PAULO ROGÉRIO SIRIANO BORGES

THE BIOACTIVE CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF TEN SELECTED BRAZILIAN CERRADO FRUITS

LAVRAS – MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós- Graduação em Ciência dos Alimentos, área de concentração em Ciência dos Alimentos, para a obtenção do título de Doutor.

Orientador

Dr. Eduardo Valério de Barros Vilas Boas

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A minha mãe, que prometeu que nada faltaria na minha educação e cumpriu a promessa com excelência. Muito obrigado por acreditar em mim e compartilhar os meus sonhos (*in memoriam*).

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RESUMO GERAL

As frutas representam uma das principais fontes de antioxidantes na alimentação humana, o que torna o seu consumo importante para a manutenção da saúde. No presente estudo, fenólicos totais, antocianinas, carotenoides, tocoferol e vitamina C foram quantificados em dez frutas do Cerrado brasileiro: araçá-boi, bacaba, bacupari, biribá, cajuí, curriola, marmelada-espinho, mirindiba, murici e puçá-preto. Cinco extratos foram preparados a partir de cada fruta utilizando-se solventes com diferentes polaridades. A atividade antioxidante total equivalente a Trolox (TEAC), capacidade de absorção do radical oxigênio (ORAC), e inibição de branqueamento no sistema β-caroteno/ácido linoleico foram determinados para cada um dos cinco extratos. Por meio de teste de média Scott-Knott e análise de agrupamento hierárquico foi observado que os frutos analisados são fontes de diferentes classes de compostos bioativos, com níveis comparáveis aos de frutas disponíveis no mercado, como ameixas, laranjas, goiabas, rosehips, e várias frutas cítricas. Para nosso conhecimento, este é o primeiro estudo dos compostos bioativos e atividades antioxidantes de bacupari, biribá, cajuí, curriola, marmelada-espinho e mirindiba. Além disso, destacamos a mirindiba como uma fonte rica em vitamina C e compostos fenólicos, com níveis consideráveis de carotenoides e tocoferóis; podendo ser classificada como uma "superfruta", junto à acerola e ao camu-camu.

Palavras-chave: Análise de agrupamento hierárquico. Frutas nativas. Tocantins. Mato Grosso.

GENERAL ABSTRACT

Fruit represents a major dietary source of antioxidant molecules that are important for health. We measured the total levels of phenolic, anthocyanin, carotenoid, and tocopherol compounds, and vitamin C in ten fruits from the Brazilian Cerrado: araçá-boi, bacaba, bacupari, biribá, cajuí, curriola, marmelada-espinho, mirindiba, murici, and puçá-preto. Five extracts were prepared from each fruit using solvents with different polarities. The Trolox equivalent antioxidant activity, oxygen radical absorbance capacity, and inhibition of β-carotene bleaching were determined for each extract. Scott-Knott test and hierarchical cluster analysis showed that the analyzed fruits were rich sources of different classes of bioactive compounds, with levels comparable to those in commonly consumed fruits such as plums, oranges, guavas, rosehips, and various berries and citrus fruits. To our knowledge, this is the first comprehensive study of the bioactive compounds and antioxidant activities of bacupari, biribá, cajuí, curriola, marmelada-espinho, and mirindiba. Moreover, mirindiba was found to be a rich source of vitamin C and phenolics, with an average level of carotenoids and tocopherols; this enabled "super fruit" classification of mirindiba, along with acerola and camu-camu.

Keywords: Hierarchical Cluster Analysis. Local food. Tocantins. Mato Grosso.

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FIRST PART

1 GENERAL INTRODUCTION

The growing interest in natural foods has raised the demand for natural ingredients which in addition to give basic nutritional and energy, are capable to contribute with additional physiological profits; the functional foods. The availability of information concerning food quality is related to the consumer preference for a healthily choice. It means that the basic knowledge about food composition may allow the consumer to choose for the best option. This relation between quality and choice seems to be obvious but the easier access to information in the last decades (e.g. internet, apps and labelling) has increased the interest about food quality. This concern comes from the increasingly occurrence of health problems such as overweight, intestinal failure, premature aging and occurrence of noncommunicable diseases.

Fruits and vegetables are well known as sources of minerals, vitamins, dietary fiber and bioactive compounds. Featuring as the most used ingredients in the healthily diets. Such popularity is related to the diet-disease relationship frequently mentioned by professionals of medicine and nutrition. In this regard, there is a current effort to investigate the Brazilian Cerrado native fruits composition due its high consume in some states of Brazil and the potential reported in recent studies.

Due the relatively new interest by the Brazilian Cerrado fruits as a source of nutrients and bioactive compounds, we can find different methodologies to evaluate the bioactive compounds and the antioxidant capacities with results shown in different units of measurement. This variety comes from the need of express the results according the researchers discretion, as well as the lack of standardization of methodologies, especially concerning the quantitation of

antioxidant activity in fruits. Moreover, native fruits are seasonal and process as freezing and freeze drying are used to available the fruits along the year as powder or frozen pulps. A new material, which shows a high antioxidant activity after freeze-drying and transport can be an important source of antioxidants in distant markets and for the industrial use.

The importance of native fruits, both for feeding local communities in Brazil and the current international market demand for new fruits with novel flavors and functional properties cannot be understated. The present study quantified the main groups of bioactive compounds (phenolics, anthocyanins, carotenoids, vitamin C, and tocopherols) in ten native fruits from the Brazilian Cerrado and assessed TEAC, ORAC, and β-carotene bleaching in the presence of five extracts of different polarities from each fruit. These Brazilian fruits were then compared with well-known sources of antioxidants found in the literature. Additionally, the fruit samples were grouped by antioxidant potential using Hierarchical Cluster Analysis.

2 THEORETICAL FRAMEWORK

2.1 Brazilian Cerrado Fruits

The traditional flora exploitation by local communities figures as a rich source of knowledge mostly regarding medicinal and feeding use (MONTEIRO et al., 2014; RUFINO et al., 2010). Selected plants are preserved along the extractive management, when considered useful by locals, resulting in floristic composition changings (FERRAZ; ALBUQUERQUE; MEUNIER, 2006). Therefore, the folk knowledge requires more studies in order to preserve the empiric information and prospect the local vegetation potential.

Brazilian Cerrado is the second largest biome of South America bordered by four other biomes: Amazon Forest, semi-arid Caatinga, Atlantic Rainforest and the wetlands Pantanal. Cerrado has a large biodiversity because of its wide area and the transition ecotones bordering the other biomes (SANO et al., 2008). Therefore it offers a large variety of plants mainly explored by the local people. The use of edible fruits, medicinal plants and ornamentals from the Cerrado region are common folk knowledge but the use of edible fruits and plants as food sources are rarely described in literature (MALTA et al., 2012; OLIVEIRA et al., 2012; RUFINO et al., 2010; SOUZA et al., 2012).

Recent studies about native fruits from Brazil reported a high functional potential of these frequently consumed but underexplored plant resources (MALTA et al., 2012; OLIVEIRA et al., 2012; PEREZ-GUTIERREZ et al., 2010; RUFINO et al., 2010). Among the native fruits known in Cerrado, araçá-boi (*Eugenia stiptata*), bacaba (*Oenocarpus bacaba*), bacupari (*Rheedia brasiliensis*), biribá (*Rollinia mucosa*), cajui (*Anacardium humile*), curriola (*Pouteria ramiflora*), marmelada (*Alibertia verrucosa*), mirindiba (*Buchenavia tomentosa*), murici (*Byrsonima crassifolia*) and puçá-preto (*Mouriri pusa*) are widely used in

culinary due its *sui generis* flavor. Moreover, stems, leafs and barks of some of these species are widely used in the folk medicine against gastric ulcers, healing and a range of diseases.

Araçá-boi is a yellow fleshy fruit from *Myrtaceae* family. Due the distinct aroma and flavor, it is highly used in the preparation of juices and jellies. Araçá-boi growing in the Colombian and Brazilian Amazon forest have been studied for their high antioxidant activity in vitro, as well as for their anti-proliferative and anti-mutagenic properties in vivo; these activities were attributed to their carotenoid and phenolic constituents (ASTRID GARZON et al., 2012; NERI-NUMA et al., 2013). However no data is available in the literature regarding araçá-boi fruits growing in the Brazilian Cerrado.

Bacaba palm is the largest fruit-producer of *Oenocarpus* gender (*Arecaceae*) due its large fruit clusters. The berries are small, round and blue-greyish or red-greyish being largest used from northern Brazil peoples to prepare juice, jams, pulps, ice-creams and fermented beverages, however this fruit is not commonly fresh consumed owing its high firmness flesh. The fruit pulp is rich in phenolic compounds mainly quercetin and ramnetin derivates, flavonols and anthocyanins and the pulp-oil fatty contains α-tocopherol and β-sitosterol (FINCO et al., 2012; LUBRANO; ROBIN; KHAIAT, 1994). Despite the large consume of this fruit a few studies mention this promising source of antioxidants (FINCO et al., 2012).

