

# Bioactivities of Brazilian Fruits and the Antioxidant Potential of Tropical Biomes

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**Abstract** The increased consumption of fruits rich in antioxidants has been associated with reduced risk of several chronic diseases caused by oxidative stress. The biological properties of these fruits have been largely attributed to their high levels of various phenolic compounds, carotenoids and ascorbic acid. Brazil is a country with appropriate climatic conditions for growing a large number of underexploited native and exotic fruit species. These fruits have great potential to the agricultural industry and can offer a future source of income for local peoples. The study of their bioactivities has been very promising and became advisable to incorporate some of these fruits in the diet of the Brazilian.

**Keywords** Antioxidant Activity, Bioactivities, Brazilian Fruits

## 1. Introduction

Epidemiological studies have shown that dietary patterns are significantly associated with the development or prevention of chronic degenerative diseases such as cancer, diabetes, coronary heart disease and Alzheimer's [1-3]. Under normal conditions, human metabolism maintains a balance between oxidants and antioxidants, an important process in maintaining appropriate physiological conditions [1],[4]. An overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can cause an imbalance, leading to oxidative and nitrosative stress, which are important factors in the development of chronic and degenerative diseases [5-8].

There is growing interest in fruit consumption primarily due to their nutritional value and medicinal properties. Fruits contain bioactive compounds such as phenolic compounds, anthocyanins, carotenoids, and ascorbic acid, among others. These compounds act by combating reactive oxygen and nitrogen species, stimulating the immune system, regulating the expression of genes involved in cell proliferation or apoptosis and modulating hormone metabolism [4-8]. They also have antibacterial and antiviral action [3],[9]. In the human body, these actions reduce the incidence of cancer, inflammation, cataracts, macular degeneration and cardiovascular disease [3],[10-14].

Brazilian native fruits or those adapted to the tropical climate are surprisingly rich in bioactive compounds and have significant antioxidant activity [15-20]. Additionally,

they are distributed across the six terrestrial biomes of Brazil. Although the majority of studies to date have focused on the fruits of the Amazon Forest, Atlantic Forest and Cerrado biomes.

The Amazon is the largest reserve of biodiversity in the world and is also the largest Brazilian biome, occupying almost half of Brazil (49.3%). It is dominated by a warm, humid climate, with an average temperature of 25 °C and torrential rains that are well distributed throughout the year. The Amazon biome is characterised by the Amazon River basin, which drains 20% of the world's fresh water, and the characteristic vegetation is tall trees. It is estimated that this biome harbours more than half of all species living in Brazil [21]. Because of these features, the Amazon is home to numerous fruit species that are consumed by the local population and distributed through the local economy at trade fairs. However, most of these fruit species are unknown to the wider Brazilian population.

The biome known as Cerrado (a type of savannah) spans approximately 2 million km<sup>2</sup> across central Brazil, representing approximately 23% of the land area of the country. In terms of area, it is only smaller than the Amazon rainforest, which covers approximately 3.5 million km<sup>2</sup>. Its rich flora has only just begun to be studied, and to date includes approximately 1000 species of trees, 3000 species of herbs and shrubs and approximately 500 vine species [22-24]. Over the past 30 years, agricultural mechanisation and the ease of cleaning and fertilising the land has contributed to accelerated devastation of native Cerrado vegetation, and it is estimated that approximately 40% of the biome has already been deforested [24]. The consumption of fruit produced from the Cerrado is quite limited in national terms. Only the fruits of a palm known as "pequi" are widely consumed in other Brazilian regions, namely, the midwest

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and parts of the north-eastern and south-eastern regions.

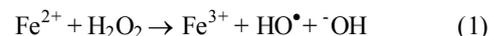
The Atlantic Forest biome is a complex environment that includes mountain ranges, valleys, plateaus and plains along the Brazilian continental Atlantic margin and continues from the Southern Plateau (“Planalto Meridional”) to the State of Rio Grande do Sul, covering 1.1 million km<sup>2</sup> of Brazilian territory. Its main vegetation type is dense rain forest, usually composed of tall trees. Some fruits from the Atlantic Forest are well known, such as the “jaboticaba” (*Myrciaria cauliflora*) and “pitanga” (*Eugenia uniflora*). However, numerous fruit species are poorly known, but have begun to be marketed in the form of frozen pulp, such as “uvaia” (*Eugenia pyriformis*), “grumixama” (*Eugenia brasiliensis*) and “jussara” (*Euterpe edulis*).

## 2. Reactive Oxygen and Nitrogen Species

In the last decade, much research has been conducted to elucidate the mechanism of formation and action of ROS and RNS in organisms. ROS and RNS are products of normal cellular metabolism, and their presence and concentration is a biological paradox[5, 25]. These radicals serve to prevent diseases, helping the immune system mediate cellular signalling and cellular regulation and acting in apoptosis. However, they can also cause significant damage to cell macromolecules and thus play an important role in carcinogenesis and the development of cardiovascular disease[26-28].

The overproduction of reactive species is controlled by an efficient antioxidant system that can control and restore the pro- and anti-oxidant balance. However, when this system is unbalanced with a predominance of oxidants, oxidative and/or nitrosative stress occurs. In biological systems, the cell membrane is one of the main sites of action of reactive species, along with the membranes of intracellular organelles such as the mitochondria, endoplasmic reticulum and the nucleus[6],[7],[26]. Table 1 shows the main reactive species of oxygen and nitrogen, their characteristics and origin.

With regard to the potential reactivity of ROS and RNS in the biological environment, the hydroxyl radical (HO•) is the most reactive and, in theory, can oxidise any organic molecule[26]. Thus, it is likely that the radical will react very close to the site where it was generated[26],[29]. Typically, reactions with guanine and thymine occur by the addition of HO• to the aromatic rings, which induce breaks in the DNA chain[26]. HO• is primarily responsible for the initiation of lipid oxidation due to its ability to remove an allylic hydrogen from the double bond of unsaturated lipids[8]. Hydrogen peroxide is more stable than the hydroxyl radical and can permeate membranes, allowing reactions to occur with biological targets in compartments distant from the site of formation. In the presence of transition metals, HO• is generated via the Fenton reaction (reaction 1).



## 3. Antioxidant Compounds Present in Fruits

Exposure to reactive oxygen and nitrogen species from different sources has led organisms to develop a series of defence mechanisms, one of which uses antioxidants[27, 29]. To neutralise the attack of ROS and RNS, cells have biological enzymatic antioxidants that convert ROS and RNS into less reactive species. For example, the superoxide anion radical is converted into molecular oxygen and hydrogen peroxide by superoxide dismutase, and H<sub>2</sub>O<sub>2</sub> is converted into water and molecular oxygen by catalase. There is also a non-enzymatic antioxidant system comprised of compounds synthesised by the human body such as bilirubin, ceruloplasmin, melatonin and others ingested through the diet or through supplementation, such as ascorbic acid, α-tocopherol, phenolic compounds and carotenoids, which deactivate reactive species[26],[30].

Antioxidants can act in different ways to protect biomolecules, such as the removal of ROS and RNS by enzymatic action, reducing the formation of reactive species, physically suppressing ROS and deactivation of ROS and RNS by so-called “agents of sacrifice”, which are molecules that are preferentially oxidised, preserving biomolecules of great biological importance[7],[26]. Compounds such as carotenoids and phenolic compounds serve this function and are degraded when they deactivate some ROS and RNS[31]. The physical deactivation mechanism of singlet oxygen by the action of carotenoids is an exception to this rule, as it involves only the transfer of energy and thus does not alter the structure of the carotenoid[32],[33].

### 3.1. Carotenoids

Carotenoids are a group of pigments that are widely distributed in nature, occur naturally in large quantities and are known for their structural diversity and the various biological functions that they serve[34].

