bioflavors and Bioactive Compounds Laboratory, Department of Food Science, School of Food Engineering, University of Campinas, Monteiro Lobato Street, 80, Campinas P.O. Box 13083-862, São Paulo, Brazil*

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### Abstract

Araticum is an underutilized Brazilian Cerrado fruit with few reports in the literature about its bioactive compounds and functional properties. This study aimed to determine the amount of total phenolic compounds, total flavonoids and antioxidant activity of araticum pulp. Total phenolic compounds and total flavonoids were determined spectrophotometrically using the Folin-Ciocalteu and aluminium chloride colorimetric assays, respectively. In addition, antioxidant activity from araticum fruit was measured by DPPH, TEAC and ORAC<sub>FL</sub> assays. The araticum pulp showed high amount of total phenolic compounds (2.62 g GAE/100 g dw) and total flavonoids (1.79 g CE/100 g dw). Flavonoids were the predominant class of phenolic compounds, representing about 68% of the phenolic compounds content. Furthermore, the fruit showed high antioxidant activity for all assays performed (93.76 µg/mL, 231.79 µmol TE/g and 902.27 µmol TE/g for DPPH IC<sub>50</sub>, TEAC and T-ORAC<sub>FL</sub>, respectively). Quercetin showed the most potent antioxidant activity in DPPH• and ABTS<sup>•+</sup> radical scavenging capacities, whereas the kaempferol was the compound with the highest scavenging capacity peroxyl radicals. Araticum fruit showed phenolic compounds content and antioxidant activity hardly found in common fruits. These results indicate that araticum is a potential source of bioactive compounds.

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### Introduction

Reactive species are highly reactive molecules containing one or more unpaired electrons in atomic or molecular orbitals which can be generated from environmental factors (smoking, certain drugs, pollution, poor diet, sedentary lifestyle and stress-inducing agents) and during normal cellular metabolism (respiration, phagocytosis, intoxication and fatty acid metabolism) (Thatoi *et al.*, 2013; Martins *et al.*, 2015). When there is an imbalance between the production of reactive species and antioxidants these species can join with cellular components (DNA, lipids and proteins) and destroy them. This condition known as oxidative stress is involved in the generation or aggravation several of pathological conditions (Schaffer *et al.*, 2016), such as metabolic, neurodegenerative, cardiovascular and mitochondrial diseases, aging process and even cancer (Martins *et al.*, 2016).

Fruits are notable sources of antioxidants that can provide protection against disorders promoted by oxidative stress (Malta *et al.*, 2012). Natural antioxidants present in food have attracted the interest of various sectors of society because of

their safety and potential nutritional and therapeutic effects (Souza *et al.*, 2012). Antioxidants are defined as one heterogeneous family of molecules, which can prevent or reduce the extension of the oxidative damage to lipids, proteins and nucleic acids as they scavenge reactive species. The most abundant antioxidants in fruits are the phenolic compounds (mostly flavonoids), carotenoids and vitamins A, B, C and E (Lim *et al.*, 2007).

Brazil boasts one of the richest biodiversity in the world, which includes a large number of fruit species. However, many native and exotic fruits species in Brazil are underexploited or unknown (Souza *et al.*, 2012). In the last years, the Cerrado fruits have attracted the attention of consumers and researchers because to their nutritional and functional properties combined with the potential to add value and preserve the biodiversity of this biome (Malta *et al.*, 2013). The Cerrado is the second largest biome in Brazil occupying approximately 25% of the country and it is considered one of the 25 sites of greatest biodiversity in the world. The Cerrado species covers about 30% of all existing species in Brazil, it is estimated the presence of approximately 11,000 plant species, 837 bird species and 199 mammal species (Arruda and

\*Corresponding author.

Email: [hsilvanoarruda@gmail.com](mailto:hsilvanoarruda@gmail.com), [henriquesilvano@live.com](mailto:henriquesilvano@live.com)

Almeida, 2015).