Cajui or caju-do-cerrado is a pseudofruit from *Annacardium* gender (*Anacardiaceae*) the same of the well-known domesticated cashew apple (COTA et al., 2012). These berries are popular in whole Cerrado area due its special flavor. They may vary between yellow and red colour, round and elongate shape, sweet and acid taste and emanates the typical cashew aroma. Studies about cajui found on literature link the leaf extracts to hypoglicemic, antibacterial and antiulcerogenic effects (FERREIRA et al., 2012; MONTANARI et al., 2012;

URZEDO et al., 2013). Despite its high consumption, there is no information about the cajui pseudofruits' bioactive compounds.

Murici (*Malpighiaceae*) is a tropical yellow berry found in Central and South America (MARIUTTI; RODRIGUES; MERCADANTE, 2013). Due to its unmistakable aroma and taste this fruit is large consumed in liquors, juices and ice-creams in northern and north-eastern Brazil (ALVES; FRANCO, 2003). The leaf and seed extracts of Byrsonima gender have been used in the folk medicine since the pre-Columbian era but the functional properties of berries had been investigated recently reporting mainly phenolics and carotenoids (GORDON et al., 2011; MALTA et al., 2012; PÉREZ-GUTIERREZ, 2010; SOUZA et al., 2008). In spite of its high consumption in Tocantins state, fruits grown on this climate conditions were not studied before.

The puçá-preto is the sweetest fruit of *Mouriri* gender (*Melostomataceae*) which has other edibles species. This round and black fruit can be consumed processed in jam, marmalades, ice-cream and juice, however puçá-preto is predominantly consumed fresh (BORGES, 2011). Despite to be well known in different states of Brazil, the literature about this specie focus in the gastroprotective activity of leaf extracts related to phenolic compounds (ANDREO et al., 2006; VASCONCELOS et al., 2008, 2010). Quantitative analysis of bioactive compounds such as total carotenoids and total phenolic had been reported before for Rufino et al. (2010) showing substantial antioxidant activity *in vitro*, however the qualitative profile of these compounds is still unexplored to puçá-preto.

There is no agreement about the use of mirindiba fruits (*Combretaceae*) between locals inhabiting the Cerrado. Some of them attribute medicinal effect to aerial parts of *Buchenavia tomentosa* trees and, usually, consume the fruit due the special flavor, while others attribute toxic effect in animals including abortion in cattle, goats and sheep. Regardless the empirical claim of toxicity in aerial parts,

accidents involving the fruit consumption are not reported in the literature. In previous tests performed in laboratory, high levels of phenolics, vitamin C and total tocopherols were detected. This values were upper than that related to strawberries, acerola and olives respectively.

Despite the high consumption of bacupari (*Clusiaceae*), biribá (*Annonaceae*), curriola (*Sapotaceae*) and marmelada-espinho (*Rubiaceae*) fruits, there is no data available in the literature regarding their bioactive compounds and antioxidant activity.

2.2 Antioxidant activity

Fruits and vegetables consume associated with a healthy lifestyle can promotes a significantly decrease in the incidence of noncommunicable diseases (GONÇALVES; LAJOLO; GENOVESE, 2010; WU; LONG; KENNELLY, 2013). This benefit is due to the antioxidant, anti-inflammatory and cytoprotective properties of bioactive compounds such as phenolics, carotenoids, tocopherols and ascorbic acid (ABE; LAJOLO; GENOVESE, 2012; GONÇALVES; LAJOLO; GENOVESE, 2010; HAMAECK et al., 2013; NASCIMENTO et al., 2011; PÉREZ-GUTIERREZ, 2010).

The oxidative process is a key process of energy production at molecular level. However, this vital reaction produce the free radicals, highly chemically reactive species, which can lead to cell injury and death, contributing to many noncommunicable diseases and premature aging (PRIOR; WU; SCHAICH, 2005). Once the free-radical oxidative damage start, a chain of oxidative reaction may occurs, engendering a cascade reaction. The antioxidants are molecules able to stop this oxidative process by neutralization of free radicals (HUANG et al., 2002a, 2002b). Such properties come from the ability of neutralizing the free radicals without generate a new one, and stopping the chain oxidative reaction.

Phenolics constitute the biggest groups of antioxidants found in food, they are responsible for the biggest part of antioxidant activity in aqueous systems, and phenolic pigments as anthocyanins are related to the red, purple and blue hues of plants. Both, colorless and colored phenolics can be correlated to the antioxidant activity in some fruits (ABADIO FINCO et al., 2012; GAIK MING et al., 2011). Carotenoids are also responsible for color in fruits ranging from yellow to red hues, some of these apolar pigments own vitamin A activity and all of them have been shown antioxidant activity in apolar systems (RODRIGUEZ-AMAYA, 2001). Apolar compounds as carotenoids can show high antioxidant activity; however they work in more complex mechanisms and may require specific methods for bioactivity detection (MARIUTTI; RODRIGUES; MERCADANTE, 2013).

Vitamin C is a small but expressive group of antioxidants represented by ascorbic acid and dehydroascorbic acid, both possess vitamin and antioxidant activity (RAMFUL et al., 2011). Tocopherols, which are lipophilic compounds located in the biological membranes, are also able to promptly neutralize chain reactions of free radicals (CHUN et al., 2006). Moreover tocopherols are easily restored by vitamin C, carotenoids and other antioxidants such as phenolic compounds (BRAMLEY et al., 2000). However, there is a lack of information about tocopherols in fruits, as fruits are not considered aliments rich in tocopherols when compared to vegetables, nuts and seeds (BRAMLEY et al., 2000). Considering that consumption of vegetables are necessary to maintain good health due their high levels of phenolic compounds, carotenoids and ascorbic acid, information regarding tocopherols in fruits are useful for a general overview of antioxidant activity.

2.3 Antioxidant activity measurements

There is no simple universal method for antioxidant activity measurement, once there are different radicals and at least four general classes of antioxidants found in food. Focusing in the small molecules, the most important group found in fruits, the large variety of antioxidant sources allow different answers in different systems *in vitro* (PRIOR; WU; SCHAICH, 2005). A fruit rich in phenolic and vitamin C, for example, can show a different answer than a fruit rich in phenolic compounds and carotenoids. Prior, Wu e Schaich (2005) reported the importance of accuracy and precision in antioxidant capacities evaluation detaching "the guidance for appropriate application of assays, meaningful comparisons of foods or commercial products, a means to control and variation within or between products and provision of quality of standards for regulatory issues and health claims".

In this respect, TEAC, ORAC are standardized methods that can detect antioxidant capacity of lipophilic and hydrophilic compounds after the necessary modification to enhance polarity in an aqueous system (HUANG et al., 2002a). TEAC is a single electron transfer based mechanism, while ORAC is a hydrogen atom transfer mechanism methodology, these two mechanisms may occur in parallel due the complex composition of fruits (PRIOR; WU; SCHAICH, 2005). ORAC is more precise but requires expensive equipment, chemicals and a long time of analyses, while TEAC allow a large sample analyses in a short time and can express reliably the antioxidant activity if performed in controlled conditions (THAIPONG et al., 2006). Regarding the quantification, Trolox is widely used in polar solvent systems as a polar standard. Trolox is a synthetic water-soluble antioxidant analogue of α-tocopherol, what make results expressed with these standards more comprehensibly and susceptible to comparisons (HUANG et al., 2002a).

Vitamin C antioxidant activity is easily detected in different methods once they are able to react directly with the free radical of different sources or restoring apolar antioxidant compounds (PRIOR; WU; SCHAICH, 2005). High correlation

between vitamin C and TEAC or ORAC is commonly found in the literature (PROTEGGENTE et al., 2002; RAMFUL et al., 2011; THAIPONG et al., 2006). The antioxidant activity of phenolic compounds is also detectable in TEAC and ORAC systems once they are commonly extractable in aqueous solutions (THAIPONG et al., 2006).

Evaluation of antioxidant activity in emulsion systems are more difficult to perform and reproduce, since the methods usually work in different mechanisms simultaneously and the response of antioxidants can be slower than in polar systems (DAPKEVICIUS et al., 1998; PRIOR; WU; SCHAICH, 2005). However, methods as β-carotene bleaching are widely used aiming to evaluate the answer of apolar antioxidants, as tocopherols and carotenoids, and polar compounds that are able to restore the tocopherol in vivo (BRAMLEY et al., 2000; DAPKEVICIUS et al., 1998). β-carotene bleaching is a standardized method relatively instable and time-consuming, however it has the advantage to be performed in a emulsion system, allowing an overview of polar and apolar compounds acting simultaneously. In this method the radical is generated by oxidation of fat acids induced by high temperature, what make the assay time consuming, running around two hours of analyses (DAPKEVICIUS et al., 1998). Regarding the quantification, tocopherol, an apolar analogue of the synthetic Trolox, is used. Tocopherol is able to act in HAT mechanism with slower time dependent answer and 50% relative answer to Trolox, figuring as good alternative for apolar systems (HUANG et al., 2002b).