In general, the basic structure of carotenoids is one tetraterpene with 40 carbon atoms, consisting of eight isoprenoid units, which are connected such that the molecule is linear with inverted symmetry in the centre[35]. This chain has a series of conjugated double bonds that generate a system of π electrons that move about the whole polyene chain via resonance, providing these compounds with high chemical reactivity and absorption in the visible region[35]. Due to this absorption of light, the colouration of carotenoids ranges from yellow to red.

The high chemical reactivity provided by the system of conjugated double bonds makes these compounds able to deactivate various reactive species and therefore confers antioxidant properties both in biological systems and in food.

**Table 1.** Characteristics of the main reactive species of oxygen and nitrogen

Reactive Species	Generation	Destination
Superoxide anion radical: $O_2^{\bullet-}$	Generated in the electron transport chain in mitochondria, in the microsomes through enzymes such as xanthine oxidase and NADPH oxidase and through the mono-electronic reduction of $O_2$ .	Can reduce $Fe^{3+}$ to $Fe^{2+}$ and accelerate the Fenton reaction.
Hydrogen peroxide: $H_2O_2$	Formed from the partial reduction of molecular oxygen by two electrons. It is an intermediate formed by dismutation of $O_2^{\bullet-}$ catalysed by the enzyme superoxide dismutase and by the action of several enzyme oxidases <i>in vivo</i> .	Weak oxidising and reducing agent. In the presence of transition metals generates the hydroxyl radical through the Fenton reaction.
Hydroxyl radical: $HO^{\bullet}$	Formed from the reduction of molecular oxygen by 3 electrons in the Fenton and Haber-Weiss reactions, catalysed by metals.	Most reactive and damaging radical known, to which the body has no defence mechanism.
Peroxyl radical: $RO_2^{\bullet}$	Formed from organic hydroperoxides.	Intermediate in membrane lipid peroxidation.
Alkoxy radical: $RO^{\bullet}$	Organic radical centred on oxygen. Formed in carbon radical reactions with oxygen, as in lipid peroxidation.	Intermediate in membrane lipid peroxidation.
Singlet oxygen: $^1O_2$	Produced by photochemical reactions or by other radiation sources. Can be generated by the transfer of energy from a sensitizer in the excited triplet state to oxygen.	Electronically excited state of oxygen.
Nitric oxide: $NO^{\bullet}$	Synthesised by the enzymatic action of nitric oxide synthase, which converts L-arginine into nitric acid and L-citrulline.	Diffuses rapidly between and within cells, is a potent vasodilator involved in regulating blood pressure and reacts with oxygen to form nitrogen dioxide.
Nitrogen dioxide: $NO_2^{\bullet}$	Formed from the exposure of $NO^{\bullet}$ in the air or the protonation of peroxy nitrite.	Potent initiator of lipid peroxidation.
Peroxy nitrite: $ONOO^{\bullet}$	Formed by the reaction of the superoxide anion radical and nitric oxide.	Properties similar to the hydroxyl radical, causing damage including to S-H protein groups. Forms $^{\bullet}HO$ regardless of the presence of transition metals.
Nitryl chloride: $NO_2Cl$	Formed from mixtures of nitric oxide and hypochlorous acid.	Nitration and chlorination agent.
Chloramines	Weaker oxidants with a longer life than $HOCl$ , react with thiols, thioethers and metallic iron centres.	Variable toxicity depending on membrane polarity and permeability.

Sources: [6-8],[26-28]

The following three mechanisms have been suggested for the deactivation of radicals such as  $ROO^{\bullet}$  and  $HO^{\bullet}$  by carotenoids: 1) electron transfer; 2) allylic hydrogen abstraction; and 3) the addition of the radical to the conjugated double bonds. Which mechanism occurs depends on the characteristics of the reaction system, the solvent polarity and the carotenoid structure [36],[37].

### 3.2. Phenolic Compounds

Phenolic compounds are secondary metabolites synthesised by plants during normal development and in response to stress, such as that caused by infections, wounds and UV radiation. These compounds are present in all plants and constitute a diverse photochemical group [38],[39].

Plants contain a variety of phenolic compounds, including the following: simple phenols (resorcinol), phenolic acids (*p*-hydroxybenzoic acid), hydroxycinnamic acids (caffeic acid), coumarins, flavonoids (quercetin), stilbenes (resveratrol), condensed tannins (procyanidin), lignans (matairesinol) and lignins. The major phenolic compounds in the human diet are phenolic acids, tannins and flavonoids, which occur in food in amounts of approximately 1-3 mg/kg. The daily intake of total flavonoids in American adults was estimated to be 189 mg/day [40] and in Brazilians the daily intake varies between 60 and 106 mg, with oranges as the main source [41].

Flavonoids are the largest group of phenolic compounds in

plants [42],[43]. These compounds are characterised by having 15 carbon atoms in their centre, which is composed of two aromatic rings joined by a chain of three carbon atoms that may or not form a third ring ( $C_6-C_3-C_6$ ). The major subclasses of flavonoids may be distinguished based on the oxidation of the central pyran ring and by the position of the B ring in flavonols, flavones, flavanol or flavan-3-ol, flavanones, isoflavones and anthocyanins [44],[45].

Individual compounds within each subclass can be differentiated by the number and position of the hydroxyl and methoxyl groups. Flavonoids occur as aglycones or are linked to sugar molecules which are known as the glycosylated form, often occurring as *O*-glucoside and more rarely as *C*-glucoside [38, 39],[44, 45].

Phenolic compounds are known to be potent deactivators of reactive species. The following two main mechanisms for this action have been observed: 1) the transfer of a hydrogen atom; and 2) the transfer of one electron. With the reduction in levels of ROS and RNS, phenolic compounds can act by modulating signal pathways that depend on the redox potential of the cells, thus modulating gene expression [46, 47].

### 3.3. Vitamin C

In biological systems (pH 7.4), 99.9% of Vitamin C is in the form of ascorbate, which is the form that acts as an antioxidant by donating an  $H^{\bullet}$  to one radical [8]. Ascorbate is

able to disable ROS and RNS in the aqueous biological environment, resulting in the formation of the semidehydroascorbic radical anion or the minimally reactive ascorbyl. It can act directly on cell membranes, preventing lipid oxidation, or indirectly by regenerating vitamin E, which acts as an antioxidant on the lipophilic surface of the membrane[8],[48].

### 3.4. Vitamin E

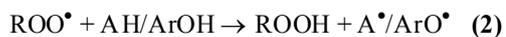
Vitamin E is the common name of two different families of lipophilic compounds, tocopherols and tocotrienols.  $\alpha$ -tocopherol is the most potent compound and is generally the predominant form found in foods. Structurally, tocopherols and tocotrienols differ only in their side chains and are subdivided into the  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  forms, depending on the number and position of the methyl groups on the chromanol ring[6],[49]. Tocopherol is a liposoluble antioxidant that acts by blocking the propagation step of lipid peroxidation of unsaturated fatty acids in membranes and lipoproteins[8]. It intercepts the peroxy radical ( $RO_2^\bullet$ ), resulting in the formation of the tocopheryl radical, which can be regenerated to tocopherol by ascorbate or glutathione[8],[50].

## 4. Methods Used to Determine Fruit Antioxidant Activity

A wide variety of chemical methods are used to determine the antioxidant activity of fruits, such as the oxygen radical absorbance capacity (ORAC)[51],[52]; inhibition of lipoprotein oxidation induced by cupric ion (CUPRAC - cupric ion reducing antioxidant capacity)[53-55]; the total capacity of oxyradical deactivation (TOSC - total oxidant scavenging capacity)[56]; the reduction of the ferric ion (FRAP - ferric reducing ability of plasma)[52],[57],[58]; ABTS/TEAC (Trolox equivalent antioxidant capacity)[52],[58],[59]; and TRAP (total radical trapping antioxidant parameter)[58]. The methods used to evaluate the antioxidant activity are classified according to the mechanism of deactivation of reactive species, which is either based on electron transfer (ET) and hydrogen atom transfer (HAT). In some cases these two mechanisms cannot be differentiated[60].