Cerrado flora holds several fruit species with great potential for food exploration, since they contain high levels of sugars, proteins, vitamins, minerals and fibers, besides attractive sensory characteristics such as peculiar and intense color, flavor and aroma (Arruda *et al.*, 2014; Arruda *et al.*, 2015). Among the Cerrado fruit species, the araticum (*Annona crassiflora* Mart.) stands out for its strong presence in this region and for its excellent sensory characteristics, much appreciated by the local population (Silva *et al.*, 2013), and it is among the 20 most commonly used species in regional food preparation (Arruda *et al.*, 2017). Araticum belongs to the Annonaceae family and receives various other popular names such as “araticum-do-Cerrado”, “ariticum”, “articum”, “marolo”, “bruto”, “cabeça-de-negro” and “pasmada” (Villela *et al.*, 2013). Several parts of the plant are used in traditional medicine to treat wounds, sexually transmitted diseases (STDs), ophidian bites, degenerative diseases, as well as antimicrobial, antidiarrheal, antitumor and antirheumatic agents and in the combat of lice (Dragano *et al.*, 2010). The fruit pulp is very popular and rich in fibers, carotenoids, vitamins A and E, ascorbic acid and folates (Cardoso *et al.*, 2013). Recently, studies have showed that the fruit presents biological properties such as antimicrobial (Silva *et al.*, 2014) and hepatoprotective activities (Roesler, 2011).

Despite the great potential presented by this fruit, there are few reports in the literature about its amount of bioactive compounds and antioxidant activity. In this context, this study aimed to determine the amount of total phenolic compounds, total flavonoids and antioxidant activity of araticum pulp in order to provide relevant information to support its application as functional food and/or ingredient.

## Material and Methods

### Chemicals

Gallic acid, (+)-catechin, (-)-epicatechin, (-)-gallocatechin, (-)-epigallocatechin, myricetin, quercetin, kaempferol, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,2'-azobis(2-methylamidinopropane)-dihydrochloride (AAPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS) and randomly methylated beta-cyclodextrin (RMBC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate, methanol and Folin-Ciocalteu reagent were provided by Dinâmica (Diadema, SP,

Brazil). All others chemical and solvents were of analytical grade.

### Plant material and sample preparation

Araticum fruits (*Annona crassiflora* Mart.) with full physiological maturity were collected during harvest season (from January to March) in the rural areas of the municipality of Carmo do Paranaíba, Minas Gerais State, Brazil. The fruits were washed with tap water to remove surface dirt. The fruit pulp was manually separated from the seeds and peel, freeze-dried (LIOTOP, model L101, São Carlos, Brazil), and ground using a knife grinder (Marconi, model MA340, Piracicaba, Brazil). The powder obtained was stored at -80°C until analysis.

### Extraction procedure

The extraction conditions were defined according to the analysis protocols of total phenolic compounds, total flavonoids and antioxidant activity (Leite-Legatti *et al.*, 2012; Pavan *et al.*, 2014) with slight modifications. Briefly, 10 mg of freeze-dried fruit pulp were transferred to a microtube and mixed with 1.0 mL of corresponding solvent to each analysis (total phenolic compounds and DPPH assays: methanol; total flavonoids and TEAC assays: ultrapure water; H-ORAC<sub>FL</sub> assay: potassium phosphate buffer (75 mM, pH 7.4); and L-ORAC<sub>FL</sub> assay: RMCD 7% (w/v; acetone:water 1:1). The mixtures were ultrasonicated (UNIQUE, model UCS-2850, 25 kHz, 120W, Brazil) for 30 minutes at 10°C and then centrifuged (Hettich Zentrifugen, model Rotanta 460R, Tuttlingen, Germany) at 10,000 rpm for 11 minutes at 5°C. The supernatants were used for the analysis of phenolic compounds and antioxidant activity.