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SECOND PART

THE BIOACTIVE CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF TEN SELECTED BRAZILIAN CERRADO FRUITS

ABSTRACT

Fruit represents a major dietary source of antioxidant molecules that are important for health. We measured the total levels of phenolic, anthocyanin, carotenoid, and tocopherol compounds, and vitamin C in ten fruits from the Brazilian Cerrado: araçá-boi, bacaba, bacupari, biribá, cajuí, curriola, marmelada-espinho, mirindiba, murici, and puçá-preto. Five extracts were prepared from each fruit using solvents with different polarities. The Trolox equivalent antioxidant activity, oxygen radical absorbance capacity, and inhibition of β-carotene bleaching were determined for each extract. Scott-Knott test and hierarchical cluster analysis showed that the analyzed fruits were rich sources of different classes of bioactive compounds, with levels comparable to those in commonly consumed fruits such as plums, oranges, guavas, rosehips, and various berries and citrus fruits. To our knowledge, this is the first comprehensive study of the bioactive compounds and antioxidant activities of bacupari, biribá, cajuí, curriola, marmelada-espinho, and mirindiba. Moreover, mirindiba was found to be a rich source of vitamin C and phenolics, with an average level of carotenoids and tocopherols, along with acerola and camu-camu.

Keywords: *Hierarchical Cluster Analysis; local food; Tocantins; Mato Grosso.*

1 INTRODUCTION

Fruit and vegetable consumption is associated with a healthy lifestyle and reduces the incidence of noncommunicable diseases (Bramley et al., 2000; Lima, Azavedo, de Souza, Nunes and Vilas Boas, 2015; Malta, Ghiraldini, Reis, Oliveira, Silva and Pastore, 2012; Muller, 1997; Perez-Gutierrez, Muiz-Ramirez, Gomez-Gomez and Bautista-Ramirez, 2010; Winkler, Orselli and Rex, 1994). These benefits are related to the antioxidant, anti-inflammatory, and cytoprotective properties of the bioactive compounds found in these foods, including phenolics, carotenoids, ascorbic acid, and tocopherols (Chun, Lee, Ye, Exler and Eitenmiller, 2006; Goncalves, Lajolo, Genovese, 2010; Proteggente et al., 2002; Ramful, Tarnus, Aruoma, Bourdon and Bahorun, 2011; Vasco, Ruales and Kamal-Edin, 2008). In this respect, the Brazilian Cerrado encompasses a large biodiversity, with a wide variety of plants that have been explored by local people (Gonçalves et al., 2010; Oliveira, Yamada, Fagg and Brandao, 2012; Rufino, Alvez, de Brito, Perez-Jimenez, Saura-Calixto and Mancini, 2010; Souza, Pimenta, Queiroz and Souza, 2012). The use of edible fruits, medicinal plants, and ornamentals from the Cerrado is common folk knowledge and some research studies regarding these edible fruits and other food sources have also been published (Abadio Finco, Kammerer, Carle, Tseng, Boser and Graeve, 2012; de Souza et al., 2012; Mariutti, Rodrigues and Mercadante, 2013). Such research has shown the great potential for Cerrado fruits to prevent noncommunicable diseases and premature aging, due to their high levels of bioactive compounds (Goncalves et al., 2010; Lima et al., 2015; Malta et al., 2012; Perez-Gutierrez et al., 2010).

Among the wide variety of native Cerrado fruits, araçá-boi (*Eugenia stipitata*), bacaba (*Oenocarpus bacaba*), bacupari (*Rheedia brasiliensis*), biribá (*Rollinia mucosa*), cajui (*Anacardium humile*), curriola (*Pouteria ramiflora*), marmelada-espinho (*Alibertia verrucosa*), mirindiba (*Buchenavia tomentosa*),

murici (*Byrsonima crassifolia*), and puçá-preto (*Mouriri pusa*) are widely used locally owing to their unique flavors and empirically attributed medicinal properties (Abadio Finco et al., 2012; Goncalves et al., 2010; Neri-Numa et al., 2013; Oliveira et al., 2012; Perez-Gutierrez et al., 2010; Rufino et al., 2010). In this respect, the key approach to assessing the potential of new fruits is to examine their antioxidant activities and determine the main groups of antioxidants present (Barros, Carvalho, Morais and Ferreira, 2010; Khoo, Clausen, Pedersen and Larsen, 2011; Neri-Numa et al., 2013; Proteggente et al., 2002; Rufino et al., 2010; Souza et al., 2012).

Phenolics constitute the largest group of bioactive compounds found in fruits, including anthocyanins, which provide the red, purple, and blue colors of fruits (Abadio Finco et al., 2012; Lee, Durst and Wrolstad, 2005; Ferreira-Zielinski, Avila, Nogueira, Wosiacki and Isidoro-Haminiuk et al., 2014). The levels of both colorless and colored phenolics with varied polarities correlates with antioxidant activity *in vitro* (Ferreira-Zielinski et al., 2014; Kajdzanoska, Petreska and Stefova, 2011; Khoo et al., 2011; Proteggente et al., 2002; Xu & Chang, 2007). Carotenoids are also responsible for fruit colors ranging from yellow to red hues; many of these nonpolar pigments show vitamin A activity and all of them have antioxidant activity in polar and nonpolar systems (Astrid-Garzon, Narvaez-Cuenca, Kopec, Barry, Rield and Schwartz, 2012; Burns, Fraser and Bramley, 2003; Ferreira-Zielinski et al., 2014; Mariutti et al., 2013; Muller, 1997). Vitamin C, comprising ascorbic and dehydroascorbic acid, is also a powerful polar antioxidant and fruits are the main source of vitamin C in common diets (Hernandez, Lobo and Gonzales, 2006; Proteggente et al., 2002; Ramful et al., 2011). Vitamin C can provide antioxidant activity *in vitro*, as well as actively restoring vitamin E *in vivo* (Proteggente et al., 2002; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos and Hawkins-Byrne, 2006; Winkler et al., 1994). Vitamin E or tocopherols are lipophilic antioxidants that promptly neutralize

imminent membrane damage caused by chain reactions involving free radicals (Bramley et al., 2000; Huang, Ou, Hampsch-Woodill, Flanagan and Deemer, 2002). Moreover, tocopherols can be restored by vitamin C, carotenoids, and phenolic compounds *in vitro* and *in vivo* (Bramley et al., 2000; Winkler et al., 1994). The tocopherol content of fleshy fruits is often overlooked, since they are not considered to be rich sources of vitamin E, however, increasing fruit consumption has complemented vitamin E intake in some diets (Burns et al., 2003; Chun et al., 2006).

There is no simple universal method to measure the antioxidant activities of bioactive compounds, since there are many free radicals and many classes of antioxidants with a range of polarities (Huang, Ou, Hampsch-Woodil, Flanagan and Prior, 2002; Kajdzanoska et al., 2011; Proteggente et al., 2002; Xu & Chang, 2007). Such complexity demands extraction of antioxidants using different solvents, resulting in a range of responses in different assays *in vitro*, which often hinders comparison of results (Hernandez et al., 2006; Kajdzanoska et al., 2011; Prior et al., 2005; Thaipong et al., 2006; Xu & Chang, 2007). To address this, standardized microplate methods for measuring the antioxidant activities of polar and nonpolar compounds, such as the determination of Trolox equivalent antioxidant activity (TEAC), oxygen radical absorbance capacity (ORAC), and β-carotene bleaching assays were revisited and standardized (Huang et al., 2002a, b; Prieto, Rodriguez-Amado, Vazquez and Murado, 2012; Prior et al., 2005). The use of standardized procedures for sample extraction and preparation can enhance the detection of particular classes of antioxidants by these assays (Huang et al., 2002a; Jensen, Blachez, Egebo and Meyer, 2007).

TEAC primarily detects the single electron transfer (SET) potential of antioxidants, while ORAC measures their potential with respect to hydrogen atom transfer (HAT) (Prior et al., 2005). When SET and HAT mechanisms occur simultaneously in foods, TEAC and ORAC are often performed in parallel to

evaluate the antioxidant activity of the fruit samples; additionally, Trolox (a water-soluble tocopherol analog) is used as a standard in both methods, facilitating data comparison (Prior et al., 2005; Proteggente et al., 2002; Thaipong et al., 2006). The β-carotene bleaching assay measures the sample's ability to prevent discoloration of β-carotene during heat-induced oxidation; this involves HAT. This method has the advantage of being performed in an emulsion system, integrating the effects of polar and nonpolar compounds acting simultaneously (Mariutti et al., 2013; Prieto et al., 2012; Prior et al., 2005; Rufino et al., 2010) and using α-tocopherol as a standard (Prieto et al., 2012). A complementary approach with hierarchical cluster analysis (HCA), clusters fruits according to their antioxidant levels and antioxidant activities, identifying similarities between fruits and indicating those with the highest antioxidant potential (Ferreira-Zielinski et al., 2014).