### 4.1. Methods Based on Hydrogen Atom Transfer (HAT)

The HAT methods measure the ability of a compound or antioxidant extract to disable free radicals (usually peroxy radicals) by donating a hydrogen atom, as shown in the reaction 2.



The aryloxy radical ( $ArO^\bullet$ ) is formed from the reaction of a phenolic antioxidant and peroxy radical and is stabilised by resonance. The effectiveness of a phenolic antioxidant comes from reacting with free radicals faster than biomolecules, thus protecting them from oxidation or nitration[60].

ORAC (which measures the absorption capacity of the hydrogen radical) is one of the principal methods employing this mechanism. It is based on the use of  $\beta$ -phycoerythrin as an indicator of oxidative damage caused by free radicals from 2,2'-azobis(2-amidinopropane dihydrochloride) (A BAP), a peroxy radical generator, and Trolox as an antioxidant reference substance[61].

The addition of an extract from tissue, serum or food samples leads to the inhibition of oxidative processes (delayed fluorescence decay over time), measured in terms of  $\mu\text{mol}$  of Trolox equivalent (TE)/litre or gram of tissue. Adaptations of the method have been developed that employ fluorescein instead of  $\beta$ -phycoerythrin[62],[63]. Other methods such as FRAP (which measures oxygen consumption), PCL (a photochemiluminescence assay measuring the chemical luminescence of the luminol radical) and the  $\beta$ -carotene/linoleic system (measures the discoloration of  $\beta$ -carotene) are also based on the deactivation of reactive species by the HAT mechanism.

### 4.2. Methods Based on Electron Transfer (ET)

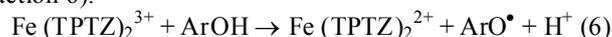
Among the methods that employ the ET mechanism, ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium) are the most widely used. The ABTS method, first suggested by Miller et al.[64] is based on metmyoglobin acting as a peroxidase in the presence of hydrogen peroxide and forming the ferryl myoglobin radical, which oxidises ABTS, forming  $ABTS^{\bullet+}$ . However, Rice-Evans et al.[65] reported another way to generate the  $ABTS^{\bullet+}$  radical by chemical reduction using manganese dioxide. Re et al.[59] proposed a modification of this method where the radical is directly generated by potassium persulfate (reaction 3). Although the main advantage of this method is its simplicity, as it can be performed in any laboratory, the results express the ability of the extract or antioxidant substance to react with the  $ABTS^{\bullet+}$  radical and not the ability to inhibit an oxidative process[66]. This is also a limitation of the DPPH method.



The use of the DPPH method was initially reported by Brand-Williams et al.[67] and involves the loss of purple colour ( $\lambda=515 \text{ nm}$ ) by deactivation of the DPPH radical by the action of antioxidant compounds or extracts according to reaction 5.

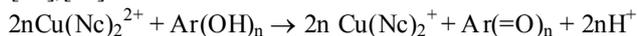


The FRAP method measures the ability of an antioxidant compound or extract to reduce the Fe (III)TPTZ complex to the ferrous form, which shows intense blue staining[68]. Therefore, the increase in absorbance measured at  $\lambda=593 \text{ nm}$  is due to the reduction of the ferric form to the ferrous form of the complex employed in the reaction system at a low pH (reaction 6).



The CUPRAC method was developed from the principle

of reducing the cupric ion and measures the formation of an orange-yellow complex ( $\lambda=450$  nm) by reduction of the chromogenic compound bis(neocuproine)copper (II) chelate to bis(neocuproine)copper (I) chelate as in reaction 7[54],[55].



The efficiency of the reduction of this complex by phenolic compounds depends on the number and position of the hydroxyl groups and the degree of conjugation of the molecule.

## 5. Bioactivity of Fruits in Brazilian Biomes

### 5.1. Amazon Forest

Recently, several studies have been conducted to assess the presence of and quantify the bioactive compounds in fruits native to the Amazon rainforest. The antioxidant potential of these fruits and the health benefits that their consumption can bring were also evaluated.

One of the most studied fruits that has reached the status of “super fruit” is “açaí” (*Euterpe oleracea*), which, due to its sweet taste and energetic properties, has gained worldwide recognition in recent years. The major bioactive components of acai are polyphenols, specifically anthocyanins[15],[69],[70], with the total content ranging from 252.9 to 303.7 mg C3G.100g<sup>-1</sup>[69],[71]. The total phenolic and carotenoid content is shown in Table 2.

**Table 2.** Bioactive compounds from acai (*Euterpe oleracea*)

Bioactive Compound	Concentration <sup>[71]</sup> (mg.100g <sup>-1</sup> FM)	Concentration <sup>[9]</sup> (mg.100g <sup>-1</sup> FM)
Total carotenoids	0.52±0.02	2.8±0.4
Total flavonoids	55.9±0.6 <sup>a</sup>	91.3±20.6
Total anthocyanins	252.9±10.1 <sup>b</sup>	111.0±30.4
Total phenolics	424.9±8.8 <sup>c</sup>	454 ±44.6 <sup>c</sup>
Ascorbic acid	n.d.	84.0±10

FM: fresh matter; <sup>a</sup> mg CE.100g<sup>-1</sup>: catechin equivalent; <sup>b</sup> mg C3G.100g<sup>-1</sup>: cyanidin 3-glucoside equivalent; <sup>c</sup> mg GAE.100g<sup>-1</sup>: gallic acid equivalent; n.d.: not detected.

Using the ORAC method, Kang et al.[16] evaluated the antioxidant activity of acai against several species of reactive oxygen and nitrogen species. The results showed that acai extract had excellent activity against the hydroxyl radical (1357.3  $\mu\text{mol TE.g}^{-1}$ , DW) and the superoxide anion radical (169.0  $3 \mu\text{mol TE.g}^{-1}$ , DW), which are two extremely reactive radicals. It also showed activity against the following radicals: peroxy (1014  $\mu\text{mol TE.g}^{-1}$ , DW), peroxy nitrite (37.2  $\mu\text{mol TE.g}^{-1}$ , DW) and singlet oxygen (71.6  $\mu\text{mol TE.g}^{-1}$ , DW), totalling 2649.1  $\mu\text{mol TE.g}^{-1}$ . The antioxidant activity measured in this study was much higher than that reported in previous studies for other dark-coloured berries.

Other authors have also evaluated the antioxidant capacity of acai. Gordon et al.[72] submitted an extract of acai to a system employing the stable radical ABTS<sup>•+</sup>, and the TEAC

values obtained were 17.0 and 2.78  $\mu\text{mol TE.100g}^{-1}$  DM for unripe and ripe acai, respectively. The antioxidant activity of the same extracts was also measured against peroxy and peroxy nitrite radicals, employing the TOSC method. The values obtained for the peroxy radical were 12.1 mg DM.100 mL<sup>-1</sup> for unripe acai and 24.0 for ripe acai, while for the radical peroxy nitrite the values obtained were 46.4 and 87.2 mg DM.100 mL<sup>-1</sup> for unripe and ripe acai, respectively.

The use of cellular models to evaluate the antioxidant activity of fruit extracts is becoming more common and may give more reliable results than chemical models. Kang et al.[16] evaluated the inhibition of oxidative stress by CAP-e assays (cell-based antioxidant protection in erythrocyte), in which an aqueous extract containing the antioxidant is introduced into living cells to quantify the protection it offers against the oxidative damage caused by peroxy radicals. The IC<sub>50</sub> of acai extract was quantified as 0.167 g.L<sup>-1</sup>.

Studies using animal models have also demonstrated the positive effect of diets supplemented with acai pulp on oxidative stress biomarkers. Oliveira de Souza et al.[73] found that a hypercholesterolemic diet supplemented with 2% (dry matter wt/wt) of acai for 6 weeks led to a decrease in total cholesterol (33%) and non-HDL cholesterol (34%) in Fischer rats when compared to animals fed only the hypercholesterolemic diet. Acai supplementation also led to a significant reduction in the activity of superoxide dismutase (30%) and increased activity of PON-arylesterase (39%), indicating that there may be a decrease in oxidative stress caused by the hypercholesterolemic diet. In addition to these results, a decrease in food intake was also observed in the group that consumed a normal diet supplemented with acai, suggesting that polyphenols may modulate appetite.