### Total phenolic compounds (TPC)

TPC were determined using the Folin-Ciocalteu method according to Cicco *et al.* (2009) with modifications. Briefly, 100 µL of extract were mixed with 100 µL of Folin-Ciocalteu reagent (50% v/v) and 800 µL of sodium carbonate (5% w/v). The mixtures were incubated at 40°C for 20 minutes. The absorbance was measured at 760 nm against a blank on a spectrophotometer (Beckman, model DU600, CA, USA). The results were expressed as g of gallic acid equivalent per 100 g of dried weight (g GAE/100g dw).

### Total flavonoids content (TFC)

TFC were measured using a colorimetric assay developed by Zhishen *et al.* (1999). Aliquots of 28 µL of extract and 110 µL of ultrapure water were homogenized and 8 µL of NaNO<sub>2</sub> (5% w/v)

was added. After 2 minutes, 8  $\mu\text{L}$  of  $\text{AlCl}_3$  (10% w/v) was added and the mixture was incubated for 3 minutes. Then, 56  $\mu\text{L}$  of NaOH (1 mol/L) was added to the mixture, and the reaction was diluted with the addition of 67  $\mu\text{L}$  of ultrapure water. The absorbance was measured at 510 nm against a blank on a NovoStar Microplate reader (New Brunswick Scientific Classic Series, model C76, NJ, USA). The results were expressed as g of catechin equivalent per 100 g of dried weight (g CE/100g dw).

#### *DPPH scavenging assay*

The DPPH assay was performed according to Leite-Legatti *et al.* (2012). Briefly, 250  $\mu\text{L}$  of DPPH (0.004% w/v) was mixed with 50  $\mu\text{L}$  of extract in different concentrations. After 30 minutes of the reaction at room temperature in the dark, the absorbance of the remaining DPPH was measured at 517 nm against blank on a NovoStar Microplate reader (New Brunswick Scientific Classic Series, model C76, NJ, USA). Trolox was used as the antioxidant standard and the results were expressed as  $\mu\text{mol TE/g dw}$  and  $\text{IC}_{50}$ .

#### *Trolox equivalent antioxidant capacity (TEAC) assay*

The TEAC assay was based on the reports of Leite-Legatti *et al.* (2012) and Pavan *et al.* (2014). The radical cation  $\text{ABTS}^{+\cdot}$  was chemically generated using 88  $\mu\text{L}$  of potassium persulfate (140 mmol/L) and 5 mL of ABTS (7 mmol/L), leaving it to react for 12 hours at room temperature in the dark. The radical was diluted with ultrapure water until reaching an absorbance of  $0.70 \pm 0.02$  at 734 nm. In brief, 250  $\mu\text{L}$  of  $\text{ABTS}^{+\cdot}$  solution was mixed with 50  $\mu\text{L}$  of extract. After 6 minutes of reaction at room temperature in the dark, the absorbance measurements were carried out at 734 nm against blank on a NovoStar Microplate reader (New Brunswick Scientific Classic Series, model C76, NJ, USA). Trolox was used as the antioxidant standard and the results were expressed as  $\mu\text{mol TE/g dw}$ .

#### *Oxygen radical absorbance capacity (ORAC<sub>FL</sub>) assay*

The automated ORAC<sub>FL</sub> (Oxygen Radical Absorbance Capacity) assays were performed on a NovoStar Microplate reader (New Brunswick Scientific Classic Series, model C76, NJ, USA) with fluorescence filters (excitation,  $\lambda$  485 nm; emission  $\lambda$  520 nm). The experiments were conducted at 37°C under pH 7.4 condition with a blank in parallel. For hydrophilic ORAC<sub>FL</sub> (H-ORAC<sub>FL</sub>) assay, 20  $\mu\text{L}$  of extract, 120  $\mu\text{L}$  of fluorescein (0.387  $\mu\text{g/mL}$ ) and 60  $\mu\text{L}$  of AAPH (108 mg/mL) were mixed. The potassium phosphate buffer (pH 7.4, 75 mM) was

used as solvent. The lipophilic ORAC<sub>FL</sub> (L-ORAC<sub>FL</sub>) assay was conducted as follows: 20  $\mu\text{L}$  of extract, 120  $\mu\text{L}$  of fluorescein (0.387  $\mu\text{g/mL}$ ) and 120  $\mu\text{L}$  of AAPH (108 mg/mL) were mixed. The randomly methylated beta-cyclodextrin (RMCD 7% w/v; acetone:water 1:1) was used as solvent. The results were expressed as  $\mu\text{mol TE/g dw}$  and were calculated using the differences of areas under fluorescence decay curves between the sample and blank.