The importance of native fruits, both for feeding local communities in Brazil and the current international market demand for new fruits with novel flavors and functional properties cannot be understated. The present study quantified the main groups of bioactive compounds (phenolics, anthocyanins, carotenoids, vitamin C, and tocopherols) in ten native fruits from the Brazilian Cerrado and assessed TEAC, ORAC, and β-carotene bleaching in the presence of five extracts of different polarities from each fruit. These Brazilian fruits were then compared with well-known sources of antioxidants found in the literature. Additionally, the fruit samples were grouped by antioxidant potential using HCA.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

2,2'-Azobis-2-methylpropanimidamide, dihydrochloride (AAPH) was purchased from Cayman Europe (Tallinn, Estonia), ethanol was purchased from Kemetyl (Haninge, Sweden), and phosphate-buffered saline-ethylenediaminetetraacetic acid (PBS-EDTA) was purchased from Lonza (Braine, Belgium). Formic acid, oxalic acid, potassium hydroxide, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). (+)-α-Tocopherol, β-carotene, methyl-β-cyclodextrin, 2,2'-azino-bis (3-ethyl-benzthiazo-line-6-sulphonic acid) (ABTS), 2-propanol, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), strong basic resin (Ambersep 900 OH), acetone, acetonitrile, ethyl acetate, fluorescein, Folin-Ciocalteu reagent, gallic acid, heptane, hexane, L(+)-ascorbic acid, linoleic acid, methanol, and Tween 20® were purchased from Sigma-Aldrich (Missouri, USA). Pure water was obtained from an SG Ultra-Pure water system (SG Water, Barsbuttel, Germany).

2.2 Plant material and sample preparation

Ten native fruits from the Brazilian Cerrado were selected for this study. Fruits of each species were harvested manually from twenty trees between November 2012 and October 2013 in Cerrado regions located in the states of Tocantins and Mato Grosso, Brazil. The common name, scientific name, family, collection site, fruit coloration, size, and dry weights are described in Table 1. Around 10 kg of each fruit was harvested and fruits that were free of visual defects were selected. These were washed, dried with paper towels, measured, weighed, frozen in liquid nitrogen, packaged in polyethylene pouches to prevent

dehydration, and kept at -18 °C in the dark during the 12-h transportation to the Federal University of Lavas, Brazil. The fruits were then stored at -80 °C until sample preparation.

Table 1 Characteristics of analyzed fruits from Brazilian Cerrado

Common name	Species	Family	Origin (City, State)	Peel color	Pulp color	Fruit size Range (cm)[a]	Dry matter[b]
Aracá-boi	*Eugenia stipitata*	Myrtaceae	Cuiabá, Mato Grosso	Yellow	Yellow	8 ±0,8	11.1 ±1.2
Bacaba	*Oenocarpus bacaba*	Arecaceae	Peixe, Tocantins	Greyish-purple	Greyish-purple	2.2 ±0,7	74.4 ±0.7
Bacupari	*Rheedia brasiliensis*	Clusiaceae	Santo Antonio do Leverger, Mato Grosso	Orange	Orange	2,3 ±0,3	17.7 ±0.6
Biribá	*Rollinia mucosa*	Annonaceae	Cuiabá, Mato Grosso	Yellow	White	8 ±0,9	19.3 ±0.4
Cajuí	*Anacardium humile*	Anacardiaceae	Dueré, Tocantins	Red	White	3.2 ±0,5	6.2 ±0.4
Curriola	*Pouteria ramiflora*	Sapotaceae	Santo Antonio do Leverger, Mato Grosso	Green	White	7 ±2	11.6 ±2.4
Marmelada-espinho	*Alibertia verrucosa*	Rubiaceae	Santo Antonio do Leverger, Mato Grosso	Yellow	White-Grey	3 ±1,4	22.6 ±1.1
Mirindiba	*Buchenavia tomentosa*	Combretaceae	Dueré, Tocantins	Yellow-green	Yellow	2,5 ±0,5	29.4 ±0.5
Murici	*Byrsonima crassifolia*	Malpighiaceae	Dueré, Tocantins	Yellow	Yellow	1,5 ±0,5	28.1 ±0.4
Puçá-preto	*Mouriri pusa*	Melastomataceae	Gurupi, Tocantins	Black	Orange	2,7 ±0,2	63.6 ±2.0 45.5 ±2.0[c]

[a] Values obtained in the present study from 20 fruits measured.

[b] Average ± standard deviation (n = 3).

[c]peel

Sample preparation was performed quickly to avoid thawing. Only the edible parts of the fruit were used. For the araçá-boi, bacaba, bacupari, cajuí, mirindiba, and murici, the peel and pulp were mashed by hand. For the biribá, curriola, and marmelada-espinho, only the flesh (endocarp) was used; the fruits were first peeled and then homogenized. Since puçá-preto fruit is consumed both with and without peel, the peel and the pulp were first separated and then homogenized, prepared, and analyzed separately throughout the study as two different samples. After homogenization, samples were immediately refrozen at -80 °C on glass plates, before freeze-drying (LIOBRAS L101 Freeze-drier; São Paulo, Brazil) at -40 °C. The dry matter content (Table 1) was calculated gravimetrically by weighing three sub-samples of each fruit before and after freeze-drying. The freeze-dried samples were subsequently milled and stored in hermetically sealed pouches at 5 ºC until chemical analyses were conducted at the Department of Food Science, Aarhus University, Årslev, Denmark.

2.3 Bioactive compounds

Bioactive compounds were extracted and quantified from each fruit (11 samples) using each of the approaches described below, in triplicate.

2.3.1 Total phenolics

Total phenolics were extracted according to Kajdzanoska et al. (2011), with slight modifications. Freeze-dried sample (0.5 g) was mixed with 10 mL of methanol solution containing acetic acid (H_2O:MeOH:HAc, 19:80:1, v/v/v) in a 15-mL argon-filled centrifuge tube (SARSTEDT, Nümbrecht, Germany) and stirred for 2 h at 8 °C in dim light on a MS2-Minishaker (IKA®, Königswinter, Germany). This mixture was centrifuged for 4 min at 13,000 rpm and 5 °C on a

Sorvall RC-5B Plus centrifuge (Buch and Holm, Herlev, Denmark) and the resulting supernatant was filtered through a 0.45-µm RR Q-Max® nylon filter (Frisenette, Knebel, Denmark) into a brown 2-mL vial (VWR® INTERNATIONAL, Radnor, Pennsylvania, USA) prior to analysis. Quantification was performed using a microplate Folin-Ciocalteu method (Magalhaes, Santos, Segundo, Reis and Lima, 2010). In brief, 50 µL of gallic acid or filtered sample was mixed with 50 µL Folin-Ciocalteu reagent (diluted 1:5 with H_2O, v/v) prior to by adding 100 µL 0.7 M sodium hydroxide. After 3 min, the absorbance was measured at 760 nm using a Synergy 2 multi-code microplate reader from BioTek (Vermont, USA). Water was used as a blank control. The total phenolic content was quantified as mg gallic acid equivalents per 100 g fresh weight (mg GAE/100 g FW).

2.3.2 Total anthocyanins

Total monomeric anthocyanin was extracted according to Jensen et al. (2007). Freeze-dried sample (0.5 g) was mixed with 20 mL ethanolic solution containing hydrochloric acid (H_2O:EtOH:HCl, 40.9:50:0.1, v/v/v) in a 50-mL argon-filled centrifuge tube (SARSTEDT) and stirred for 15 min at 8 °C in dim light. The mixture was centrifuged for 15 min at 13,000 rpm and 4 °C. The supernatant was filtered through a 0.22-µm Q-Max® acetate filter (Frisenette) directly into a 2-mL brown vial prior to analysis. Anthocyanins were quantified as the difference between sample absorption at 510 nm in pH 1.0 buffer (0.025 M KCl) (aq) and sample absorption at 700 nm in pH 4.5 buffer (0.4 M sodium acetate) (aq), as described by Lee et al. (2005). In brief, two 75-µL aliquots of the filtered sample were mixed with either 125 µL pH 1 buffer or 125 µL pH 4.5 buffer. After 15 min, the absorbance was measured using a microplate reader. The total anthocyanin content was quantified as cyanidin-3-glucoside equivalents per 100 g FW (mg/100 g FW).