A reduction in oxidative stress was also observed in a study by Ribeiro et al.[71], who investigated the genotoxic and antigenotoxic effects of acute and subacute treatments with acai pulp in Swiss albino rats. It was found that acai protected the DNA of liver and kidney cells from damage induced by the drug doxorubicin, with harm reduction values reaching 98.1% in the kidneys and 92.7% in the livers of rats that underwent the subacute treatment for 14 days, with acai administered at 16.67 g.kg<sup>-1</sup> bw.

There are few studies evaluating the effect of consuming acai in humans. One was performed by Jensen et al.[74] and evaluated the antioxidant activity and lipid peroxidation levels in 12 volunteers in a randomised, placebo-controlled study. Blood samples were taken before and after (1 and 2 hours) ingestion of 120 ml of a juice whose main component was acai (Mona Vie<sup>®</sup>). The levels of the serum antioxidant and lipid peroxidation assays were analysed using CAP-e and TBARS (thiobarbituric acid reactive substances). The results showed that the levels of antioxidants in the serum increased significantly in 11 of the 12 volunteers at 2 hours after ingestion of Mona Vie<sup>®</sup>. A significant decrease in lipid peroxidation was also observed 2 hours after administration of acai.

Another fruit native to the Amazon region that has been studied due to its elevated quantity of phytochemicals,

notable for their actions related to promoting health, is the “camu-camu” (*Myrciaria dubia*). The bioactive composition of this fruit is shown in Table 3.

**Table 3.** Bioactive compounds from camu-camu (*Myrciaria dubia*)

Bioactive compound	Concentration <sup>[75],[76],[77]</sup>
Total Carotenoids*	354.8 – 1095.3 $\mu\text{g}\cdot 100\text{g}^{-1}$
all- <i>trans</i> -lutein*	160.5 – 601.9 $\mu\text{g}\cdot 100\text{g}^{-1}$
Ascorbic acid	2010 - 2061.01 $\text{mg}\cdot 100\text{g}^{-1}$
Total Anthocyanins	30.3 – 54.0 $\text{mg}\cdot 100\text{g}^{-1}$
Delphinidin 3-glucoside	4.3 – 5.1% (peak area)
Cyanidin 3-glucoside	88.0 – 89.5% (peak area)
Polyphenols Totais	1176–1320 $\text{mg GAE}\cdot 100\text{g}^{-1}$ FM

GAE: gallic acid equivalent; FM: Fresh Matter;

The camu-camu contains a large variety of bioactive compounds, including ascorbic acid, at varying levels depending on the maturation stage. According to Chirinos et al.[77] the ascorbic acid contents in ripe camu-camu was found  $2,010 \pm 65 \text{ mg}\cdot 100 \text{ g}^{-1}$  FM, while levels in unripe fruits reached  $2,280 \pm 65 \text{ mg}\cdot 100 \text{ g}^{-1}$  FM. Other authors also reported similar values for the ascorbic acid content in the camu-camu, including  $2,061 \text{ mg}\cdot 100 \text{ g}^{-1}$  FM at the ripe stage and  $1,910 \text{ mg}\cdot 100 \text{ g}^{-1}$  FM at the unripe stage[78]. The ascorbic acid content of camu-camu was found to be 1.5 and 11 times that of acerola and cashew, two other Brazilian fruits, respectively[19].

Regarding the presence of natural pigments, it has been found that anthocyanins and carotenoids are present mostly in the fruit exocarp[75],[76]. The content of anthocyanins in the fresh peel of camu-camu ranged from 30 to 54  $\text{mg}\cdot 100 \text{ g}^{-1}$  in fruit collected from Iguape and Mirandópolis, respectively[75]. Two studies have reported total carotenoid levels in camu-camu, and the fresh peel content quantified by Zanatta and Mercadante[76] ranged from 354.8 to 1,095  $\mu\text{g}\cdot 100 \text{ g}^{-1}$  in fruit also collected from Iguape and Mirandópolis. Rufino et al.[19] found similar carotenoid levels ( $400 \mu\text{g}\cdot 100 \text{ g}^{-1}$  FM).

Together with ascorbic acid, phenolic compounds make up the highest concentrations of bioactive compounds in camu-camu. In a recent study, Chirinos et al.[77] reported that the stage of ripening influenced the total levels of phenolic compounds. Unripe fruits showed values of  $1,120 \text{ mg GAE}\cdot 100\text{g}^{-1}$  FM, while the partially ripe and fully ripe fruits showed levels of  $1,420$  and  $1,320 \text{ mg GAE}\cdot 100\text{g}^{-1}$  FM, respectively. Similar content was reported by Rufino et al.[19] for camu-camu in natura ( $1,176 \pm 14.8 \text{ mg GAE}\cdot 100\text{g}^{-1}$  FM), and dry pulp levels have been quantified at  $11,615 \pm 384 \text{ mg GAE}\cdot 100 \text{ g}^{-1}$  by Rufino et al.[19] and  $10,100 \pm 25 \text{ mg GAE}\cdot 100 \text{ g}^{-1}$  by Reynertson et al.[79].

The major phenolic compounds identified in camu-camu include the following: ellagic acid ( $45 \text{ mg}\cdot 100 \text{ g}^{-1}$  DM), quercetin ( $24 \text{ mg}\cdot 100 \text{ g}^{-1}$  DM), quercitrin ( $6 \text{ mg}\cdot 100 \text{ g}^{-1}$  DM) and rutin ( $13 \text{ mg}\cdot 100 \text{ g}^{-1}$  DM)[79]. Kaempferol ( $0.4$  to  $2.5 \text{ mg}\cdot 100 \text{ g}^{-1}$  DM) and quercetin ( $42 \text{ mg}\cdot 100 \text{ g}^{-1}$  DM) have also been quantified in camu-camu fruit[80].

Different methods have been used to establish the antioxidant activity of camu-camu, including bleaching of

the ABTS<sup>•+</sup> radical, DPPH, ORAC and FRAP. Rufino et al.[19] found that camu-camu antioxidant activity measured by the DPPH method was  $\text{IC}_{50} = 42.6 \text{ g DM}\cdot \text{g}^{-1}$ , suggesting a positive association between the antioxidant activity and the ascorbic acid content. Chirinos et al.[77] also using the DPPH method, demonstrated that ascorbic acid is responsible for 70% of the antioxidant capacity of camu-camu ( $153 - 167 \mu\text{mol TE}\cdot \text{g}^{-1}$  FM). Another determination of the  $\text{IC}_{50}$  for camu-camu by the DPPH method gave  $57.2 \pm 5.61 \mu\text{g}\cdot \text{mL}^{-1}$ [79]. Genovese et al.[18] showed that the antioxidant activity of camu-camu pulp was the highest of the 5 fruits and 7 pulps studied. Those authors also found that the antioxidant capacity was 10 times higher when compared with pulps from other exotic fruits such as “cagaita” (*Stenocalyx dysentericus*), which had the second highest antioxidant activity.

De Souza Schmidt Gonçalves et al.[80] found that the antioxidant capacity of camu-camu measured by ORAC and DPPH was the highest among 16 fruits analysed, and a positive correlation between the total polyphenol content and antioxidant capacity was also observed for ORAC ( $r = 0.795$ ;  $p < 0.001$ ) and DPPH ( $r = 0.989$ ;  $p < 0.001$ ). The high correlation between the Folin-Ciocalteu and DPPH methods was attributed to the similarity of the mechanisms of action of the two methods, which are based on electron transfer. The ORAC method is based on the transfer of a hydrogen atom from the antioxidant to the peroxy radical formed by the thermal decomposition of AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride).