#### *Data analysis*

Results were statistically evaluated for significance by using one-way analysis of variance (ANOVA) followed by the Tukey's HSD test ( $p \leq 0.05$ ) using STATISTICA software (Statsoft, Oklahoma, USA) version 12.0. All determinations were carried out in triplicate, and the values were reported as the mean  $\pm$  standard deviation.

## **Results and Discussion**

#### *Total phenolic compounds (TPC) and total flavonoids content (TFC) in araticum pulp*

The Cerrado fruits are known for being rich in phenolic compounds. According to Vasco *et al.* (2008) the levels of phenolic compounds in fruits can be arranged into three categories: low (<100 mg GAE/100g fw), intermediate (100-500 mg GAE/100g fw) and high (>500 mg GAE/100g fw). As seen in Table 1, our results showed that araticum pulp was rich in phenolic compounds (2.62 g GAE/100g dw or 739.92 mg/100g fw). Furthermore, the TPC content found in the araticum pulp was similar or higher than previously reported by Souza *et al.* (2012) (739.37 mg GAE/100g fw), Villela *et al.* (2013) (423.94 mg GAE/100g fw and 1,245.44 mg GAE/100g of pulp flour), Damiani *et al.* (2011) (211.50 and 260.50 mg GAE/100g dw in the hydro-ethanolic and aqueous extracts, respectively), Siqueira *et al.* (2013) (580 and 1,095 mg GAE/100g dw in the ethyl acetate and aqueous extracts, respectively) and Roesler *et al.* (2006) (2.03-3.11 and 1.69-1.70 g GAE/100g dw in the hydro-ethanolic and aqueous extracts, respectively).

Among the various groups of phenolic compounds present in nature the flavonoids stand out for being the most common and widely distributed in the plant kingdom, being found in practically all plant parts, especially in photosynthetic cells (Kumar and Pandey, 2013). TFC in araticum pulp was 1.79 g CE/100g dw or 505.98 mg GAE/100g fw, accounting for approximately 68% ( $R_{\text{TFC-TPC}} = 0.68$ ) of the TPC from araticum pulp (Table 1). Data of the current study were higher than those reported by Villela *et*

Table 1. Total phenolic compounds (TPC), total flavonoid content (TFC) and antioxidant activity by DPPH, TEAC and ORAC<sub>FL</sub> assays found in araticum pulp.

Antioxidant assays <sup>a</sup>	Content <sup>b</sup>
TPC (g GAE/100 g dw)	2.62±0.11
TFC (g CE/100 g dw)	1.79±0.03
R <sub>TFC-TPC</sub>	0.68±0.02
DPPHIC <sub>50</sub> (µg/mL)	93.76±2.74
DPPH (µmol TE/g dw)	306.04±9.93
TEAC (µmol TE/g dw)	231.79±8.65
H-ORAC <sub>FL</sub> (µmol TE/g dw)	337.25±5.78
L-ORAC <sub>FL</sub> (µmol TE/g dw)	565.02±13.25
T-ORAC <sub>FL</sub> (µmol TE/g dw)	902.27±11.26

<sup>a</sup>TPC: total phenolic compounds; TFC: total flavonoids content; R<sub>TFC-TPC</sub>: relationship between total flavonoids and total phenolic compounds; DPPH IC<sub>50</sub>: radical scavenging activity expressed as final concentration of sample in the cuvettes defined as µg/mL of the araticum pulp powder required to decrease or inhibit 50% of the initial DPPH concentration; DPPH: DPPH radical scavenging activity; TEAC: trolox equivalent antioxidant capacity; ORAC<sub>FL</sub>: oxygen radical absorbance capacity; H-ORAC<sub>FL</sub>: Hydrophilic ORAC<sub>FL</sub>; L-ORAC<sub>FL</sub>: Lipophilic ORAC<sub>FL</sub>; T-ORAC<sub>FL</sub>: Total ORAC<sub>FL</sub> calculated as the sum of H-ORAC<sub>FL</sub> and L-ORAC<sub>FL</sub>.