2.3.3 Total carotenoids

Extraction of carotenoids was performed according to Larsen & Christensen (2005). Freeze-dried sample (1 g) was homogenized with 20 mL cold acetone (100%) in a 50-mL argon-filled centrifuge tube and left for 20 min. The mixture was then centrifuged for 4 min at 13,000 rpm and 4 °C. Ten milliliters of supernatant was stirred with 1 g Ambersep 900 OH for 30 min to saponification. The supernatant was then filtered through a 0.45-µm RR Q-Max® nylon filter directly into a 2-mL brown vial. High-performance liquid chromatography (HPLC) analysis was performed on a Dionex Ultimate 3000 Series HPLC equipped with a DAD-3000 (RS) diode array detector, a TCC-300SD column compartment, a WPS-3000SL autosampler and a 3000 Pump Series, all obtained from Dionex (Dionex Softron GmbH, Germering, Germany). Carotenoids were separated on a Hypersil Gold C_{18} analytical column (250 × 4.6 mm; 5-µm particle size) that was protected by a Hypersil Gold C_{18} guard cartridge (15 × 4 mm); both were obtained from Agilent Technologies (Santa Clara, California, USA). The mobile phase, gradient, and further chromatographic conditions were described previously (Larsen & Christensen, 2005). Identification and quantification of β-carotene was conducted using a calibration curve constructed using an authentic standard; other carotenoids were quantified according to Rodriguez-Amaya (2001), using the extinction coefficient in acetone. Results were expressed as mg total carotenoids per 100 g FW (mg/100 g FW). Data were processed with Chromeleon® version 6.8 software (Dionex Corporation).

2.3.4 Vitamin C

Extraction of vitamin C was performed according to Hernandez et al. (2006), with slight modifications. Freeze-dried sample (1 g) was homogenized with 10 mL cold (8 °C) aqueous solution (H_2O:OA, 99.5:0.5, v/w) in a 15-mL argon-filled centrifuge tube for 60 sec and subsequently stirred for 15 min in dim light. Homogenates were centrifuged for 5 min at 13,000 rpm and 4 °C and the resulting supernatant was filtered through a 0.22-µm Q-Max® acetate filter directly into a brown 2-mL vial prior to analysis. Separation and quantification of vitamin C were performed according to Li (2013), with slight modifications, using the HPLC apparatus previously described. The system was equipped with a Kinetex XB-C_{18} (4.6 × 150 mm; 2.6-µm particle size) column from Phenomenex (Torrance, California, USA). The mobile phase, gradient, and further chromatographic conditions were described previously (Li, 2013). Peaks were processed using Chromeleon® version 6.8 software and results were expressed as mg vitamin C per 100 g FW (mg/100 g FW), using an authentic ascorbic acid standard.

2.3.5 Total tocopherols

The extraction procedure was performed according to the European standard EN 12822. Freeze-dried sample (1 g) was saponified in a mixture of 5 mL ascorbic acid (EtOH:AA, 99.9:0.1, v/w), 5 mL ethanol (H_2O:EtOH, 96:4, v/v), 4.5 mL absolute methanol, and 3.5 mL saturated KOH, directly into a 25-mL argon-filled brown glass vial (VWR® INTERNATIONAL). First, samples were homogenized for 60 sec, saponified for 90 min at 70 °C in the dark, and then quickly cooled to -25 ºC. One milliliter of this mixture was mixed with 2.5 mL heptane in an argon-filled 15-mL centrifuge tube, stirred for 30 sec, and centrifuged for 5 min at 13,000 rpm and 4 °C. The heptane fraction containing the

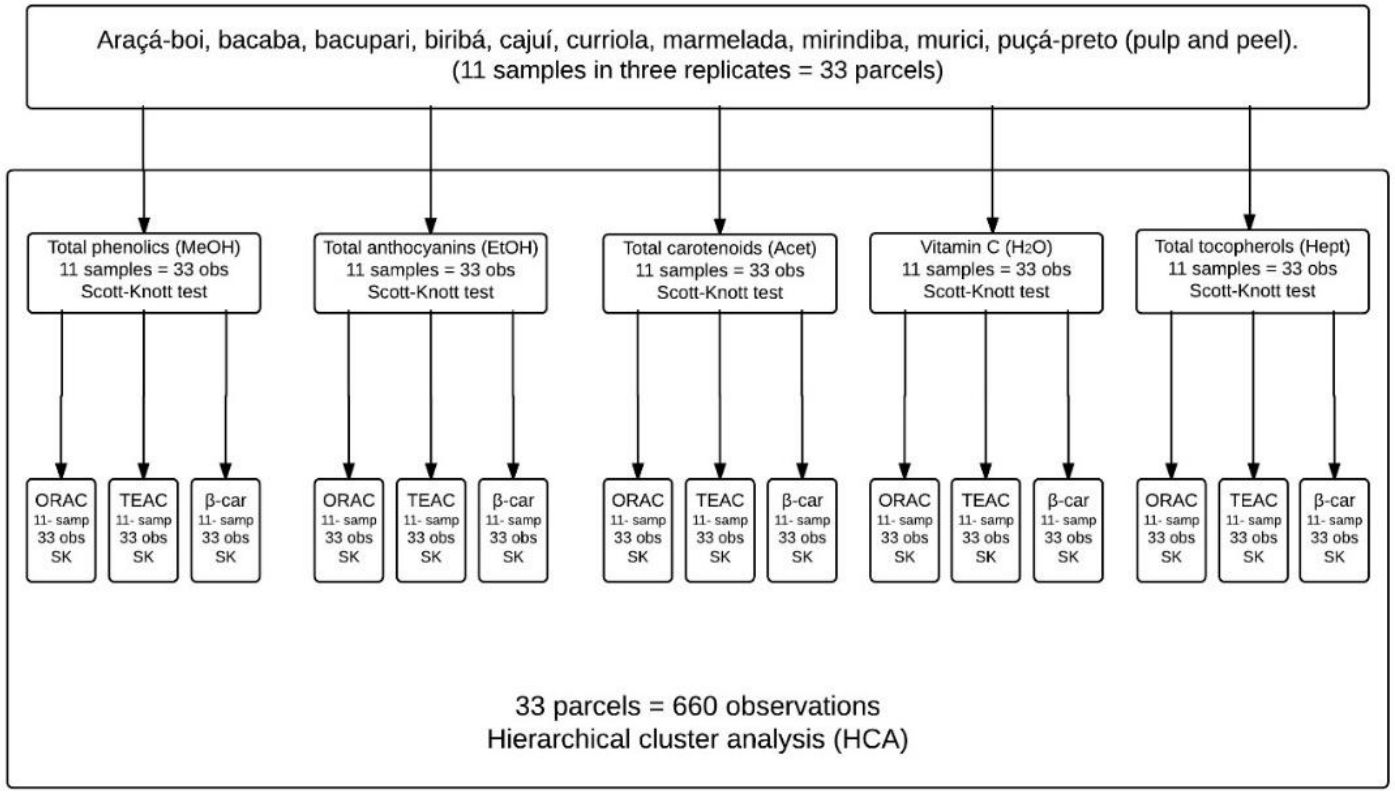
tocopherols was transferred to a brown argon-filled vial and the mixture was extracted a second time with 2.5 mL heptane, as described above. The resulting supernatants were combined and filtered through a 0.45-µm RR Q-Max® nylon filter directly into a 2-mL brown vial prior to analysis. Chromatographic separation was performed according to Kamal-Eldin, Gorgen, Petterson and Lampi, (2000) with the HPLC system described previously, equipped with an RF-2000 fluorescence detector connected to a ZORBAX RX-SIL (4.6 mm × 150 mm; 5 µm particle size) column (Agilent Technologies). The mobile phase consisted of hexane modified with 2-propanol (1.5 % v/v). The flow rate was 0.5 mL/min, and the oven temperature was 30 °C. A 50-µL sample was injected and the total run time was 20 min with isocratic elution. The excitation wavelength was 295 nm and the emission wavelength was 327 nm. Identification and quantification of α-tocopherol were achieved by comparison with authentic standard. Other tocopherol isomers, beta-, gamma-, delta-tocopherols and alpha-, beta-, gamma-, and delta-tocotrienols, were identified according to Kamal-Eldin et al. (2000) and quantified according to EN 12822 methodology using the fluorescence response relative to α-tocopherol. Peaks were processed with Chromeleon® version 6.8 software and results were expressed as mg tocopherols per 100 g FW (mg/100 g FW).

2.4 Antioxidant activity

ORAC, TEAC, and β-carotene bleaching inhibition were assessed in triplicate in the eleven samples prepared using the five approaches described in section 2.3, as shown in Figure 1. These samples were stored at -80 °C in brown vials filled with argon prior to antioxidant activity assessments. For each fruit, the extracts were named according to the extraction solution used, as follows: phenolic compounds in methanol (MeOH), anthocyanins in aqueous ethanol (EtOH), carotenoids in acetone (Acet),

ascorbic acid in water (H_2O), and tocopherols in heptane (Hept) (Figures 1 and 2). Additionally, the respective pure extraction solution was used as a blank control for the antioxidant activity measurements. For the carotenoid (Acet) and tocopherol (Hept) extracts, pre-treatment with methyl-β-cyclodextrin was performed as described by Huang et al. (2002a) to increase the solubility of the bioactive compounds.