Using the TOSC method to establish the antioxidant capacity of camu-camu juice, Rodrigues et al.[81] found that its action against peroxy and peroxy nitrite radicals was significantly higher than that of apple, acai, blueberry and orange juice. This study also noted the significant contribution of ascorbic acid to the antioxidant activity of camu-camu juice.

In addition to the potent chemical antioxidant activity attributed to the high levels of phenolic compounds and ascorbic acid, camu-camu showed a significant antigenotoxic effect on the blood cells of mice exposed to subacute, acute and chronic treatments with camu-camu juice at concentrations of 25, 50 and 100%[82]. The results of the comet assay (*ex vivo* analysis using  $\text{H}_2\text{O}_2$ ) showed a significant decline in the damage index for all groups receiving acute treatment with camu-camu juice by gavage, regardless of the concentration of the juice. When subacute treatment was used, there was a significant decrease in the damage index in the group treated with 50% juice. In the case of chronic treatment, the damage index was reduced only in the groups treated with 100% juice. These results were attributed to the phytochemical compounds of the juice, which could have acted through two distinct mechanisms depending on the treatment the animals underwent. In the case of acute treatment, the antigenotoxic effect was attributed to the deactivation of free radicals by bioactive compounds. In the longer subacute and chronic treatments, it is possible that the antigenotoxic effect was

due to the induction of antioxidant enzymes triggered by the presence of the anthocyanin flavonoids present in the juice[82].

Inoue et al.[83] reported that camu-camu juice (70 mL containing 1,050 mg of vitamin C) showed a significant effect in reducing markers of oxidative stress and anti-inflammatory activity in humans. Urine (8-hydroxy-deoxyguanosine) and 10 proinflammatory cytokines present in the blood, including interleukin 6 and 8, were analysed in 20 smokers who consumed the juice for 7 days. In this study, it was also shown that the control group (who received a 1,050 mg vitamin C supplement) did not exhibit the same effects as those individuals supplemented with the juice. Thus, the observed effect was attributed to other antioxidant substances present in the juice.

Due to the promising results obtained in studies of the bioactive compounds from acai and camu-camu, other fruits from the Amazon biome are being studied, including the "pequiá" (*Caryocar villosum*). Recent studies have investigated the bioactive composition of pequiá[84], its ability to deactivate reactive oxygen and nitrogen species[20] and its antigenotoxic effect in multiple mouse organs[85].

The major phenolic compounds identified and quantified by HPLC-PDA-MS/MS in pequiá were gallic acid (182.4  $\mu\text{g.g}^{-1}$  FM), ellagic acid (104  $\mu\text{g.g}^{-1}$  FM) and ellagic acid-rhamnose (107  $\mu\text{g.g}^{-1}$  FM)[84]. As for carotenoid levels, Chisté & Mercadante[84] reported that the major components were all-*trans*-antheraxanthin (3.4  $\mu\text{g.g}^{-1}$  FM), all-*trans*-zeaxanthin (2.9  $\mu\text{g.g}^{-1}$  FM) and lutein-like (2.8  $\mu\text{g.g}^{-1}$  FM). Using the ORAC method, Chisté & Mercadante[84] showed that the antioxidant activity of pequiá pulp (3.74 $\pm$ 1.09 mmol TE.100 g<sup>-1</sup>) is greater than the average value (2.7 mmol TE.100 g<sup>-1</sup>) reported for both 41 fruits consumed in the United States[86] and two Amazonian fruits[87], *B. crassifolia* (2.65 mmol TE.100 g<sup>-1</sup>) and *I. edulis* (2.34 mmol TE.100 g<sup>-1</sup>). However, these values were much lower than the activity of freeze-dried acai pulp (*Euterpe oleracea* Mart) (99.7 mmol TE.100 g<sup>-1</sup>)[88].

An evaluation of the potential of pequiá to deactivate reactive oxygen and nitrogen species, Chisté et al.[20] demonstrated that aqueous (aq.) and aqueous/alcoholic extracts (aq/alc.) contained a high content of phenolic compounds, totalling 5,163 and 1,745  $\mu\text{g.g}^{-1}$  of the extract, respectively. In turn, the antioxidant activity of these extracts against superoxide radicals (IC<sub>50</sub>=15 $\pm$ 5  $\mu\text{g.mL}^{-1}$  aq.; 37  $\pm$ 8  $\mu\text{g.mL}^{-1}$  aq/alc.), hydrogen peroxide (IC<sub>50</sub>=19 $\pm$ 4  $\mu\text{g.mL}^{-1}$  aq.; 23 $\pm$ 2  $\mu\text{g.mL}^{-1}$  aq/alc.), hypochlorous acid (IC<sub>50</sub>=6.3 $\pm$ 2.3  $\mu\text{g.mL}^{-1}$  aq.; 3.6 $\pm$ 1.0  $\mu\text{g.mL}^{-1}$  aq/alc.), singlet oxygen (IC<sub>50</sub>=156 $\pm$ 11  $\mu\text{g.mL}^{-1}$  aq.; 74 $\pm$ 8  $\mu\text{g.mL}^{-1}$  aq/alc.), nitric oxide (IC<sub>50</sub>=4.8 $\pm$ 0.6  $\mu\text{g.mL}^{-1}$  aq.; 2.8 $\pm$ 0.8  $\mu\text{g.mL}^{-1}$  aq/alc.) and peroxy nitrite (IC<sub>50</sub>=17 $\pm$ 0.8  $\mu\text{g.mL}^{-1}$  aq.; 4.8 $\pm$ 8  $\mu\text{g.mL}^{-1}$  aq/alc.) was positively correlated with the content of phenolic compounds. Additionally, the extracts (ethanol, ethanol/ethyl acetate and ethyl acetate) that primarily contained carotenoids had less or no antioxidant activity when compared to other aqueous and alcoholic extracts[20].

The ingestion of pequiá pulp also resulted in a significant reduction in DNA damage induced by doxorubicin in the liver, kidney, heart, and bone marrow cells of rats[85]. Furthermore, it was observed that small pulp doses of 75 mg.kg<sup>-1</sup> bw administered by gavage for 14 days caused a reduction in DNA damage of 81.1% in liver cells, 56.5% in kidney cells and 42.8% in heart cells. Those authors also reported that higher doses of pequiá pulp (150 and 300 mg.kg<sup>-1</sup> bw) resulted in a smaller reduction in DNA damage, showing an inverse dose-response relationship[85].

#### 4.2. Cerrado

Bioactive compounds from the Cerrado may be the least explored of the Brazilian biomes, especially when compared with Amazonian fruits. However, some studies have evaluated the bioactive compounds and antioxidant activity of fruits such as "marolo", "jenipapo", "murici", soursop, sweet passion fruit[89], araticum/marolo[90],[91], "lobeira", "cagaita", "banha de galinha"[91] and pequi (*Caryocar brasiliense*)[91],[92]. Some results of these studies are shown in Table 4.

Among the fruits studied by De Souza et al.[89], the pulp of araticum or marolo showed the greatest antioxidant potential (131.58  $\mu\text{mol TE.g}^{-1}$ ), which was attributed to the high content of polyphenols (739.37 mg GAE.100 g<sup>-1</sup>) and ascorbic acid (59.05 mg.100 g<sup>-1</sup>) present in this fruit. Araticum is a member of the *Annonaceas* family, which contains a variety of exotic fruit-producing species and whose fruits have a rustic appearance and the characteristic shape of the conde fruit (*Annona squamosa*). This family also contains the soursop (*Annona muricata*), which had an antioxidant activity 3.5 lower than araticum. These two fruits are eaten by the local population in natura, but can also be used to prepare juices, ice creams and jams.