<sup>b</sup>All determinations were carried out in triplicate, and the values were reported as the mean ± standard deviation.

*al.* (2013) (63.14 mg CE/100g fw and 201.32 mg CE/100g pulp flour) and Siqueira *et al.* (2013) (4.58 mg/100g fw). Julián-Loeza *et al.* (2011) reported values between 107.41 and 152.04 mg CE/100g fw in varieties of *Annona diversifolia* (ilama), while Paz *et al.* (2015) found the amount of 252 mg CE/100g dw in *Annona muricata* (soursop).

#### Antioxidant activity in araticum pulp

The antioxidant activity of food is determined by a mixture of different antioxidants that have different action mechanisms. Therefore, the antioxidant activity of food products must be evaluated by more than one method so that the different action mechanisms can be addressed and we can thus obtain useful information about the antioxidant activity of all compounds present in the matrix (Moo-Huchin *et al.*, 2014). The antioxidant activity of a compound and/or complex mixtures is often evaluated through methods based on the stable free radical scavenge (for example, DPPH• and ABTS<sup>•+</sup>) by antioxidants and/or on the ability of the antioxidant in protecting a target molecule exposed to a source of free radicals (for example, ORAC<sub>FL</sub>) (López-Alarcón and Denicola, 2013). To characterize the antioxidant potential of the araticum pulp, the DPPH, TEAC and ORAC<sub>FL</sub> assays were used, and the results are shown in Table 1.

The DPPH assay is widely used to evaluate the antioxidant activity of aqueous/organic extracts of food matrices with hydrophilic and lipophilic

compounds (Moo-Huchin *et al.*, 2014). This assay is based on the ability of antioxidants of the sample in reducing the DPPH• radical by transferring an electron, which is measured by the decrease in absorption at 517 nm. Results may be expressed in µmol TE/g and IC<sub>50</sub>. Low values of IC<sub>50</sub> indicate high antioxidant activity. The amount of extract required to reduce the initial concentration of the DPPH• radical by 50% (IC<sub>50</sub>) was 93.76 µg/mL (Table 1). The extract obtained in this study had higher antioxidant activity than previously reported by Roesler *et al.* (2006) (148.82 and 1,321.93 µg/mL dw for the ethanol and aqueous extracts, respectively) and Damiani *et al.* (2011) (894, 1,996 and 552 µg/mL dw for the ethereal, ethanol and aqueous extracts, respectively). The variation of the IC<sub>50</sub> values between studies may be related to differences in the parameters of extraction processes such as extraction mechanism, solvent-solid ratio, type of solvent, extraction time and number of re-extractions (Damiani *et al.*, 2011). The DPPH IC<sub>50</sub> assay was also used to evaluate the antioxidant activity of seven phenolic compounds commonly present in fruit pulp (Table 2). The ability of phenolic compounds in scavenging DPPH• radicals presented the following order: quercetin > myricetin > (-)-epigallocatechin > (-)-epicatechin > (-)-gallocatechin > (+)-catechin > kaempferol. The DPPH IC<sub>50</sub> values for the phenolic compounds varied between 3.02 and 7.43 µg/mL, being quercetin the compound with higher antioxidant activity and kaempferol the least active. These values were much lower than that one found in araticum pulp extract (93.76 µg/mL). However, taking into consideration that the concentration of phenolic compounds in fruit extracts is relatively low compared to the individual phenolic compounds (tested in pure form) and the wide variety of antioxidant compounds in the araticum pulp with different action mechanisms, the extracts obtained can be considered as efficient free radical scavengers. The araticum pulp also showed high antioxidant activity by the DPPH assay (306.04 µmol TE/g dw). A previous study with araticum pulp reported values of 54.3 and 67.3 µmol TE/g fw for the ethyl acetate and aqueous extracts, respectively (Siqueira *et al.*, 2013).