Figure 1 Schematic presentation of experimental data achievement for Scott-Knott test and hierarchical clustering analysis (HCA). All chemical analyses were performed as described in Material and Methods



*obs = observations; samp = samples; SK = Scott-Knott test; β-car = β-carotene bleaching assay.

2.4.1 ORAC

The ORAC assay was performed as described by Huang et al. (2002b). Twenty-five microliters of sample extract or standard solution were placed in a Nunc 96-well black plate (Thermo Fisher Scientific, Leicestershire, UK), 150 µL of fluorescein solution (1.2×10^{-8} mM in 75 mM phosphate buffer, pH 7.42) was added, and the plates were incubated for 30 min at 37 °C. The assay was initiated by adding 25 µL AAPH solution (15 mM in 75 mM phosphate buffer, pH 7.42). Fluorescence was recorded every minute for one hour at an excitation wavelength of 485 nm and an emission wavelength of 515 nm using the microplate reader previously described. The net area under the curve (net-AUC) in relation to the blank control was calculated (Prior et al., 2005). Trolox was diluted in pure ethanol and results were expressed as Trolox equivalents per gram of FW (µM TEq/g FW).

2.4.2 TEAC

TEAC was measured according to Khoo et al. (2011). In brief, 50 µL filtered sample or standard solution was mixed with 200 µL ABTS radical buffer solution and the absorbance was read after 10 min at a wavelength of 414 nm, using the microplate reader previously described. Trolox was diluted in pure ethanol and results were expressed as µM TEq/g FW.

2.4.3 β-carotene bleaching

Discoloration of β-carotene was measured according to Prieto et al. (2012), with slight modifications. One milligram of beta-carotene was dissolved in 10 mL dichloromethane prior to dissolving 25 µL linoleic acid and 200 mg Tween-20 in 1 mL of this mixture. Dichloromethane was then removed under vacuum using a rotary evaporator operating at 40 °C in dim light. Oxygenated pure water (50 mL at 50 ºC)

was added to the mixture and vigorously shaken by hand to form an emulsion. A second emulsion that lacked β-carotene was prepared at room temperature (18 °C) following the procedure described above. The assay was started by adding a 50-μL aliquot of emulsion without β-carotene to the 96-well microplate followed by 30 μL sample extract or standard solution, gently mixing to avoid evaporation of solvents. Next, a 200 μL aliquot of the β-carotene/linoleic acid emulsion at 50 ºC was applied, stirred, and immediately read at 492 nm every 5 min for 3 h at 50 ºC using a microplate reader that was pre-heated to 50 ºC. Data were plotted as fixed-percent inhibition in relation to the first measurement, and discoloration of β-carotene between 120 and 180 min was used for net-AUC integration (Prieto et al., 2012; Prior et al., 2005). α-Tocopherol authentic standard was diluted in heptane and results were expressed as μM TPEq/g FW.

2.5 Data analysis

A schematic representation of the data analysis is shown in Figure 1. Bioactive compound levels and antioxidant activity measurements were obtained in three replicates. These were initially compared by one-way analysis of variance ($p < 0.05$), followed by the Scott-Knott test, to identify significant differences between the fruits. A multivariate analysis approach comprising HCA was implemented using Statistica 7.0 software (Stat-Soft Inc., Tulsa, Okla., USA). The dependent variables were autoscaled to standardize their statistical importance, and no detected results were considered zero. HCA was applied to asses similarities between fruit samples (11 samples × 3 replications; n = 33 parcels) according to the bioactive compound levels (33 samples × 5 measurements = 165 parcels) and the antioxidant activity measurements (165 extracts × 3 measurements = 495 parcels), as shown in Figure 1. Sample similarities were calculated based on the Euclidean metric.

3 RESULTS AND DISCUSSION

3.1 Bioactive compounds

All of the analyzed fruits had detectable total phenolic levels in the following order: mirindiba > bacaba > puçá-preto (peel) > puçá-preto (pulp) > bacupari > murici > biribá = marmelada-espinho = cajuí = araçá-boi = curriola (Table 2). Souza et al. (2012) classified native Cerrado fruits according to their total phenolic levels, following the scale proposed by Vasco et al. (2008). Following these parameters, we found mirindiba, bacaba, and puçá-preto peel and pulp to be high-level sources of phenolic compounds (> 500 mg GAE/100 g), comparable to "super fruits" such as acerola, camu-camu (Rufino et al., 2010), blackberries, raspberries, and blueberries (Abadio Finco et al., 2012). In the second group of medium phenolic compound sources (100-500 mg GAE/100 g), we classified bacupari and murici alongside strawberries, red plums (Proteggente et al., 2002), guavas (Vasco et al., 2008), and sour cherries (Khoo et al., 2011). The fruits considered to represent low sources of phenolic compounds (< 100 mg GAE/100 g), namely biribá, marmelada-espinho, cajuí, araçá-boi, and curriola were comparable to highly consumed fruits, including apples, peaches, pears (Proteggente et al., 2002), passion fruit, and mangoes (Vasco et al., 2008). Since consumption of phenolic compounds is important for proper health, these findings indicated that the Brazilian Cerrado fruits analyzed here represent important food sources for local consumers and provide promising raw materials to meet the market demand for new products (Barros et al., 2010; Goncalves et al., 2010).

Table 2 Total phenolics, total anthocyanins, total carotenoids, Vitamin C, and total tocopherols in 10 Brazilian native fruits from cerrado (mg/100 fresh matter).

Fruit	Total phenolic[a]	Total anthocyanins[b]	Total carotenoids	Vitamin C	Total tocopherols
Araçá-boi	35,7 g	nd	0.9 d	8.3 c	0,60 e
Bacaba	1244,6 b	116.7 a	nd	nd	1,20 c
Bacupari	208,4 e	nd	0.5 e	1.6 c	1,19 c
Biribá	46,4 g	nd	nd	1.9 c	0,87 d
Cajui	36,3 g	110.6 a	nd	7.5 c	0,13 g
Curriola	34,2 g	nd	nd	3.5 c	0,43 f
Marmelada-espinho	46,3 g	nd	nd	2.0 c	1,12 c
Mirindiba	2827,1 a	nd	1.4 c	2018.4 a	4,05 a
Murici	143,9 f	nd	1.4 c	36.4 b	0,78 d
Puçá-preto (pulp)	512,3 d	nd	1.5 b	2.9 c	1,15 c
Puçá-preto (peel)	610,1 c	36.4 b	1.8 a	2.7 c	1,94 b
CV (%)	5.6	6.7	5.4	5.4	4.7

Values followed by the same letter in the column are equal accordingly the Scott-Knott means test ($p<0.05$); n = 3; nd = not detected.
[a] galic acid equivalent (GAE)
[b] cyanidin-3-glucoside equivalents

The bacaba, cajuí, and puçá-preto peel had detectable levels of total anthocyanins (Table 2), giving them greyish-purple, red, and black colors, respectively (Table 1). The anthocyanin content in bacaba detected in this study was three-fold higher than that reported by Abadio Finco et al. (2012) for fruits that were also collected in Tocantins State, Brazil. The anthocyanin levels in bacaba, cajuí, and puçá-preto peel (36.4-116.7 mg/100 g FW) are comparable to those reported in the literature for açaí, camu-camu (Rufino et al., 2010), strawberries (Kajdzanoska et al., 2011), red grapes (Jensen et al., 2007), and sour cherries (Khoo et al., 2011). Apart from their total phenolic content, bacaba, cajuí, and puçá-preto are thus also rich sources of anthocyanins.