Among the fruit studied by Roesler et al.[91], the best antioxidant activity results as measured by the DPPH method were found for the peel of pequi (IC<sub>50</sub> of 9.44  $\mu\text{g.mL}^{-1}$  for the ethanol extract and 17.98  $\mu\text{g.mL}^{-1}$  for the aqueous extract). However, when comparing the edible fractions of the fruit (pulp, peel+pulp, pulp+seed), it was found higher antioxidant activity in araticum (IC<sub>50</sub> of 148.82  $\mu\text{g.mL}^{-1}$  for the ethanol extract) and lobeira (IC<sub>50</sub> of 162.97  $\mu\text{g.mL}^{-1}$  for the ethanol extract). This study also reported a significant correlation between IC<sub>50</sub> values and the total phenolic compound content in fruits, with an  $r = 0.9966$ [91].

The pequi is the Cerrado fruit that is most consumed by the local population. This fruit is an important part of local dishes such as chicken with pequi, and it is also eaten with rice, beans and "farinha" (yuca flour). The characteristic colour of dishes cooked with this fruit comes from the high concentration of carotenoids (7.25 mg.100 g<sup>-1</sup>)[93] mostly violaxanthin, lutein and zeaxanthin, plus smaller amounts of neoxanthin,  $\beta$ -cryptoxanthin and  $\beta$ -carotene[94]. Roesler et al.[92] reported that ethanol extracts of the pequi peel (IC<sub>50</sub> 0.78  $\mu\text{g.mL}^{-1}$ ) inhibited lipid peroxidation of rat liver microsomes at concentrations similar to those reported using the same TBARS method for highly reactive polyphenols

such as gallic acid ( $IC_{50}$  1.01  $\mu\text{g}\cdot\text{mL}^{-1}$ ) and quercetin ( $IC_{50}$  1.18  $\mu\text{g}\cdot\text{mL}^{-1}$ ). The powerful antioxidant action of the pequi peel was attributed to the polyphenols gallic acid, quinic acid, quercetin and quercetin 3-arabinoside, which were identified in the alcoholic extract of the fruit by ESI-MS fingerprinting[92]. Aqueous and organic extracts obtained from pequi pulp demonstrated a protective effect against oxidative damage to DNA caused by two antineoplastic drugs, indicating the ability to inhibit chemical mutagenesis *in vivo*[95]. However, this same study found that the aqueous extract of pequi led to greater lipid peroxidation in rats of both sexes *in vivo*, as measured by the TBARS method, while the organic extract caused an increase in lipid peroxidation only in male rats.

A paradoxical effect was observed by Aguilar et al.[96] in rats treated for 6 weeks with a hypercholesterolemic diet supplemented with 7% soybean oil in the control group and 7% pequi oil in the treated group. The group treated with pequi showed a greater atherogenic lipid profile and more advanced atherosclerotic lesions in the aortic root than the control group. However, the pequi group also had less advanced lesions in the aorta and showed less lipid peroxidation in the liver. These results suggest that a diet rich in pequi oil slows atherogenesis in the early stages, possibly due to the antioxidant activity of pequi oil, which is extremely rich in carotenoids. However, the increased serum

cholesterol induces a prominent migration of LDL through the inside of the arteries, increasing the advance of atherosclerotic plaque[96].

### 5.3. Atlantic Forest

Jaboticaba (*Myrciaria cauliflora*) belongs to the Myrtaceae family, is native to Brazil and is distributed throughout the Atlantic Forest biome. Recent studies have demonstrated the presence of bioactive compounds with strong antioxidant activity. Abe et al.[97] reported that jaboticaba had the highest levels of total ellagic acid ( $3.11\pm 0.19$   $\text{g}\cdot\text{kg}^{-1}$  FW) when compared with the 34 other fruits analysed. Variation in the levels of total and free ellagic acid was also evaluated in the pulp, peel and seeds of jaboticaba in relation to the ripening stage. It was observed that in the unripe stage, the levels of total ellagic acid were higher in all fruit parts. In ripe fruits, the seeds showed the highest level ( $40.18\pm 0.60$   $\text{g}\cdot\text{kg}^{-1}$  FW) followed by the peel ( $22.5\pm 1.3$   $\text{g}\cdot\text{kg}^{-1}$  FW). The evaluation of the antioxidant activity of the 10 fruits richest in ellagic acid against the DPPH radical demonstrated that jaboticaba exhibits excellent activity ( $62\pm 6$   $\text{mmol TE}\cdot\text{kg}^{-1}$  FW), second to only camu-camu ( $141\pm 6$   $\text{mmol TE}\cdot\text{kg}^{-1}$  FW), which had a high concentration of ascorbic acid[97].

**Table 4.** Bioactive compounds and antioxidant activity from Brazilian Cerrado fruits

Name	Fruit Scientific Name	Bioactive Compounds	Antioxidant Activity
Jenipapo[89]	<i>Genipa americana</i>	Total phenols ( $47.94\pm 1.81$ $\text{mg GAE}\cdot 100\text{g}^{-1}$ FW) Ascorbic acid ( $27.01\pm 2.84$ $\text{mg}\cdot 100\text{g}^{-1}$ FW) Carotenoids ( $0.93\pm 0.03$ $\text{mg}\beta\text{-carotene}\cdot 100\text{g}^{-1}$ FW)	ABTS ( $7.31\pm 1.74$ $\mu\text{mol TE}\cdot\text{g}^{-1}$ FW)
Araticum or Marolo[89]	<i>Annona crassiflora</i>	Total phenols ( $739.37\pm 7.92$ $\text{mg GAE}\cdot 100\text{g}^{-1}$ FW) Ascorbic acid ( $59.05\pm 0.46$ $\text{mg}\cdot 100\text{g}^{-1}$ FW) Carotenoids ( $0.57\pm 0.01$ $\text{mg}\beta\text{-carotene}\cdot 100\text{g}^{-1}$ FW)	ABTS ( $131.5\pm 19.6$ $\mu\text{mol TE}\cdot\text{g}^{-1}$ FW)
Murici[89]	<i>Byrsonima crassifolia</i> L. RICH	Total phenols ( $334.37\pm 9.07$ $\text{mg GAE}\cdot 100\text{g}^{-1}$ FW) Ascorbic acid ( $47.44\pm 3.26$ $\text{mg}\cdot 100\text{g}^{-1}$ FW) Carotenoids ( $1.25\pm 0.12$ $\text{mg}\beta\text{-carotene}\cdot 100\text{g}^{-1}$ FW)	ABTS ( $57.25\pm 4.05$ $\mu\text{mol TE}\cdot\text{g}^{-1}$ FW)
Soursop[89]	<i>Annona muricata</i> L.	Total phenols ( $281.00\pm 5.40$ $\text{mg GAE}\cdot 100\text{g}^{-1}$ FW) Ascorbic acid ( $21.83\pm 3.99$ $\text{mg}\cdot 100\text{g}^{-1}$ FW) Carotenoids ( $1.21\pm 0.17$ $\text{mg}\beta\text{-carotene}\cdot 100\text{g}^{-1}$ FW)	ABTS ( $35.95\pm 2.04$ $\mu\text{mol TE}\cdot\text{g}^{-1}$ FW)
Sweet passion fruit[89]	<i>Passiflora alata</i> Dryand	Total phenols ( $245.36\pm 3.70$ $\text{mg GAE}\cdot 100\text{g}^{-1}$ FW) Ascorbic acid ( $24.66\pm 4.29$ $\text{mg}\cdot 100\text{g}^{-1}$ FW) Carotenoids ( $1.31\pm 0.03$ $\text{mg}\beta\text{-carotene}\cdot 100\text{g}^{-1}$ FW)	ABTS ( $10.84\pm 2.20$ $\mu\text{mol TE}\cdot\text{g}^{-1}$ FW)
Araticum or Marolo[90]	<i>Annona crassiflora</i>	Total phenols ( $31.08\pm 1.23$ $\text{g GAE}\cdot\text{kg}^{-1}$ DM – ethanolic extract; $17.01\pm 1.87$ $\text{g GAE}\cdot\text{kg}^{-1}$ DM – aqueous extract)	DPPH – $IC_{50}$ ( $1204.22\pm 27.43$ $\mu\text{g}\cdot\text{mL}^{-1}$ – ethanolic extract; aqueous extract not measured)
Banha de galinha[91]	<i>Swartzia langsdorfi</i>	Total phenols ( $4.68\pm 0.57$ $\text{g GAE}\cdot\text{kg}^{-1}$ pulp DM – ethanolic extract; $1.59\pm 0.50$ $\text{g GAE}\cdot\text{kg}^{-1}$ pulp DM – aqueous extract)	not measured in pulp
Cagaita[91]	<i>Eugenia dysenterica</i>	Total phenols ( $18.38\pm 0.81$ $\text{g GAE}\cdot\text{kg}^{-1}$ pulp+peel DM – ethanolic extract; $16.23\pm 1.36$ $\text{g GAE}\cdot\text{kg}^{-1}$ pulp+peel DM – aqueous extract)	DPPH – $IC_{50}$ ( $387.47\pm 8.7$ $\mu\text{g}\cdot\text{mL}^{-1}$ – ethanolic extract; $879.33\pm 11.7$ $\mu\text{g}\cdot\text{mL}^{-1}$ – aqueous extract)
Lobeira[91]	<i>Solanum lycocarpum</i>	Total phenols ( $35.58\pm 19.72$ $\text{g GAE}\cdot\text{kg}^{-1}$ pulp+seed DM – ethanolic extract; $25.81\pm 2.22$ $\text{g GAE}\cdot\text{kg}^{-1}$ pulp+seed D.M. – aqueous extract)	DPPH – $IC_{50}$ ( $162.97\pm 2.05$ $\mu\text{g}\cdot\text{mL}^{-1}$ – ethanolic extract; $199.34\pm 2.75$ $\mu\text{g}\cdot\text{mL}^{-1}$ – aqueous extract)
Pequi[91]	<i>Caryocar brasiliense</i>	Total phenols ( $27.19\pm 1.25$ $\text{g GAE}\cdot\text{kg}^{-1}$ pulp+seed DM. – ethanolic extract; $20.88\pm 3.45$ $\text{g GAE}\cdot\text{kg}^{-1}$ pulp+seed D.M. – aqueous extract)	DPPH – $IC_{50}$ ( $298.75\pm 3.80$ $\mu\text{g}\cdot\text{mL}^{-1}$ – ethanolic extract; $534.43\pm 7.32$ $\mu\text{g}\cdot\text{mL}^{-1}$ – aqueous extract)