The TEAC method is another antioxidant assay widely used to determine the antioxidant activity of extracts of food matrices and is indicated for the assessment of hydrophilic compounds (Moo-Huchin *et al.*, 2014). This assay is based on the ability of antioxidants of the sample in reducing the ABTS<sup>•+</sup> radical by transferring an electron and/or hydrogen atom, which is measured by the decrease in absorption at 734 nm (Gülçin, 2012). As seen in

Table 2. Antioxidant activity of phenolic compounds commonly found in fruits.

Standard	Antioxidant assays <sup>a,b</sup>			
	DPPH IC <sub>50</sub> (µg/mL)	DPPH (mol TE/mol standard) <sup>c</sup>	TEAC (mol TE/mol standard) <sup>c</sup>	H-ORAC <sub>FL</sub> (mol TE/mol standard) <sup>c</sup>
(+)-Catechin	4.18±0.03 <sup>f</sup>	2.10±0.06 <sup>d</sup>	4.42±0.12 <sup>e</sup>	12.96±0.70 <sup>cc</sup>
(-)-Epicatechin	3.72±0.06 <sup>d</sup>	2.20±0.06 <sup>cd</sup>	4.63±0.17 <sup>e</sup>	12.43±0.51 <sup>c</sup>
(-)-Gallocatechin	3.94±0.04 <sup>e</sup>	2.71±0.24 <sup>ab</sup>	6.25±0.08 <sup>ab</sup>	2.85±0.08 <sup>e</sup>
(-)-Epigallocatechin	3.53±0.06 <sup>c</sup>	2.72±0.05 <sup>ab</sup>	5.94±0.04 <sup>b</sup>	2.80±0.11 <sup>e</sup>
Myricetin	3.24±0.06 <sup>b</sup>	2.56±0.05 <sup>bc</sup>	6.02±0.13 <sup>b</sup>	5.53±0.30 <sup>d</sup>
Quercetin	3.02±0.07 <sup>a</sup>	3.10±0.28 <sup>a</sup>	6.39±0.21 <sup>a</sup>	14.02±0.11 <sup>b</sup>
Kaempferol	7.43±0.09 <sup>g</sup>	1.10±0.04 <sup>e</sup>	1.51±0.03 <sup>d</sup>	15.60±0.37 <sup>a</sup>

<sup>a</sup>DPPH IC<sub>50</sub>: radical scavenging activity expressed as final concentration of sample in the cuvettes defined as µg/mL of the araticum pulp powder required to decrease or inhibit 50% of the initial DPPH concentration; DPPH: DPPH radical scavenging activity; TEAC: trolox equivalent antioxidant capacity; ORAC<sub>FL</sub>: oxygen radical absorbance capacity; H-ORAC<sub>FL</sub>: Hydrophilic ORAC<sub>FL</sub>.

<sup>b</sup>All determinations were carried out in triplicate, and the values were reported as the mean ± standard deviation. Equal letters in the same column do not differ significantly by Tukey's test ( $p > 0.05$ ).

<sup>c</sup>The highest values of mol TE/mol standard represent the standards with higher antioxidant activity.