According to their carotenoid levels, the fruits were ranked as follows: puçá-preto peel > puçá-preto pulp > murici = mirindiba > araçá-boi > bacupari (Table 2). Bacaba, biribá, cajuí, curriola, and marmelada-espinho did not have detectable levels of carotenoids, an expected outcome, considering the colors of their edible parts (Table 1). In the context of the screening study performed by Muller (1997) to analyze carotenoid levels in 28 fruit species, araçá-boi, bacupari, mirindiba, murici, and puçá-preto were classified as average sources of carotenoids for human consumption, similar to apricots, plums, and clementines. We also observed three major carotenoids in the samples analyzed: (*all-E*)-zeaxanthin, (*all-E*)-lutein, and (*all-E*)-β-carotene were present in araçá-boi, as well as (*all-E*)-zeaxanthin and (*all-E*)-β-carotene in mirindiba, murici, and puçá-preto pulp and peel. Astrid Garzon et al. (2012) found similar major carotenoid isomers in araçá-boi growing in the Colombian Amazon forest: (*all-E*)-zeaxanthin, (*all-E*)-lutein, and (*all-E*)-β-carotene. Moreover, these authors found similar total carotenoid levels (0.81 mg/100 g FW) as those identified in the present study (Table 2). Furthermore, a detailed description of the carotenoid composition of murici collected from Belém, Pará State, Brazil (Mariutti et al., 2013) showed similarities with the present findings, with predominantly (*all-E*)-lutein and (*all-E*) zeaxanthin

detected. The total carotenoid content in murici from Tocantins (Table 2) was higher than that found in the fruit from Pará State, Brazil (0.7 mg/100 g; Mariutti et al., 2013) or Ceará State, Brazil (1.1 mg/100 g; Rufino et al., 2010). For puçá-preto, Rufino et al. (2010) found higher levels of total carotenoids in a mix of pulp and peel from fruits collected from the Ceará State (4.2 mg/100 g) than was detected in the present analysis of peel and pulp separately (Table 2). No published data were found in relation to the carotenoid profiles of puçá-preto, mirindiba, or bacupari. The detailed characterization of carotenoids in these fruits represents an important step toward the accurate and precise identification of isomers, vitamin A activity, and bioactivity potential (Astrid Garzon et al., 2012; Burns et al., 2003; Mariutti et al., 2013). However, due the complexity of this detailed analysis, the present screening study focused on the total carotenoid content and its antioxidant activity. Further studies detailing the carotenoid composition of puçá-preto, mirindiba, and bacupari are necessary.

The vitamin C content was as follows: mirindiba < murici < aracá-boi = bacupari = biribá = cajuí = curriola = marmelada-espinho = puçá-preto pulp and peel (Table 2). There was no detectable vitamin C in the bacaba samples. Fruits were grouped according to their vitamin C content, following the classification proposed by Ramful et al. (2011) for citrus fruits. Mirindiba was classified as a high source of vitamin C (> 50 mg/100 g FW), comparable to oranges, papayas (Hernandez et al., 2006), guavas, and passion fruits (Vasco et al., 2008); this fruit had values similar to the two richest sources of vitamin C found in the literature, acerola and camu-camu (Goncalves et al., 2010; Rufino et al., 2010). Murici was classified as a medium source of vitamin C (30-50 mg/100 g FW), similar to mandarins, clementines (Ramful et al., 2011), rosehips (Barros et al., 2010), strawberries (Vasco et al., 2008) and raspberries (Proteggente et al., 2002). Fruits with low vitamin C levels (< 30 mg/100 g FW), including araçá-boi, bacupari, biribá, cajuí, curriola, marmelada-espinho, and pulp and peel of puçá-preto, still had

similar levels to those reported in highly consumed fruits such as red plums, green grapes, pears, apples (Proteggente et al., 2002), lemons (Vasco et al., 2008), pineapples, and mangoes (Hernandez et al., 2006). These results indicated that seasonally available fruit in the Cerrado provided the same daily intake of vitamin C for locals as commercially available fruit.

The total tocopherol content was as follows: mirindiba < puçá-preto peel < puçá-preto pulp = bacaba = bacupari = marmelada-espinho < biribá = murici < araçá-boi < curriola < cajuí (Table 2). At least one tocopherol isomer was found in each sample analyzed; the most frequent were α-tocopherol and β-tocopherol, followed by other isomers (data not shown). Bacaba, bacupari, marmelada-espinho, mirindiba, and the peel and pulp of puçá-preto contained similar total tocopherol levels (1.12-4.05 mg/100 g FW) as blackberries, red raspberries, bottled green olives (Chun et al., 2006), barley, oats, and white rice (Bramley et al., 2000). Other fruits from this study contained the same level (0.60-0.87 mg/100 g FW) as apples, figs, grapes, peaches (Chun et al., 2006), coconuts, carrots, broccoli, cabbages, and asparagus (Bramley et al., 2000). These results indicate that native fruits from the Brazilian Cerrado also provide a complementary source of vitamin E. Although fresh fruits, vegetables, and grains are not considered the richest tocopherol sources, they contribute to total vitamin E intake if they are consumed more than primary sources (e.g., nuts and vegetable oils) in some diets (Bramley et al., 2000; Burns et al., 2003; Chun et al., 2006).

3.2 Antioxidant activity

Bacaba and mirindiba extracts were found to have the highest antioxidant activities in the three assays (Table 3). All five bacaba extracts showed higher ORAC antioxidant activity values than the other fruit extracts, and the acetone and aqueous extracts showed the highest TEAC signal (Table 3), indicating a

possible predominance of HAT over SET mechanisms (Huang et al., 2002; Prior et al., 2005). The high content of total phenolics in bacaba fruits (including the second highest anthocyanin level) (Table 3) were primarily responsible for these ORAC and TEAC activities, confirming results reported by Abadio Finco et al. (2012). Furthermore, we found up to six times more ORAC activity (Table 3) and three times more anthocyanins (Table 2) than were observed by Abadio Finco et al. (2012). These results highlight the influence of total phenolics, including anthocyanins, on the antioxidant activity of bacaba fruits, and its potential as a rich source of bioactive compounds for consumers.

Table 3 Oxygen radical absorbance capacity (ORAC), trolox equivalent oxidant activity (TEAC) and inhibition of β-carotene bleaching (β-car) in five extracts obtained; total phenolics in methanol (MeOH), total anthocyanins in aqueous ethanol (EtOH), total carotenoids in acetone (Acet), Vitamin C in water (H2O), and total tocopherols in heptane (Hept).

	ORAC (µM TEq/g FW)[a]					TEAC (µM TEq/g FW)[a]					β-car (µM TPEq/g FW)[b]				
Fruit	MeOH	EtOH	Acet	H_2O	Hept	MeOH	EtOH	Acet	H_2O	Hept	MeOH	EtOH	Acet	H_2O	Hept
Araçá-boi	8,7i	54,3h	39,3g	44,5i	24,9e	11,7h	33,6g	4.5e	8,3h	25,7h	2.0e	nd	3,6c	12,6e	nd
Bacaba	85,3a	394,3a	367,9a	284,4a	210,6a	71,7a	198,1a	nd	55,3b	175,4a	nd	nd	13,0c	72,7b	nd
Bacupari	nd	nd	75,3e	53,5h	31,4e	11,0h	34,1g	12.2b	8,7h	27,0h	6.0d	nd	14,0c	5,4e	nd
Biribá	14,3g	95,3f	99,6d	80,2g	40,3d	18,7f	55,1f	10.1c	14,2f	44,9g	nd	10.7b	1,5d	24,4d	21.8b
Cajuí	4,3j	28,1i	26,8h	24,2j	11,4f	6,3j	18,1i	5.4d	4,6i	14,1i	nd	6.41b	12,5c	4,6e	0.55d
Curriola	11,7h	56,0g	53,0f	46,6i	23,1e	12,1h	35,0g	nd	8,7h	27,6h	7.2d	4.95b	11,8c	8,3e	nd
Marmelada	16,3f	111,0e	72,9e	93,2f	46,4d	16,1g	26,9h	1.5e	11,4g	54,6f	11.2c	5.63b	38,3b	17,2d	10.5c
Mirindiba	34,8c	195,8c	211,8b	167,0c	88,8b	33,6c	105,9d	61.7a	294,4a	93,1c	104.7a	47.5a	136,5a	97,1a	93.8a
Murici	24,4e	nd	126,2c	113,2e	53,4c	29,2e	84,6e	nd	21,2e	67,0e	24.6b	nd	3,6c	19,9d	nd
Puçá-preto (pulp)	31,4d	175,2d	77,3e	144,7d	54,2c	32,3d	109,3c	1.2e	27,4d	87,8d	nd	nd	nd	nd	nd
Puçá-preto (peel)	39,5b	230,7b	38,8g	177,9b	42,7d	44,9b	131,6b	nd	34,1c	110,3b	nd	nd	13.9c	56.0c	nd
CV (%)	3.4	2.75	4.7	2.4	9.4	3.8	1.9	2.3	1.06	2.5	9.8	18.1	6.3	10	11.2

Values followed by the same letter in the column are equal accordingly the Scott-Knott means test ($p<0.05$); n = 3; nd = not determined.