FW: Fresh Weight; DM: Dry Matter; GAE: Gallic Acid Equivalent

**Table 5.** Bioactive compounds and antioxidant activity from Atlantic Rainforest fruits

Name	Fruit Scientific Name	Bioactive Compounds	Antioxidant Activity
Jussara[104]	<i>Euterpe edulis</i>	Total monomeric anthocyanins (14.84 – 409.85 mg C3G.100g <sup>-1</sup> FM) Total phenolic acids (24.87 – 73.60 mg.100g <sup>-1</sup> FM) Total flavonoids (30.83 – 63.33 mg.100g <sup>-1</sup> FM)	DPPH (EC <sub>50</sub> : 0.85 – 4.83 mg.mL <sup>-1</sup> )
Jussara[19]	<i>Euterpe edulis</i>	Ascorbic acid (186±43.3 mg.100g <sup>-1</sup> FM) Total anthocyanins (192±43.2 mg.100g <sup>-1</sup> FM) Yellow flavonoids (375±87.6 mg.100g <sup>-1</sup> FM) Total carotenoids (1.9±0.5 mg.100g <sup>-1</sup> FM) Chlorophyll (21.5±4.1 mg.100g <sup>-1</sup> FM)	DPPH EC <sub>50</sub> : 1711±46 g.g <sup>-1</sup> ABTS 78.3±13.3 μmol trolox. g <sup>-1</sup> FRAP 84.9±16.1 μmol Fe <sub>2</sub> SO <sub>4</sub> .g <sup>-1</sup> β-carotene bleaching: 70.8±7.9%
Pitanga[100]	<i>Eugenia uniflora</i>	Total phenolics: 2.45±0.08 11g FAE.100g <sup>-1</sup> DM in red variety; 3.09±0.11g FAE.100g <sup>-1</sup> DM in purple variety; Total flavonoids glycosides: 698.91±18.59 μg AE.g <sup>-1</sup> DM in red variety; 2857.22±46.05 μg AE.g <sup>-1</sup> DM in purple variety;	DPPH (%) 35.5±1.2 in red variety; 43.5±0.7 in purple variety;
Guabiroba[105]	<i>Campomanesia xanthocarpa</i> O. Berg	Total phenolic compounds: 9033.19±1428.3 mg GAE.100g <sup>-1</sup> DM Total Carotenoid: 305.53±22.98 μg.g <sup>-1</sup> DM Ascorbic acid: 30.58±3.91 mg.g <sup>-1</sup> DM	ABTS: 507.49±29.17 μmol Trolox.g <sup>-1</sup> DM DPPH (EC <sub>50</sub> ): 161.29±12.09 g DM.g <sup>-1</sup> DPPH
Uvaia[105]	<i>Eugenia pyriformis</i> Cambess	Total phenolic compounds: 3482.04±74.1 mg GAE.100g <sup>-1</sup> DM Total Carotenoid: 909.33±270.90 μg.g <sup>-1</sup> DM Ascorbic acid: 0.7±0.37 mg.g <sup>-1</sup> DM	ABTS: 336.29±38.19 μmol Trolox.g <sup>-1</sup> DM DPPH (EC <sub>50</sub> ): 170.26±13.21 g DM.g <sup>-1</sup> DPPH
Uvaia[19]	<i>Eugenia pyriformis</i>	Ascorbic acid (39.3±5.2 mg.100g <sup>-1</sup> FM) Total anthocyanins (1.13±0.1 mg.100g <sup>-1</sup> FM) Yellow flavonoids (17.5±1.6 mg.100g <sup>-1</sup> FM) Total carotenoids (1.7±0.1 mg.100g <sup>-1</sup> FM) n.d.	DPPH EC <sub>50</sub> : 3247±392 g.g <sup>-1</sup> ABTS: 18±0.8 μmol trolox. g <sup>-1</sup> FRAP: 38.4±4.1 μmol Fe <sub>2</sub> SO <sub>4</sub> .g <sup>-1</sup> β-carotene bleaching: 79.8±5.9%
Jambolão[106]	<i>Syzygium cumini</i>	Total phenols: 148.3±32.4 mg GAE.100g <sup>-1</sup> FM Total flavonoids: 91.2±15.7 mg CE.100g <sup>-1</sup> FM Monomeric anthocyanins: 210.9±0.91 mg C3G.100g <sup>-1</sup> FM Total Carotenoids: 89.2±5.4 μg 100g <sup>-1</sup> FM	ABTS 4.8±0.6 TEAC % Singlet Oxygen Protection 60.6±4.1 ORAC 16.4±0.1 TEAC
Jaboticaba[97]	<i>Myrciaria cauliflora</i>	Total phenolics: 7.44±0.32 g GAE.kg <sup>-1</sup> FW Total ellagic acid: 3.11±0.19 g.kg <sup>-1</sup> FW Anthocyanins: 0.32±0.02 g C3G.kg <sup>-1</sup> FW Ascorbic acid: 0.25±0.01 g.kg <sup>-1</sup> FW	DPPH: 62±6 mmol Trolox.kg <sup>-1</sup> FW