Table 1, the araticum pulp showed high antioxidant activity through the TEAC assay (231.79 µmol TE/g dw). Souza *et al.* (2012) previously reported a value of 131.58 µmol TE/g fw. In addition, the TEAC values found in this study were similar, lower or higher, depending on the fruit under study, to other non-traditional Brazilian fruits rich in antioxidants, such as carnaúba (16.4 µmol TE/g), bacuri (18.1 µmol TE/g), cajá (40.7 µmol TE/g), açaí (64.5 µmol TE/g), mangaba (65.6 µmol TE/g), umbu (77 µmol TE/g), caju (79.4 µmol TE/g), jambolão (125 µmol TE/g), gurguri (136 µmol TE/g), puçá-coroa-de-frade (161 µmol TE/g), murta (166 µmol TE/g), uvaia (182 µmol TE/g), jabuticaba (317 µmol TE/g), puçá-preto (346 µmol TE/g), murici (412 µmol TE/g), juçara (606 µmol TE/g), acerola (953 µmol TE/g) and camu-camu (1,237 µmol TE/g) (Rufino *et al.*, 2010).

The ORAC<sub>FL</sub> assay has advantages over other *in vitro* antioxidant methods as it is conducted under physiological conditions of temperature (37°C) and pH (7.4), besides acting on peroxy radicals (ROO•), which are produced naturally by the human metabolism. The ORAC<sub>FL</sub> is based on a competitive system in which antioxidant and substrate compete kinetically for peroxy radicals and can be adapted for both hydrophilic and lipophilic antioxidants by altering the radical and/or solvent source. This assay measures the antioxidant ability of a compound by inhibiting peroxy radicals through the transfer of

hydrogen atoms, which is determined through the fluorescence decay curve over time (Gülçin, 2012). In this study we determined the ORAC<sub>FL</sub> in the hydrophilic (H-ORAC<sub>FL</sub>) and lipophilic (L-ORAC<sub>FL</sub>) fractions of the araticum pulp. The lipophilic fraction of araticum pulp (565.02 µmol TE/g dw) had greater ability to inhibit peroxy radicals as compared to the fraction hydrophilic (337.25 µmol TE/g dw). Previous study have shown that, besides phenolic compounds, the araticum pulp is rich in lipophilic antioxidant compounds such as carotenoids (lycopene, α- and β-carotene) and vitamin E (Cardoso *et al.*, 2013). These lipophilic compounds can synergistically interact with phenolic compounds explaining the highest antioxidant activity of the lipophilic fraction.

The araticum pulp presented compounds with high antioxidant potential. Several studies have shown a high correlation between the amount of TPC and the antioxidant activity (Roesler *et al.*, 2007; Vasco *et al.*, 2008; Rufino *et al.*, 2010; Almeida *et al.*, 2011; Luzia and Jorge, 2014). However, other compounds such as carotenoids, tocopherols, minerals, organic acids and ascorbic acid, which were not determined in this study, may be present in the extracts studied and thus may have contributed to the antioxidant potential of the araticum pulp.

The highest antioxidant value for araticum pulp was obtained through the ORAC<sub>FL</sub> assay, followed by TEAC and DPPH, which had similar results, suggesting that the antioxidant compounds from araticum pulp act more efficiently through the mechanism of hydrogen atoms transfer than electrons transfer (Rebello *et al.*, 2014). Moreover, the ORAC<sub>FL</sub> assay analyzes both the hydrophilic and the lipophilic fractions, being able to assess a wide range of compounds with different polarities, which may contribute with an increased antioxidant activity through synergistic effects between these compounds. The hydrophilic or lipophilic characteristics of the reaction system, the polarity and the type of antioxidants present in the extract, the extracting solvent and the radical source (for example, some compounds that are highly reactive with peroxy radicals may react slowly or not react with the DPPH• radical) can explain the difference in the results between the antioxidant assays (Roesler *et al.*, 2006; Vasco *et al.*, 2008). Another fact that may explain the high values in the ORAC<sub>FL</sub> assay is its ability to measure the antioxidant activity of compounds that have a long lag phase, making it possible to determine the activity of compounds of slow action, especially in complex matrices (Gülçin, 2012). Thus, the use of more than one method in the evaluation of antioxidant properties of food products

allows us to obtain more information regarding the antioxidant capacity of the matrix as they address different action mechanisms.