[a] trolox equivalent per 100 g of fresh matter

[b] tocopherol equivalent per 100 g of fresh matter

All five Mirindiba extracts showed greater inhibition of β-carotene bleaching than the other fruit extracts (Table 3). The high content of total tocopherol found in heptane extracts of mirindiba fruits (Table 2) may relate to this high activity in the β-carotene bleaching assay (Table 3). Similarly, methanol and acetone, which extract tocopherols (Burns et al., 2003; EN 12822, 2000; Prieto et al., 2012), produced high levels of inhibition of β-carotene bleaching. These results were also consistent with the high levels of phenolic compounds (extractable in methanol, ethanol, acetone, or water; Jensen et al., 2007; Kajdzanoska et al., 2011; Khoo, Clausen, Pedersen and Larsen, 2012; Thaipong et al., 2006), and vitamin C (primarily extractable in aqueous solutions; Hernandez et al., 2006; Thaipong et al., 2006) found in mirindiba fruits (Table 2). Similarly, methanolic, ethanolic, and heptane extracts of mirindiba had the highest TEAC values (Table 3). High levels of total tocopherol, total phenolics and vitamin C combined, as found in mirindiba fruits, can act either in polar or nonpolar media (Prior et al., 2005; Thaipong et al., 2006; Xu & Chang, 2007), resulting in the high antioxidant activities observed in this study. The results indicate a possible predominance of HAT over SET mechanism in mirindiba, as previously reported in bacaba samples.

Methanolic, ethanolic, and aqueous extracts of the puçá-preto pulp and peel showed high antioxidant activity in the ORAC assay, as did these and the heptane extracts in the TEAC assay (Table 3). Rufino et al. (2010) assessed TEAC in a mix of puçá-preto pulp and peel and found values similar to those observed in this study for ethanol and heptane extracts of pulp and peel analyzed separately (Table 3). These authors associated the high antioxidant activity with the total phenolic content (868 ± 51 mg GAE/100 g), which was similar to that observed in the present study (Table 2). Remarkably, the levels of bioactive compounds (Table 2) and antioxidant activity (Table 3) were higher in the puçá-preto peel than in the pulp. Moreover, anthocyanins were only detected in the peel (Table 2).

In this respect, we only observed inhibition of β-carotene bleaching in the acetone and aqueous extracts of the peel (Table 3). Given that anthocyanins are extractable in acetone and aqueous solutions with or without acidification (Jensen et al., 2011; Kajdzanoska et al., 2011; Khoo et al., 2012) and are often considered to be the major contributors to high antioxidant activity *in vitro* in polyphenol-rich fruits (Abadio Finco et al., 2012; Khoo et al., 2011; Proteggente et al., 2002), we suggest that the high antioxidant activity of the peel was due to the anthocyanins present.

The methanol murici extract showed considerable inhibition of β-carotene degradation, and its acetone, aqueous, and heptane extracts showed good ORAC antioxidant activity (Table 3). Murici fruits collected from the Brazilian states of Pará (Mariutti et al., 2013) and Goiás (Malta et al., 2012) have been studied for their high peroxyl radical scavenging potential *in vitro*, as well as for their anti-genotoxic and anti-mutagenic properties *in vivo*. Such properties are primarily attributed to carotenoids and phenolic constituents (Malta et al., 2012; Mariutti et al., 2013; Perez-Gutierrez et al., 2010), both of which are extractable in acetone or methanol (Jensen et al., 2007; Larsen and Christensen, 2005; Xu and Chang, 2007). Moreover, the vitamin C content of murici is also associated with antioxidant activity *in vitro* for fruits collected from Ceará (Rufino et al., 2010) and Minas Gerais State (Souza et al., 2012). In this study, tocopherol-rich extracts (Hept) of murici collected in Tocantins State showed ORAC activity (Table 3), indicating that this fruit provides a complementary source of tocopherols, in addition to carotenoids, phenolics, and vitamin C.

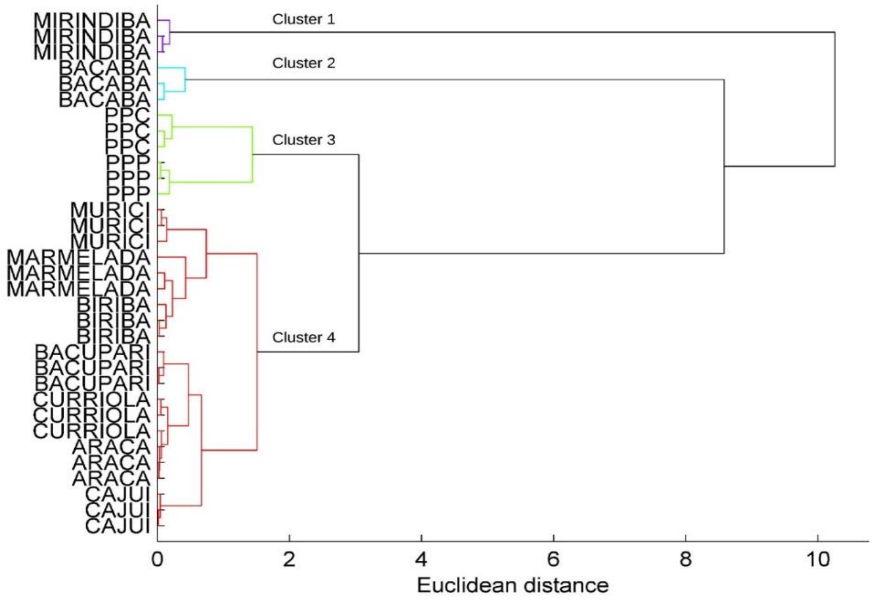
The other fruits studied, including araçá-boi, bacupari, biribá, cajuí, curriola, and marmelada-espinho, had lower antioxidant activity values (Table 3). However, even these lower TEAC values were in the same range as those observed by Khoo et al. (2011) in 34 cultivars of sour cherry, and by Ramful et al. (2011) in 21 citrus varieties. Moreover, their ORAC values were in the same range as those found by Thaipong et al. (2006) for four varieties of guava, and

those reported by Khoo et al. (2012) in fifteen cultivars of blackcurrant. These results confirm that native fruits from the Brazilian Cerrado provide sources of bioactive compounds with high antioxidant potentials *in vitro* that are on a par with other well-known fruits. To our knowledge, out of the six fruits mentioned above, only araçá-boi fruit growing in the Colombian (Astrid Garzon et al., 2012) and Brazilian (Neri-Numa et al., 2013) Amazon have been studied for their high antioxidant activity *in vitro*, as well as for their anti-proliferative and anti-mutagenic properties *in vivo*; these activities were attributed to their carotenoid and phenolic constituents. The present study found similar total carotenoid levels (Astrid Garzon et al., 2012), higher TEAC activity (Astrid Garzon et al., 2012), lower total phenolic levels (Astrid Garzon et al., 2012; Neri-Numa et al., 2013), and lower ORAC (Neri-Numa et al., 2013) in araçá-boi growing in the Brazilian Cerrado, as compared with previous studies.

3.3 Hierarchical cluster analyses

HCA analysis distinguished four groups (Figure 2). The first group comprises mirindiba samples, which had abundant bioactive compounds and excellent antioxidant activities (Table 2, 3) and thus emerged as a rich source of antioxidants. The second group consisted of bacaba samples, as the second richest source of bioactive compounds. These results were consistent with the antioxidant potential reported by Abadio Finco et al. (2010). Puçá-preto pulp and peel samples were in the third cluster (Figure 2). In view of these results, we also consider both the peel and pulp of puçá-preto to represent rich sources of bioactive compounds.

Figura 2 Dendrogram for araçá-boi, bacaba, bacupari, biribá, cajuí, curriola, marmelada, mirindiba, murici, pulp (PPP) and peel (PPC) of puçá-preto samples, obtained from the hierarchical cluster analysis accordingly them total phenolic, total anthocyanins, total carotenoids, vitamin C, total tocopherols and antioxidant activities measured by β-carotene bleaching, ORAC and TEAC assays in five extracts obtained; phenolic compounds in methanol (MeOH), anthocyanins in aqueous ethanol (EtOH), carotenoids in acetone (Acet), Vitamin C in water (H_2O), and tocopherols in heptane (Hept).



The fourth HCA group comprised fruits with relatively low antioxidant levels, such as bacupari, biribá, cajuí, curriola, and marmelada-espinho, in addition to araçá-boi and murici, which have both been reported to show high bioactivities (Malta et al., 2012; Mariutti et al., 2013; Neri-Numa et al., 2013; Perez-Gutierrez et al., 2010; Rufino et al., 2010; Souza et al., 2012). The present holistic analysis of fruit extracts prepared using solvents with different polarities indicated that fruits with relatively low levels of antioxidants had considerable antioxidant potential.

4 CONCLUSION

Brazilian native fruits from the Cerrado provide rich sources of bioactive compounds, comparable to commonly consumed fruits such as plums, oranges, guavas, rosehips, and various berries and citrus fruits. To our knowledge, this is the first comprehensive study of the bioactive compounds and antioxidant activities of bacupari, biribá, cajuí, curriola, marmelada, and mirindiba. Bacaba, puçá-preto, and murici were found to be rich sources of bioactive compounds, as previously described in the literature. Moreover, we report that mirindiba contained above-average levels of carotenoids and tocopherols and can be classified as a “super fruit”, similar to camu-camu, acerola, and various berries, owing to its high vitamin C and total phenolic levels.

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