FM: Fresh Matter

In another study, Wu et al.[98] determined the composition of phenolics in fresh fruit extracts and the juice of jaboticaba using HPLC-PDA-HR-ESI-TOF-MS, successfully identifying twenty-two compounds. In the extract, the phenolics identified were mainly cyanidin 3-glucoside (29.8±1.73 mg.10 g<sup>-1</sup> FW), quercetin (11.57±0.66 mg.10 g<sup>-1</sup> FW), delphinidin 3-glucoside (7.36±0.64 mg.10 g<sup>-1</sup> FW) and gallic acid (5.07±0.48 mg.10 g<sup>-1</sup> FW). The majors constituents found in the juice were casuarictin (7.08±0.30 mg.10 g<sup>-1</sup> FW) and casuarinin (2.49±0.09 mg.10 g<sup>-1</sup> FW), both of which are derived from gallic acid and were reported in jaboticaba for the first time[98]. The same authors also found that processed products such as juice and jam lacked the anthocyanins cyanidin 3-glucoside and delphinidin 3-glucoside, which are major compounds of the fresh jaboticaba fruit. Another interesting point was the detection of citric acid and tributyl

citrate only in processed products. The authors also compared the antioxidant activity of fresh fruit and processed jaboticaba products against the ABTS and DPPH radicals. The results showed that the extracts from fresh fruits were more active against the DPPH radical (IC<sub>50</sub>= 0.282±0.009 mg.mL<sup>-1</sup> in fruit extract, 0.453±0.00 mg.mL<sup>-1</sup> in juice, 0.618±0.023 mg.mL<sup>-1</sup> in jam). The results obtained by the ABTS method showed higher antioxidant activity in the juice than in fresh fruit extract and jam. The difference between the results obtained by each method was attributed to overlapping bands of DPPH and anthocyanins in the UV-Vis spectra[98].

The extraction of bioactive compounds from the peel of jaboticaba was studied by Santos et al.[99] who evaluated levels of total phenolic compounds, monomeric anthocyanins and antioxidant activity of extracts obtained using ultrasound, orbital shaking, combining ultrasound and

orbital shaking, soxhlet with ethanol and soxhlet with acidified ethanol (pH 3.0). The highest level of total phenolic compounds was obtained by the soxhlet with acidified ethanol method (35.85 mg GAE.g<sup>-1</sup>), while the highest content of monomeric anthocyanins was obtained by the orbital shaking method (6.18 mg C3G.g<sup>-1</sup>). When the antioxidant activity of the extracts was compared, all types of extract showed less activity than the reference substances used (butylated hydroxytoluene (BHT) and quercetin). As expected, the extract with the highest concentration of phenolic compounds showed the highest antioxidant activity, measured by the method of  $\beta$ -carotene/linoleic acid discoloration. However, the per cent inhibition of oxidation by the extracts was significantly different, and thus the authors indicated that the establishment of the composition of the extracts was fundamental for the choice of the extraction method to be used[99].

The “pitanga” (*Eugenia uniflora*) is a tree native to the Atlantic Forest of southern and south-eastern Brazil, where the fruit is eaten in natura and is also consumed in the form of juices, jams and ice creams. The study by Celli et al.[100] found that the levels of phenolic compounds and antioxidant activity against DPPH decreased as the cherry ripened in both the red and purple varieties; however, even in the intermediate stages of ripening these values were much lower than in the unripe stage. When the two varieties were compared (Table 5), the purple variety had a higher antioxidant activity than the red; however, this difference was not significant.

Another study compared the levels of bioactive compounds and antioxidant activity of three varieties of pitanga grown in the State of Rio Grande do Sul[101]. It was found that the antioxidant activity against the DPPH radical was similar among the three varieties (37 mmol TE.100 g<sup>-1</sup> in the purple variety and 41 mmol TE.100 g<sup>-1</sup> in the red and orange varieties). However, against the FRAP radical, the purple variety (8.2 mmol TE.100 g<sup>-1</sup>) had twice the antioxidant activity of the orange (4.4 mmol TE.100 g<sup>-1</sup>) and red (4.2 mmol TE.100 g<sup>-1</sup>) varieties. The difference between the results was attributed to the higher concentration of anthocyanins in the purple variety (136±6 mg.100 g<sup>-1</sup>) than in the red (69±3 mg.100 g<sup>-1</sup>) and orange (25±1 mg.100 g<sup>-1</sup>) varieties. The antioxidant activity (determined by DPPH and FRAP) was positively correlated with the levels of phenolic compounds, but not with the levels of carotenoids, and lycopene was the major carotenoid identified in the red (166±7  $\mu$ g.g<sup>-1</sup>) and orange (151±30  $\mu$ g.g<sup>-1</sup>) varieties[101].

The Surinam cherry has huge potential as a source of carotenoids through the use of supercritical extraction. Furthermore, the use of different processing conditions (temperature, pressure and using ethanol as cosolvent) enabled the selective extraction of the following major carotenoids: all-*trans*-rubixanthin, all-*trans*-lycopene and all-*trans*- $\beta$ -cryptoxanthin[102]. At 40°C and 300 bar, rubixanthin was mostly extracted, and at 60°C and 250 bar lycopene was the carotenoid extracted at the highest concentration. However, the study did not evaluate the

antioxidant potential of the extracts.

Among the fruits native to the Atlantic Forest, jussara has begun to gain prominence. The fruit has a round shape and dark purple colour due to the presence of anthocyanins. It has nutritional and organoleptic properties similar to those of acai and can be consumed the same way. Some studies have evaluated its bioactive composition. De Brito et al. [103] reported the HPLC-MS identification and quantification of six anthocyanins (cyanidin 3-sambubioside, cyanidin 3-glucoside, cyanidin 3-rutinoside, perlagonidin 3-glucoside, perlagonidin 3-rutinoside and cyanidin 3-ramnoside) in jussara pulp from the State of São Paulo. Da Silva Campelo Borges et al.[104] tentatively identified and quantified four phenolic acids (ferulic, gallic, protocatechuic and *p*-coumaric) and three flavonoids (catechin, epicatechin and quercetin) in fruit pulp from the state of Santa Catarina. Rufino et al.[19] reported the quantification of ascorbic acid (186 ± 43.3 mg.100 g<sup>-1</sup>), total anthocyanins (192 ± 43.2 mg.100 g<sup>-1</sup>), yellow flavonoids (375 ± 87.6 mg.100 g<sup>-1</sup>), total carotenoids (1.9 ± 0.5 mg.100 g<sup>-1</sup>) and chlorophyll (21.5 ± 4.1 mg.100 g<sup>-1</sup>) in jussara pulp from the State of São Paulo. In addition to establishing the composition of some bioactive compounds, Rufino *et al.*[19] and Da Silva Campelo Borges et al.[104] also evaluated the antioxidant activity of juçara against the ABTS and DPPH radicals (Table 5).

The bioactive levels and antioxidant activity of other fruits such as “uvaia”[105], “guabiroba”[105], and jambolão[106] have also been analysed. The results are shown in Table 05.

## 6. Conclusions

The results of studies investigating Brazilian tropical fruits demonstrate the incredible potential of the biodiversity in the different biomes in terms of the variety and quantity of bioactive compounds. It is also worth noting that the methods employed to measure the antioxidant activity of these compounds and extracts from fruits are still very limited when trying to establish relationships with their activity *in vivo*. Despite the extensive use of chemical methods, there are still several questions regarding their results, as most of the studies used radicals that are not found in biological systems, did not reproduce the physiological conditions of the cells and did not consider the bioavailability and metabolism of the bioactive compounds.

Biological systems are much more complex than the chemical mixtures applied in the chemical methods employed. In addition, antioxidant compounds can act through diverse mechanisms. Animal models and human studies are the best methods to determine the actual antioxidant activity of bioactive compounds in the body, but such studies are expensive and time-consuming. Therefore, more effective methods, such as Cellular Antioxidant Activity, are being developed to determine the antioxidant potential of foods and their ingredients. Furthermore, the great chemical diversity of antioxidant compounds complicates the separation and evaluation of individual

antioxidant activity. Thus, most methods measure the total antioxidant capacity of the fruit using extracts prepared with different aqueous or organic solvents that contain antioxidant species.

It is therefore necessary to use methods that measure the antioxidant activity in cell systems and *in vivo* to establish the effect of diet (i.e., the matrix of food items) on the bioavailability of these compounds. When combined, the results from these two areas may facilitate the recommendation of fruit types and quantities in the Brazilian diet, taking into account the benefits that each variety can offer.

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