#### *Antioxidant activity of phenolic compounds commonly found in fruits*

The DPPH, TEAC and H-ORAC<sub>FL</sub> assays were performed to assess the antioxidant potential and individual behavior of seven phenolic compounds commonly present in fruit pulp in relation to several antioxidant methods available (Table 2). The phenolic compounds analyzed showed the following descending order of antioxidant activity by the DPPH assay: quercetin  $\geq$  (-)-epigallocatechin = (-)-galocatechin  $\geq$  myricetin  $\geq$  (-)-epicatechin  $\geq$  (+)-catechin > kaempferol. For the TEAC assay, we observed the following order: quercetin  $\geq$  (-)-galocatechin  $\geq$  (-)-epigallocatechin = myricetin > (-)-epicatechin = (+)-catechin > kaempferol, while for the H-ORAC<sub>FL</sub> assay we observed: kaempferol > quercetin  $\geq$  (+)-catechin  $\geq$  (-)-epicatechin > myricetin > (-)-galocatechin = (-)-epigallocatechin.

Flavonoids are among the most potent plant antioxidants as they present one or more of the following structural elements that contribute to the antioxidant activity: (i) a *o*-diphenolic group in the B ring, (ii) a double (2-3) bond coupled with a 4-oxo function, and (iii) hydroxyl groups at the 3 and 5 positions (Hur *et al.*, 2014). The efficiency of phenolic compounds as antioxidants depends largely on their chemical structure, and the antioxidant activity of these compounds increases with the degree of hydroxylation and substitution of hydrogen atoms by ethyl or *n*-butyl groups, also depending on the steric hindrance caused by the orientation of the hydroxyl groups (Landete, 2012).

The results of the TEAC assay for the phenolic compounds were approximately 2 times greater than for the DPPH assay, except for kaempferol. However, the phenolic compounds showed an antioxidant capacity order similar for both assays, while there was no relation between the values and behavior of the antioxidant activities of these compounds between the H-ORAC<sub>FL</sub> assay and the others (DPPH and TEAC). The lower antioxidant activity values presented by the DPPH assay compared to TEAC can be explained by two reasons. First, the action mechanism of the DPPH assay is influenced by the structural conformation of the antioxidant; thus, small molecules have higher antioxidant activity because of the easier access to the active site of the radical (Almeida *et al.*, 2011). The second explanation is that the DPPH• radicals can reversibly react with certain phenolic compounds resulting in low levels

of antioxidant activity (Moo-Huchin *et al.*, 2014).

The similar behavior of the antioxidant activities of the phenolic compounds by the DPPH and TEAC assays may be due to the action mechanism involved in these assays, since both are based on the electrons transfer, whereas the H-ORAC<sub>FL</sub> assay is based on the hydrogen atoms transfer. Apak *et al.* (2007) reported that methods based on the electrons transfer cannot be compared to methods based on the hydrogen atoms transfer. Moreover, the H-ORAC<sub>FL</sub> assay has some peculiarities compared to the other assays that might influence the results, such as: (i) the assay is conducted in physiological temperature and pH; (ii) by acting competitively it measures the ability of the antioxidant to protect a molecule that is the target of action of free radicals, while the other methods act directly on a reference radical; (iii) the quantification of the results combines inhibition time and inhibition degree of the free radical, while other similar methods use inhibition time at a fixed inhibition degree or inhibition degree at a fixed time; and (iv) the measurements are performed using fluorescence decay curves, while the other ones use absorption at a fixed wavelength (Apak *et al.*, 2007; Gülçin, 2012).

#### **Conclusion**

The araticum Brazilian Cerrado fruit contains a high amount of phenolic compounds mainly flavonoids, which may contribute to its ability to reduce free radicals, such as DPPH•, ABTS<sup>+</sup> and peroxy radicals. This study may contribute significantly to the awareness of consumers and to the encouragement of future biological research studies. In addition, the araticum pulp could serve as a valuable natural antioxidant source for potential application in the production of functional foods. Therefore, this is a fruit with promising economic valorization.

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